SUPPLEMENTARY INFORMATION

Astrocytic Ensemble at vHip-NAc Synapses Modulates Cognitive Impairments Induced by Chronic THC Exposure

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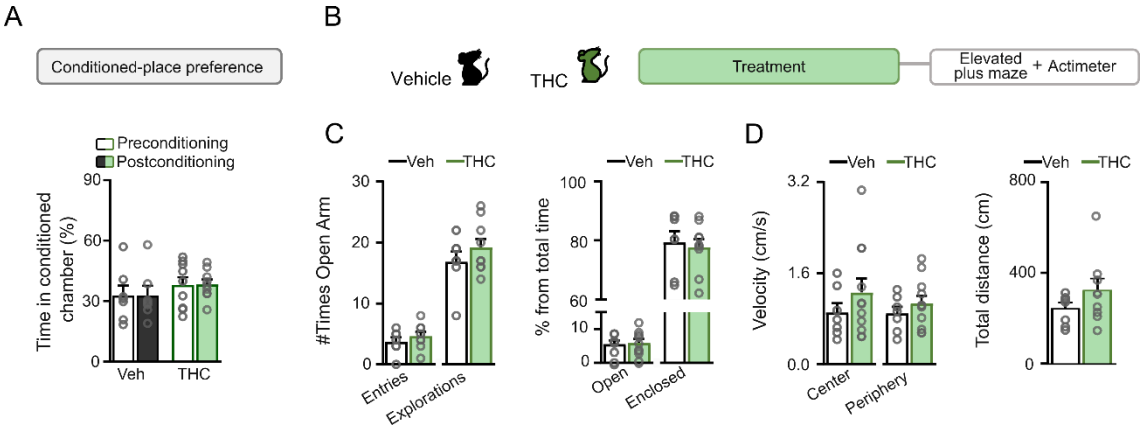
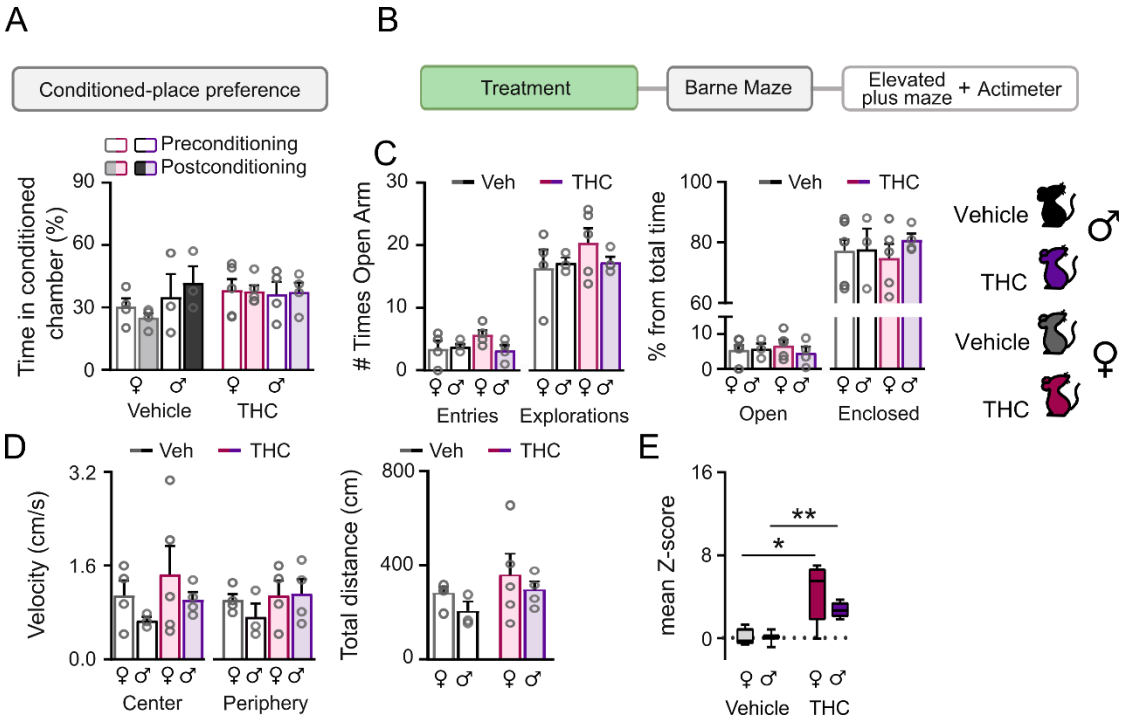
Includes supplementary figures 1 – 9

Figure S1. Effects on behavior of THC exposure during adolescence



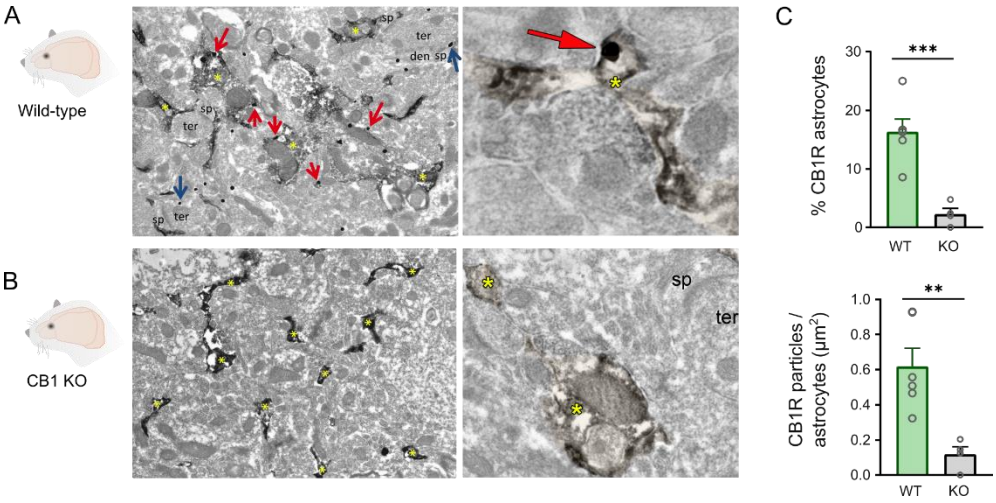
(**A**) Conditioned place preference (CPP) paradigm to study rewarding effects of 1mg/kg THC treatment using mice treated with vehicle (white and black, n = 7 mice) or THC (green, n = 9 mice). Percentage of the time spent in the conditioned chamber before (preconditioning) and after (postconditioning) the treatment. Difference between among pre-and post-conditioning within each treatment was determined by two-tailed paired t-test. Difference between treatments was determined using two-tailed unpaired t-test. (**B**) Timeline of the experimental approach of mice treated with vehicle (white and black, n = 7 mice) or THC (green, n = 9 mice). (**C**) Analysis of the elevated plus maze test. Left: quantification of the number of entries and explorations into the open arm of the maze. Right: percentage of the time spend in the open or enclosed arms of the maze, relative to the total time. Difference between treatment were determined using two-tailed unpaired t-test. (**D**) Analysis of locomotor activity in the actimeter. Left: Quantification of the velocity of the mice in the center and periphery of the actimeter. Difference between treatments were determined using one-way ANOVA and Holm-Sidak test for multiple comparisons. Right: Total distance traveled during the test. Difference between treatments were determined using two-tailed unpaired t-test. All errors bars express SEM.

Figure S2: There is no sexual dimorphism in the behavioral effects of THC treatment.



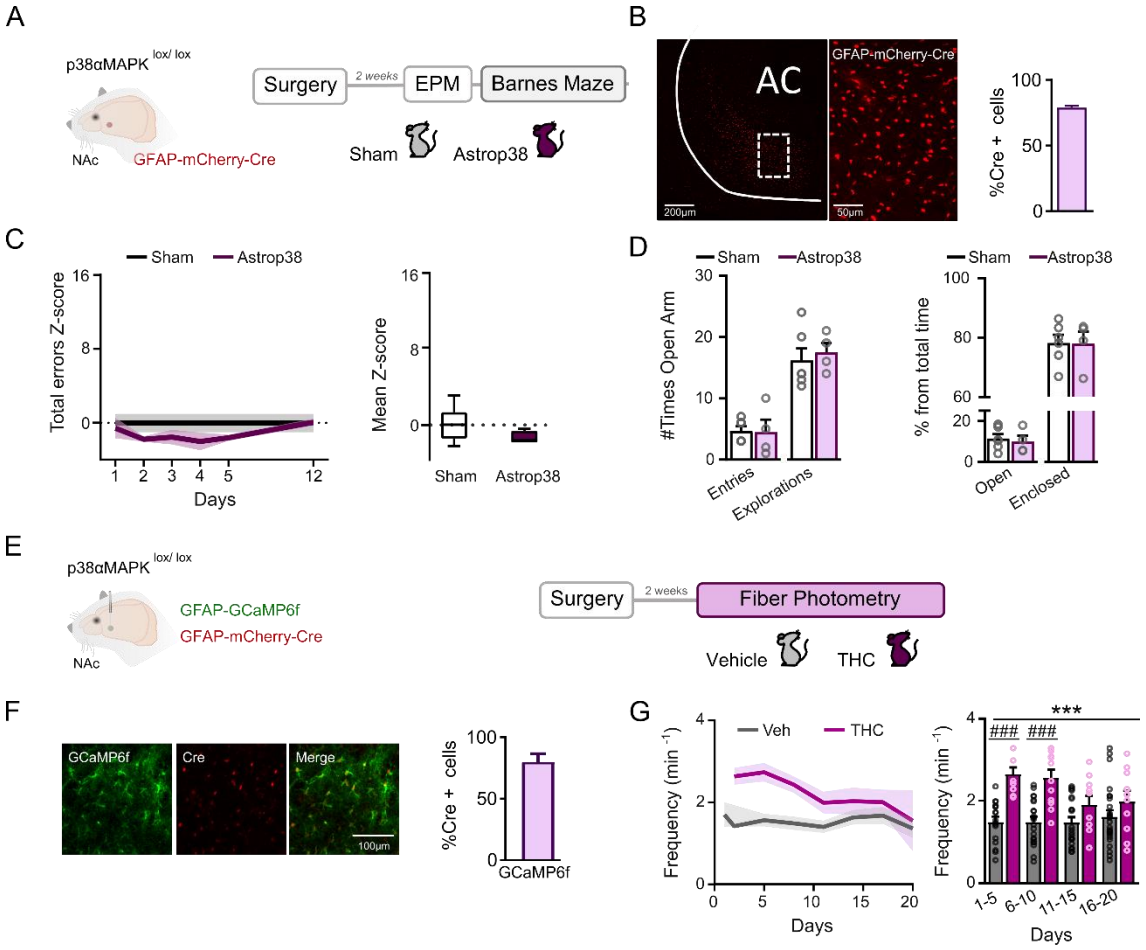
(**A**) Conditioned place preference (CPP) paradigm to study differential rewarding effects of 1mg/kg THC treatment using female and male mice treated with vehicle (female grey, n = 4 mice; male black, n = 3 mice) or THC (female pink, n = 5 mice; male purple, n = 4 mice). Percentage of the time spent in the conditioned chamber before (preconditioning) and after (postconditioning) the treatment. Differences were determined using one-way ANOVA and Holm-Sidak test for multiple comparisons. (**B**) Timeline of the experimental approach of mice treated with vehicle female grey, n = 4 mice; male black, n = 3 mice) or THC (female pink, n = 5 mice; male purple, n = 4 mice). (**C**) Analysis of the elevated plus maze test. Left: quantification of the number of entries and explorations into the open arm of the maze. Right: percentage of the time spend in the open or enclosed arms of the maze, relative to the total time. Differences were determined using one-way ANOVA and Holm-Sidak test for multiple comparisons (**D**) Analysis of locomotor activity in the actimeter. Left: Quantification of the velocity of the mice in the center and periphery of the actimeter. Right: Total distance traveled during the test. Differences were determined using one-way ANOVA and Holm-Sidak test for multiple comparisons. (**E**) Mean z-score of total errors performed during the spatial acquisition phase (days 1 – 4) of Barnes Maze test. *:P < 0.05; **:P < 0.01; All errors bars express SEM. Boxes represent 25 -75 percentiles and median, whiskers represent minimum and maximum data points

Figure S3: CB1R expression in Nucleus Accumbens.



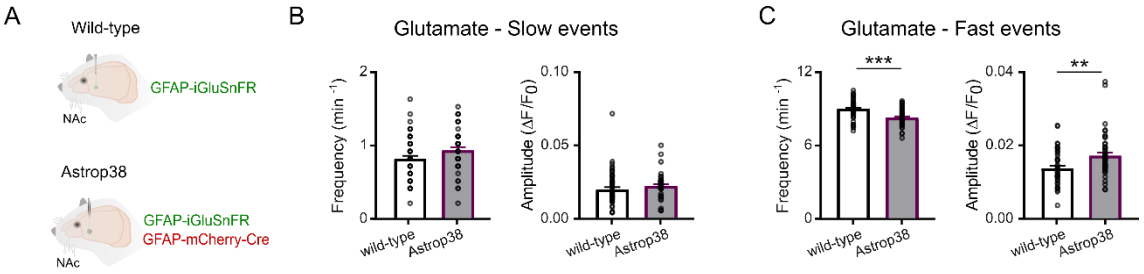
(**A-B**) Representative electron immunogold images of the detection of CB1R on astrocytic processes (identified with anti-GLAST immunoperoxidase staining) in wild-type (**A**) and CB1KO (**B**) mice. Red arrows indicate CB1R in astroglial processes (*); blue arrows indicate CB1R in excitatory terminals. (**C**) Quantification of the % CB1R present in astrocytes and the amount of CB1R particles in area of astrocytes in wild-ype (green, n = 6 mice) and CB1 KO (grey, n = 4 mice). **: $P < 0.01$; ***: $P < 0.001$. All error bars express SEM.

Figure S4: Characterization of Astrop38 mice.



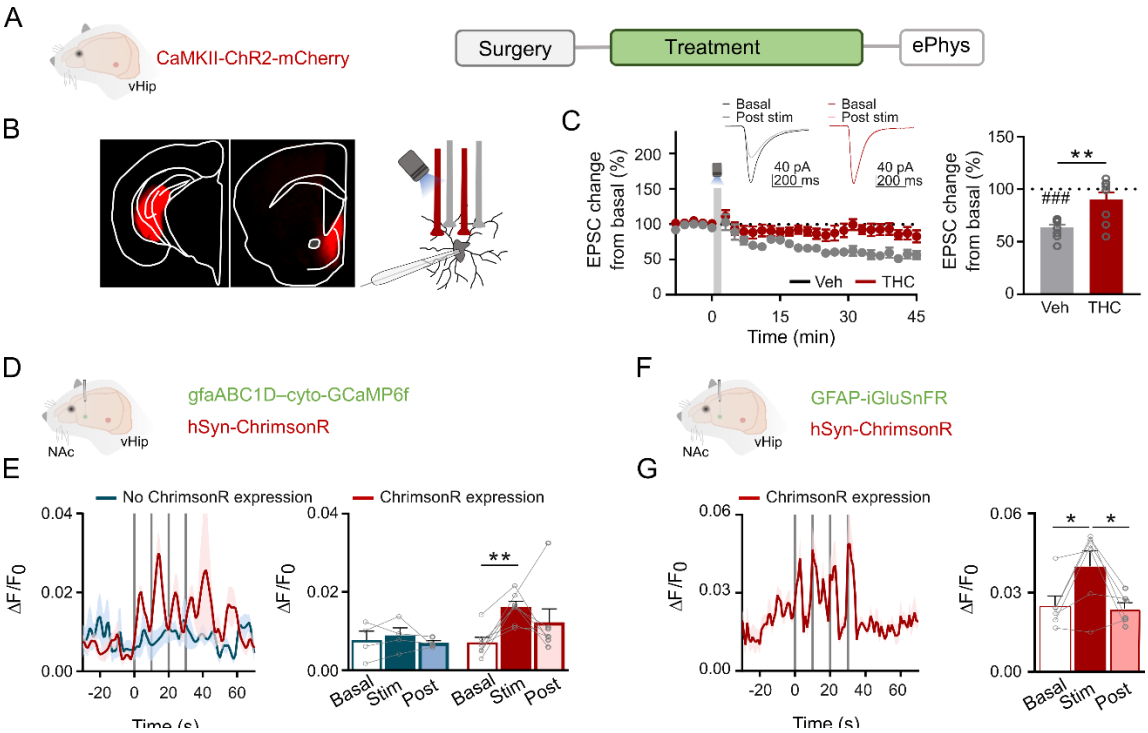
(**A**) Experimental timeline: p38αMAPK$^{lox/lox}$ mice underwent surgery to inject GFAP-mCherry-Cre in NAc astrocytes (purple, n = 4) or received a sham (grey, n = 6) procedure as a control. (**B**) Representative confocal images of the NAc astrocytes expressing Cre and quantification of the percentage of cells expressing the recombinase (n = 6 slices). (**C**) Z-score from total errors performed during the spatial acquisition phase (days 1 – 4) of Barnes Maze test by Astrop38 mice (purple, n = 4) or sham (black, n = 6) and mean z-score of total errors. Differences were determined using two-tailed unpaired t-test. (**D**) Analysis of the elevated plus maze test. Left: quantification of the number of entries and explorations into the open arm of the maze. Right: percentage of the time spend in the open or enclosed arms of the maze, relative to the total time. Difference between treatment were determined using two-tailed unpaired t-test. (**E**) Timeline of the experimental approach in which p38αMAPK$^{lox/lox}$ underwent stereotaxic surgeries to coexpress Cre recombinase and the Ca^{2+} sensor GCaPM6f. (**F**) Representative confocal images and quantification of the co-expression of GCaMP6f and the Cre recombinase (n = 9 slices). (**G**) Frequency of the Ca2+ events during the treatment (left) and mean quantification (right) of vehicle-treated (grey, n = 5 mice) and THC treated (purple, n = 3 mice) mice. Difference between treatments was determined by one-way ANOVA and Holm-Sidak test for multiple comparisons. ***:P < 0.001, ###:P < 0.001; All errors bars express SEM. Boxes represent 25 -75 percentiles and median, whiskers represent minimum and maximum data points.

Figure S5: Analysis of glutamate activity of Astrop38 mice in basal conditions



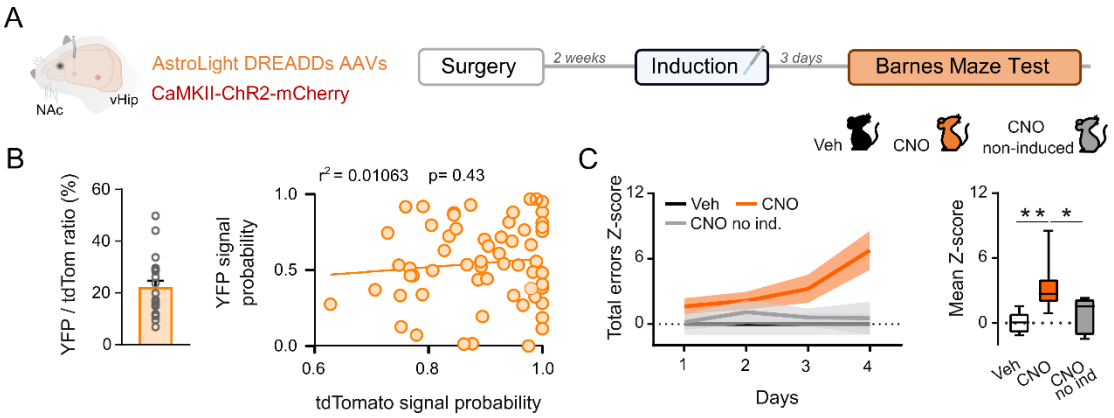
(**A**) Schematic representation of the experimental approach. (**B**) Mean frequency (left) and amplitude (right) of glutamate slow events in wild-type (white, n = 3) and Astrop38 (grey, n = 3) mice under basal conditions. Differences were determined using two-tailed unpaired t-test. (**C**) Mean frequency (left) and amplitude (right) of glutamate fast events in wild-type (white, n = 3) and Astrop38 (grey, n = 3) mice under basal conditions. Differences were determined using two-tailed unpaired t-test. **: P< 0.01; ***: P < 0.001. All errors bars express SEM.

Figure S6: Characterization of vHip-NAc circuit



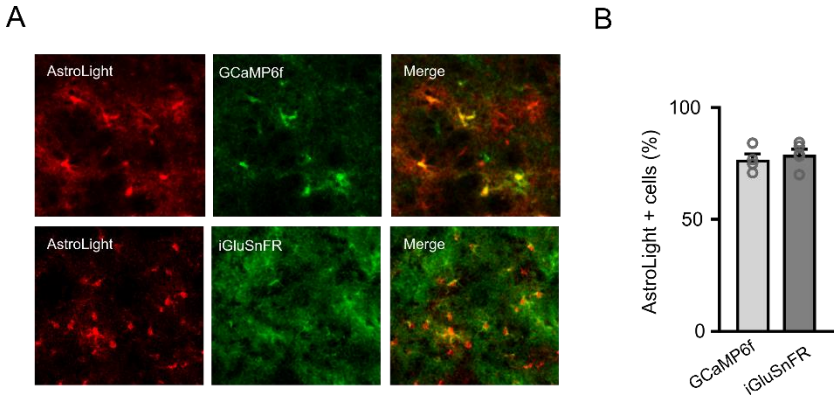
(**A**) Timeline of the experimental approach. Mice underwent stereotaxic surgeries to express ChR2 in vHip. (**B**) Left: Representative images of ChR2-mCherry expression in vHip and the hippocampal afferents in the NAc. Right: schematic representation of the electrophysiology recordings, where NAc neurons are recorded during optostimulation of hippocampal afferents. (**C**) Electrophysiology recording of NAc neurons from mice treated with THC or vehicle during adolescence. Top; Representative EPSCs before and 30 min after optostimulation at 5Hz in acute NAc slices. Relative EPSC amplitudes (left) and average relative changes (right) versus time during LTD experiment with slices from mice treated with vehicle (black, n = 9) and THC (green, n = 8). Differences between groups were determined by two-tail t-test. One sample t-test to compare baseline versus 30 minutes after 5Hz protocol; Time zero corresponds to the onset of the LTD protocol. (**D**) Schematic representation of the experimental approach. (**E**) Average trace (left) and quantification (right) of fiber photometry recordings of Ca^{2+} activity before, during and after optostimulation of hippocampal afferents in mice expressing ChrimsonR in vHipp neurons (red, n = 3 mice) or control animals that not express ChrimsonR (blue, n = 4 mice). Differences were determined using one-way ANOVA and Holm-Sidak test for multiple comparisons. (**F**) Schematic representation of the experimental approach. (**G**) Average trace (left) and quantification (right) of fiber photometry recordings of glutamate activity before, during and after optostimulation of hippocampal afferents in mice expressing ChrimsonR in vHipp neurons (red, n = 4 mice). Differences were determined using one-way ANOVA and Holm-Sidak test for multiple comparisons. *: P< 0.05, **: P< 0.01, ###: P < 0.001. All errors bars express SEM.

Figure S7: Characterization of AstroLight-Gq-DREADDs expression in vHip->NAc astrocytic ensemble



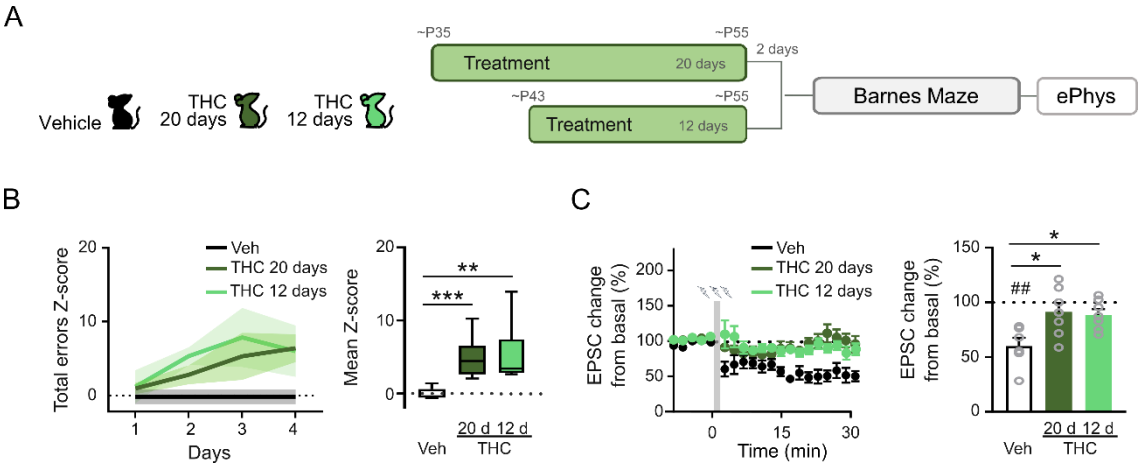
(**A**) Timeline of the experimental approach. (**B**) Left: Quantification of the percentage of particles expressing YFP versus total amount of tdTomato particles. Right: Correlation of the signal probability of YFP fluorescence versus tdTomato signal probability. (**C**) Left; Z-score from total errors performed during the spatial acquisition phase (days 1 – 4) of Barnes Maze test by AstroLight-Gq-DREADDs induced mice treated with vehicle (black/white, n = 5) or CNO (orange, n = 10) and non-induced mice treated with CNO (grey, n = 5) and mean z-score of total errors. Differences were determined using two-tailed unpaired t-test. *: P< 0.05, **: P< 0.01. All errors bars express SEM. Boxes represent 25 -75 percentiles and median, whiskers represent minimum and maximum data points.

Figure S8: Coexpression of AstroLight-tdTomato and the fluorescent sensors



(**A-B**) Representative confocal images (A) and quantification (B) of the coexpression of AstroLight-tdTomato and the fluorescent sensors GCaMP6f (n = 4) and iGluSnFr (n = 5). All errors bars express SEM.

Figure S9: Comparison of different treatment duration during adolescence



(**A**) Timeline of the duration of treatment during adolescence prior to asses behavioral and synaptic consequences using Barnes Maze test and electrophysiology respectively. (**B**) Left; Z-score from total errors performed during the spatial acquisition phase (days 1 – 4) of Barnes Maze test by vehicle-treated mice (black, n = 7), mice treated with THC during 20 days (dark green, n = 9) and mice treated with THC during 12 days (light green, n = 6) and mean z-score of total errors. Differences were determined using two-tailed unpaired t-test. (**C**) Electrophysiology recording of NAc neurons from mice treated with THC or vehicle during adolescence. Relative EPSC amplitudes (left) and average relative changes (right) versus time during LTD experiment with slices from vehicle-treated mice (black, n = 6), mice treated with THC during 20 days (dark green, n = 7) and mice treated with THC during 12 days (light green, n = 7) Differences between groups were determined by two-tail t-test. One sample t-test to compare baseline versus 30 minutes after 5Hz protocol; Time zero corresponds to the onset of the LTD protocol. *: P< 0.05, **: P< 0.01, ***: P < 0.001, ##: P < 0.01. All errors bars express SEM. Boxes represent 25 -75 percentiles and median, whiskers represent minimum and maximum data points