Δ 9 -Tetrahydrocannabinol Promotes Functional Remyelination in the Mouse Brain

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Research article

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Abstract

Background: Research on demyelinating disorders aims to find novel molecules able to induce oligodendrocyte precursor cell differentiation to promote CNS remyelination and functional recovery. Δ9-Tetrahydrocannabinol (THC), the most prominent active constituent of the hemp plant *Cannabis sativa*, confers neuroprotection in animal models of demyelination. However, the possible effect of THC on myelin repair has never been studied.

Methods: By using oligodendroglia-specific reporter mouse lines in combination with 2 models of toxin-induced demyelination, we analyzed the effect of THC on the processes of oligodendrocyte regeneration and functional remyelination.

Results: We show that THC administration enhanced oligodendrocyte regeneration, white matter remyelination, and motor function recovery. THC also promoted axonal remyelination in organotypic cerebellar cultures. THC remyelinating action relied on the induction of oligodendrocyte precursor differentiation upon cell cycle exit and *via* CB1 cannabinoid receptor activation.

Conclusions: Overall, our study identifies THC administration as a promising pharmacological strategy aimed to promote functional CNS remyelination in demyelinating disorders.

Full Text

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Figures

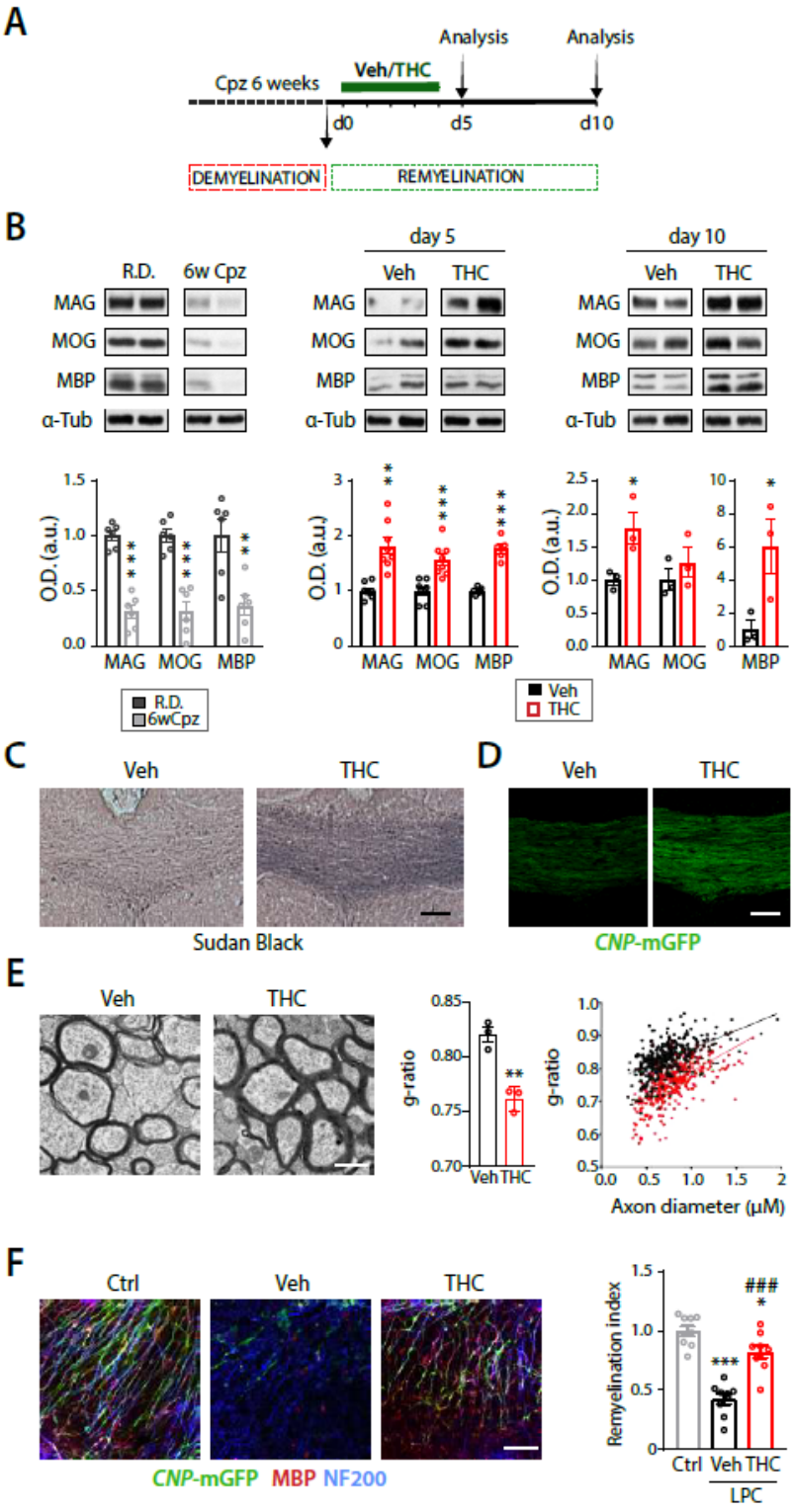

Figure 1

THC enhances CNS remyelination. A) Diagram represents the timeline of treatments and time points of analysis. Mice were fed with a Cpz diet for 6 weeks and returned to regular diet for allowing remyelination. At 24 hours after Cpz removal, THC (3 mg/Kg) or vehicle (Veh) was administered for 5 consecutive days and mice were sacrificed at 5 and 10 days following the first THC administration. Some control animals were fed with regular diet (R.D.), or cuprizone 6 weeks (Cpz 6w). B) Representative scans

of western blot analysis of myelin-associated proteins of CC extracts. Optical densities values are relative to those of loading controls and expressed as arbitrary units (a.u.). C) Representative images of Sudan black staining in the CC at day 5 of recovery. D) Representative confocal images in the CC of CNP-mGFP mice at day 5 of recovery. E) Representative electron microscopy images of axonal myelination in the CC at day 5 of recovery. Quantitative analysis of tmyelin g-ratio and scatter plots of g-ratio versus axon diameter. F) Slices were incubated with THC or Veh after Lysolecithin (LPC)-induced demyelination. Representative confocal images of z-stack projection of CNP-mGFP (green), MBP (red), and NF200 (blue) immunoreactivities in non-LPC treated and THC or vehicle-LPC-treated cerebellar organotypic slice cultures and scatter plot histogram analysis of the remyelination index (NFH+ fibers covered by MBP staining). Data are shown as mean +/- SEM; n=3-6 (B), n=3 mice (E), n=9 slices (F). *p<0.05, **p<0.01, ***p<0.001 from R.D. or vehicle-treated groups, by unpaired Student's t-test or unpaired t-tests with Welch correction for MAG values (B); **p<0.01 from vehicle-treated group, by unpaired Student's test (E); *p<0.05, ***p<0.001 form control group or ###p<0.001 from vehicle-treated group by one-way ANOVA with Tukey´s post-hoc test (F). Scale bars C, D= 70 µm, E= 4 µm, F =50µm.

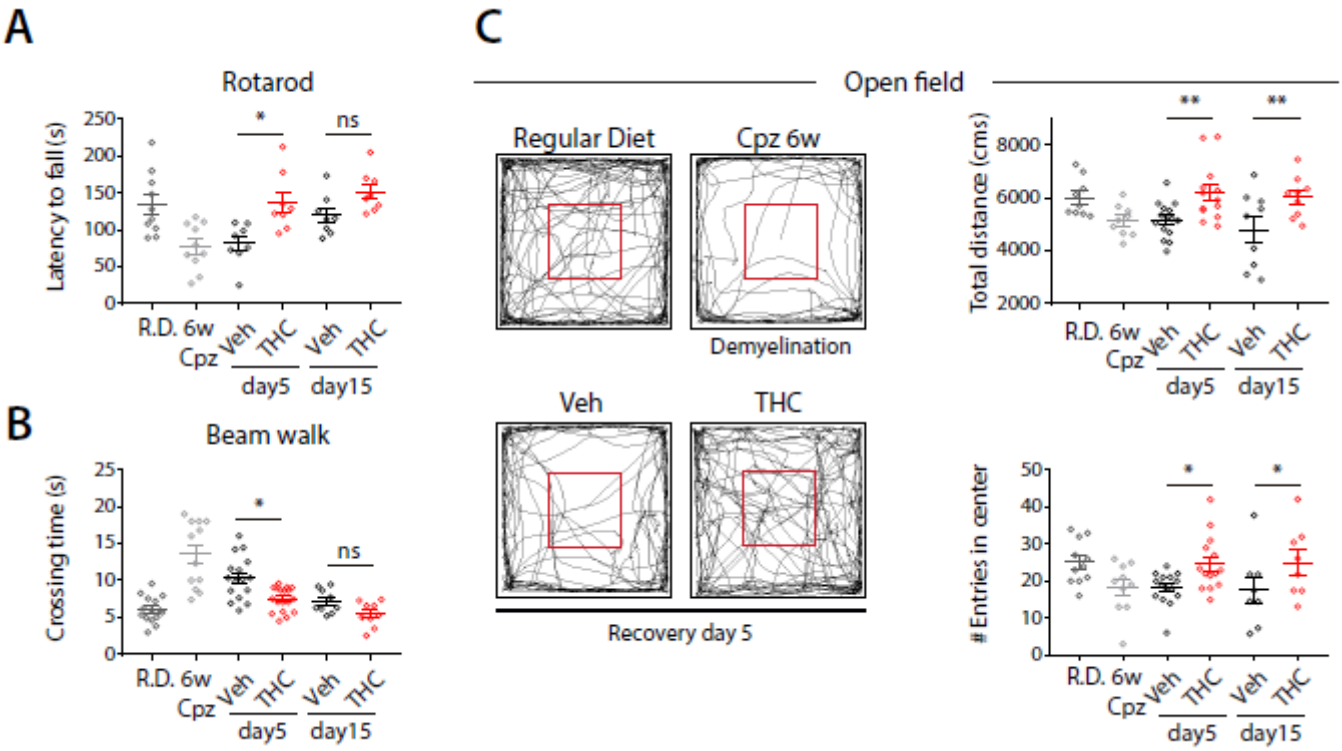

Figure 2

THC enhances functional recovery during remyelination. Mice were fed with a 0.2% Cpz diet for 6 weeks, followed by a regular diet, and Tetrahydrocannabinol (THC) (3mg/Kg) or vehicle (Veh) was administered for 5 consecutive days and a battery of behavioral tests was performed at 5 and 15 days of recovery. Some control animals were fed with regular diet (R.D.), or cuprizone 6 weeks (Cpz 6w). A) Animals were trained to perform the rotarod test the first week after weaning and reevaluated during remyelination. Bar graph shows the mean latency to fall (sec) at day 5, or 15 of remyelination. B) Beam walk test quantified

as the mean time spent to cross the beam C) Representative images of trajectories in the open field test. Representation of total distance, number of entries in the center of the arena. Data are shown as mean +/- SEM; n=8-15 mice for each experimental group or time point of analysis. *p 0.05, **p<0.01 from vehicle-treated group by one-way ANOVA, with uncorrected Fisher's LSD (A, C upper panel), Kruskal–Wallis one-way ANOVA with uncorrected Dunn's post hoc test (B,C lower panel).

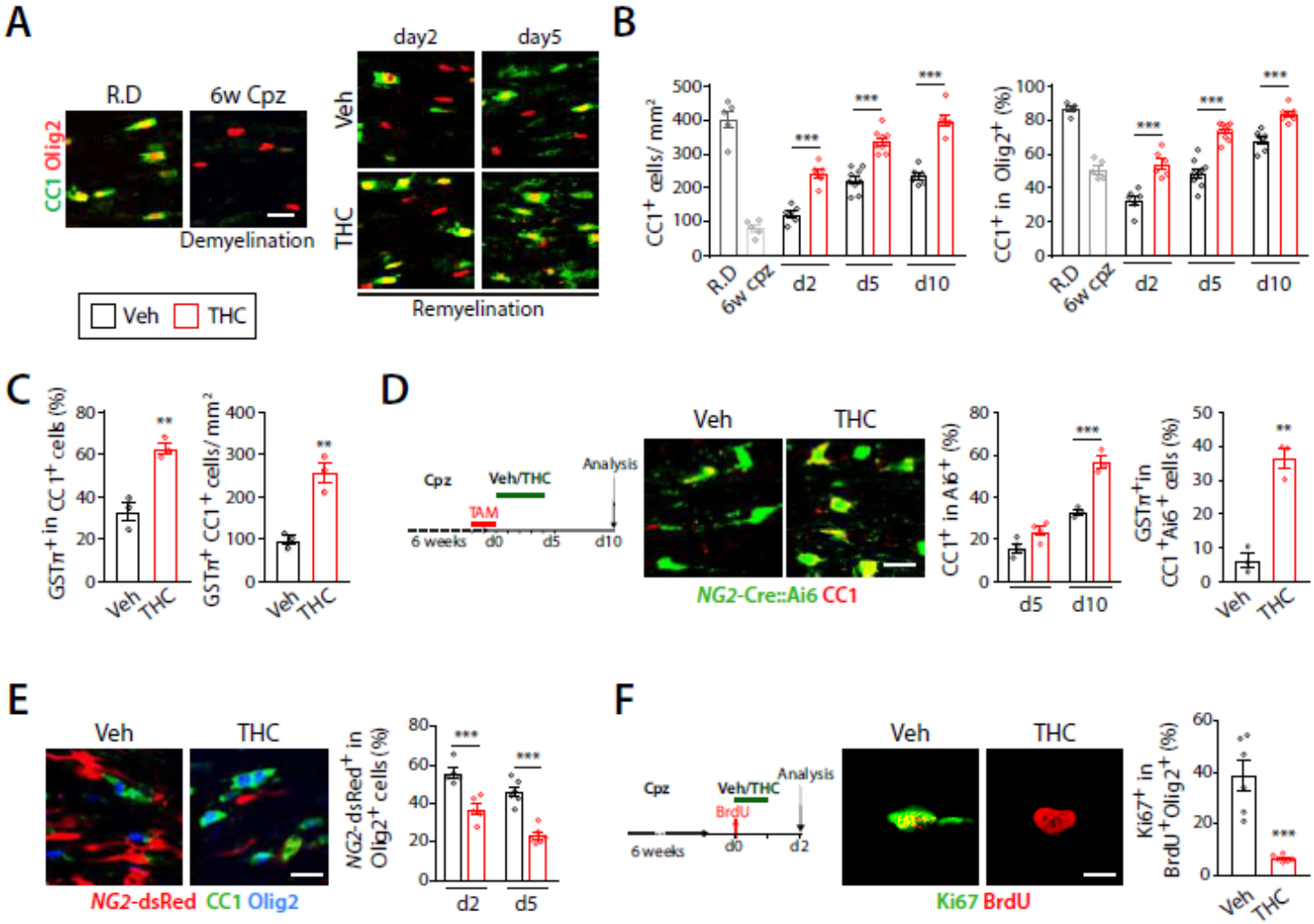

Figure 3

THC enhances oligodendrocyte regeneration during remyelination. Mice were fed with a 0.2% Cpz diet for 6 weeks, followed by a regular diet, and Tetrahydrocannabinol (THC) (3mg/Kg) or vehicle (Veh) was administered for 2 or 5 consecutive days. Oligodendrocyte regeneration was analyzed by immunofluorescence analysis in the CC 2, 5, or 10 days later. Some control animals were fed with regular diet (R.D), or cuprizone for 6 weeks (Cpz 6w). A) Representative images of CC1 and Olig2 staining in the CC. B) Quantification of CC1+ oligodendrocyte cell densities and the percentage of CC1+ oligodendrocytes among Olig2+ cells at day 5 and 10 of recovery. C) Quantification of glutathione-S-transferase GSTπ+CC1+ mature oligodendrocyte cell densities and the percentage of GSTπ+ cells among CC1+ cells at day 10 of recovery. D) NG2-Cre:Ai6 mice were administered with tamoxifen starting 1 day before Cpz removal (3 times, 24h apart). THC or Veh administration was initiated the day following Cpz

withdrawal and was given for 5 consecutive days. Representative confocal images and quantification of the percentage of CC1+ oligodendrocytes among the recombinant Ai6+ population or the percentage of GSTπ+ mature oligodendrocytes among CC1+Ai6+ cells at day 10 of recovery. E) NG2-dsRed received THC or Veh administrations the day following Cpz withdrawal and was given for 5 consecutive days. Immunofluorescence analysis and quantification of the percentage of NG2-dsRed+ among Olig2+ cells. F) Wild-type mice received BrdU injections the day following Cpz removal Representative confocal images and quantification of the percentage of Ki67+ cells among BrdU+Olig2+ cells. Data are shown as mean +/- SEM; n=5-9 mice (B), and n=3-6 (C,D,E,F). **p 0.01, ***p<0.001 from vehicle-treated group, by one-way ANOVA with Tukey's post hoc test (B,D,E), by unpaired Student's t-test (C,D) or unpaired Student's t-tests with Welch correction (F). Scale bars A,D,E=18μm, F=8μm.

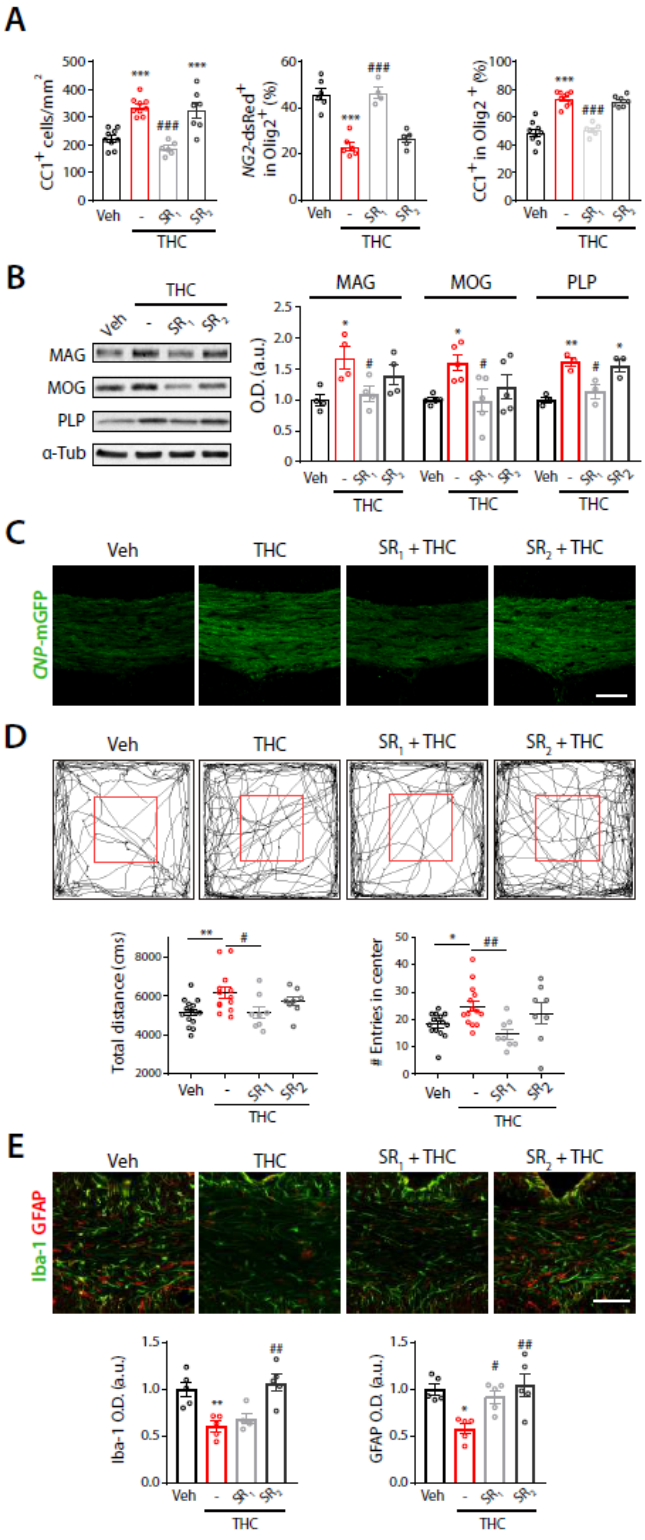

Figure 4

CB1 receptors antagonism prevents the THC-enhanced oligodendrocyte regeneration and functional remyelination. Wild type c57/BL6 (B,D,E), NG2-dsRed (A) or CNP-mGFP (C) mice were fed with a 0.2% Cuprizone (Cpz) diet for 6 weeks, followed by a regular diet. At 24hrs after Cuprizone removal, SR1 (SR-141716A, 2 mg/kg) or SR2 (SR- 144528, 2 mg/kg), selective CB1 or CB2 respective cannabinoid receptors antagonists, were administered 30 min before THC (3 mg/Kg) for 5 consecutive days and

oligodendrocyte regeneration and CNS remyelination was analyzed in the CC 5 days after the first THC administration. A) Quantification of APC, anti-adenomatous polyposis coli (CC1)+ oligodendrocyte cell density and the percentage of NG2-dsRed+ OPC or CC1+ cells among Olig2+ cells. B) Representative scans and optical density (O.D.) values of western blot analysis of myelin-associated protein levels of dissected CC extracts at day 5 of recovery. O.D. are relative to those of their respective loading controls and expressed as arbitrary units (a.u.). Myelin associated glycoprotein (MAG), Myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP). C) Representative confocal images of the CC of CNP-mGFP mice at day 5 of recovery. D) Representative images of trajectories in the open field test. E) Representative confocal images and quantification of microglial (Iba1) and astroglial (GFAP) markers. Data are shown as mean +/- SEM; n=6-9 (A), n=3-5 (B,E), n=8-15 (D), for each experimental group. *p<0.05, **p<0.01, ***p<0.001 from vehicle-treated group or #p<0.05, ##p<0.01###<p 0.001, by one-way ANOVA with Tukey’s post hoc test (A,B,E), and with uncorrected Fisher's LSD (D). Scale bars C,E=60 µm

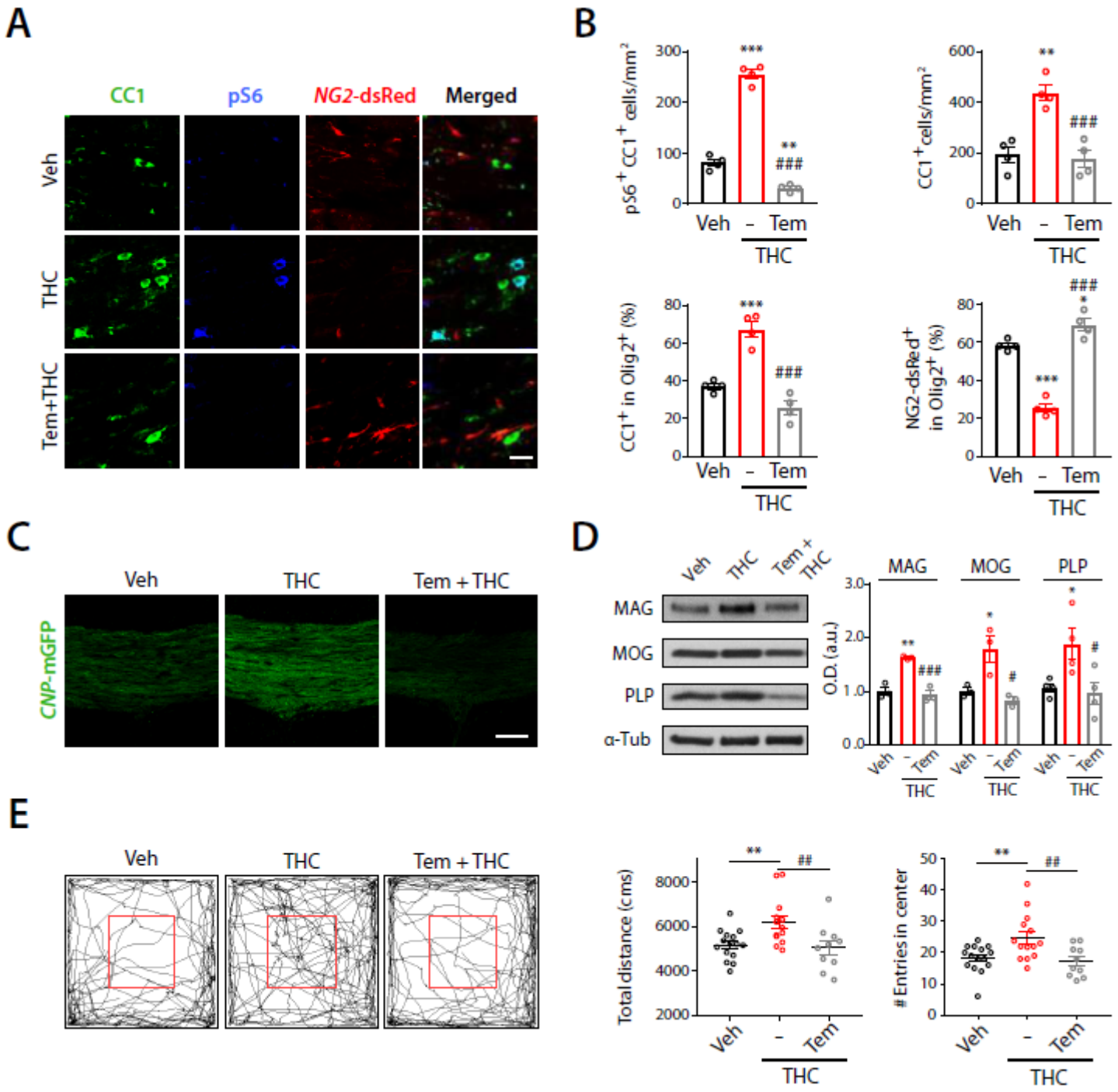

Figure 5

mTORC1 blockade prevents the THC-enhanced oligodendrocyte regeneration and functional remyelination. Adult NG2-dsRed or CNP-mEGFP mice were fed with a 0.2% Cpz diet for 6 weeks, followed by regular diet. At 24hrs after Cuprizone removal, the mTORC1 selective inhibitor Temsirolimus (5mg/kg), was administered at 30 min before THC (3 mg/Kg) for 5 consecutive days, and oligodendrocyte regeneration and remyelination was analyzed in CC 5 days after the first THC administration. A,B) Immunofluorescence analysis of mTORC1 activation levels in oligodendroglial cells. Confocal representative images and quantification of pS6+CC1+ and CC1+ oligodendrocyte cell densities, the

percentage of NG2-dsRed+ or CC1+ cells among Olig2+ cells. S6, Ribosomal protein; CC1, APC, anti-adenomatous polyposis coli. C) Representative confocal images of the CC of CNP-mGFP mice at day 5 of recovery. D) Representative scans and optical density (O.D.) values of western blot analysis of myelin-associated protein levels of CC extracts at day 5 of recovery. O.D. are relative to those of their respective loading controls and expressed as arbitrary units (a.u.). Myelin associated glycoprotein (MAG), Myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP). E) Representative images of trajectories in the open field test. Graph representation of total distance travelled and the number of entries in the center of the arena at day 5 of recovery. Data are shown as mean +/- SEM; n=3-4 mice (B,D), and n=10-15 (E), for each experimental group or time point of analysis. *p<0.05, **p<0.01, ***p<0.001 from vehicle-treated group or #p<0.05, ##p<0.01###<p 0.001, from THC-treated group, by one-way ANOVA with Tukey’s post hoc test (B,D), and with uncorrected Fisher's LSD (E). Scale bars A =18 μm; C=60 μm