Delta-9-Tetrahydrocannabinol

Subjects: Neurosciences

Contributor: Qingyu Zhou

Delta-9-tetrahydrocannabinol (THC) is the main phytocannabinoid found in plants of the Cannabis genus. Although THC has exactly the same chemical formula as cannabidiol (CBD) (i.e., C21H30O2), there is a slight difference in their atomic arrangement in that THC contains a cyclic ring, whereas CBD contains a hydroxyl group. THC is considered the main psychotropic constituent of cannabis, acting as a partial agonist at cannabinoid type 1 (CB1) and type 1 (CB2) receptors of the endocannabinoid system.

delta-9-tetrahydrocannabinol cannabidiol Alzheimer's disease radial arm water maze test amyloid-β APP/PS1 transgenic mice

1. Introduction

Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the two main phytocannabinoids found in plants of the Cannabis genus [1]. Although THC has exactly the same chemical formula as CBD (i.e., $C_{21}H_{30}O_2$), there is a slight difference in their atomic arrangement in that THC contains a cyclic ring, whereas CBD contains a hydroxyl group [1][2]. Due to the difference in chemical structure, THC and CBD are found to have different pharmacological effects. THC is considered the main psychotropic constituent of cannabis, acting as a partial agonist at cannabinoid type 1 (CB1) and type 1 (CB2) receptors of the endocannabinoid system [3]. CBD is non-psychotropic and interacts with many receptors and proteins other than the CB1 and CB2 receptors in the body [2]. Currently, the synthetically produced THC, dronabinol (Marinol®), has received FDA approval to treat nausea and vomiting caused by cancer chemotherapy. Plant-based pharmaceutical grade CBD (Epidiolex®) has been approved by the U.S. FDA for the treatment of seizures associated with Lennox–Gastaut syndrome and Dravet syndrome. Given the specific effect of THC on the endocannabinoid system and the diverse receptor profile of CBD, both THC and CBD have been implicated as potential neuroprotectants for mental and motor dysfunctions in neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD) [4][5].

Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting over five million Americans and the annual direct medical costs have exceeded $300 billion (https://www.alz.org/aaic/downloads2020/2020_Facts_and_Figures_Fact_Sheet.pdf accessed on 1 March 2022). The incidence of AD increases exponentially with age and is shown to be similar for males and females [6]. Even though AD is the sixth leading cause of death in the United States, there is no treatment available to halt or cure AD (https://www.nia.nih.gov/health/alzheimers-disease-fact-sheet accessed on 1 March 2022). Neurologically, AD is characterized by the extracellular deposition of β-pleated assemblies of the amyloid β (Aβ) peptide in the form of

diffuse plaques and neuritic plaques as well as the intracellular aggregation of insoluble hyperphosphorylated tau protein into neurofibrillary tangles (NFTs) within the perikarya of neurons [7][8]. These changes cause progressive memory loss as well as cognitive and behavioral impairments that ultimately lead to dementia. Mounting evidence demonstrates that Aβ accumulation can start as early as age 40 [9] and the major toxic isoform linked to neuron death is oligomeric Aβ [10][11]. Therefore, it is conceivable that the prevention or reduction in oligomeric Aβ formation may delay the onset or progression of AD.

Of the two main phytocannabinoids in cannabis, CBD has been extensively assessed for its potential therapeutic effect on AD in a number of preclinical studies [12]. In in vitro studies, CBD was found to protect against Aβ neurotoxicity [13], reduce Aβ production by promoting amyloid precursor protein ubiquitination [14], inhibit Aβ-induced tau protein hyperphosphorylation [15], modulate microglial cell functions [16], and improve cell viability [17]. In transgenic mouse models of AD, treatment with CBD alone was shown to prevent the development of social recognition memory deficits without influencing the anxiety parameter [18]. Results of in vivo studies using the Aβ-inoculated naïve mouse and rat models of AD-related neuroinflammation suggest that the neuroprotective effect of CBD is partly attributable to its anti-inflammatory property [19][20]. Treatment with CBD in combination with THC has been shown to improve memory deficits and prevent learning deficits in APP/PS1 transgenic mice that express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) [21][22] and to improve certain neurological deficits in parkin-null and human tau overexpressing PK$^{-/-}$/TauVLW mice [23]. In APP/PS1 mice, combined CBD and THC treatment reduced the glial reactivity associated with aberrant Aβ deposition through normalizing the pre-synaptic synaptosome associated protein 25 (SNAP25), decreasing the expression of glutamate receptors 2 and 3 (GluR2/3), and increasing the expression of γ-aminobutyric acid receptor A subunit α1 (GABA-A Rα1) in mouse brain cortex [22]. In PK$^{-/-}$/TauVLW mice, treatment with a mixture of CBD and THC reduced the stress-related abnormal behaviors observed in vehicle-treated PK$^{-/-}$/TauVLW mice, decreased the gliosis and deposition of tau and Aβ in the hippocampus and cerebral cortex, and increased the ratio of reduced/oxidized glutathione and autophagy [23]. In contrast to the putative effect of CBD on improving cognitive performance in preclinical AD models, the therapeutic potential of THC alone in the treatment of AD has not been widely documented. In the previous in vitro study using N2a-variant Aβ protein precursor cells, researchers observed that THC treatment inhibited the production and aggregation of Aβ40, the production of phosphorylated tau protein, decreased the intracellular phospho-GSK3β expression level, and enhanced mitochondrial function [24]. Two recent in vivo studies by Zimmer's group demonstrated that treatment with 1 and 3 mg/kg/d of THC through osmotic minipumps for 28 consecutive days significantly improved the special learning performance of male C57BL6/J mice at the ages of 12 and 18 months [25][26] whereas a 1:1 combination of THC and CBD (1 mg/kg/d for each) failed to achieve the same effect as THC monotherapy [26]. Another study showed that single-dose intraperitoneal (IP) administration of THC at 0.002 mg/kg restored the cognitive function in 24-month-old female wild-type mice [27].

2. THC and CBD Reduced the Production of Aβ1–42 Peptide in N2a/APPswe Cells

The 40-residue peptide Aβ (Aβ1–40) represents the most abundant Aβ isoform in the brain [28] while the C terminally extended variant Aβ1–42, able to form insoluble fibril-like structures rapidly, is considered to be highly associated with AD pathology [29][30]. Early studies have demonstrated that CBD treatment significantly reduced Aβ1–40 production in vitro [14], while CBD–THC combination decreased soluble Aβ1–42 levels in vivo [21].

In this regard, treatment with CBD alone and in combination with THC were included as positive controls was the in vitro study to examine whether the inhibitory effect of THC alone on Aβ production in N2a/APPswe cells was comparable to that of CBD alone and CBD + THC. In this experiment, ELISA was used to examine the effect of THC and CBD treatment alone or in combination on the production of Aβ1–40 and Aβ1–42 in N2a/APPswe cells that constitutively produce high levels of Aβ protein due to the transfected mutant APP gene. As shown in **Figure 1A**, treatment with 10 nM THC, 100 nM THC, 100 nM CBD, and 100 nM of THC and CBD in combination for 24 h significantly decreased the Aβ1–40 production in N2a/APPswe cells by 23% ($p < 0.05$), 27% ($p < 0.01$), 21% ($p < 0.05$), and 32% ($p < 0.001$), respectively. The mean Aβ1–40 level in N2a/APPswe cells treated with 10 nM CBD was not significantly different from that of the vehicle control (**Figure 1A**.). Treatment with 10 nM THC, 100 nM THC, 10 nM CBD, 100 nM CBD, and 100 nM of THC and CBD in combination for 24 h significantly decreased the Aβ1-42 production in N2a/APPswe cells by 28% ($p < 0.001$), 35% ($p < 0.001$), 18% ($p < 0.05$), 19% ($p < 0.05$), and 31% ($p < 0.001$), respectively (**Figure 1B**). Moreover, the inhibitory effect of 100 nM THC on Aβ1–42 production was significantly greater than that of 10 nM CBD ($P < 0.05$. **Figure 1**). Treatment with THC and CBD alone or in combination for 42 h had no significant effect on the production of Aβ1–40 and Aβ1–42 in N2a/APPswe cells ($p > 0.05$ for all, **Figure 1C,D**) although treatments with 10 and 100 nM of THC for 42 h were able to decrease the Aβ1–42 production in N2a/APPswe cells by 16% and 24%, respectively (**Figure 1D**).

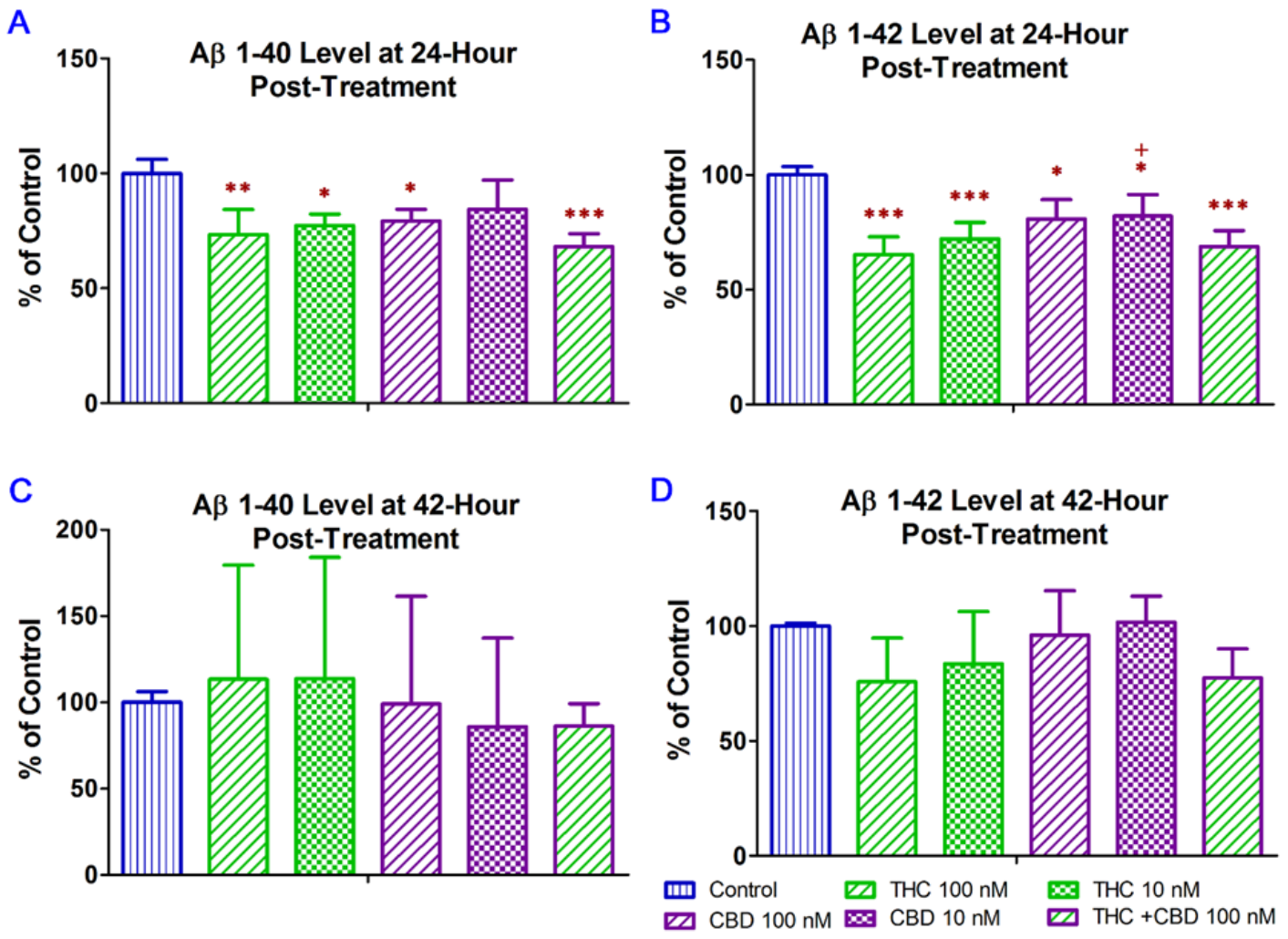

Figure 1. Effect of THC and CBD treatment alone or in combination on Aβ1–40 and Aβ1–42 production in N2a/APPswe cells at 24 (**A)** for Aβ1–40; (**B)** for Aβ1–42) and 42 (**C)** for Aβ1–40; (**D)** for Aβ1–42) hours after the treatment. The production of Aβ1–40 and Aβ 1–42 in the cell culture supernatant was determined by ELISA. After N2a/APPswe cells were treated with THC and CBD alone or in combination for 24 h, Aβ1–40 and Aβ 1–42 levels in the supernatant reduced significantly by 16~32% and 18~35%, respectively, compared with those in the non-treated control samples. No significant changes in the Aβ1–40 and Aβ 1–42 levels in the supernatant were found between the non-treated and treated samples after the 42-h treatment. Data are expressed as mean ± SD (N = 4). SD is denoted by the error bars. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the control group and +$p < 0.05$ compared with the 100 nM THC treatment group using one-way ANOVA followed by the Tukey–Kramer post hoc multiple comparison test.

3. THC Treatment Improved Impaired Spatial Memory Performance of Aged APP/PS1 Mice

To evaluate the effect of low-dose THC exposure on the spatial reference memory of aged APP/PS1 transgenic mice, individual single-housed aged APP/PS1 transgenic mice were evaluated for the time taken to locate the

escape platform (latency) and the number of errors using the RAWM test before and after vehicle or THC treatment and compared with the age-matched control of the non-transgenic (NTG) mice (Table S1). To evaluate the effect of low-dose THC exposure on the spatial reference memory of aged APP/PS1 transgenic mice, individual single-housed aged APP/PS1 transgenic mice were evaluated for the time taken to locate the escape platform (latency) and the number of errors using the RAWM test before and after vehicle or THC treatment and compared with the age-matched control NTG mice. There was no significant effect of sex on either the latency or number of errors before the start of the treatments, suggesting the insignificance of effect modification by sex (**Figure 2A,B**). Therefore, the data from male and female mice were pooled for subsequent statistical analyses including the two-sample *t* test, one-way ANOVA with the post-hoc Bonferroni's multiple comparisons test, and two-way ANOVA. Results of the two-way ANOVA for the baseline latency revealed a significant effect of time ($F(2, 46) = 7.61$, $p = 0.0014$) and group interaction ($F(3, 23) = 7.24$, $p = 0.0014$), while there was no significant effect for the time × group interaction ($F(6, 46) = 0.759$, $p = 0.606$). The post-hoc analysis indicated a significant difference in latency between the non-transgenic (NTG) control and individual transgenic groups, but no difference among the three transgenic groups in Trial 3 of the last block on Day 3 (**Figure 2C**). Results of two-way ANOVA for the baseline number of errors also showed a significant effect of time ($F(2, 46) = 3.84$, $p = 0.0286$) and group interaction ($F(3, 23) = 9.65$, $p = 0.0003$), but not for the time × group interaction ($F(6, 64) = 0.393$, $p = 0.88$). The post hoc analysis indicated a significant difference in the number of errors between the NTG control and transgenic 0.02 mg/kg THC group in Trial 3 of the last block on day 2 and between the NTG control and transgenic 0.02 mg/kg and 0.2 mg/kg THC groups in Trial 3 of the last block on day 3. No difference was found among the three transgenic groups in Trial 3 of the last block on day 3 (**Figure 2D**). Taken together, it was demonstrated that the APP/PS1 mice displayed significant spatial memory deficits compared to the NTG mice before the THC treatment was initiated. The APP/PS1 mice were grouped based on the RAWM results so that there was no overt detectable difference in spatial memory among the three study groups.

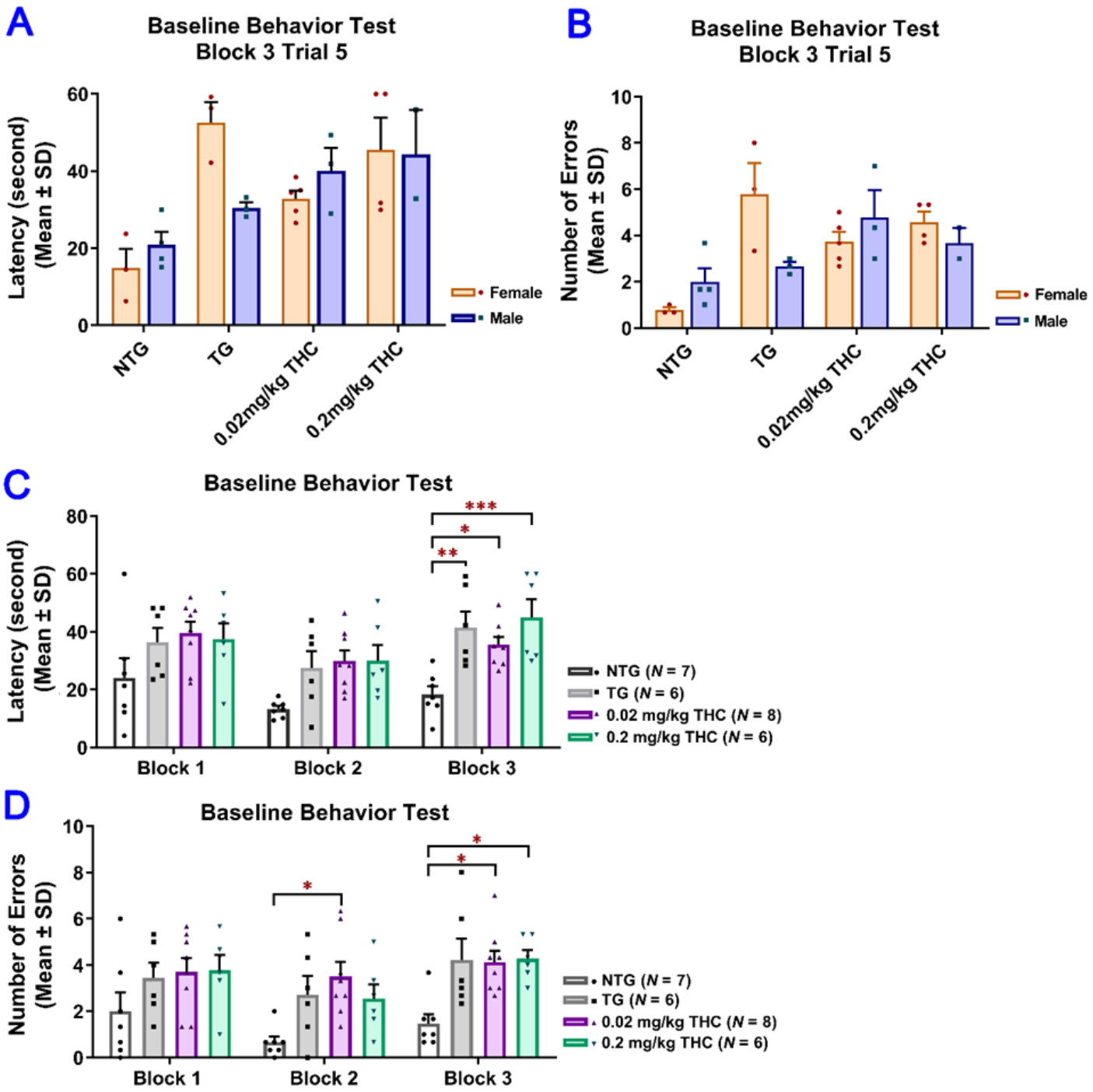

Figure 2. Evaluation of the baseline spatial reference memory of 14-month-old APP/PS1 mice in three study groups (i.e., the control transgenic control (TG), 0.02 mg/kg and 0.2 mg/kg THC treatment groups as well as 14-month-old non-transgenic (NTG) mice using the radial arm water maze (RAWM) test. Individual mice were subjected to five blocks of trials each day for three days with each block containing three trials. (**A**) No significant difference in baseline latency between male and female mice within individual study groups (p = 0.56, p = 0.06, p = 0.51 and p = 0.947 for NTG, TG, 0.02 mg/kg and 0.2 mg/kg THC groups, respectively, using the Independent sample t test). (**B**) No significant difference in baseline number of errors between male and female mice within individual study groups (p = 0.356, p = 0.299, p = 0.513, and p = 0.513 for NTG, TG, 0.02 mg/kg, and 0.2 mg/kg THC groups, respectively, using the Independent sample t test). (**C**) Significant increase in the baseline latency in

TG ($p < 0.01$), 0.02 mg/kg THC ($p < 0.05$), and 0.2 mg/kg THC ($p < 0.001$) groups compared with the NTG control group in Trial 3 of the last block on day 3. (**D**) Significant increase in the baseline latency in TG ($p < 0.01$), 0.02 mg/kg THC ($p < 0.05$), and 0.2 mg/kg THC ($p < 0.001$) groups compared with the NTG control group in Trial 3 of the last block on day 3. Data are expressed as mean ± SD. SD is denoted by the error bars. A comparison of mean latency and number of errors between female and male animals in individual study groups was made with multiple t-tests with correction for multiple comparisons using the Holm–Sidak method. Comparison of mean baseline latency and number of errors among different study groups were conducted using one-way ANOVA with the post hoc Bonferroni's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared between the control NTG ($N = 7$), control TG ($N = 6$), 0.02 mg/kg THC ($N = 8$), and 0.2 mg/kg THC ($N = 6$) groups.

Since the results of the RAWM test obtained after the 3-month vehicle or THC treatment indicated that the effect of sex on either the latency or number of errors was not significant, the data from male and female mice were pooled for subsequent statistical analyses (**Figure 3A,B**). Results of two-way ANOVA for the latency revealed a significant main effect of treatment ($F(3, 23) = 10.51$, $p < 0.001$) and time ($F(4, 92) = 10.41$, $p < 0.001$), but not for the time × treatment interaction ($F(12, 92) = 1.14$, $p = 0.338$). Results of two-way ANOVA for the number of errors showed a significant main effect of treatment ($F(3, 23) = 16.74$, $P < 0.001$) and the time × treatment interaction ($F(12, 92) = 2.28$, $p = 0.014$), but not for the time ($F(4, 92) = 1.30$, $p = 0.277$). Results of one-way ANOVA with post hoc Bonferroni's multiple comparisons test demonstrated that the vehicle-treated aged APP/PS1 mice took a significantly longer time to locate the escape platform and made more errors than the aged NTG mice during the last three interval-separating blocks of training trials ($p < 0.001$. **Figure 3C,D**). In contrast, in Trial 5 Block 5, APP/PS1 mice treated with 0.02 and 0.2 mg/kg THC showed a significant decrease in the latency and number of errors compared with the vehicle group ($p < 0.01$ and $p < 0.001$ for 0.02 mg/kg and 0.2 mg/kg THC groups, respectively). No significant differences in the latency and number of errors were observed between the THC-treated APP/PS1 mice and aged NTG control mice (**Figure 3C,D**). These observations indicate that the spatial learning and memory of THC-treated APP/PS mice were superior to those of the vehicle-treated APP/PS1 mice and comparable to those of the NTG control mice. Overall, the superiority of THC-treated APP/PS1 mice over the control APP/PS1 mice was evident in that the control APP/PS1 mice consistently exhibited inferior acquisition with little improvement in the spatial memory throughout the RAWM test sessions, while the performance of THC-treated APP/PS1 mice improved markedly over the same training period. In addition, no significant difference in latencies and number of errors was found between the NTG control mice and THC-treated APP/PS1 mice ($p > 0.05$. **Figure 3C,D**), implicating that the memory deficits in aged APP/PS1 mice are reversed with THC treatment.

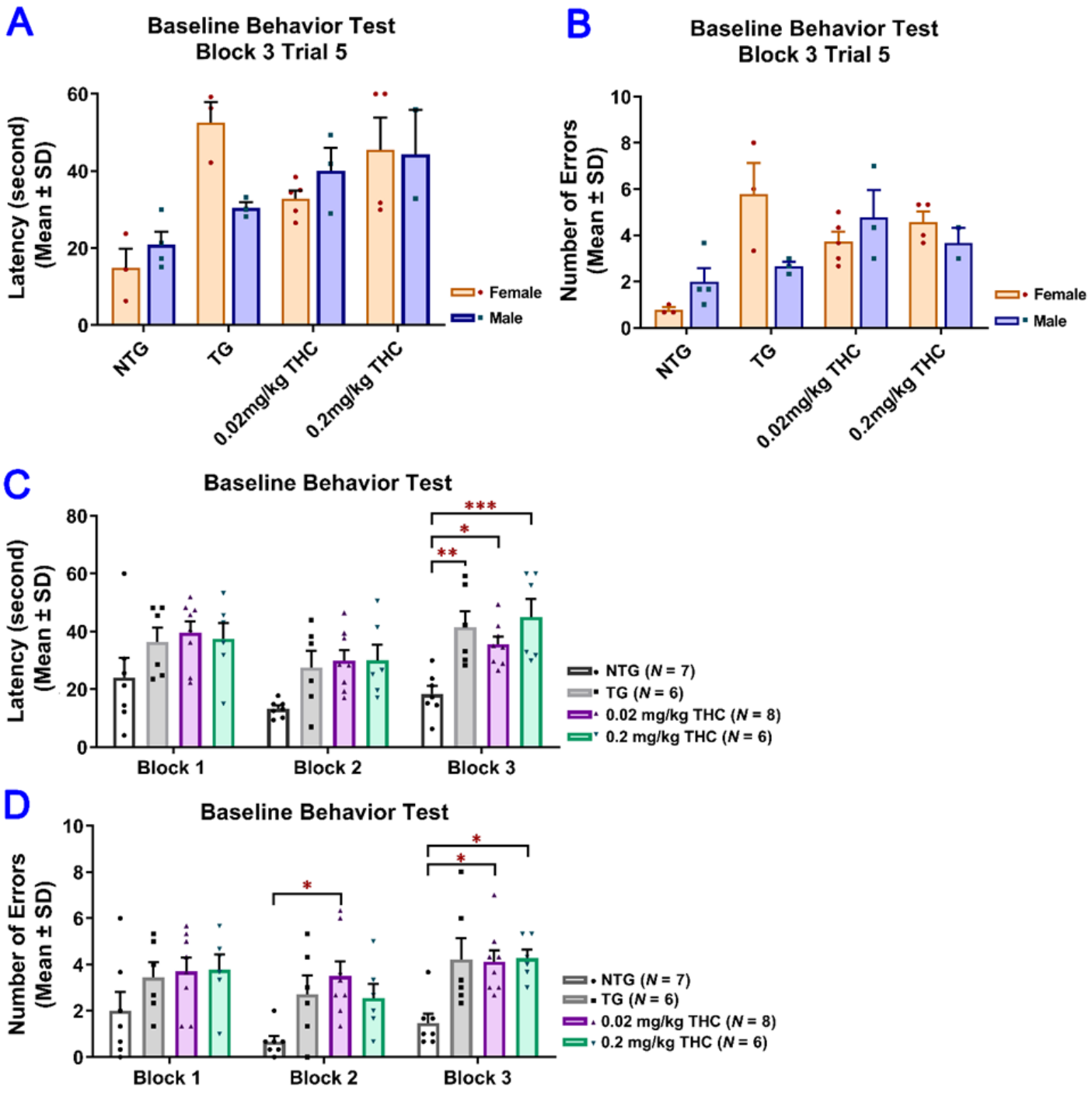

Figure 3. Evaluation of the effect of THC treatment on improving the spatial reference memory of 14-month-old APP/PS1 mice using the RAWM test. Individual mice were subjected to five trials per day for 15 consecutive days with each block containing 15 trials. (**A**) No significant difference in latency between male and female mice within individual study groups in Trial 5 of Block 5 (p = 0.998, p = 0.996, p = 0.998, and p = 0.996 for NTG, TG, 0.02 mg/kg, and 0.2 mg/kg THC groups, respectively, using the Independent sample t test). (**B**) No significant difference in number of errors between male and female mice within individual study groups in Trial 5 of Block 5 (p = 0.992, p = 0.979, p = 0.992, and p = 0.992 for NTG, TG, 0.02 mg/kg, and 0.2 mg/kg THC groups, respectively, using the Independent sample t test). (**C**) Significant decrease in the latency in NTG control (p < 0.001), 0.02 mg/kg THC (p < 0.01, and 0.2 mg/kg THC (p < 0.001) groups compared with the TG control group in Block 5 Trial 5. Significant

decrease in latency was also found in the NTG control ($p < 0.001$) and 0.2 mg/kg THC ($p < 0.05$) groups compared to the TG control group in Block 4 Trial 5. (**D**) Significant decrease in the number of errors in 0.02 mg/kg THC ($p < 0.01$) and 0.2 mg/kg THC ($p < 0.001$) groups compared with the TG control group in Block 5 Trial 5. Significant decrease in the number of errors was also found in the NTG control ($p < 0.001$) and 0.2 mg/kg THC ($p < 0.01$) groups compared with the TG control group in Block 4 Trial 5. Data are expressed as mean ± SD. SD is denoted by the error bars. Comparison of mean latency and number of errors between female and male animals in individual study groups was made by multiple t-tests with correction for multiple comparisons using the Holm–Sidak method. Comparison of mean latency and number of errors among different study groups were made using one-way ANOVA with post hoc Bonferroni's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared between the control NTG ($N = 7$), control TG ($N = 6$), 0.02 mg/kg THC ($N = 8$), and 0.2 mg/kg THC ($N = 6$) groups.

4. THC Treatment Resulted in Decreased Oligomeric Aβ Levels in Hippocampi of APP/PS1 Mice

It is well documented that the spatial learning and memory abilities and brain chemical levels of APP/PS1 mice are different from those of wild-type non-transgenic mice due to the increased secretion of Aβ peptides in the APP/PS1 mouse brain, which causes neurotoxicity and triggers memory impairment [31][32]. In this regard, researchers dissected the mice brains after the three-month THC treatment. Aβ plaques on the mouse hippocampus sections were examined using Congo Red staining. Results of the Congo Red staining showed that the Aβ plaque areas and number of Aβ plaques on the APP/PS1 mouse brain hippocampus sections were significantly higher than those on control NTG mouse brain hippocampus sections irrespective of treatment (**Figure 4**). Although treatment with 0.2 mg/kg of THC appeared to decrease the Aβ plaque areas and number of Aβ plaques by 28% and 27%, respectively, compared with the vehicle treatment, the difference was not statistically significant (**Figure 4**B,C). Nonetheless, the difference in Aβ plaque areas was statistically significant between the 0.02 mg/kg and 0.2 mg/kg THC treatment groups ($p < 0.01$, **Figure 4**B). The overall result of Congo Red staining of APP/PS1 mouse hippocampus sections revealed the limited effect of 3-month THC treatment on reducing the amyloid deposits in the hippocampi.

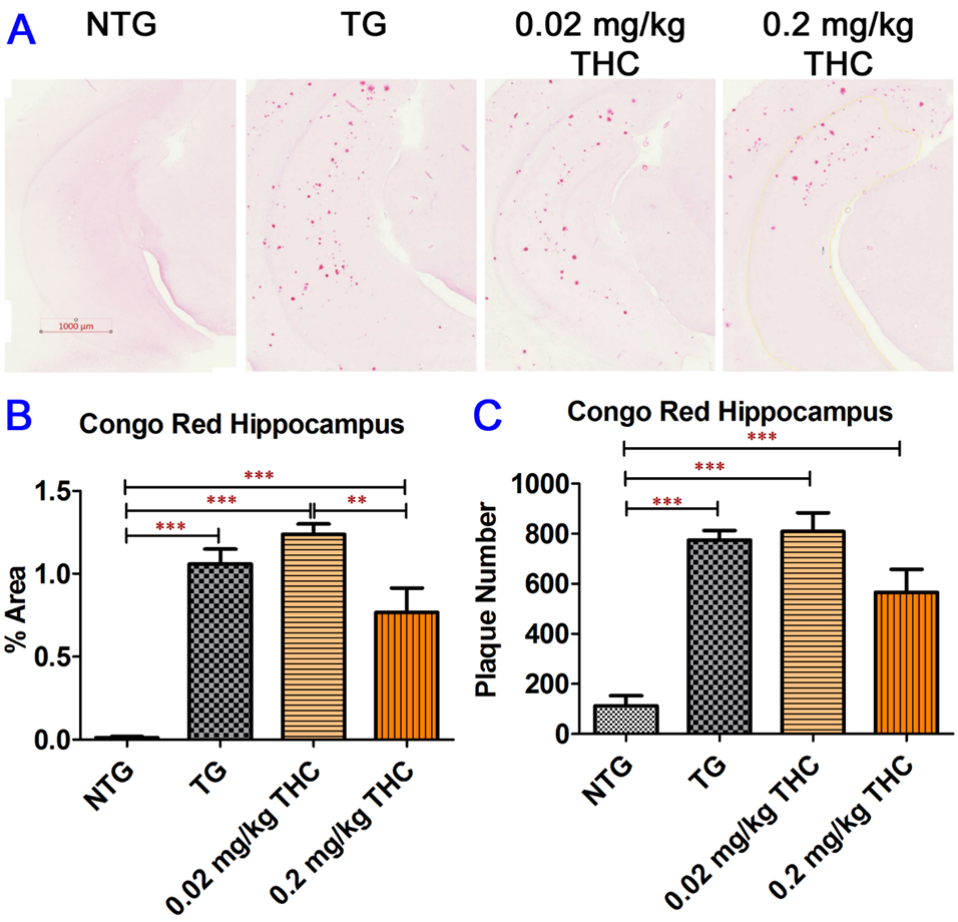

Figure 4. Congo Red staining of Aβ plaques in mouse hippocampi. (**A**) Representative Congo Red staining images acquired under light microscopy. (**B**) Quantification of Congo red staining shown as the percentage of Congo red-positive area compared to the hippocampus tissue area per field. (**C**) Quantification of Congo red staining shown as the number of Congo red stained plague in the hippocampus area. The non-transgenic (NTG) mice had significantly fewer Aβ plaques than all the APP/PS1 transgenic (TG) mice regardless of treatment ($p < 0.001$ for all). No significant differences in Aβ plaque area and number of Aβ plaques were found between the vehicle control and 0.02 or 0.2 mg/kg THC treated APP/PS1 mice. However, the Aβ plaque area in hippocampi sections of APP/PS1 mice treated with 0.2 mg/kg THC was significantly lower than those treated with 0.02 mg/kg THC ($p < 0.01$). Data are expressed as mean ± SD ($N = 7$ for the control NTG group, $N = 6$ for the control TG, and 0.2 mg/kg THC groups, and $N = 8$ for the 0.02 mg/kg THC group). Error bars denote the SD. ** $p < 0.01$ and *** $p < 0.001$ compared between the control NTG mice, control APP/PS1 mice, and APP/PS1 mice treated with 0.02 and 0.2 mg/kg THC using one-way ANOVA followed by the Tukey–Kramer post hoc multiple comparison test.

5. Conclusions

Treatment with low-dose THC at 0.2 and 0.02 mg/kg improved the spatial learning of aged APP/PS1 mice by reducing the expression levels of oligomeric Aβ, phospho-tau and total tau and decreasing the activity of GSK-3β without eliciting any psychotropic or immunomodulatory effects, suggesting that low-dose THC is a safe and effective treatment for AD.

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