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# Chronic Treatment with DCPCX, an Adenosine $A_1$ Antagonist, Worsens Long-Term Memory

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## Abstract

Alzheimer's disease is characterized by progressive cognitive disturbances and neurotransmitter dysfunction. Previous studies targeting the adrenergic $A_1$ pathway suggest that this plays a role in cognitive impairment in Alzheimer's disease. Previous studies have reported that acute treatment with $A_1$ antagonists appears to improve behavioral deficits in rodent models of memory and behavioral impairment. In this study, we addressed whether the chronic administration of 8-cyclopentyl-1,3-dipropylxanthine, a potent and selective adenosine A1 antagonist, could reverse the memory deficits found in aged APPswe/PS1dE9 mice. Chronic treatment did not improve memory in the APPswe/PS1dE9 mouse model and resulted in reduced exploratory behavior, suggestive of reduced anxiety, and a worsening of long-term memory in nontransgenic mice. These results have important implications for understanding the mechanisms of A1 receptor modulation as a target in Alzheimer's disease therapy.

### Keywords

Alzheimer’s disease; Adenosine A1 antagonist; Chronic treatment; Impaired learning; Anxiolytic

## 1. INTRODUCTION

Alzheimer’s disease (AD) is a neurodegenerative disorder resulting in progressive cognitive impairment and the loss of short term memory. The development of AD is characterized by significant alterations in neurotransmitter pathways. In the CNS, adenosine acts as a neuromodulator, controlling the release of several neurotransmitters including, acetylcholine and glutamate that regulate synaptic transmission and neuronal excitability [7]. The effect of adenosine is mediated by four main G protein coupled receptors (GPCRs), namely $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$, which have been cloned and pharmacologically characterized. $A_1$ and $A_3$ receptors couple mainly to the inhibitory $G_I$ family of proteins, whereas $A_{2A}$ and $A_{2B}$ receptors activate adenylate cyclase by coupling to stimulatory $G_s$ proteins [5]. In AD, GPCR function in amyloid precursor protein (APP) processing and tau phosphorylation is compromised [10].



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A number of studies have suggested that adenosine receptors are a potential pharmacological target for the treatment of AD. In neuropathological studies of patients with late-onset AD, there is a clustering of $A_1$ receptors in association with the degenerating neurons containing neurofibrillary tangles and within dystrophic neurites [3]. In this study, the involvement of $A_1$ receptors in the production of soluble secreted form of APP as well as tau phosphorylation and its translocation to the cytoskeleton was demonstrated; Albasanz et al [1] similarly demonstrated an up-regulation of $A_1$ receptors in the frontal cortex of AD patients.

In rodent models, short-term activation of the $A_1$ receptor has been associated with disruptions of learning and memory, whereas selective blockade of the receptor has shown improvement in various behavioral tasks [6, 11, 12, 15, 17, 18, 21, 22, 25]. Treatment of mice with $N^6$-Cyclopentyladenosine (CPA), a highly selective agonist for the $A_1$ receptor, lead to deficits in retention performance. The CPA-elicited deficits in retention performance were, however, blocked by pretreatment with 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective $A_1$ receptor antagonist [16]. In studies conducted by Homayoun et al. [13] to determine the effects of adenosine receptor ligands on amnesia induced by pentylenetetrazole in mice, acute administration of adenosine receptor antagonists, theophylline and 8-phenyltheophylline resulted in a dose dependent reduction in the amnestic effect of pentylenetetrazole.

Although inhibition of A1 receptors is suggested to be a potent target for treatment of memory loss in AD and other cognitive disorders, all of the reported studies on pharmacological modulation of A1 receptors have employed acute pharmacological manipulation to study effects on cognitive behavior. The present study was designed to assess whether chronic administration of a selective adenosine receptor antagonists in an aged mouse model of Alzheimer's disease had a positive impact on learning and memory.

## 2. MATERIALS AND METHODS

### 2.1. Animals

This study used 15-month old APPswe/PS1dE9 (APdE9) double transgenic mice with a C57BL/6J background that were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Genotypes were confirmed by PCR. Treatment groups and number of mice were as follows: non-transgenic littermates (control; n = 12), non-transgenic DPCPX (N=5), transgenic APdE9 (n = 7), APdE9/DPCPX (n = 7). Male mice and female mice were randomized across groups. All mice used in the present study were housed in a room maintained at 23 °C ± 1 °C with a 12 h light–dark cycle and free access to food and water.

### 2.2. Drug treatment

Animals were injected with 0.9% sterile saline solution with 5% DMSO or 1 mg/kg DPCPX (Tocris Bioscience) dissolved in 5% DMSO once daily intra-peritoneally (IP) for 60 days, prior to conducting behavioral tests. Animals received daily injections during the course of the behavioral studies.

### 2.3. Open Field test

Anxiety-like and non-cognitive behavior was assessed by the open field test. Each mouse was allowed to explore a novel environment in a clear chamber for 30 min while being monitored (OptoMax, Columbus Instruments) with standard room lighting conditions [23].

### 2.4. Morris Water Maze test

Spatial learning and memory acquisition was assessed by a modified Morris Water Maze (MWM) paradigm, as previously described [8]. On the 5$^{th}$ day, 24 hours after the last trial, a second probe test was used to assess long-term spatial memory.

### 2.5. Rotarod test

Motor coordination and balance were evaluated by a motarized rotarod. Each mouse was placed on a horizontal accelerating rod (4–40 rpm) and subjected to 4 trials (5-min/trial) for one day with 15 min. intervals between each trial. A trial ended when the mouse either fell off the rod or time elapsed 5 minutes.

### 2.6. Statistical analyses

Quantitative results were expressed as mean ± standard error of mean (SEM). Data was analyzed using one-way ANOVA and Fisher LSD post hoc test to determine significant differences between groups. $p \leq 0.05$ was considered statistically significant.

## 3. RESULTS

### 3.1. Effect of DPCPX on mouse weight

Mouse weight was measured each week during treatment to determine if chronic DPCPX treatment might be toxic. No significant difference in mice weight was observed during treatment in APdE9 and nontransgenic mice (Fig. 1A).

### 3.2. Effect of DPCPX on non-cognitive behavior

Because impaired performance in learning tests can be affected by non-cognitive functions emotional-motivational and sensory-motor processes were assessed. Measures of open field performance, DPCPX treatment had no significant effect on exploratory stereotypic and resting behavior (Fig. 2A.). DPCPX treatment caused no significant difference in motor coordination, and balance on a rotarod (Fig. 2B).

### 3.3. Analysis of anxiety-like behavior in mice treated with DPCPX

To assess anxiety-like behavior in mice time spent in the center and in the margins was measured. DPCPX treatment had a significant increase in time spent in the center of the open field (Zone B), and in the margins (Zone A) in the nontransgenic, but not the APdE9, mice (Fig. 3A, $p < 0.05$).

### 3.4. Effect of DPCPX on Morris water maze test

In acquisition trials of the Morris water maze test from days 1 to 4, DPCPX treatment had no significant effect on escape latency between groups. APdE9 mice showed a significantly increased escape latency on days 2 and 3 when compared to nontransgenic vehicle treated animals, indicating impaired learning (Fig. 3A, $p < 0.05$). DPCPX had no effect on escape latency during the visual acuity trials ruling out visual impairment as a confounding variable (Fig. 3B). In the probe trial to assess short-term memory (STM), 1 hour after the last acquisition trial, there was no significant difference between groups in platform frequency (Fig. 3C). However, in the probe trial to assess long-term memory (LTM), 24 hours after the last acquisition trial, DPCPX treatment significantly decreased platform frequency in nontransgenic mice, indicating an impairment in long-term memory (Fig. 3D, $p < 0.05$).

## 4. DISCUSSION

The present paper describes the effect of chronic administration of DPCPX, a selective $A_1$ receptor antagonist on cognitive and non-cognitive properties as well as motor functions in wild type and in a transgenic mice model of AD. Studies that were conducted to test for changes in the behavioral patterns of the mice included the rotarod for effects on general motor performance, open field to monitor non-cognitive and anxiety-like behaviors, and the Morris water maze for spatial learning and memory. Animals treated during the course of the study did not appear to show detectable signs of toxicity from DPCPX treatment; there were no statistically significant changes in weight, movement in the open field and appearance in the treated animals. Treated mice did not exhibit any signs of toxicity or significant difference in motor deficits as was measured by motor coordination and balance on a motorized rotarod.

We observed, however, that DPCPX treatment did have significant effects on non-cognitive behavior. In the open field test, treated mice showed a reduced degree of exploration, suggestive that chronic administration of DPCPX may have an anxiolytic effect. This finding is in contrast to previous literature that showed acute treatment exerted an anxiogenic effect. A study conducted in mice showed that exposure to ethanol produced anxiolytic effects with pronounced hangover-induced anxiety between 12 and 18 hours after ethanol administration. The hangover-induced anxiety-like effect of ethanol could be reduced after acute administration of the selective $A_1$ receptor agonist 2-chloro-N(6)-cyclopentyladenosine. However, the anxiolytic property of CCPA was reversed by pretreatment with DPCPX.[19] Similarly, selective $A_1$ receptor blockade with cyclopentyltheophylline, as well as targeted disruption of the second coding exon of the $A_1$ receptor ($A_1R-/-$), also reduce anxiety in mice [9, 14].

In this study, chronic administration of DPCPX also had an impact on learning and memory. In the Morris water maze (MWM) test, which is designed to test hippocampal-based spatial learning, we found that nontransgenic mice treated with the drug demonstrated statistically significant reductions in crosses over the target platform in the long-term memory component of the MWM, suggesting that there was a mild cognitive impairment in long-term spatial memory with chronic DPCPX treatment. Previous studies using acute blockade of the $A_1$ receptor reported that drug treatment resulted in a significant improvement in learning and memory. For instance, in a study on the role of transient hypoxia in synaptic function impacting on spatial learning and memory, it was observed that brief hypoxia impaired Morris water maze performance, an effect that could be rescued with acute treatment with DPCPX [20]. Another study provided evidence that in an animal model of learning and memory, caffeine a nonspecific adenosine receptor antagonist, enhances memory retention [2]. Our results, however, are consistent with a previous study by Von Lubitz and colleagues [24] that demonstrated that C57BL/6 mice, tested for spatial memory acquisition in Morris water maze, failed to develop spatial preference after chronic inhibition of the $A_1$ receptor using 8-cyclopentyl-1,3-dipropylxanthine.

In summary, chronic administration of DPCPX exerts effects that are substantially different from those in acute treatment. Our data suggest that the effect of acute and chronic inhibition of the $A_1$ receptor produce significantly different behavioral patterns. The opposing effects of acute versus chronic dosing have also been observed in other diseases such as congestive heart failure and asthma [4]. Given the divergence between the short and long-term effects, the development of therapeutic approaches to chronically target the $A_1$ receptor for management of neurodegenerative diseases must be approached with care.

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## HIGHLIGHTS

- APPswe/PS1dE9 transgenic mice were treated with the A1-antagonist DPCPX for 60 days.
- Treated mice did not show overt signs of toxicity or demonstrate impaired motor performance.
- DPCPX reduced exploratory behavior, consistent with a general anxiolytic effect.
- Treatment worsened long-term memory in nontransgenic mice.

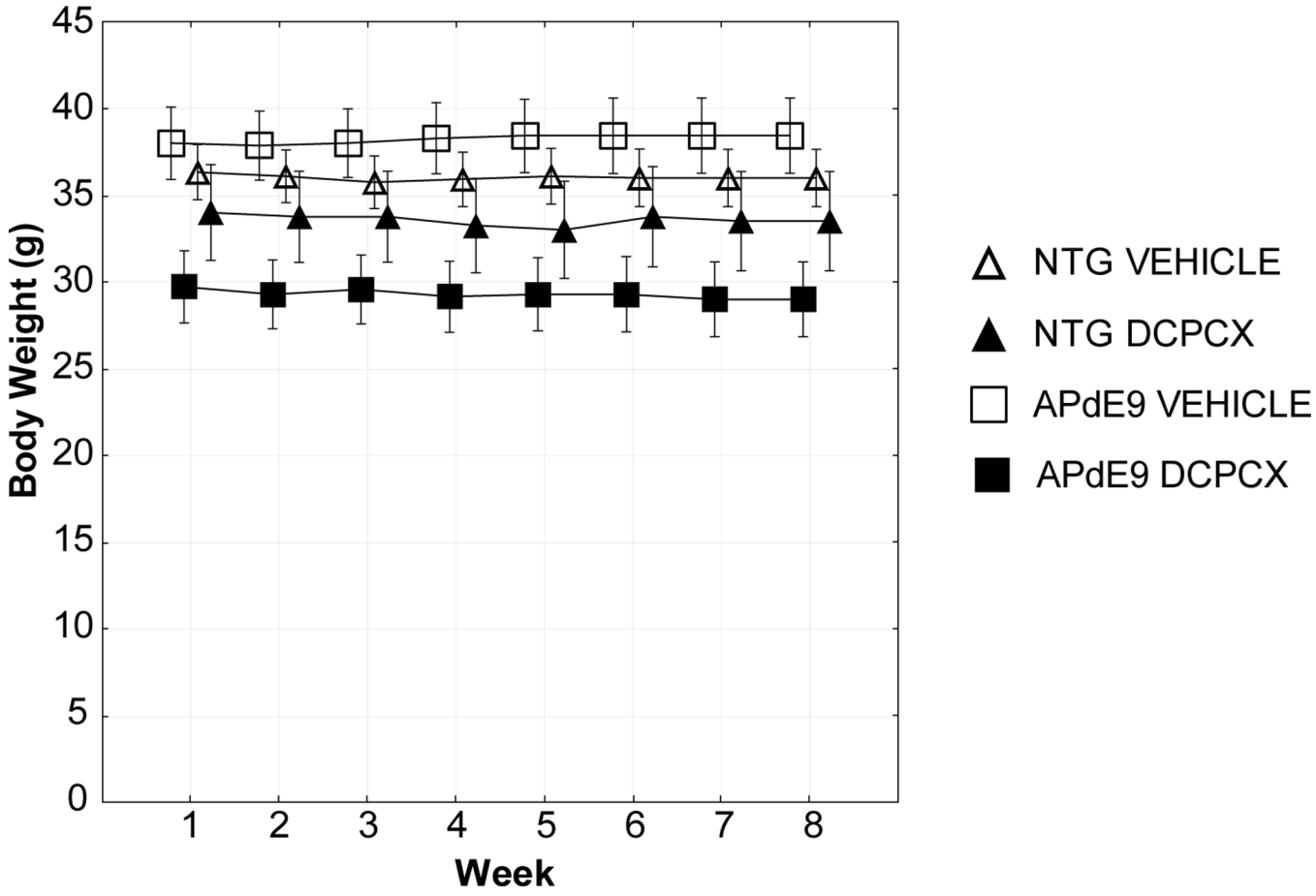


**Fig. 1. Effect of DPCPX treatment on weight**

Mice were weighed on a weekly basis; no change in weight in any group was seen over the 8 week treatment period. Mean ± SEM.

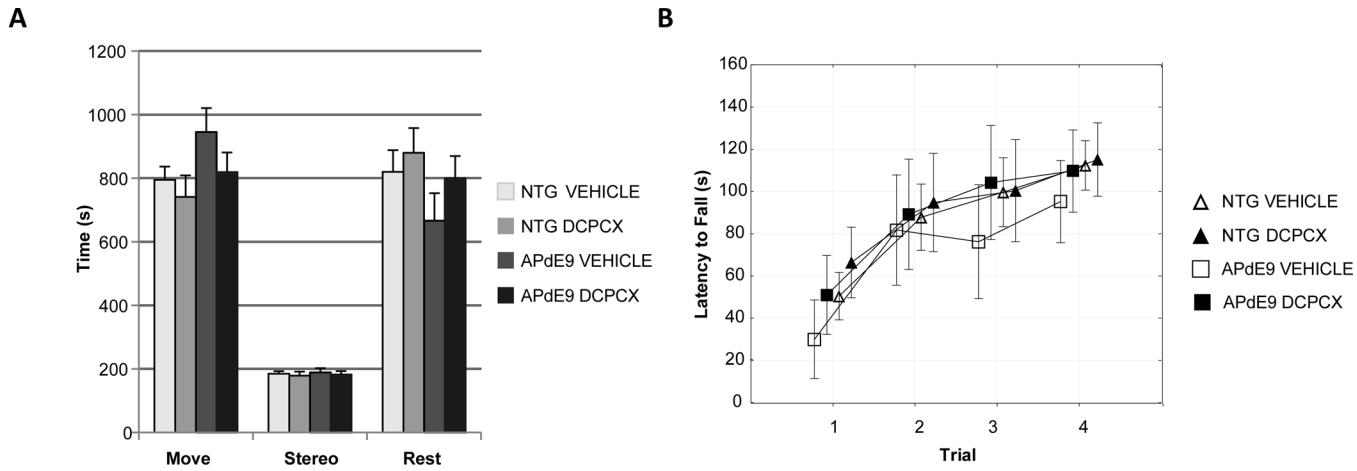


**Fig. 2. Effect of DPCPX treatment on non-cognitive behavior**

(A) Exploratory behavior measured by the open field activity was unaffected by treatment. (Move: Total activity time; Stereo: time moving in stereotypic fashion; Rest: Time at rest). (B) Motor coordination and balance with accelerating rotarod were unchanged with DCPCX treatment. * $p < 0.05$; mean ± SEM.

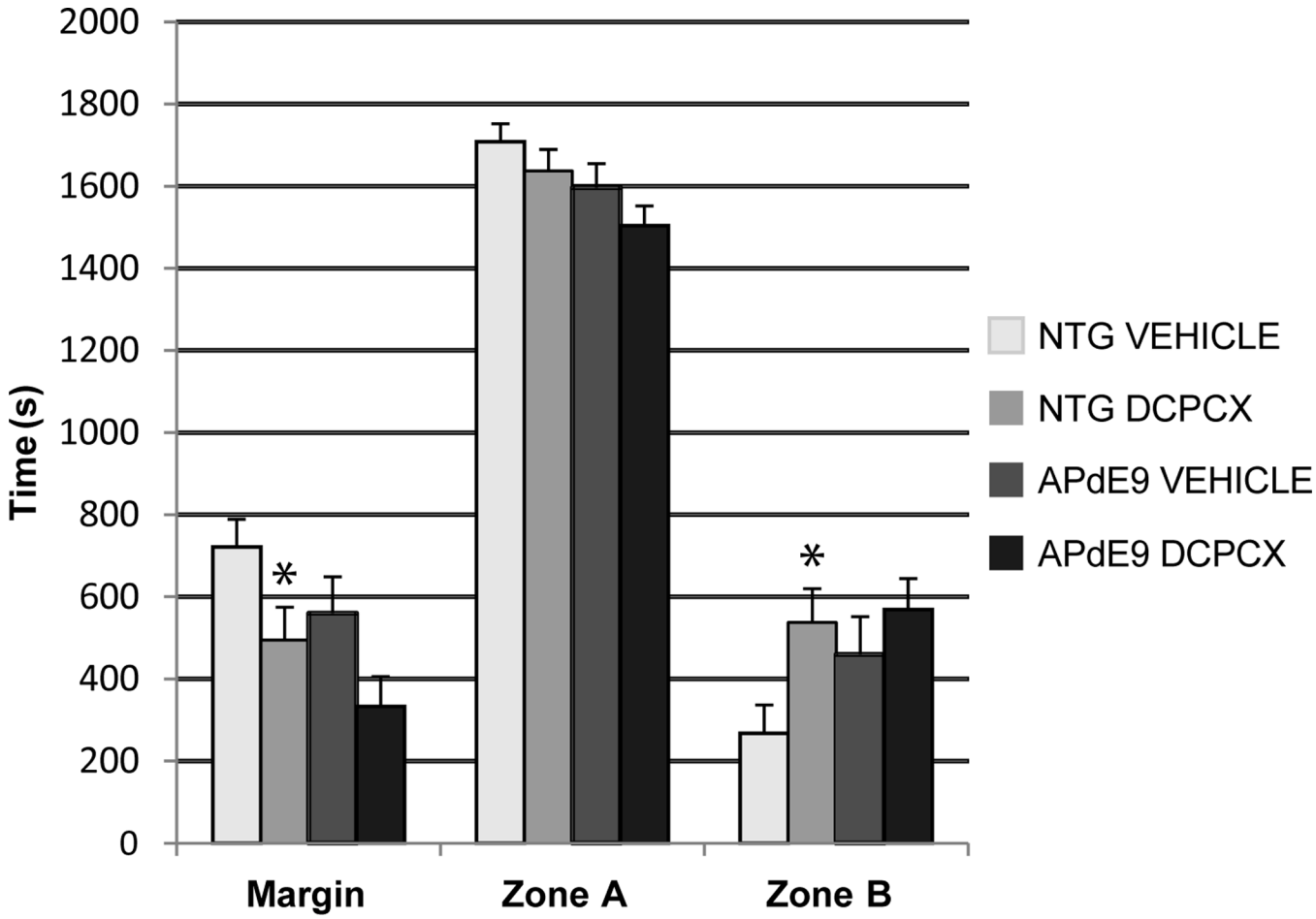


**Fig. 3. Effect of DPCPX on anxiety-like behavior**

Nontransgenic mice treated with DCPCX spent significantly less time in the margin and more time in the center of the open field, compared to nontransgenic vehicle-treated mice. APP mice showed a similar trend, although the results were non-significant. (Zone A: perimeter of the open field; Zone B: center of the open field). * $p < 0.05$; mean ± SEM.

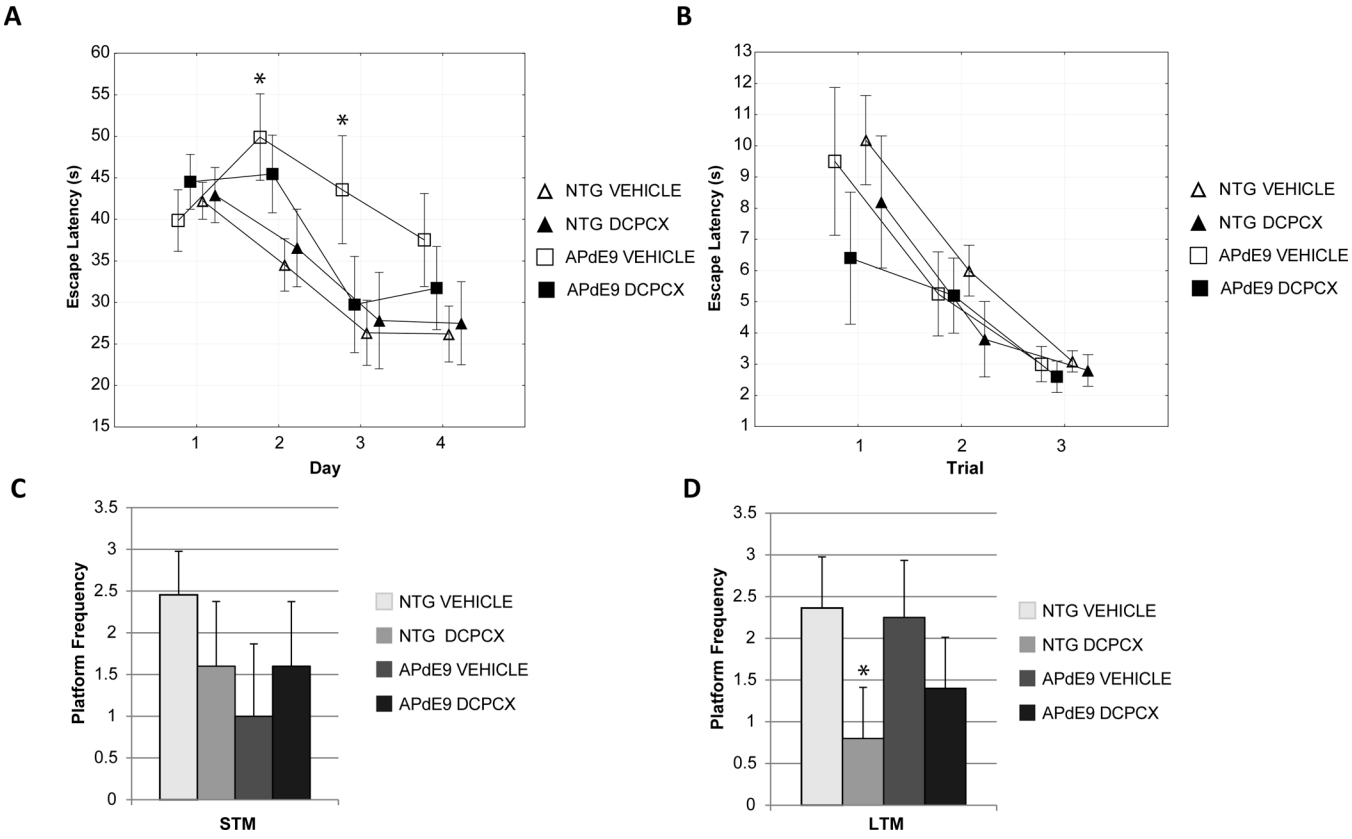


**Fig. 4. Effect of DPCPX treatment on long-term memory in the Morris Water Maze**

(A) APdE9 mice had significantly increased escape latency compared to nontransgenic mice on day 2 and 3 during acquisition trials. (B) Escape latency during visual acuity trials. (C) Platform frequency during short-term memory (STM) probe test. (D) DCPCX treated nontransgenic mice had significantly fewer platform crosses than nontransgenic vehicle indicating impaired memory in the long-term memory (LTM) probe test. * $p < 0.05$; mean ± SEM.