

SUPPLEMENTARY INFORMATION

An astrocytic ensemble at vHip-NAc synapses modulates cognitive impairments induced by chronic tetrahydrocannabinol exposure

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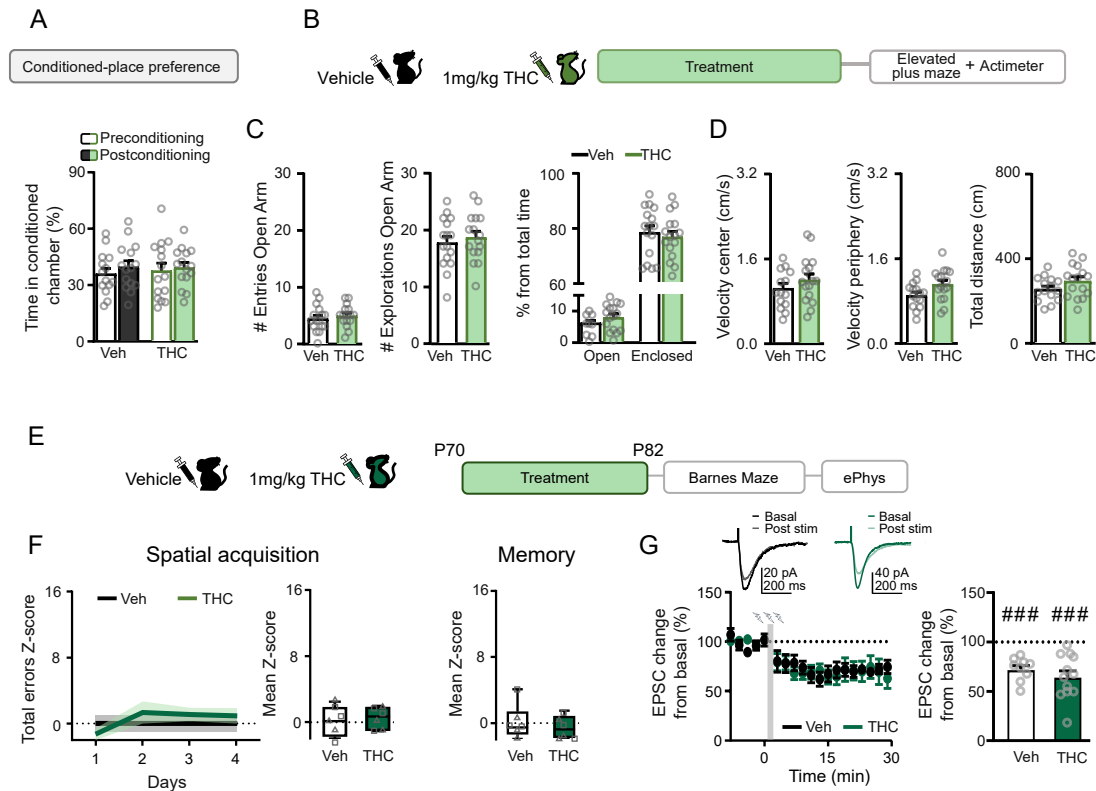
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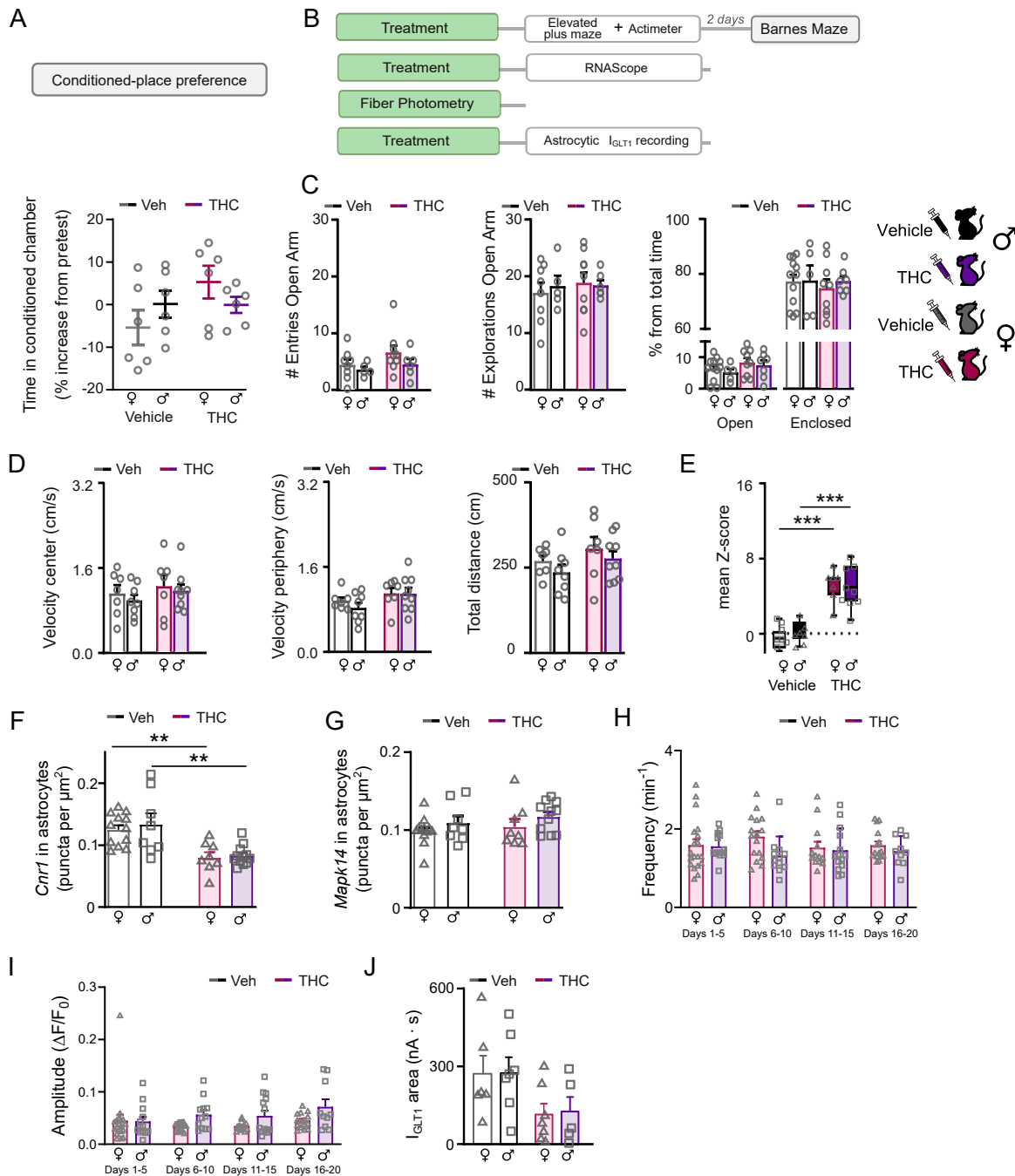
Includes supplementary figures 1 – 13

Figure S1. Behavioral effects of THC exposure during adolescence and adulthood



(A) Conditioned place preference (CPP) paradigm to study rewarding effects of 1mg/kg THC treatment using mice treated with vehicle (white and black, $n = 15$ mice) or THC (green, $n = 15$ mice). Percentage of time spent in the conditioned chamber before (preconditioning) and after (postconditioning) the treatment. Difference between 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, $n = 15$ mice) or THC (green, $n = 16$ 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 spent in the open or enclosed arms of the maze, relative to the total time. Differences between treatments 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. Right: total distance traveled during the test. Differences between treatments were determined using two-tailed unpaired t-test. (E) Timeline of the experimental approach: adult mice (P70) received THC treatment and were tested for spatial memory and electrophysiological recordings. (F) Left: z-score of total errors performed during the spatial acquisition phase (days 1–4) of Barnes Maze test by adult mice treated with THC (green, $n = 6$) or vehicle (black, $n = 6$) and mean z-score of total errors. Differences were determined using two-tailed unpaired t-test. Right: mean z-score of errors during memory test. Two-tailed unpaired t-test. (G) Top: representative EPSCs before and 30 min after electrical 5 Hz stimulation in acute NAC slices. Bottom: electrophysiological recording of NAC neurons from adult mice treated with THC or vehicle during adulthood. Relative EPSC amplitudes (left) and average relative changes (right) versus time during LTD experiment with slices from vehicle-treated (black, $n = 9$ cells) and THC-treated (green, $n = 12$ cells) mice. Differences between groups were determined by two-tailed t-test (*). One sample t-test was used to compare baseline versus 30 minutes after 5 Hz protocol (#). Time zero corresponds to the onset of 5 Hz stimulation *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. All error bars express SEM. Triangles represent females. Squares represent males.

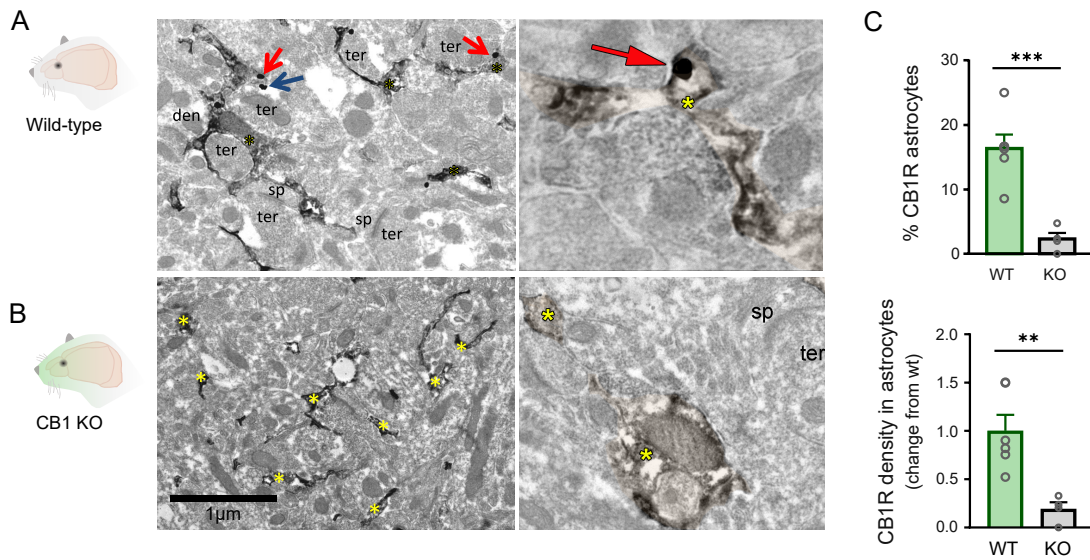
Figure S2: There is no sexual dimorphism in the behavioral effects of THC treatment, *Cnr1* expression, THC-induced astrocytic Ca^{2+} activity nor GLT-1 dependent current in NAC astrocytes



(A) Conditioned place preference (CPP) paradigm to study differential rewarding effects of 1 mg/kg THC treatment using female and male mice treated with vehicle (female, $n = 6$ mice; male, $n = 6$ mice) or THC (female, $n = 6$ mice; male, $n = 6$ mice). Increase in percentage of time spent in the conditioned chamber during postconditioning. Differences were determined using one-way ANOVA and Holm-Sidak test for multiple comparisons. (B) Timeline of the different experimental approaches. (C) Analysis of the elevated plus maze test after vehicle (female, $n = 9$ mice; male, $n = 7$ mice) or THC (female, $n = 9$ mice; male, $n = 7$ mice). Left: quantification of the number of entries and explorations into the open arm of the maze. Right: percentage of time spent in the open or enclosed arms of the maze, relative to the total time. Differences were determined using two-way ANOVA and Holm-Sidak test for multiple comparisons. (D) Analysis

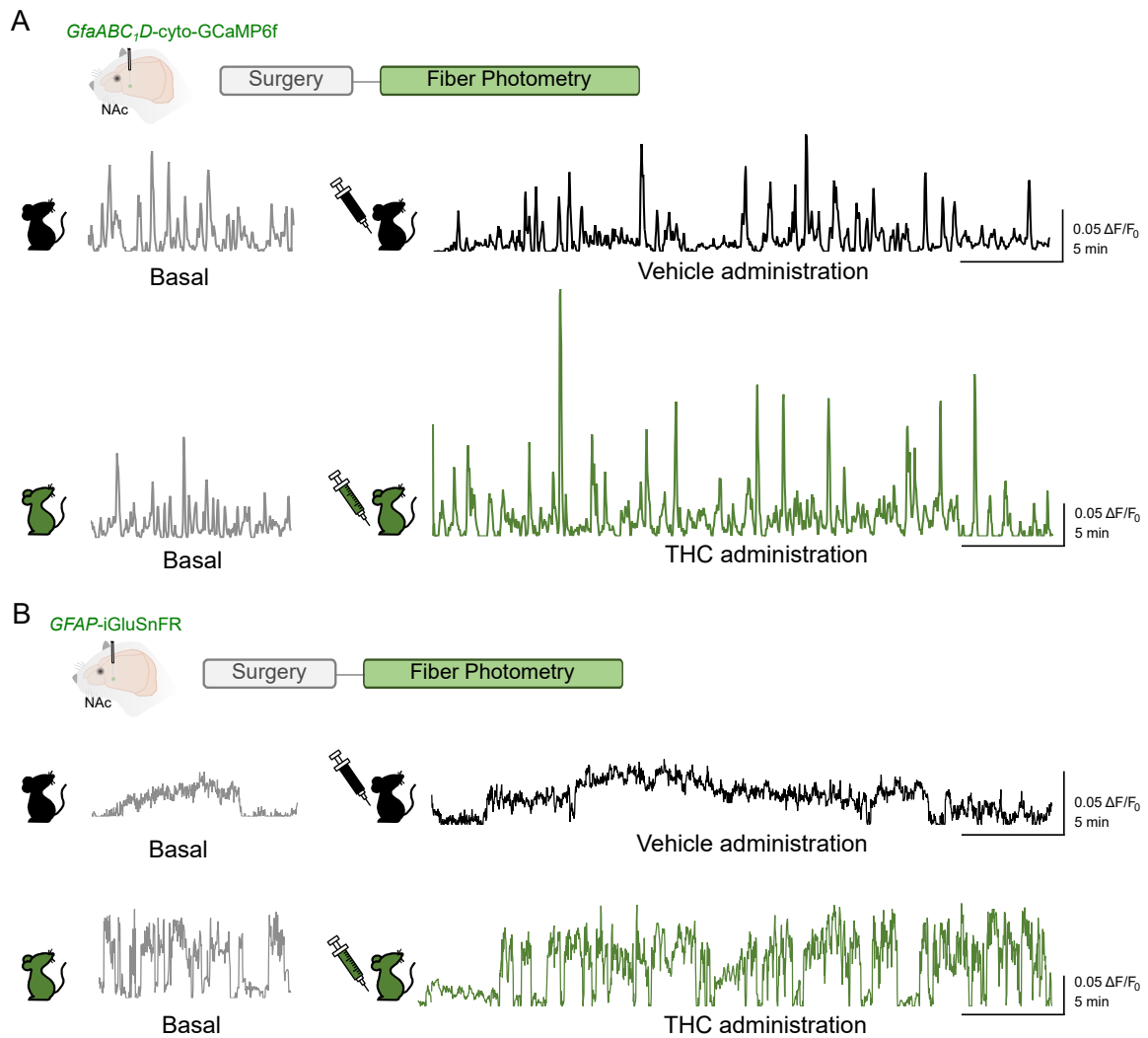
of locomotor activity in the actimeter in mice treated with vehicle (female, n = 7; male, n = 8) or THC (female, n = 7, male, n = 9). Left: Quantification of the velocity of mice in the center and periphery of the actimeter. Right: Total distance traveled during the test. Differences were determined using two-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 after vehicle (female, n = 7; male, n = 7) or THC (female, n = 7; male, n = 9) treatment. **(F-G)** *Cnr1* (F) and *Mapk14* (G) puncta per μm^2 of astrocyte area in vehicle (female, n = 12 slices, 3 mice; male, n = 8 slices, 2 mice) and THC-treated mice (female, n = 8 slices, 2 mice; male, n = 12 slices, 3 mice). Differences between groups were determined using two-way ANOVA and Holm-Sidak test for multiple comparisons. **(H-I)** Comparison of THC-effect in frequency (H) and amplitude (I) in females and males during fiber photometry recordings (females, n = 4; males, n = 3). Differences between groups were determined a mixed-effect model and Holm-Sidak test for multiple comparisons. **(J)** GLT-1-mediated current ($I_{\text{GLT-1}}$), in astrocytes from vehicle (females, n = 6 cells; males, n = 7 cells) and THC-treated mice (females, n = 7 cells; males, n = 5 cells). Differences between groups were determined using two-way ANOVA and Holm-Sidak test for multiple comparisons. **:P < 0.01; ***:P < 0.001. All error bars express SEM. Boxes represent 25-75 percentiles and median, whiskers represent minimum and maximum data points. Triangles represent females. Squares represent males.

Figure S3: CB1R expression in nucleus accumbens.



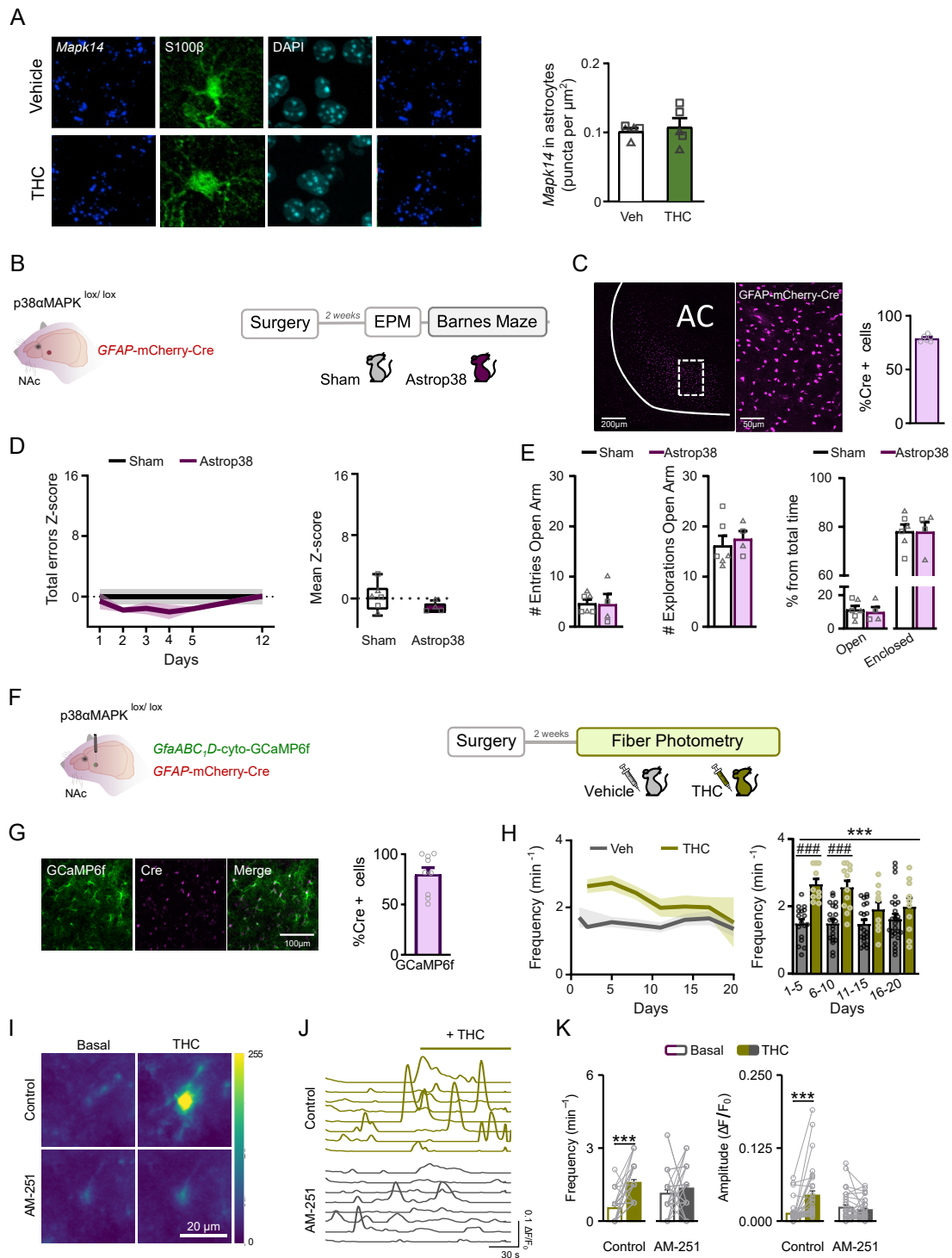
(A-B) Representative images and quantification (C) of labeling by preembedding immunogold method for electron microscopy of CB1R in wild-type (A; $n = 6$ mice) and CB1R KO (B; $n = 4$ mice) mice. Quantification indicates the percentages of CB1R immunopositive astrocytic processes in WT versus KO (top) and CB1R immunoparticle density on membranes of astrocytic processes (particles/ μm^2) (bottom). Data are expressed as mean $\pm$ SEM. *** $p < .001$; ** $p < .01$. Red arrows indicate CB1R in astroglial processes (*); blue arrow indicates CB1R in excitatory terminal. ter: terminal; den: dendrite; sp: dendritic spine. Scale bar: 1 μm .

Figure S4: Ca^{2+} and glutamate activity before and after treatment administration



(A-B) Representative traces of fiber photometry recordings monitoring astrocytic Ca^{2+} activity (A) and extracellular glutamate (B) before (basal) and after treatment administration on day 12 of treatment.

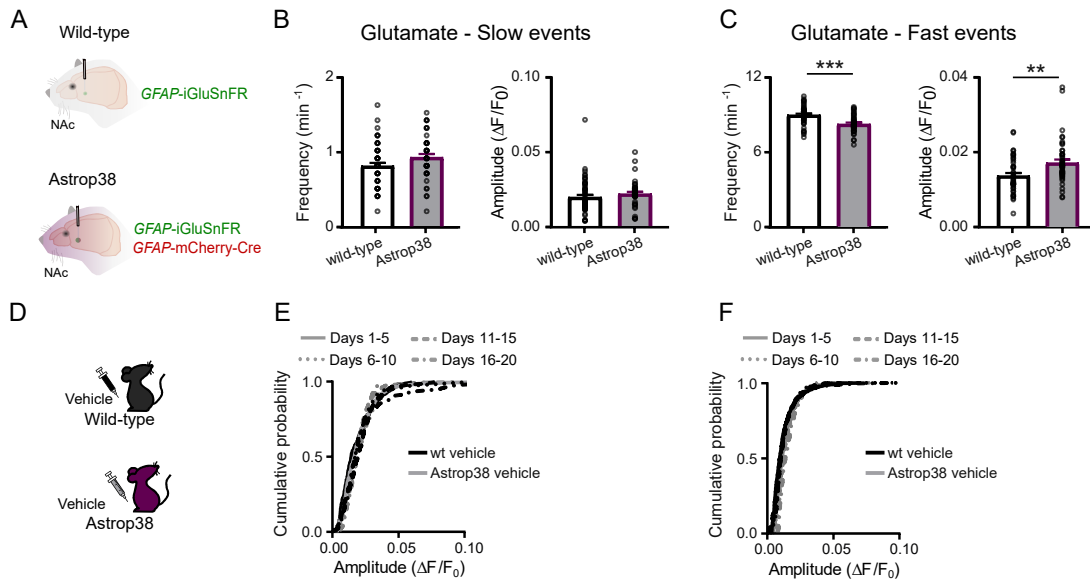
Figure S5: Characterization of Astrop38 mice.



(A) Left: RNAscope detection of *Mapk14* mRNA in astrocytes immunostained for S100 β and labeled with DAPI from vehicle or THC-treated mice. Right: *Mapk14* puncta per μm^2 of astrocyte area in vehicle ($n = 5$ mice) and THC-treated mice ($n = 5$ mice). Differences between groups were determined by two-tailed t-test. (B) Experimental timeline: p38 α MAPK^{lox/lox} mice underwent surgery to inject *GFAP*-mCherry-Cre in NAc astrocytes ($n = 4$) or received a sham procedure ($n = 6$) as a control. (C) Representative confocal images of the NAc astrocytes expressing Cre and quantification of the percentage of cells expressing the recombinase ($n = 6$ slices, 3 mice). (D) Z-score of total errors performed during the spatial acquisition phase (days 1–4) of Barnes Maze test by Astrop38 mice ($n = 4$) or sham ($n = 6$) and mean z-score of total errors. Differences were

determined using two-tailed unpaired t-test. (E) 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 between treatments were determined using two-tailed unpaired t-test. (F) Timeline of the experimental approach in which p38 α MAPK^{lox/lox} underwent stereotaxic surgeries to co-express Cre recombinase and the Ca²⁺ sensor GCaMP6f. (G) Representative confocal images and quantification of the co-expression of GCaMP6f and the Cre recombinase (n = 9 slices). (H) Frequency of Ca²⁺ events during the treatment (left) and mean quantification (right) of vehicle-treated (n = 5 mice) and THC-treated (n = 3 mice) mice. The difference between treatments was determined by one-way ANOVA and Holm-Sidak test for multiple comparisons. (I) Representative images of GCaMP6f fluorescence of NAc astrocytes before and after THC in control condition (top) and in the presence of CB1R inverse agonist AM-251 (bottom; from n = 4 mice). (J) Representative Ca²⁺ signals from NAc astrocytes before and after a THC puff in control slices (top) and in the presence of AM-251 (bottom). (K) Quantification of the frequency (left) and amplitude (right) of the Ca²⁺ events before and after THC in control and in the presence of AM-251 (n = 32 ROIs). Differences between basal and THC administration were determined by two-tailed paired t-test. ***: P < 0.001, ###: P < 0.001; All error bars express SEM. Boxes represent 25-75 percentiles and median, whiskers represent minimum and maximum data points. Triangles represent females. Squares represent males.

Figure S6: 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. (D-F) Comparison of cumulative probability of slow (E) and fast (F) glutamate events during vehicle treatment in wild-type ($n = 3$ mice) and Astrop38 ($n = 3$ mice) mice. Differences were determined using two-tailed unpaired t-test. **: $P < 0.01$; ***: $P < 0.001$. All error bars express SEM.

Figure S7: Characterization of the vHip-NAc circuit

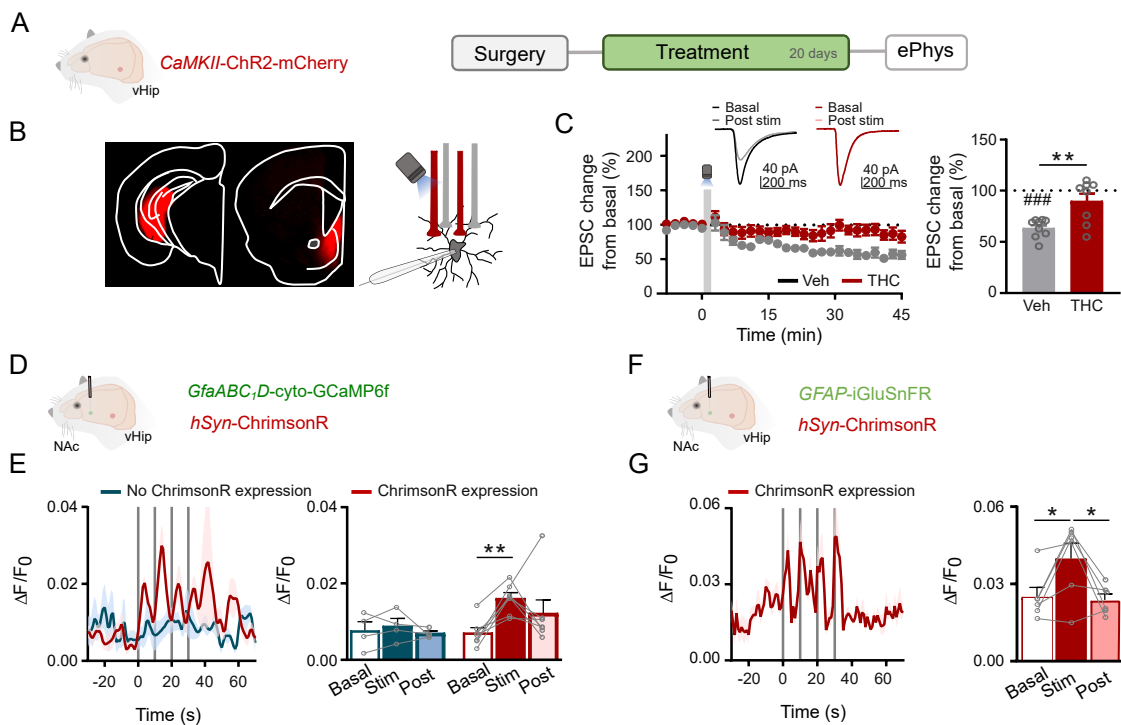
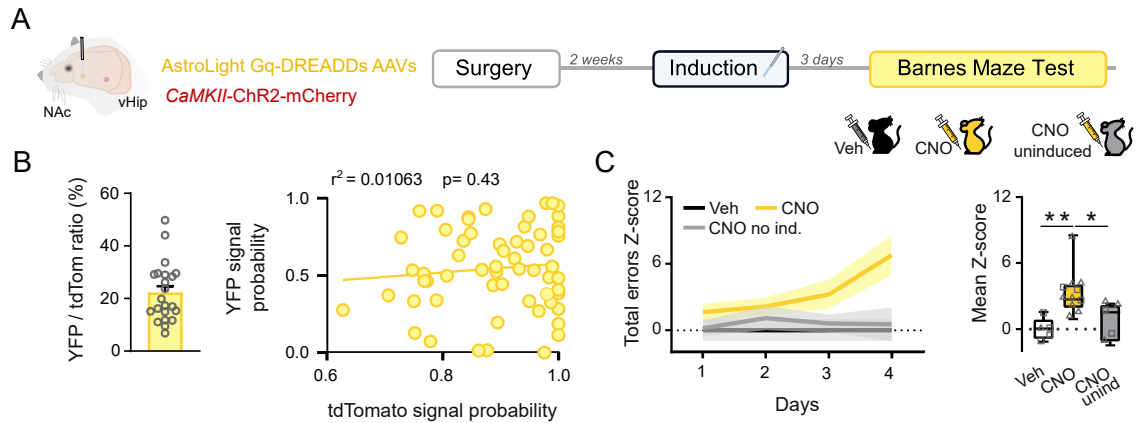
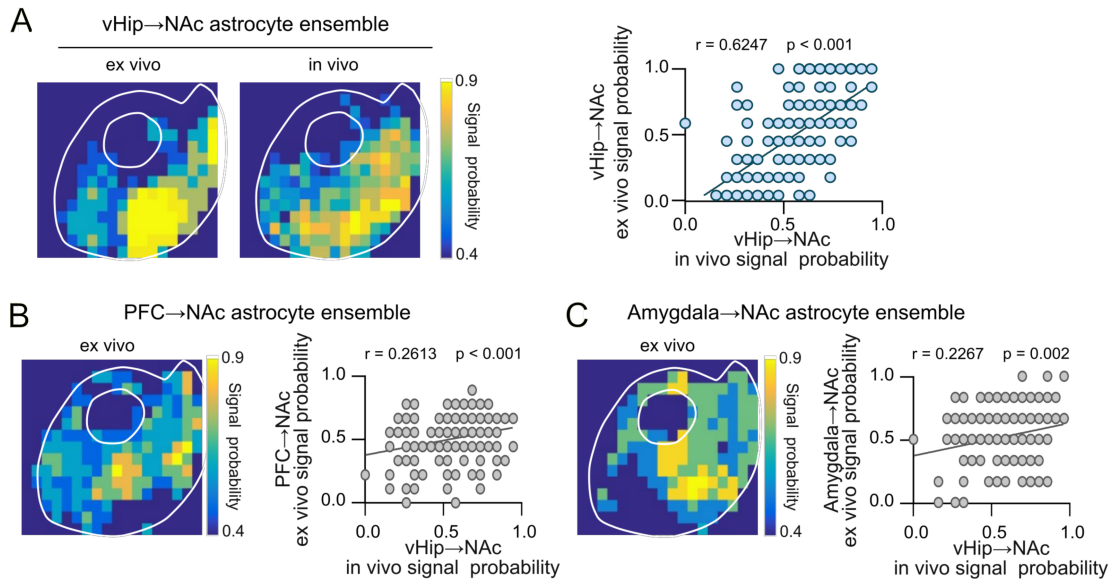


Figure S8: Characterization of AstroLight-Gq-DREADDs expression in vHip->NAc astrocytic ensemble



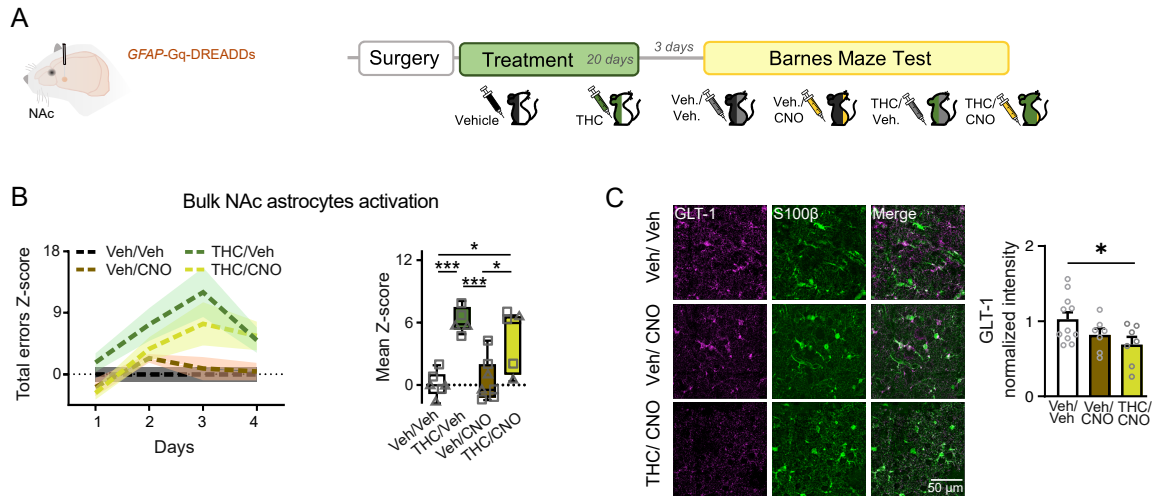
(A) Timeline of the experimental approach. (B) Left: quantification of the percentage of particles expressing YFP versus the total amount of tdTomato particles. Right: correlation of the signal probability of YFP fluorescence versus tdTomato signal probability. (C) Left: z-score of total errors performed 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 (yellow, $n = 10$) and uninduced mice treated with CNO (grey, $n = 6$). Right: mean z-score of total errors. Differences were determined using two-tailed unpaired t-test. *: $P < 0.05$, **: $P < 0.01$. All error bars express SEM. Boxes represent 25-75 percentiles and median, whiskers represent minimum and maximum data points. Triangles represent females. Squares represent males.

Figure S9: Comparison of the in vivo and ex vivo vHip→NAc astrocytic ensemble



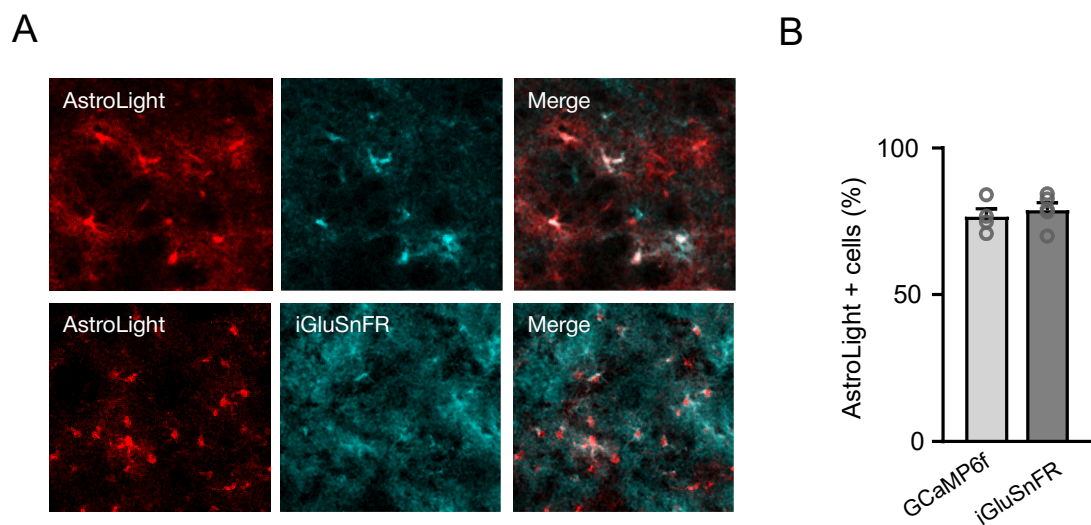
(A) Representative images of fluorescent signal probability across the NAc for vHip-NAc astrocyte ensemble for ex vivo (left, $n = 8$ slices) and in vivo (right, $n = 19$ slices) experiments and linear pixel-wise correlation of the ex vivo and in vivo signal probability. (B-C) Representative images of signal probability across the NAc for ex vivo astrocyte ensemble identification engaged in the prefrontal cortex (PFC, B) and amygdala (C) circuits and the corresponding linear correlation with in vivo vHip-NAc astrocyte ensemble. Correlation was determined by Pearson's test. Ex vivo ensemble data were obtained from Serra et al. (2022) *Nat Commun*.

Figure S10: Bulk reactivation of NAc astrocytes with Gq-DREADDs does not restore THC-induced impairments.



(A) Timeline of the experimental approach. (B) Left: z-score of total errors performed during the spatial acquisition phase (days 1–4) of Barnes Maze test by mice expressing Gq-DREADDs under a pan-astrocytic promoter treated with vehicle/vehicle ($n = 6$), THC/vehicle ($n = 5$), vehicle/CNO ($n = 7$) or THC/CNO ($n = 5$). Right: mean z-score of total errors. Differences were determined using one-way ANOVA followed by Holm-Sidak test for multiple comparisons. (C) Representative images of GLT-1 immunolabeling (left) and quantification of GLT-1 staining intensity (right) in vehicle/vehicle ($n = 11$), vehicle/CNO ($n = 7$) or THC/CNO-treated mice ($n = 8$). The difference between treatments was determined using one-way ANOVA followed by Holm-Sidak test for multiple comparisons. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. All error bars express SEM. Boxes represent 25-75 percentiles and median, whiskers represent minimum and maximum data points. Triangles represent females. Squares represent males.

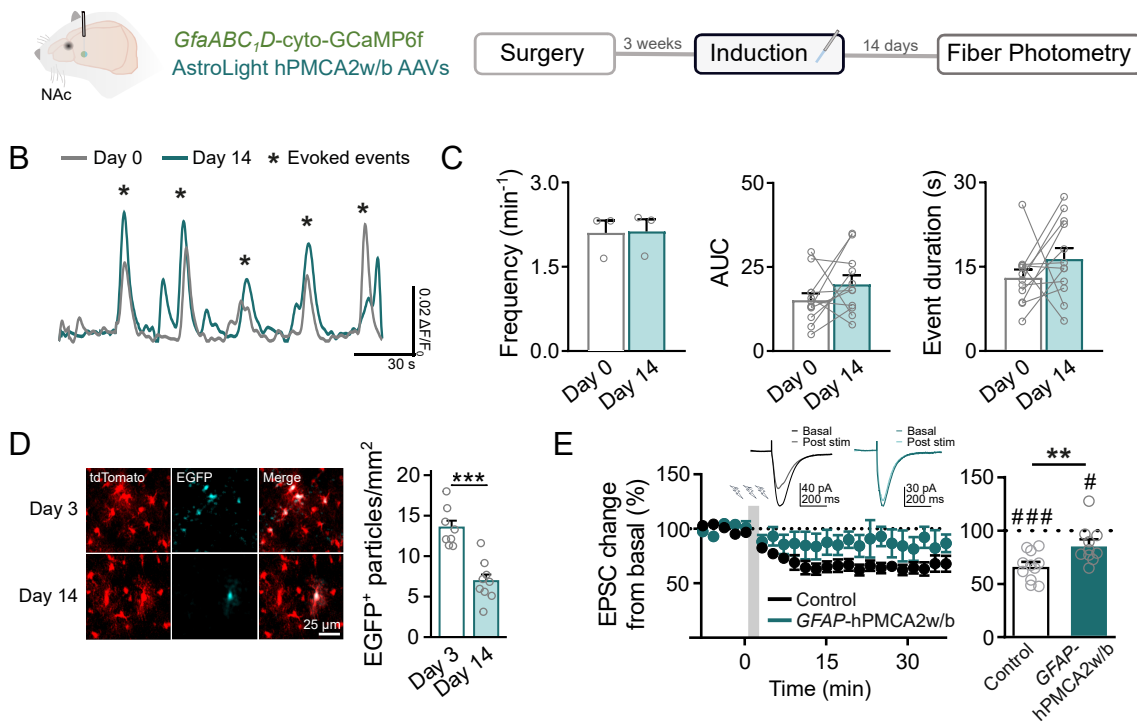
Figure S11: Co-expression of AstroLight-tdTomato and fluorescent sensors



(A-B) Representative confocal images (A) and quantification (B) of the co-expression of AstroLight-tdTomato and the fluorescent sensors GCaMP6f ($n = 4$) and iGluSnFR ($n = 5$). All errors bars express SEM.

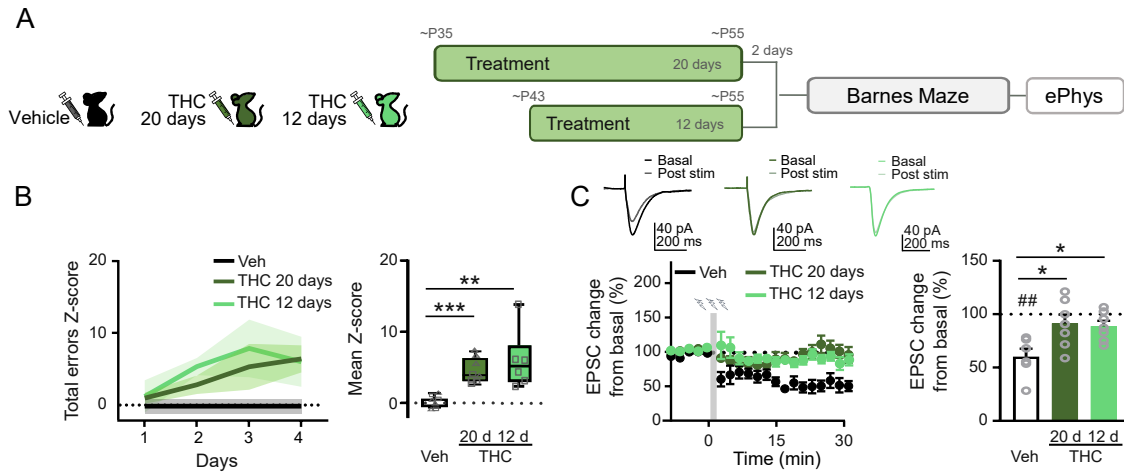
Figure S12: AstroLight-hPMCA2w/b expression is transient and has different effects from bulk hPMCA2w/b expression in NAc astrocytes.

A



(A) Timeline of the experimental approach. (B) Representative traces of fiber photometry recordings of Ca^{2+} activity in mice expressing GCaMP6f in astrocytes before (day 0, grey) or 14 days (blue) after AstroLight-hPMCA2w/b induction. Stars (*) indicate evoked Ca^{2+} events. (C) Quantification of the Ca^{2+} event frequency (left), area under the curve (AUC, middle) and duration (right) on day 0 ($n = 3$) and day 14 ($n = 3$) after AstroLight-hPMCA2w/b induction. Differences were determined using paired two-tailed t-test. (D) Left: representative confocal images of AstroLight-hPMCA2w/b expression at the time of behavioral experiments (day 3, $n = 8$) and 14 days after induction ($n = 9$). Right: quantification of hPMCA2w/b-expressing particles per mm^2 . Differences were determined using two-tailed t-test. (E) Top: representative EPSCs before and 30 min after electrical 5 Hz stimulation in acute NAc slices. Bottom: electrophysiological recording of NAc neurons from control mice or mice expressing hPMCA2w/b under a pan-astrocytic promoter. Relative EPSC amplitudes (left) and average relative changes (right) versus time during LTD experiment with slices from control ($n = 11$ cells) and hPMCA2w/b-expressing ($n = 10$ cells) mice. Differences between groups were determined by two-tailed t-test (*). One sample t-test was used to compare baseline versus 30 minutes after 5 Hz protocol (#). Time zero corresponds to the onset of 5 Hz stimulation. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. All error bars express SEM.

Figure S13: Comparison of different treatment durations during adolescence



(A) Timeline of the duration of treatment during adolescence prior to assessing behavioral and synaptic consequences using Barnes Maze test and electrophysiology. (B) Left: z-score of 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) Top: representative EPSCs before and 30 min after electrical 5 Hz stimulation in acute NAc slices. Bottom: electrophysiological 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 using two-tailed t-test (*). One sample t-test was used to compare baseline versus 30 minutes after 5 Hz protocol (#). Time zero corresponds to the onset of the LTD protocol. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ##: $P < 0.01$. All error bars express SEM. Boxes represent 25–75 percentiles and median, whiskers represent minimum and maximum data points. Triangles represent females. Squares represent males.