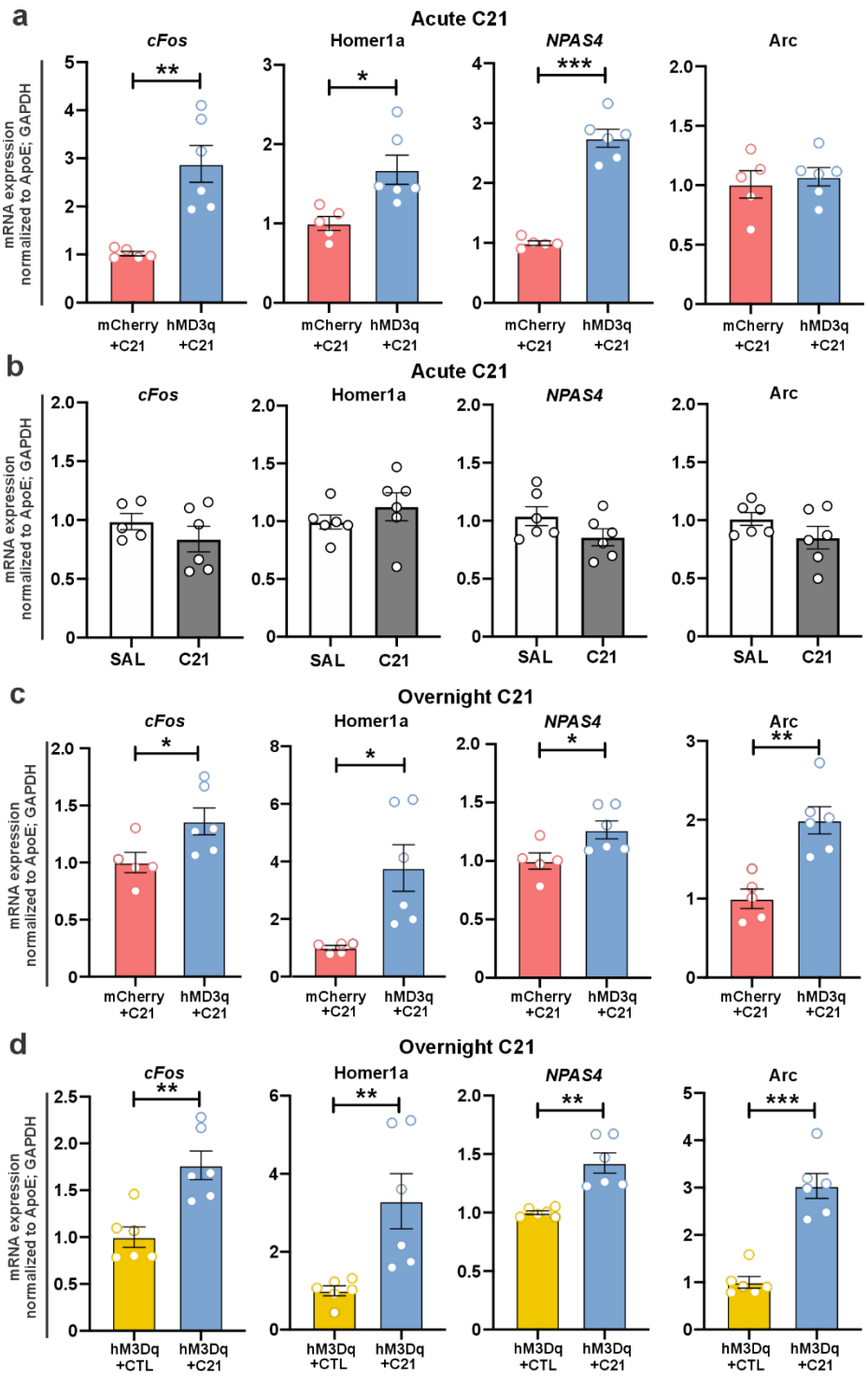
Supplementary Data for

Linking Brain Circuitry and Neural Plasticity in Antidepressant Response: The mPFC-Reuniens-Hippocampus Pathway.

Veleanu et al.

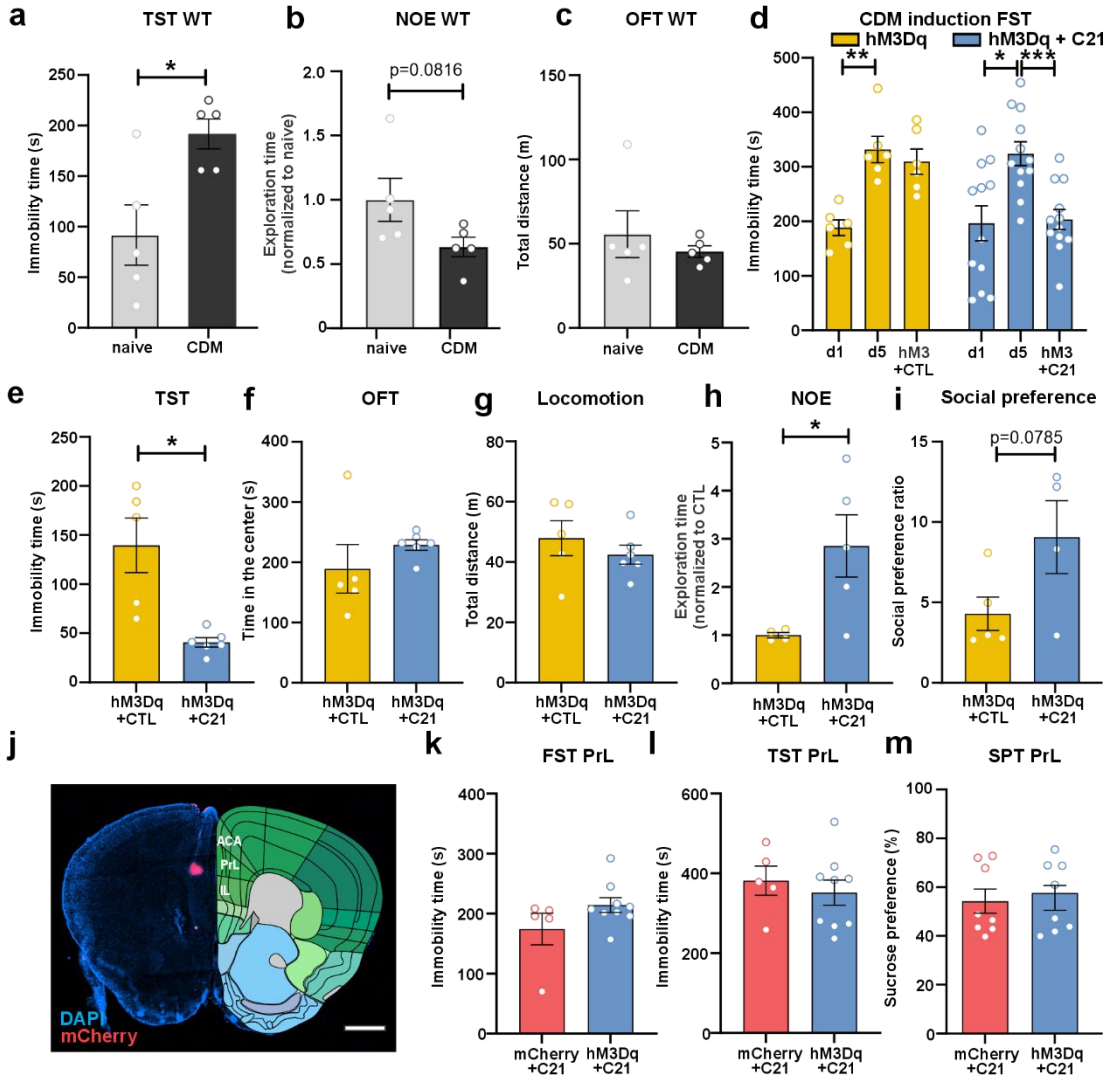
Includes :

Supplementary Figures 1-7



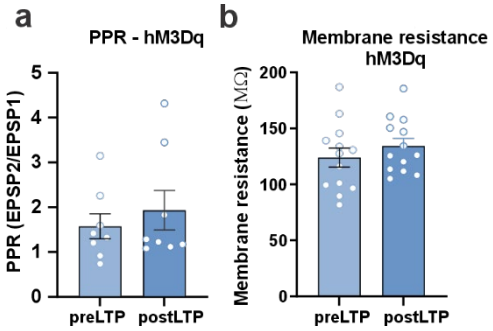
Supplementary Fig. 1 | a mRNA expression of immediate early genes (normalized to ApoE and GAPDH) in the mPFC of mCherry (n=5) and hM3Dq (n=6) mice 2h after acute C21 treatment (cFos: *P*=0.0044, Welch's t-test, Homer1a: *P*=0.0127, NPAS4: *P*<0.0001, Arc: *P*=0.6465). **b** mRNA expression of immediate early genes in the mPFC of mCherry (n=6) and hM3Dq (n=6) mice 2h after acute saline or C21 i.p. treatment in wild-type mice (cFos: *P*=0.3028, Homer1a: *P*=0.3514, NPAS4: *P*=0.1322, Arc: *P*=0.1835). **c** mRNA expression in the mPFC of mCherry (n=5) and hM3Dq (n=6) mice following overnight C21 treatment (cFos: *P*=0.0426, Homer1a: *P*=0.0185, Welch's t-test, NPAS4: *P*=0.0320), Arc: *P*=0.0015). **d** mRNA

expression of immediate early genes in the mPFC of hM3Dq mice (n=6 per group) after overnight C21-treated and untreated controls (cFos: P=0.0021, Homer1a: P=0.0096, Welch's t-test, NPAS4: P=0.0040, Welch's t-test, Arc: p<0.0001). Unless otherwise stated, statistical comparisons were performed using unpaired two-tailed Student's t-test. Data are presented as mean ±SEM and the individual data points are depicted. P<0.05, *P<0.01, **P<0.001. Source data are provided as a Source Data file.

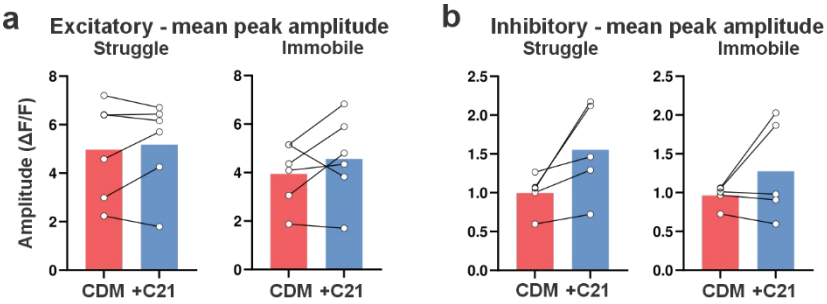


Supplementary Fig. 2 | a Immobility time in tail suspension test (TST) of wild type (WT) CDM mice and naïve mice (n=5, *P*=0.0171, unpaired Student's t-test). **b** Total time spent exploring two novel objects in novel object exploration test (NOE) between CDM and naïve WT mice. Data normalized to naïve group (n=5, *P*=0.0816, unpaired Student's t-test). **c** Locomotion measured by distance travelled in open field test (OFT) of CDM and naïve WT mice (n=5, *P*=0.5057, Welch's t-test). **d** Immobility time in CDM of hM3Dq-injected mice receiving either overnight C21 in drinking water or untreated controls (repeated measures two-way ANOVA with Bonferroni post-hoc test). **e** Immobility time in TST of hM3Dq-injected mice treated overnight with C21 (n=6) and controls (n=5) (*P*=0.0225, Welch's t-test). **f** Time spent in center of OFT of hM3Dq-injected mice treated with C21 (n=6) and untreated controls (n=5) (*P*=0.3862, Welch's t-test). **g** Locomotion measured by distance travelled in OFT between hM3Dq-injected mice treated overnight with C21 (n=6) and untreated controls (n=5) (*P*=0.4074, unpaired Student's t-test). **h** Total time spent exploring two novel objects of hM3Dq-injected mice overnight treated with C21 (n=5) and untreated controls (n=4) (*P*=0.0454, Welch's t-test). **i** Social interaction measured in three-chamber task between hM3Dq-injected

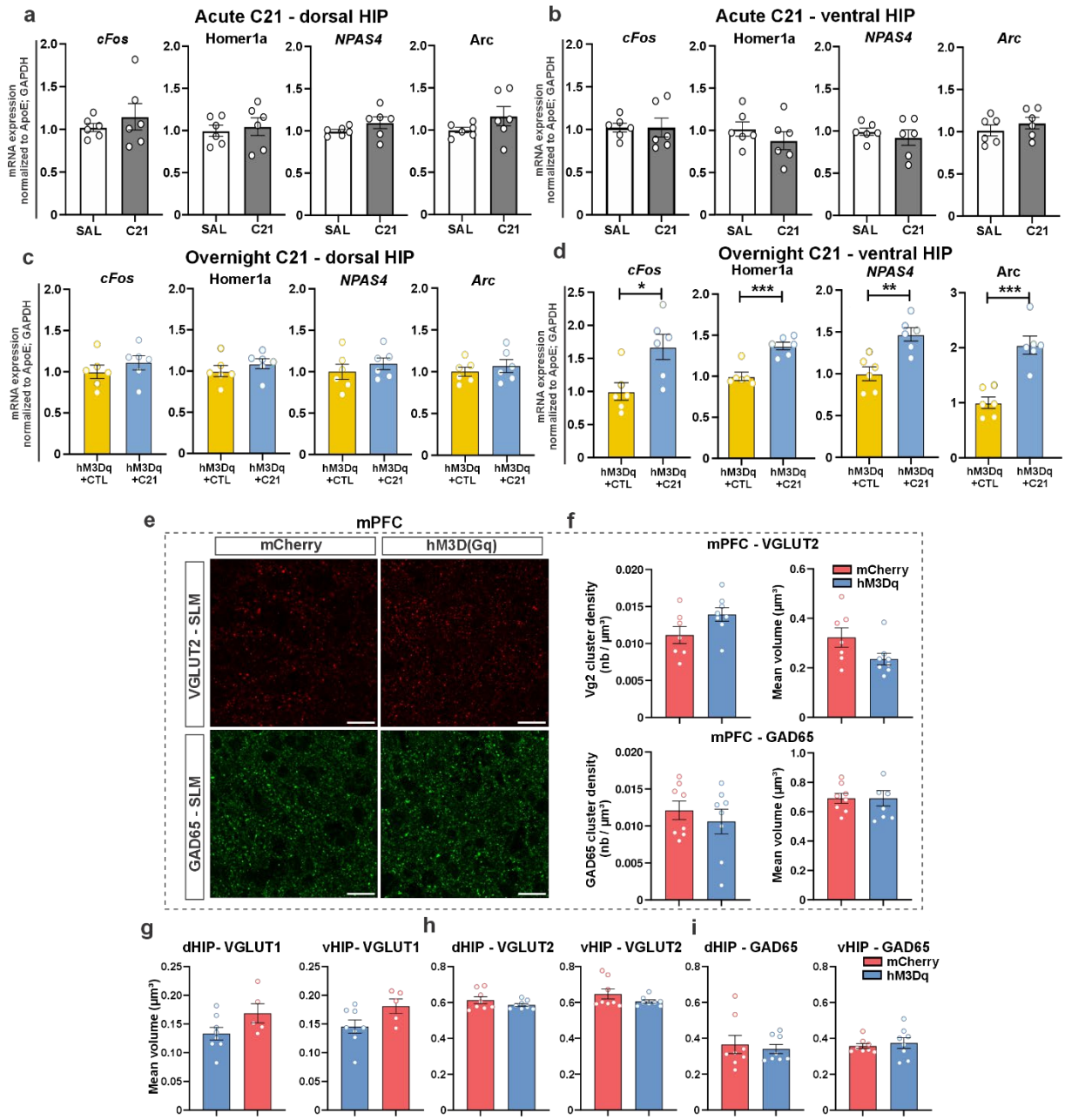
mice overnight treated with C21 (n=5) and untreated controls (n=4) (*P*=0.0785, unpaired Student's t-test). **j** Representative image of AAV-CaMKIIa-hM3D(Gq)-mCherry injection in lateral prelimbic cortex (PrL). Scale bar: 1000 µm. **k** Immobility time in FST of PrL-injected mice with mCherry (n=5) or hM3Dq (n=9) treated overnight with C21 (*P*=0.5641, unpaired Student's t-test). **l** Immobility time in TST of PrL-injected mice with mCherry (n=5) or hM3Dq (n=9) treated overnight with C21 (*P*=0.1563, Mann-Whitney test). **m** Sucrose preference of C21-treated hM3Dq and mCherry mice (n=8, *P*=0.8785, Mann-Whitney test). Data are presented as mean ±SEM and the individual data points are depicted. *P*<0.05, **P*<0.01, ***P*<0.001. Source data are provided as a Source Data file.



Supplementary Fig. 3 | a Analysis of PPR pre- vs. post aLTP induction in hM3Dq mice treated with C21 overnight (n=8, *P*=0.6406, Wilcoxon matched-pairs signed test). **b** The input resistance (Rm) after aLTP in hM3Dq mice treated with C21 overnight (n=13, *P*=0.0743, paired Student's t-test). Data are presented as mean ±SEM and the individual data points are depicted. **P*<0.05, ***P*<0.01, ****P*<0.001. Source data are provided as a Source Data file.

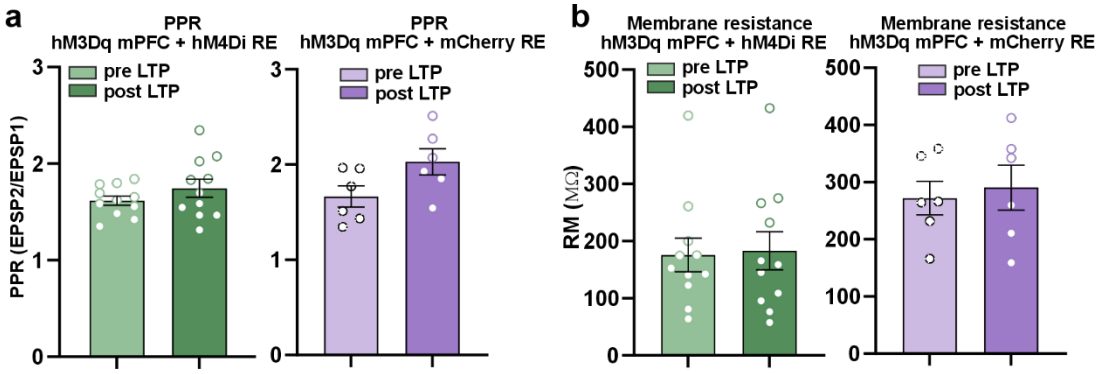


Supplementary Fig. 4 | a Mean peak amplitude of hippocampal excitatory neurons during struggle (left, n=6, *P*=0.5480) and immobile (right, n=6, *P*=0.2766) between post-CDM (red) and post-C21 (blue) conditions. **b** Mean peak amplitude of hippocampal inhibitory neurons during struggle (left, n=5, *P*=0.0631) and immobile (right, n=5, *P*=0.2600) periods between post-CDM (red) and post-C21 (blue) conditions. Statistical comparisons were performed using paired Student's t-test. Data are presented as mean ±SEM and the individual data points are depicted. **P*<0.05, ***P*<0.01, ****P*<0.001. Source data are provided as a Source Data file.

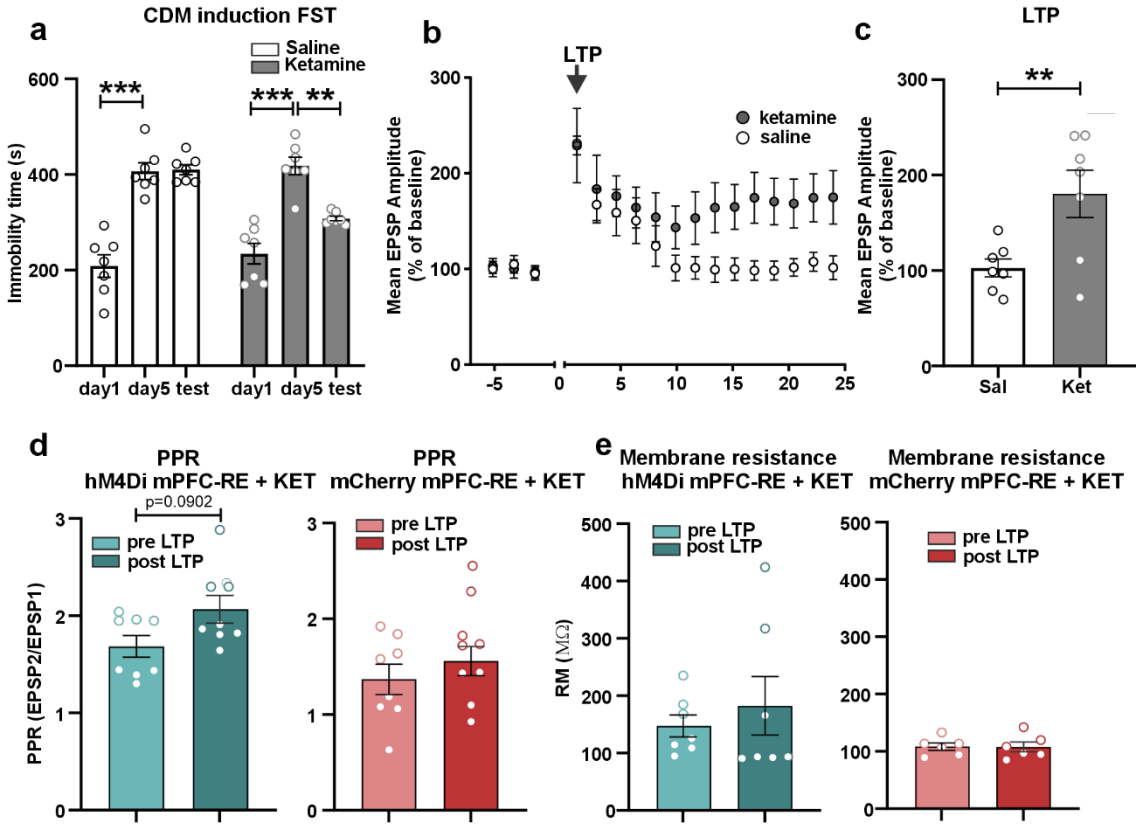


Supplementary Fig. 5 | a-b mRNA expression of immediate early genes (normalized to ApoE and GAPDH) in the dorsal hippocampus (**a**) (n=6 per group; *cFos*: *P*=0.4661, Welch's t-test, Homer1a: *P*=0.7027, *NPAS4*: *P*=0.2164, Welch's t-test, *Arc*: *P*=0.2179, Welch's t-test) and ventral hippocampus (**b**) (n=6 per group; *cFos*: *P*=0.9989, Homer1a: *P*=0.3380, *NPAS4*: *P*=0.5117, *Arc*: *P*=0.3805) of wild type mice 2h after acute C21 or saline i.p. treatment. **c-d** mRNA expression of immediate early genes in dorsal hippocampus (**c**) (n=6 per group, *cFos*: *P*=0.1565, Homer1a: *P*=0.3442, *NPAS4*: *P*=0.4262, *Arc*: *P*=0.4874) and ventral hippocampus (**d**) (n=6 per group, *cFos*: *P*=0.0160, Homer1a: *P*=0.0003, *NPAS4*: *P*=0.0022, *Arc*: *P*=0.0003) of hM3Dq-injected mice overnight treated with C21. **e** Representative confocal images

showing VGLUT2 and GAD65 immunostaining in IL layer V/VI of mCherry and hM3Dq mice after overnight C21 treatment. Scale bar: 20µm. **f** Left: quantification of VGLUT2 and GAD65 cluster density comparisons between hM3Dq mice treated with C21 and control (VGLUT2: mCherry n=7, hM3Dq n=8, *P*=0.0766; GAD65: n=8, *P*=0.4825). Right: quantification of VGLUT2 and GAD65 cluster mean volume comparisons (VGLUT2: mCherry n=7, hM3Dq n=8, *P*=0.0712; GAD65: n=8, *P*=0.4987). **g** VGLUT1 presynaptic clusters mean volume comparisons between hM3Dq mice treated with C21 and controls in the dorsal (mCherry n=5, hM3Dq n=8, *P*=0.0903) and ventral (mCherry n=5, hM3Dq n=8, *P*=0.0711) hippocampus. **h** VGLUT2 presynaptic clusters mean volume comparisons in the dorsal (n=8, *P*=0.2312, Welch's t test) and ventral (n=8, *P*=0.3953, Mann-Whitney test) hippocampus. **i** GAD65 presynaptic clusters mean volume comparisons in the dorsal (n=8, *P*=0.8785, Mann-Whitney test) and ventral (n=8, *P*=0.6454, Mann-Whitney test) hippocampus. Unless otherwise stated, statistical comparisons were performed using unpaired two-tailed Student's t-test. Data are presented as mean ±SEM and the individual data points are depicted. *P*<0.05, **P*<0.01, ***P*<0.001. Source data are provided as a Source Data file.



Supplementary Fig. 6 | a PPR comparison pre- vs. post aLTP induction. Left: hM3Dq mPFC + hM4Di RE mice treated with C21 overnight (n=10, *P*=0.2736, paired Student's t-test). Right: hM3Dq mPFC + mCherry RE mice treated with C21 overnight (n=6, *P*=0.1760, paired Student's t-test). **b** Input resistance (Rm) comparison before and after aLTP. Left: hM3Dq mPFC + hM4Di RE mice treated with C21 overnight (n=11, *P*=0.8355, paired Student's t-test). Right: hM3Dq mPFC + mCherry RE mice treated with C21 overnight (n=6, *P*=0.5786, paired Student's t-test). Data are presented as mean ±SEM and the individual data points are depicted. Source data are provided as a Source Data file.



Supplementary Fig. 7 | a Immobility time of CDM mice treated with saline or ketamine i.p. (10mg/kg) (n=7 per group, repeated measure two-way ANOVA). **b-c** LTP measurements in CDM mice with different treatments. **b** Time course of aLTP inducibility of ketamine (10mg/kg) and saline i.p. treated groups. **c** Group analysis (n=7, *P*=0.02, unpaired Welch's t-test). **d-e** Electrophysiological recordings. **d** Paired-pulse ratio (PPR) comparison before and after LTP induction in Ketamine + hM4Di mPFC-RE (left, n=8, *P*=0.2831) and Ketamine + mCherry mPFC-RE (right, n=8, *P*=0.0902, paired Student's t-test) groups. **e** Input resistance comparison before and after LTP induction in Ketamine + hM4Di mPFC-RE (left, n=6, *P*=0.8708) and Ketamine + mCherry mPFC-RE (right, n=7, *P*=0.3600), paired Student's t-test. Data are presented as mean ±SEM and the individual data points are depicted. *P*<0.05, **P*<0.01, ***P*<0.001. Source data are provided as a Source Data file.