

# **Activation of CB2 Receptor System Reverses Amyloid-Induced Memory Deficiency and Restores SOX2 Activity in a Transgenic Mouse Model of Alzheimer's Disease**

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory and cognitive function. The brains of patients with AD are characterized by extensive deposits of extracellular aggregation of amyloid-beta peptides. These peptides form senile plaques and intracellular aggregation of hyperphosphorylated tau protein. In addition, amyloid fibrils activate the inflammatory pathway, characterized by the activated microglia and astrocytes seen in the brains of patients with AD.

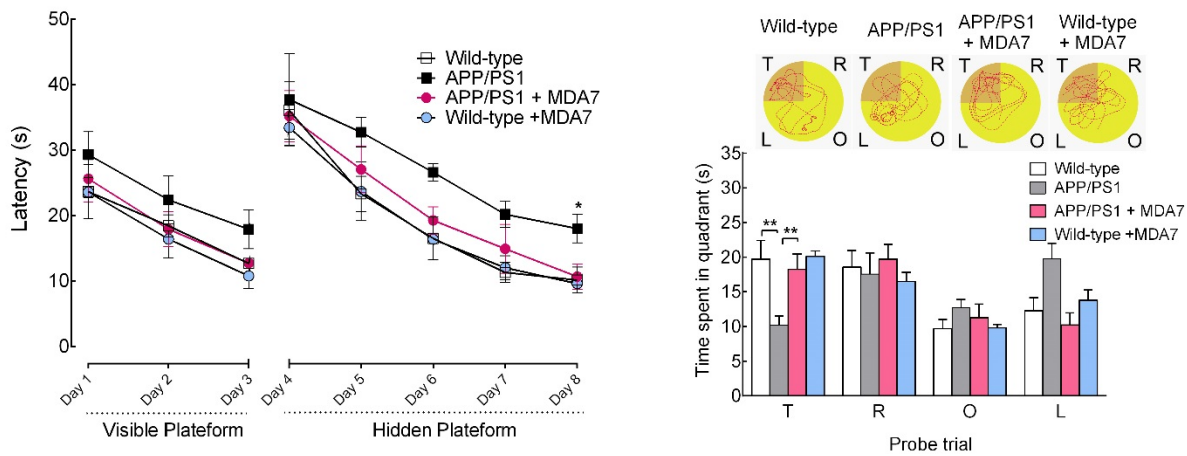
Cannabinoid type 2 (CB2) agonists are neuroprotective and appear to play modulatory roles in neurodegenerative processes in AD. CB<sub>2</sub> receptors are upregulated in reactive microglial cells in AD. This upregulation of CB<sub>2</sub> receptors tends to attenuate the activation of early pro-inflammatory microglial signaling pathways associated with AD.

Sox2 (sex-determining region Y (SRY)-box 2) is a transcriptional factor that is essential for maintaining self-renewal/proliferation of undifferentiated embryonic stem cells (ESCs) and multipotency of neural stem cells (NSCs). Sox2 behaves as a protective factor during the development of Alzheimer's disease.

We have studied the effect of 1-((3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl)carbonyl) piperidine (MDA7) a novel, blood brain barrier-permeant, and highly selective CB2 agonist that lacks psychoactivity on ameliorating the neuroinflammatory process, synaptic dysfunction, and cognitive impairment in the Tg-APPsw/PSEN1DE9 (APP/PS1) mouse model of AD. APP/PS1 mice are well suited for our investigations because they exhibit high production of A $\beta$  peptides in the brain, accumulation of amyloid plaques, and demonstrate cognitive impairments. Senile plaques can be detected by thioflavin S or 3D6 as early as 4 months of age, and there is an overall increase in plaque burden with age. PAPP/PS1 mice displayed significantly impaired glutamatergic long-term potentiation (LTP) in the hippocampal CA1 neurons, indicating an impaired synaptic plasticity. LTP is an experimental phenomenon that takes place at excitatory glutamatergic synapses and is believed to play a central role in learning and memory.

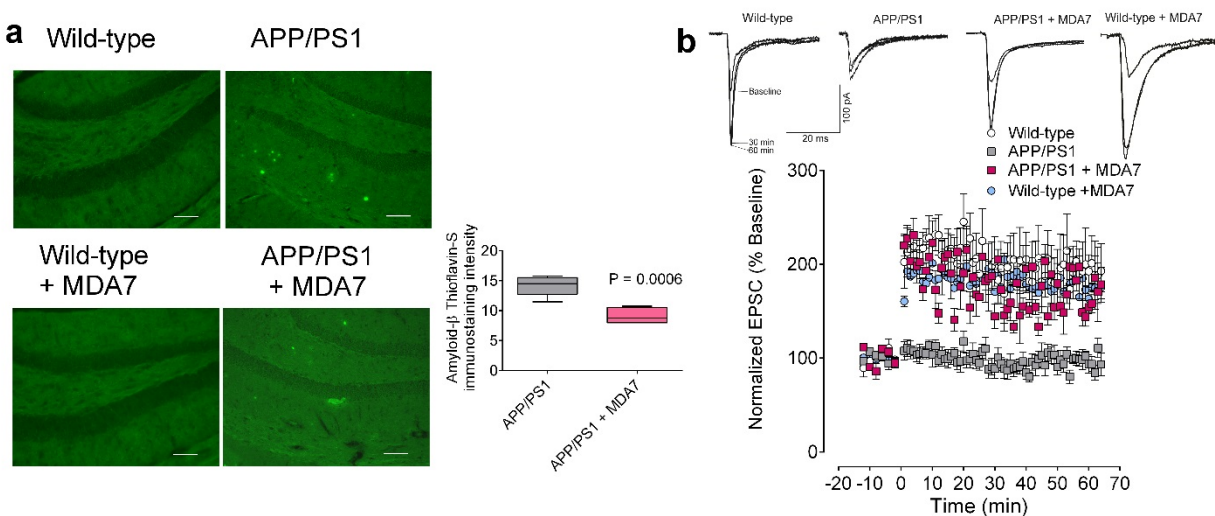
At 3 mo of age, MDA7 14 mg/kg was administered intraperitoneally (i.p.) every other day for 5 mo. Another cohort of APP/PS1 received i.p. injections of the vehicle at alternate days for 5 mo. In the APP/PS1 transgenic mice, compared to wild type mice, treatment with MDA7 (i) ameliorated the expression of Iba1 (microglia marker), (ii) promoted amyloid-beta clearance in the hippocampal CA1, (iii) restored the expression of SOX2 (stem cell marker) in the hippocampal dentate gyrus, and (iv) restored synaptic plasticity, cognition and memory. Our findings suggest that MDA7 is an innovative therapeutic approach for Alzheimer's disease.

**Summary:** Activation of CB2 receptor system represents a novel therapeutic target for the treatment of neurodegenerative disorders.



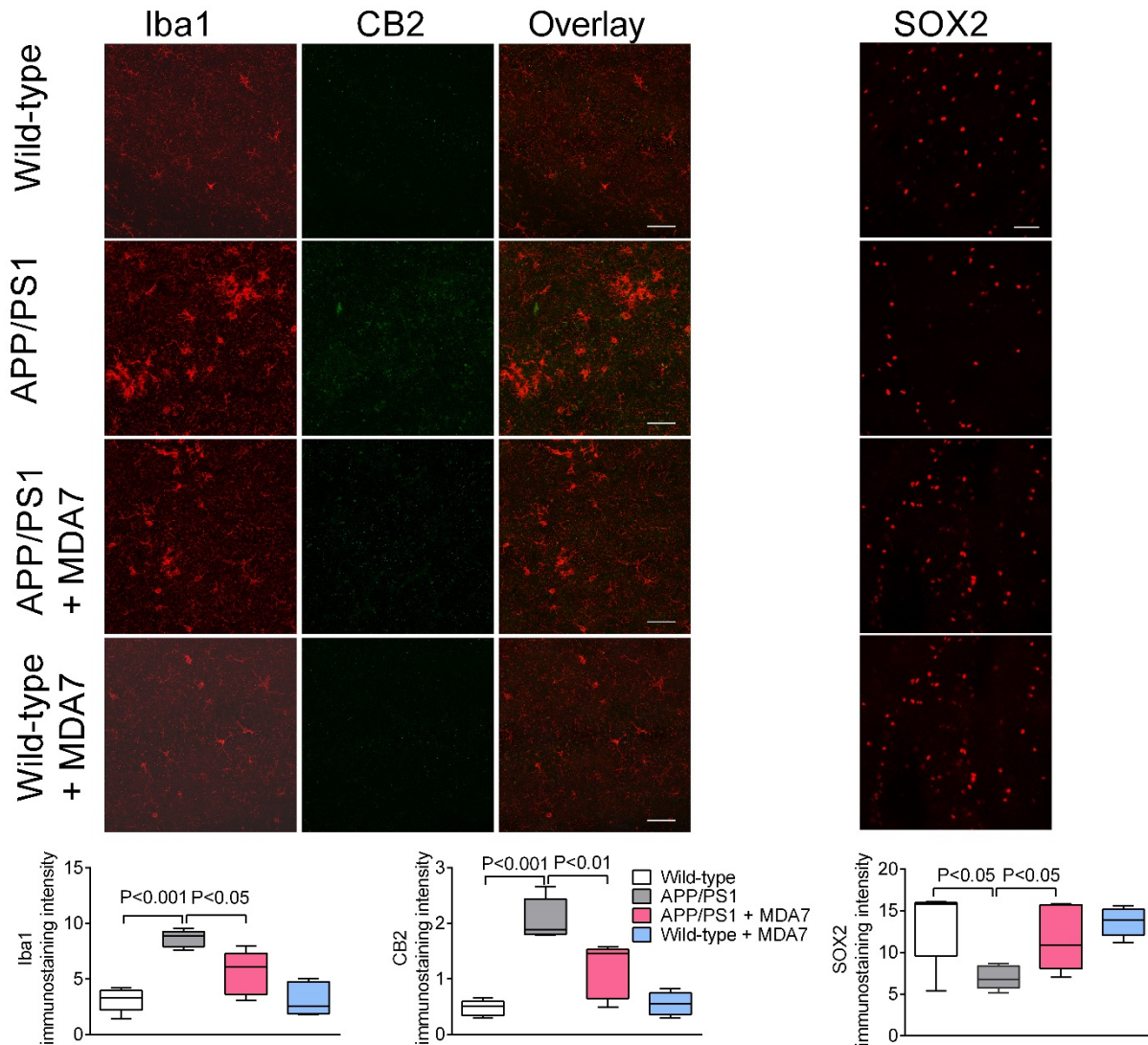
**Figure 1. In the APP/PS1 transgenic mice, compared to wild type mice, systemic administration of MDA7 significantly mitigated the memory deficiency.**

In APP/PS1 mice, significantly increased escape latency was noted, indicating memory deficiency (a). Treatment of animals at 3 mo of age with MDA7 14 mg/kg i.p. every other day for 5 mo (a) shortened the escape latency ( $n = 6-7$  mice in each group,  $P < 0.05$ ) and (b) increased the time in spent in the target quadrant ( $n = 6-7$  mice in each group,  $P < 0.05$ ) in the APP/PS1 transgenic mice. Representative path tracings in each quadrant during the probe trial on day 8.



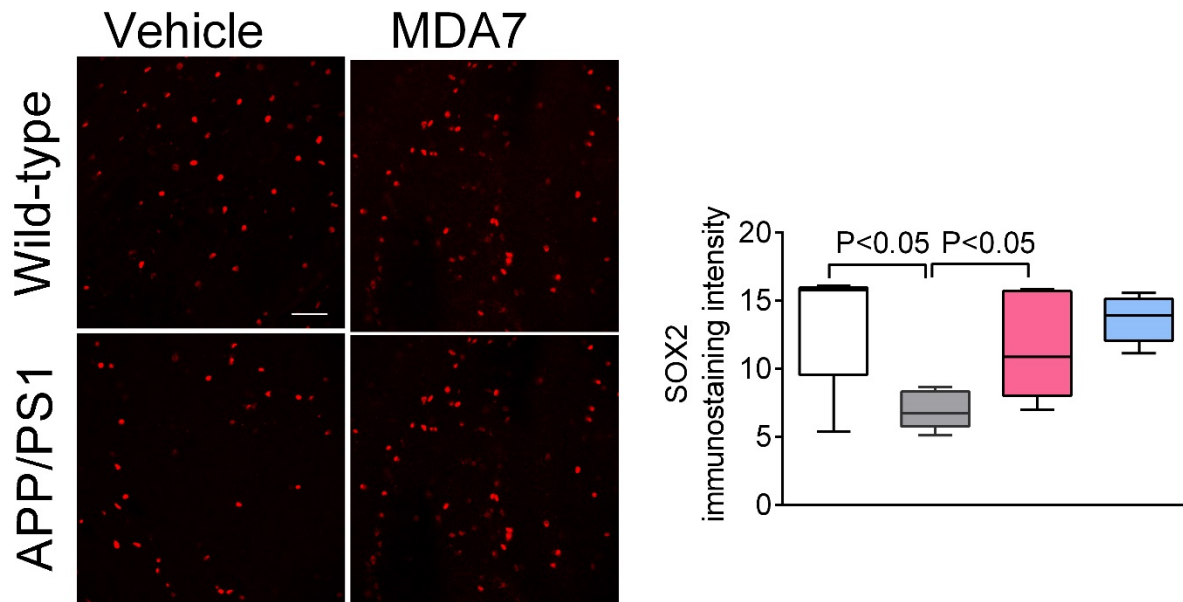
**Figure 2. In the APP/PS1 transgenic mice, compared to wild type mice, systemic administration of MDA7, promoted amyloid -beta ( $A\beta$ ) plaque clearance in the hippocampal CA1 area and restored synaptic plasticity in CA1.** (a) Thioflavin-S staining of amyloid plaques in the hippocampus. Treatment of animals at 3 mo of age with MDA7 14

mg/kg i.p. every other day for 5 mo significantly decreased the A $\beta$  burden in the hippocampal CA1 neurons. Scale bar = 50mm. (b) APP/PS1 mice displayed significantly impaired glutamatergic long-term potentiation (LTP) in the hippocampal CA1 neurons, indicating an impaired synaptic plasticity. Treatment of animals at 3 mo of age with MDA7 14 mg/kg i.p. every other day for 5 mo significantly recovered the electric stimuli-induced LTP in the hippocampal CA1 neurons.



**Figure 3. In the APP/PS1 transgenic mice, compared to wild type mice, systemic administration of MDA7, significantly attenuated the upsurge of CB2 and Iba1 expression in the hippocampal CA1 area.** APP/PS1 mice displayed significantly increased the expression of Iba1 and CB2; Treatment of animals at 3 mo of age with MDA7 14 mg/kg i.p. every other day for 5 mo significantly attenuated upsurge of CB2 and Iba1

expression in hippocampal CA1 (n = 24 sections from 5 animals in each group). Scale bar = 50  $\mu$ m.



**Figure 4. In the APP/PS1 transgenic mice, compared to wild type mice, systemic administration of MDA7, significantly restored SOX expression in the hippocampal CA1 area.**

APP/PS1 mice displayed significantly decreased the expression of SOX2; Treatment of animals at 3 mo of age with MDA7 14 mg/kg i.p. every other day for 5 mo significantly restored SOX2 expression in hippocampal CA1 (n = 24 sections from 5 rats in each group). Scale bar = 50  $\mu$ m.