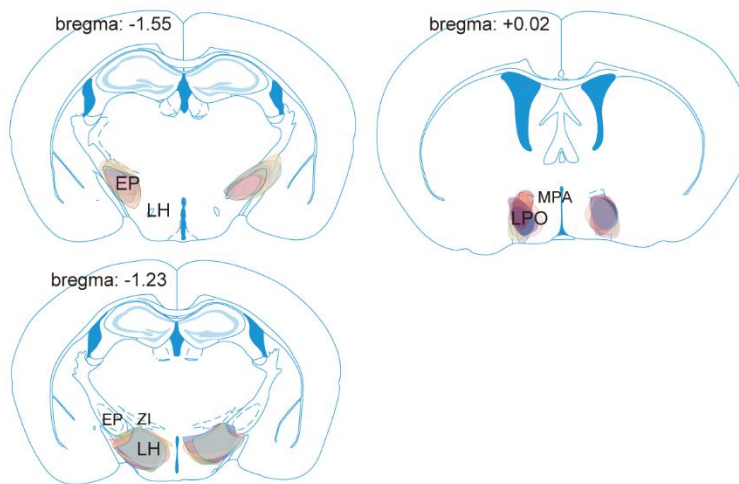


A AAV2/9-hSyn-oChIEF for behavioral test and electrophysiological recording



B AAV2/8-hSyn-ChrimsonR and AAV2/9-hSyn-eCB2.0 for fiber photometry recording

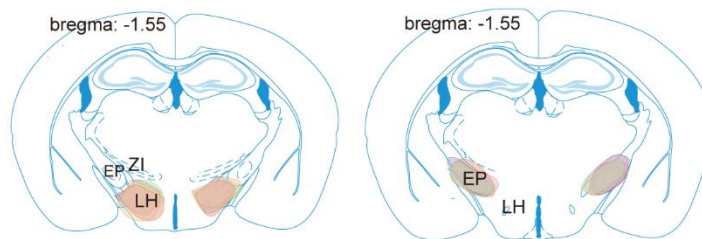


Figure S1. Viral expression profiles under different conditions, related to Figures 2-6.

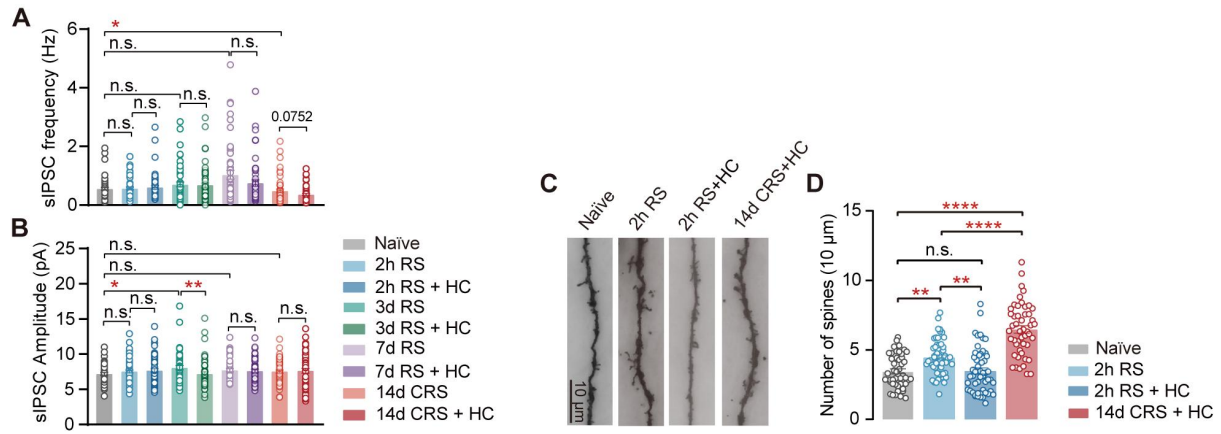


Figure S2. Inhibitory synaptic transmission onto the LHb neurons at different stages of chronic stress, related to Figure 1. (A and B) Quantification of sIPSCs recorded in LHb neurons from mice under naïve condition or treatment of different doses of RS ($n_{\text{neuron}} = 50$ to 60, $n_{\text{mice}} = 5$ to 6; Kruskal-Wallis test). (C and D) Representative images (C) and quantification (D) of spine density in LHb neurons from mice under naïve condition or treatment of different doses of RS ($n_{\text{dendrite}} = 46$ to 52, $n_{\text{mice}} = 4/\text{group}$, One-way ANOVA). Data are indicated as mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$; n.s., not significant.

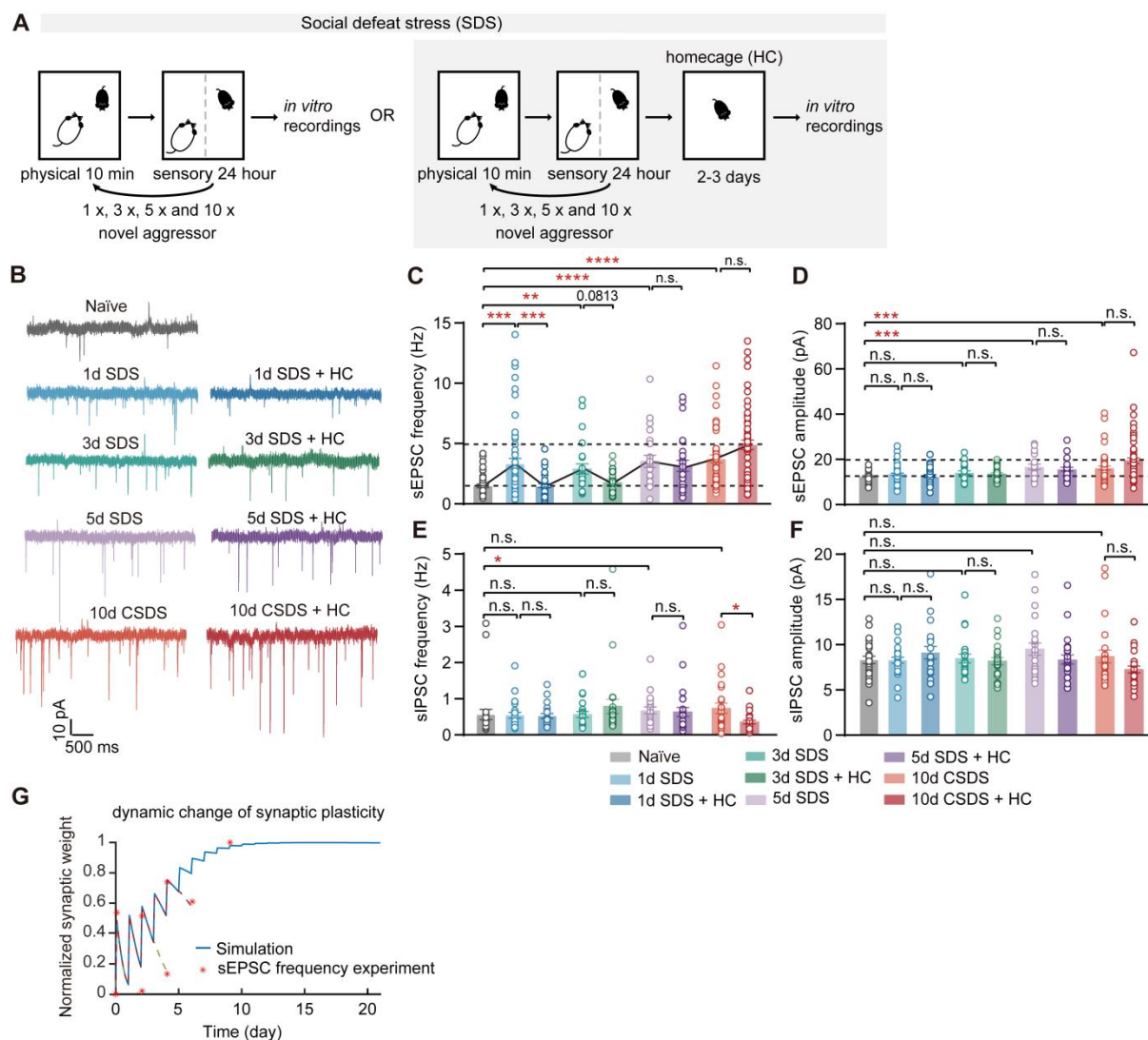


Figure S3. Verification of synaptic dynamics in the LHB under SDS model, related to Figure 1. (A) Experimental paradigm for SDS. (B-F) Representative traces (B) and quantification (C-F) of synaptic transmission dynamics in LHB during SDS ($n_{\text{neuron}} = 22$ to 61, $n_{\text{mice}} = 3$ to 7; Kruskal-Wallis test). (G) Mathematical simulation showing the LHB neurons response to SDS in an oscillating way. Data are indicated as mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; n.s., not significant.

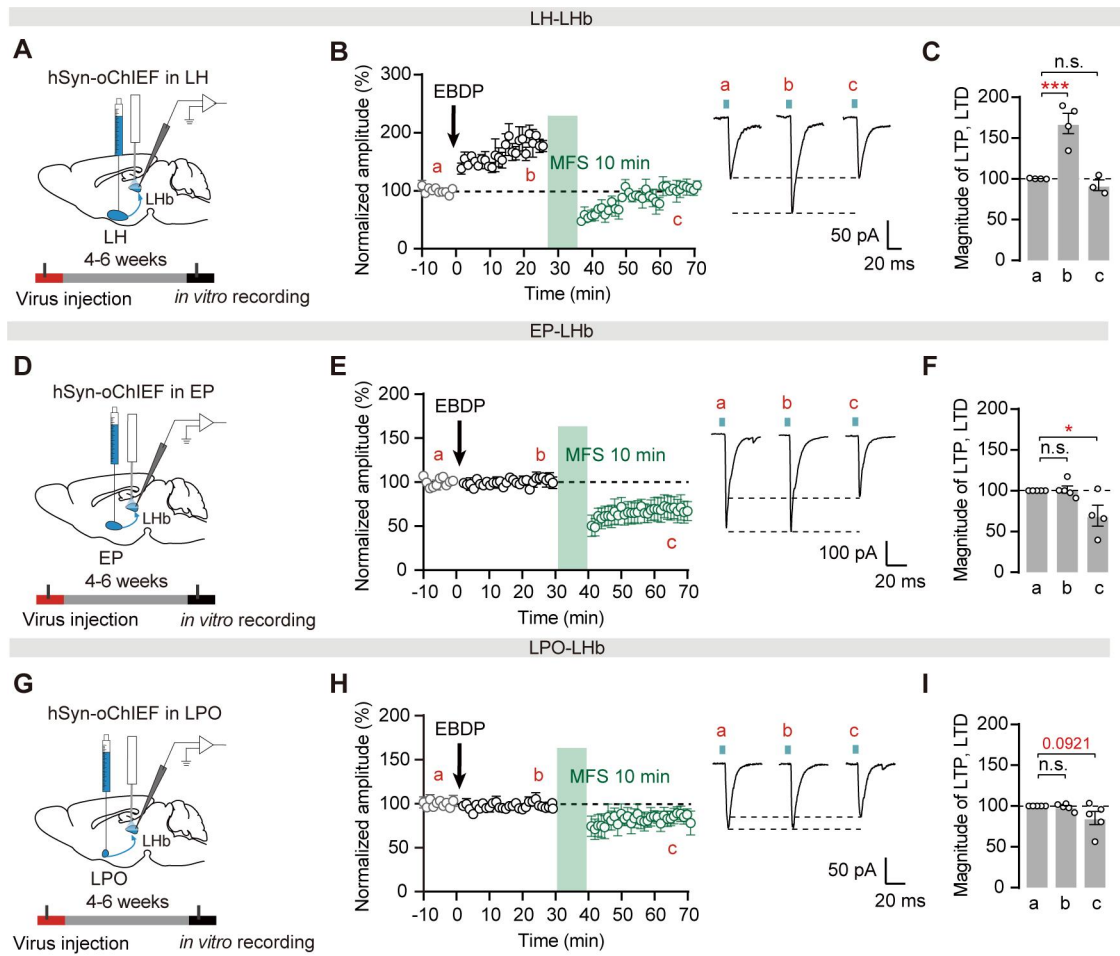


Figure S4. Bidirectional plasticity occurs specifically at LH-LHb pathway, related to Figure 2. (A) Viral injections and experimental design. (B and C) Normalized amplitude (B, left), representative traces (B, right) and quantification (C) of normalized EPSCs showing EBDP-induced LTP and MFS-induced synaptic depotentiation at LH-LHb pathway ($n_{\text{neuron}} = 3$ to 4, $n_{\text{mice}} = 2$; One-way ANOVA). (D) Viral injections and experimental design. (E and F) Normalized amplitude (E, left), representative traces (E, right) and quantification (F) of normalized EPSCs showing unidirectional MFS-induced LTD at EP-LHb pathway ($n_{\text{neuron}} = 4$ to 5, $n_{\text{mice}} = 2$; One-way ANOVA). (G-I) LPO-LHb synapses exhibit no plasticity ($n_{\text{neuron}} = 5$, $n_{\text{mice}} = 2$; One-way ANOVA). Data are indicated as mean $\pm$ SEM; * $P < 0.05$, *** $P < 0.001$; n.s., not significant.

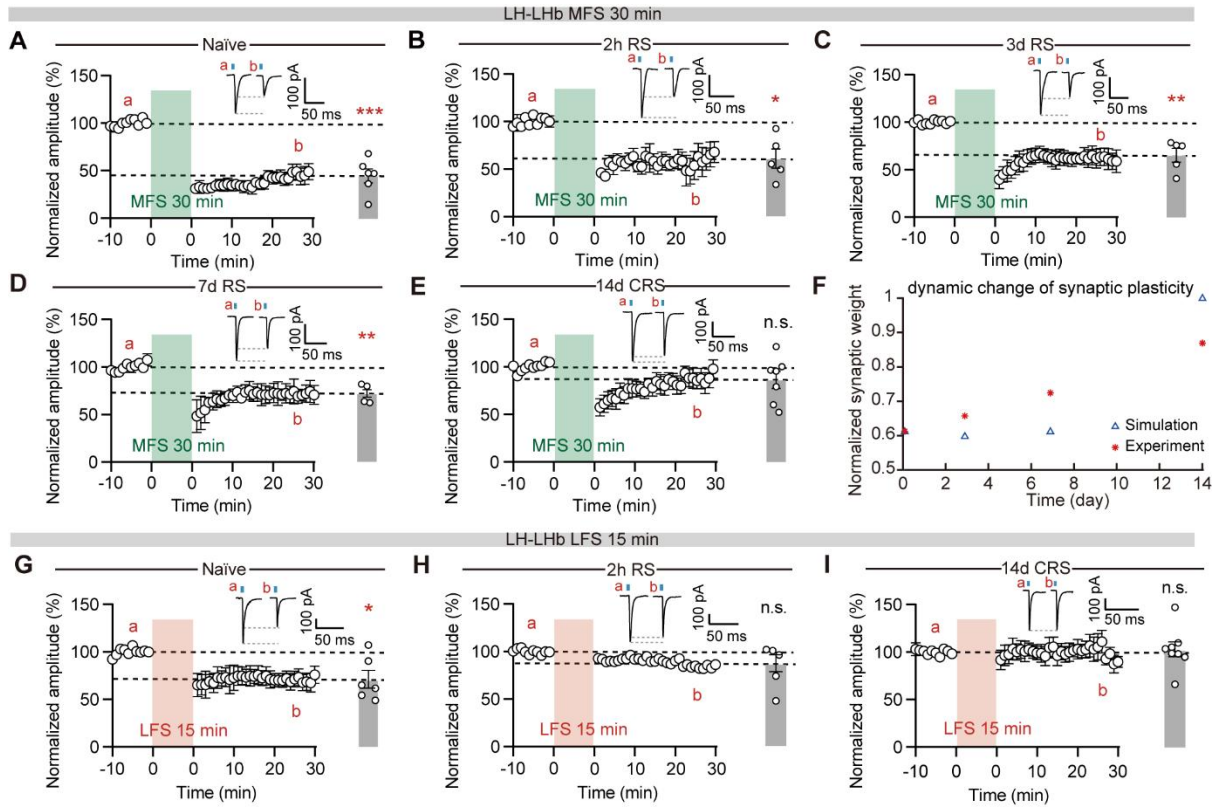


Figure S5. Effects of stress on synaptic depression/depotentiation at LH-LHb synapses, related to Figure 2. (A) 3 trains of MFS (30 min) induces LTD at LH-LHb pathway in naïve mice. (B-E) Stress exposure progressively impairs the capability and capacity of SPSTD in LHb neurons from mice treated with 2h (B), 3d (C), 7d (D) or 14d (E) CRS ($n_{\text{neuron}} = 5$ to 7, $n_{\text{mice}} = 2$ to 5; Paired t -test). (F) Mathematical simulations of the capacity of LH-LHb synaptic depotentiation during chronic stress. (G-I) LFS induces LH-LHb synaptic depression in mice under naïve condition (G) or treatment of 2h RS (H) or 14d CRS (I) ($n_{\text{neuron}} = 6$ to 8, $n_{\text{mice}} = 3$ to 4; Paired t -test). Data are indicated as mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n.s., not significant.

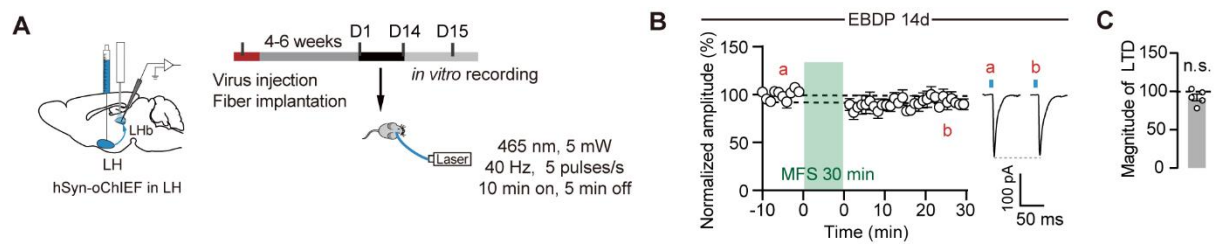


Figure S6. 14 days of EBDP photostimulation dampens SPSP at LH-LHb pathway, related to Figure 2. (A) Viral injections and experimental design. (B and C) Normalized amplitude (B, left), representative traces (B, right) and quantification (C) of normalized EPSCs showing impaired LH-LHb SPSP by EBDP photostimulation ($n_{\text{neuron}} = 5$, $n_{\text{mice}} = 2$; Paired t -test). Data are indicated as mean $\pm$ SEM; n.s., not significant.

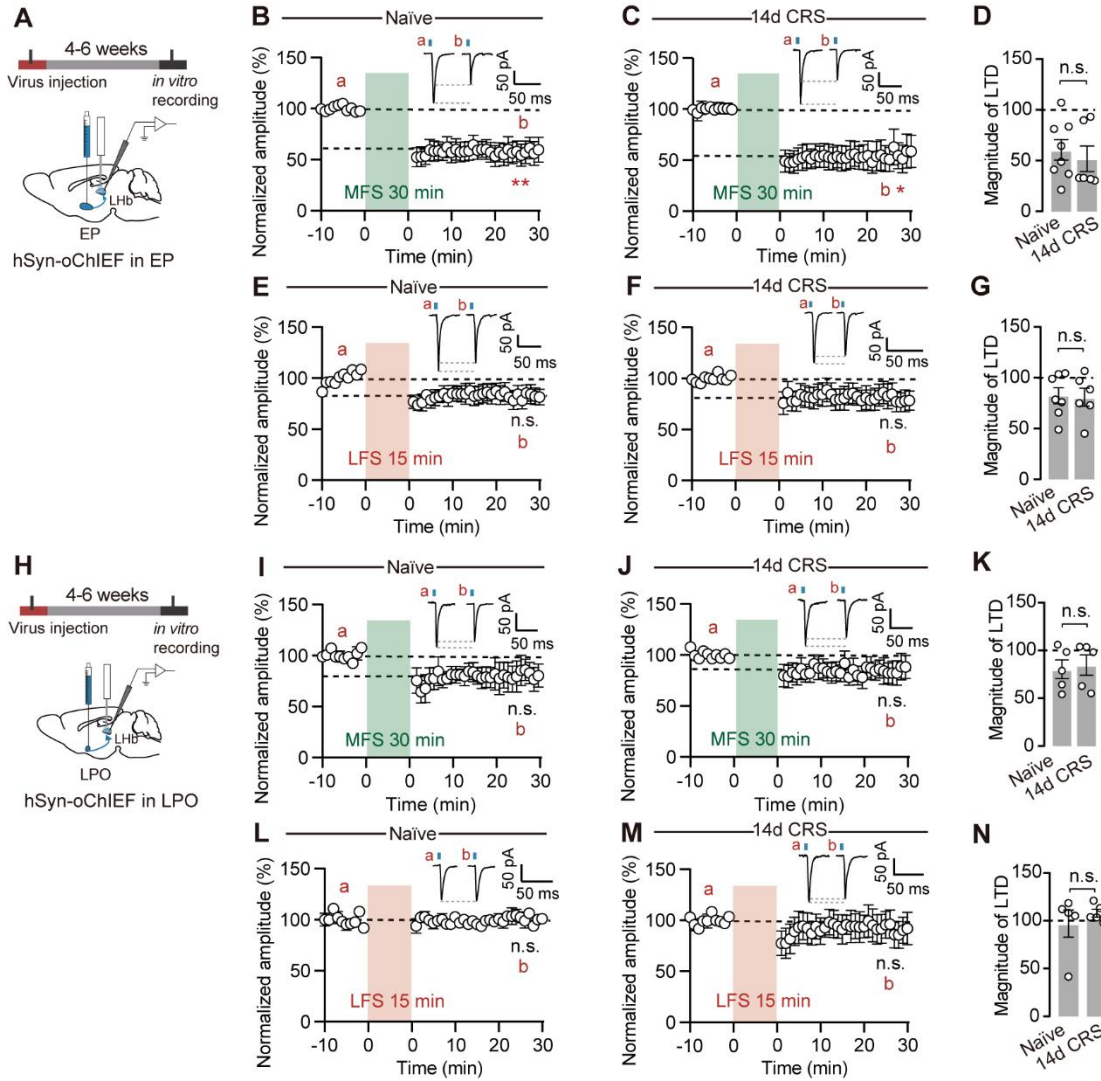


Figure S7. Distinct property of synaptic depression at other major excitatory afferents onto LHB, related to Figure 2. (A) Viral injections and experimental design. (B-D) Normalized amplitude (left), representative traces (right) and quantification (D) of normalized EPSCs showing a more robust EP-LHB LTD induced by 3 trains of MFS in both naïve mice and 14d CRS mice ($n_{\text{neuron}} = 6$ to 8, $n_{\text{mice}} = 5/\text{group}$; Paired t -test, Wilcoxon test and Mann-Whitney test). (E-G) Normalized amplitude (left), representative traces (right) and quantification (G) of normalized EPSCs showing that LFS fails to induce EP-LHB LTD in both naïve mice and 14d CRS mice ($n_{\text{neuron}} = 6$ to 7, $n_{\text{mice}} = 3$ to 5; Paired t -test and Unpaired t -test). (H) Viral injections and experimental design. (I-N) Normalized amplitude (left), representative traces (right) and quantification (K, N) of normalized EPSCs showing that MFS or LFS fails to elicit LPO-LHB synaptic depression in both naïve and 14d CRS mice (MFS, 3 trains: $n_{\text{neuron}} = 5/\text{group}$, $n_{\text{mice}} = 2$ to 3; Paired t -test; LFS: $n_{\text{neuron}} = 5/\text{group}$, $n_{\text{mice}} = 2$ to 4; Paired t -test, Wilcoxon test and Mann-Whitney test). Data are indicated as mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$; n.s., not significant.

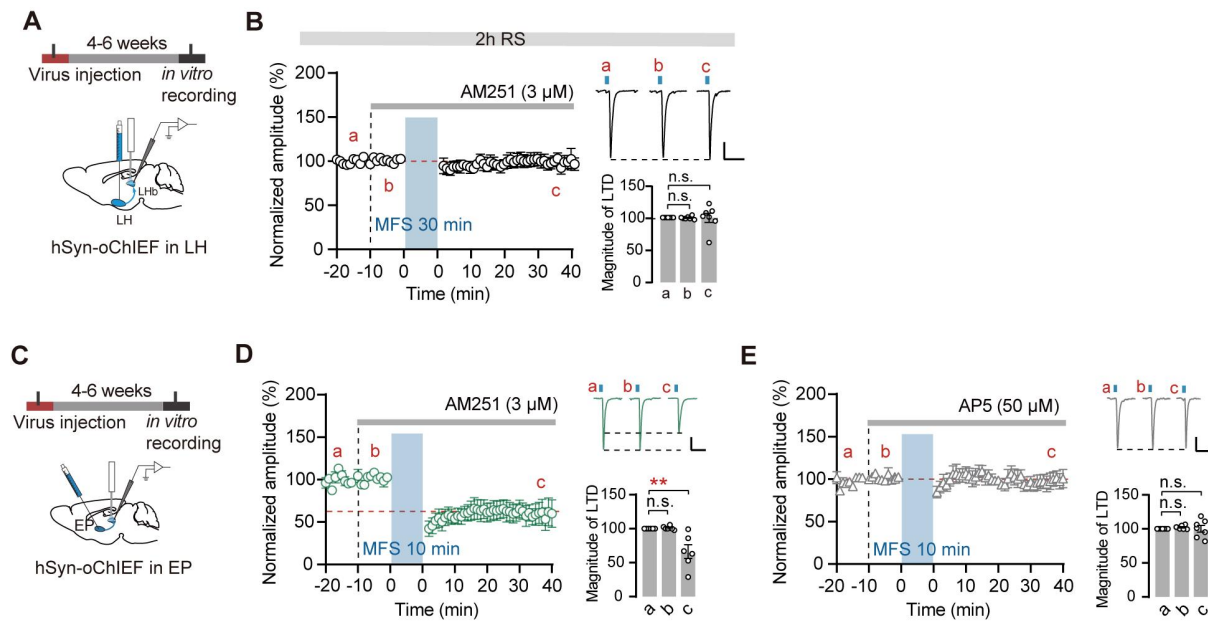


Figure S8. MFS-induced SPSP at LH-LHb pathway is CB₁R-dependent, related to Figure 3. (A) Viral injections and experimental design. (B) Normalized amplitude (left), representative traces (top right) and quantification (bottom right) of normalized EPSCs showing eCB-dependent LH-LHb SPSP in mice treated with 2h RS ($n_{\text{neuron}} = 7$, $n_{\text{mice}} = 3$; One-way ANOVA). Scale bar: 50 pA and 20 ms. Vertical dashed line indicates the arrival of drugs. (C) Viral injections and experimental design. (D and E) Normalized amplitude (left), representative traces (top right) and quantification (bottom right) of normalized EPSCs showing NMDAR-dependent EP-LHb LTD (E) but not eCB-dependent LTD (D) ($n_{\text{neuron}} = 6$ to 7 , $n_{\text{mice}} = 4$; One-way ANOVA). Scale bar: 50 pA and 20 ms. Vertical dashed line indicates the arrival of drugs. Data are indicated as mean $\pm$ SEM; $**P < 0.01$; n.s., not significant.

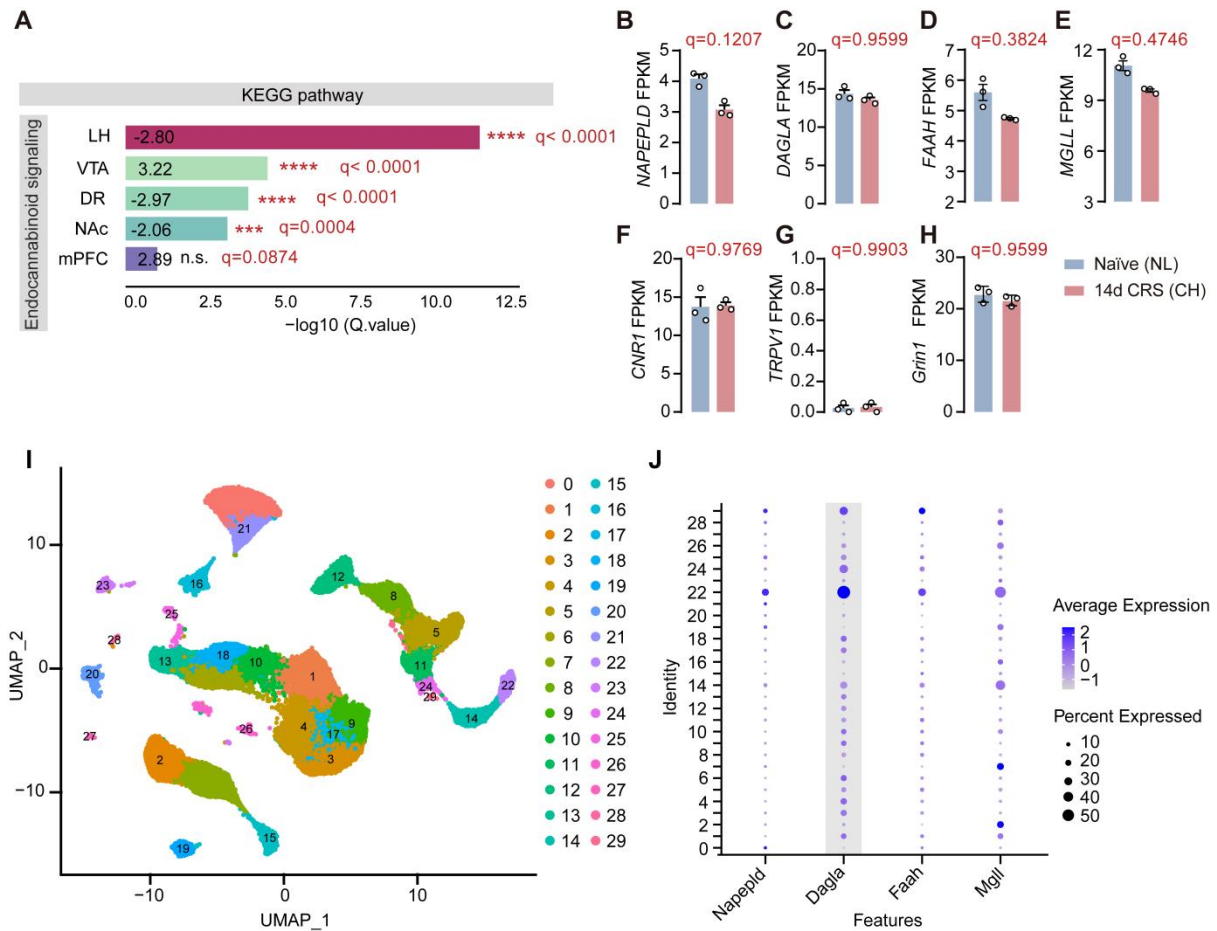


Figure S9. Integrated transcriptomic analysis of eCB signaling and KEGG pathways across brain regions, related to Figure 3. (A) KEGG analysis for bulk RNA-sequencing in the medial prefrontal cortex (mPFC), ventral tegmental area (VTA), nucleus accumbens (NAc), dorsal raphe (DR), and LH (mice = 3 per group; DESeq2 were used for data analysis; Z-score within the column; *** $q < 0.001$, **** $q < 0.0001$). (B-G) Quantification of eCB signaling-related mRNA levels (*NAPEPLD*, *DAGLA*, *FAAH*, *MGLL*, *CNR1* and *TRPV1*) in LHb from naïve (NL) and 14d CRS (CH) mice (mice = 3 per group; DESeq2 were used for data analysis). (H) Quantification of *Grin1* (gene of NMDAR subunit NR1) mRNA in LHb (mice = 3 per group; DESeq2 were used for data analysis). (I) UMAP visualization of 29 clusters in snRNA-seq. (J) Marker genes expression for eCB signaling-related clusters identified. NL, naïve mice with low FST scores; CH, 14d CRS mice with high FST scores. UMAP, uniform manifold approximation and projection. Data are indicated as mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; n.s., not significant.

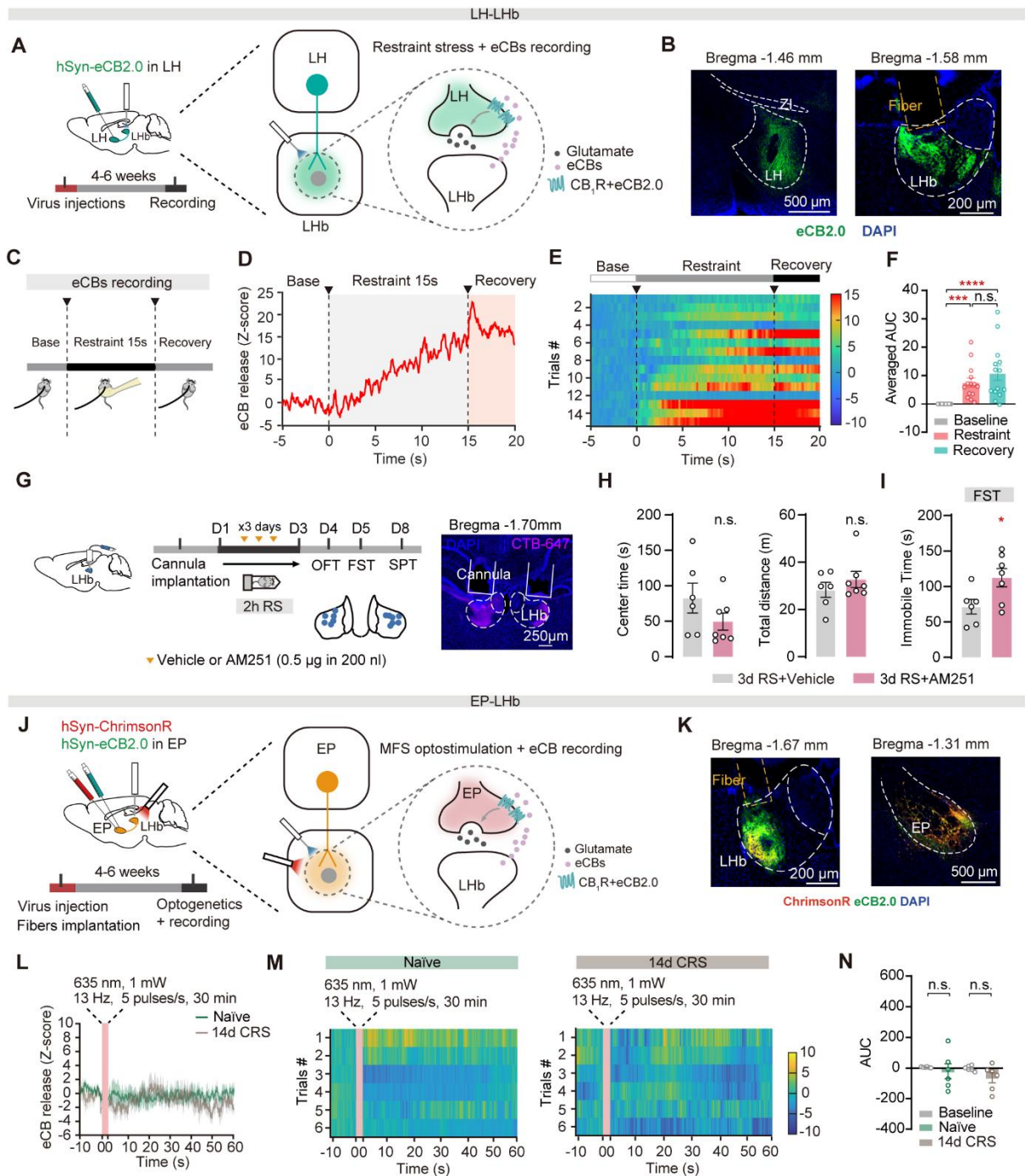


Figure S10. Functional verification of eCB synthesis in RS period at LH-LHb circuits, while EP-LHb MFS optostimulation fails to evoke eCB release in LHb, related to Figure 4. (A and B) Schematic drawing showing the *in vivo* fiber photometry procedure for LH-LHb GRAB_{eCB2.0} biosensor recordings (A) and the verification of eCB2.0 in the LHb (B). (C-F) Experimental design (C), representative trace (D), heatmaps (E) and quantification (F) showing *in vivo* eCB release during RS or in the post-stress phase in naïve mice (trial = 14 mice = 4; Kruskal-Wallis test). (G) Experimental design, cannula implantations (left) and sites of drugs were verified by CTB (right). (H-I) Local infusion of AM251 in LHb after RS increased

immobile time in FST (I) without affecting locomotion in OFT (H) (mice = 6 to 7, Unpaired t test). (J and K) Schematics of EP-LHb GRAB_{eCB2.0} biosensor recordings (J) and verification of viral expression with optic fiber implantation (K). (L-N) averaged traces (L), heat maps (M) and quantification (N) showing no release of eCB *in vivo* after EP-LHb MFS (trial = 6,6; mice = 4,4; Unpaired t -test). Data are indicated as mean $\pm$ SEM; * P < 0.05, *** P < 0.001, **** P < 0.0001; n.s., not significant.

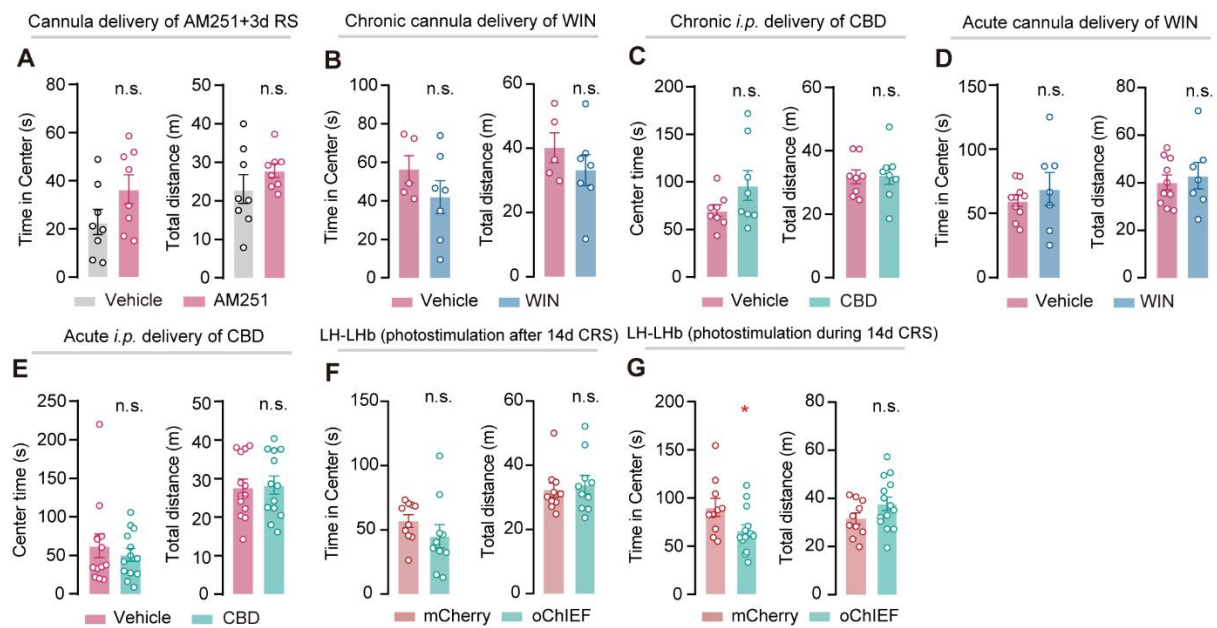


Figure S11. OFT under different circumstances, related to Figures 4-6 and S12, S13. (A) OFT behavioral testing after LHb local infusion of AM251, related to Figure 4. (B) OFT behavioral testing after chronic delivery of WIN in the LHb, related to Figure 5. (C) OFT behavioral testing after chronic *i.p.* delivery of CBD in the LHb, related to Figure S13. (D) OFT behavioral testing after acute delivery of WIN in the LHb, related to Figure 5. (E) OFT behavioral testing after acute *i.p.* delivery of CBD in the LHb, related to Figure S13. (F) OFT behavioral testing after LH-LHb photostimulation after a 14d CRS, related to Figure S14. (G) OFT behavioral testing after LH-LHb photostimulation during the 14d CRS, related to Figure 6.

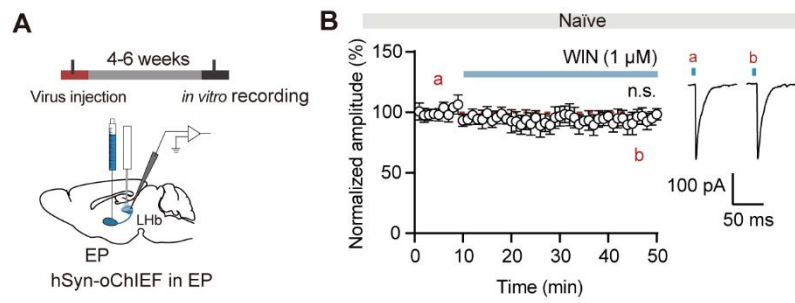


Figure S12. CB₁R agonist fails to induce synaptic depression at EP-LHb pathway, related to Figure 5. (A) Viral injections and experimental design. (B) Normalized amplitude (left), representative traces (right) of normalized EPSCs showing no LTD at EP-LHb circuit after WIN perfusion in naïve mice ($n_{\text{neuron}} = 5$; $n_{\text{mice}} = 3$; Paired t -test). n.s., not significant.

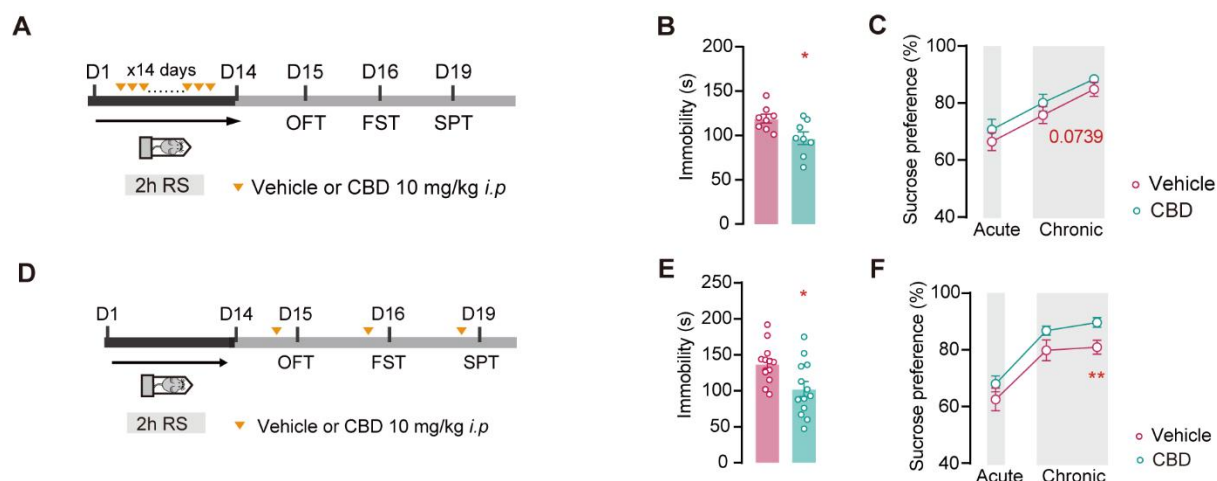


Figure S13. CBD exerts antidepressant effects, related to Figure 5. (A) Experimental design. (B and C) Quantification of the immobile duration in FST and sucrose preference in SPT behavioral outcomes of daily administration of CBD right after 2h RS during 14d CRS ($n_{\text{mice}} = 8/\text{group}$; Unpaired t -test and Two-way ANOVA). (D) Experimental design. (E and F) Quantification of the immobile duration in FST and sucrose preference in SPT behavioral outcomes after acute CBD *i.p.* treatment in 14d CRS mice ($n_{\text{mice}} = 13/\text{group}$; Unpaired t -test and Two-way ANOVA). Shading part indicate acute or chronic phases for SPT. Data are indicated as mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$; n.s., not significant.

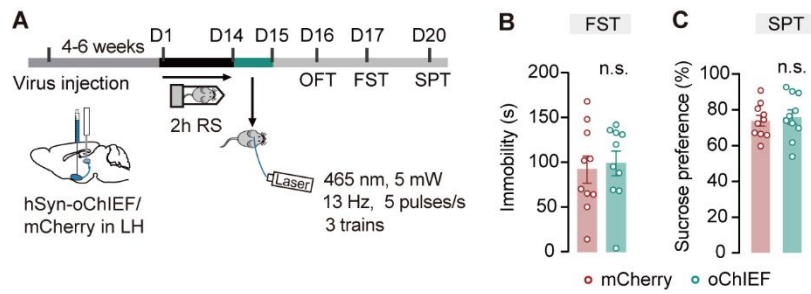


Figure S14. LH-LHb MFS delivered after 14d CRS does not reverse depression-like behaviors, related to Figure 6. (A) Viral injections and experimental design. (B and C) MFS at LH-LHb pathway applied after 14d CRS fails to alleviate depression-like behaviors ($n_{\text{mice}} = 10/\text{group}$; Unpaired t -test). Data are indicated as mean $\pm$ SEM. n.s., not significant.

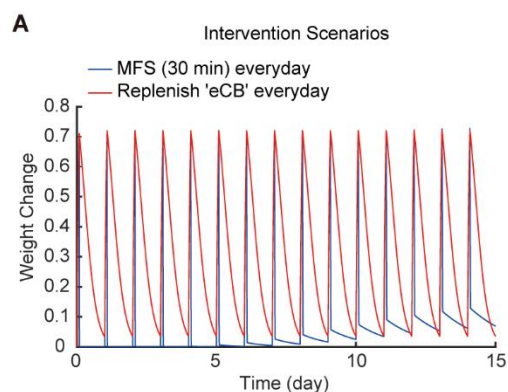


Figure S15. Mathematical simulations of interventional effects of MFS or eCB replenishment on synaptic dynamics, related to Figure 6. (A) Simulated effects of two interventional approaches (MFS for 30 min and eCB) on the dynamic change of synaptic weight during chronic stress. Blue line indicates the dynamics of synaptic weight under MFS delivered right after daily restraint stress. Red line indicates the dynamics of synaptic weight under pharmacological replenishment of eCB delivered right after daily restraint stress.

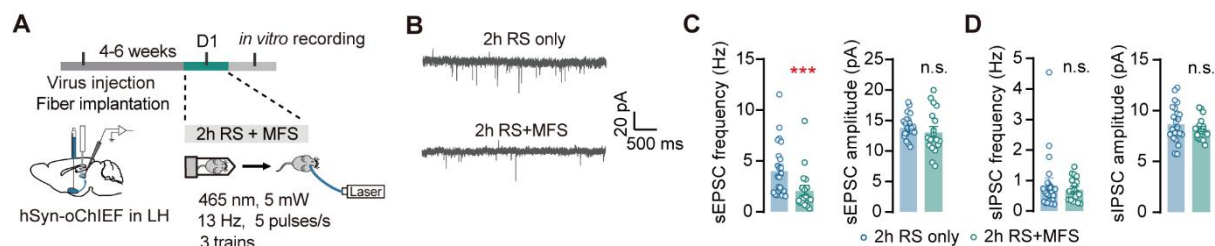


Figure S16. LH-LHb MFS induces synaptic depotentiation, related to Figure 6. (A) Viral injections and experimental design. (B-D) Representative traces (B) and quantification (C and D) showing that LH-LHb MFS after 2h RS exposure attenuates sEPSCs (C) in LHb neurons, whereas sIPSCs (D) are unaltered ($n_{\text{neuron}} = 18$ to 23; $n_{\text{mice}} = 2$ to 3; Unpaired *t*-test and Mann Whitney test). Data are indicated as mean $\pm$ SEM; *** $P < 0.001$; n.s., not significant.

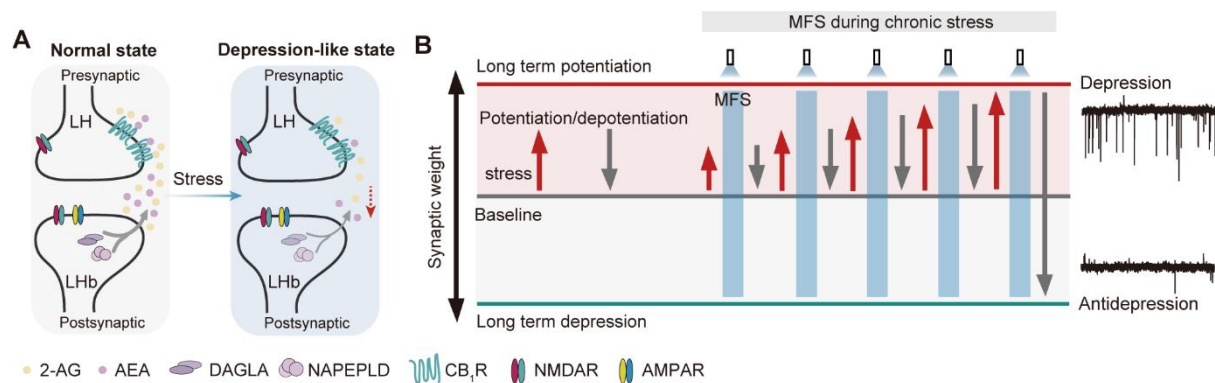


Figure S17. Hypothesized model of dynamic bidirectional synaptic plasticity underlying stress allostasis.

(A) In the LHb, eCB signaling exhibits impaired synthesis of 2-AG and AEA after chronic stress, leading to a reduction in LTD capacity specifically in the LH-LHb circuit and an enhancement of synaptic potentiation and then turning the normal state to depression-like state. (B) The phenomenon of synaptic potentiation/depotential specifically occurs in the LH-LHb circuit. During chronic stress, MFS stimulation can reverse stress-induced synaptic potentiation back to baseline levels, thereby maintaining stress resilience.