Synaptic potentiation onto habenula neurons in learned helplessness model of depression

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SUPPLEMENTAL INFORMATION

Supplemental Figure 1. Major brain areas that send projections to the LHb. Alexa 488-cojugated Cholera toxin was injected into the LHb of a male, 55-day-old WT rat (a; red arrow). The animal was transcardially perfused with 4% PFA 8 days later and the retrograde labeling by cholera toxin was examined in 200 μm sagittal brain sections. Prominent labeling (see arrowheads) was found in the medial prefrontal cortex (mPFC; green boxes in a), bed nucleus of the stria terminalis (BNST; red boxes in a), lateral hypothalamus area (LHA; green boxes in b), and the entopeduncular nucleus (EP; green boxes in c). Images on the right side are higher magnification pictures of the boxed areas on the left. Scale bar: 1 mm.

Supplemental Figure 2. Retrograde labeling of LHb neurons. a. VTA-projecting LHb neurons labeled by *in vivo* injection of a retrograde tracer in VTA. Left: a parasagittal section of a rat brain that contains both LHb (red box) and VTA (arrow); middle: the same brain section viewed under fluorescence mode. A retrogradely-transported HSV virus expressing GFP was injected into VTA (arrow) 2 days before the animal was perfused and fixed with 4% PFA. Right: higher magnification of the LHb region to visualize the GFP-labeled, VTA-projecting neurons. Scale bar: 1 mm. b. Left: a diagram of habenula in a coronal section (MHb: medial habenula; LHbM: the medial section of lateral habenula; LHbL: the lateral section of lateral habenula). Right panels: cholera toxin (ChTx; conjugated with Alexa 488) was injected into VTA 2 to 3 days before the animal was perfused and coronal brain sections containing habenula were prepared.

Sections were stained with an anti-NeuN antibody to visualize all the neurons. Typically there were only a small fraction of ChTx positive, VTA-projecting neurons in LHb. Scale bar: 200 μm. c. An image of the LHb with neurons labeled by ChTx injected into both the VTA (Alexa488 conjugated; green) and RMTg (Alexa594 conjugated; red). The green and red cells, representing VTA-projecting and RMTg-projecting neurons, respectively, are largely non-overlapping. The coordinates (mm) for the injections are: VTA -5.3 (AP), 0.9 (ML), and -8 to -8.2 (DV); RMTg -6.8 (AP), 0.4 (ML), and -7.7 to -8 (DV). Injections were also made in different locations within RMTg (from 0 to 1 ML), which resulted in similar non-overlapping labeling of LHb neurons (not shown). Scale bar: 50 μm.

Supplemental Figure 3. Glutamatergic neurons are dominant in the LHb. a. Injection was done as described in supplemental figure 2. Images were taken using a two-photon laser scanning microscope. Sections were stained with an antibody against the glutamate transporter EAAC1 (red). On the right are higher magnification pictures of the boxed region in the left. About 90% of the ChTx positive, VTA-projecting neurons (green) co-localized with EAAC1 (arrowheads). Scale bar: 50 μm. b. Left: a diagram of habenula in a parasagittal section. Right panels: Two days after *in vivo* injection of the HSV-GFP virus into VTA, parasagittal brain sections containing LHb (left) were prepared and stained with an anti-GABA antibody. The co-localization of VTA-projecting LHb neurons (GFP) and GABA-positive neurons (arrow head) was examined. Among the 391 GFP positive-neurons examined from 9 LHb sections, which contained 58 GABA-positive neurons, no co-localization of GFP and GABA expression in the same neuron was found. Scale bar: 100 μm c. Coronal brain sections containing LHb and

dentate gyrus (DG) were stained with an anit-GAD67 antibody. On the same brain sections, many GAD67 positive cells were found in the DG, whereas very few GAD67 positive neurons could be found in the LHb. On the right are higher magnification pictures of the boxed regions in the left. Scale bar: 200 μm.

Supplemental Figure 4. Synaptic properties of LHb neurons. a. Sample mEPSC traces recorded from LHb neurons before (left) and after (right) application of CNQX. Similar results were obtained from 4 cells. b. Left: examples of the mIPSCs recorded from VTA-projecting LHb neurons of control or cLH animals. Right: Quantification of mIPSC frequency (control: 2.3±0.7, n=12; cLH: 1.9±0.7, n=10; $p>0.05$) and amplitude (control: 25.5±1.6 pA, n=11; cLH: 27.9±2.8 pA, n=10; $p>0.05$). c. Sample mIPSC traces recorded from LHb neurons before (left) and after (right) application of picrotoxin. Similar results were obtained from 3 cells. d. Evoked EPSCs from both VTA-projecting and randomly recorded LHb neurons contained very little NMDAR-mediated component (NMDA/AMPA ratio: VTA-projecting: 0.05±0.01, n=8; random: 0.06±0.03, n=9; $p>0.05$). e. Evoked EPSCs from both VTA-projecting and randomly recorded LHb neurons showed similarly strong inward rectification (rectification index: VTA-projecting: 5.6±1, n=5; random: 5±0.8, n=5; $p>0.05$). All error bars represent s.e.m.

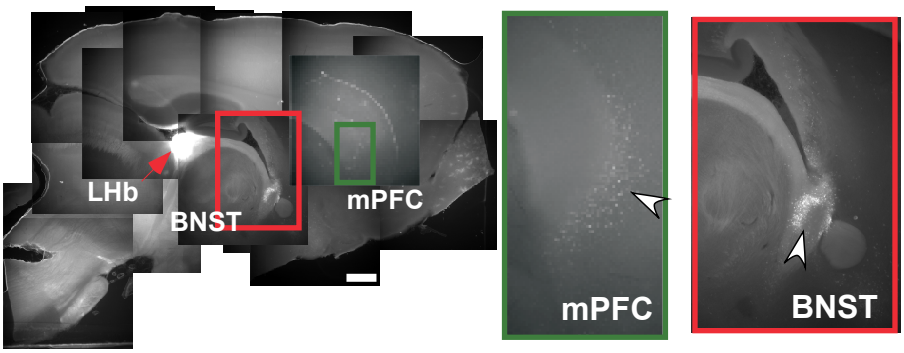
Supplemental Figure 5. The volume of tissue affected by DBS in the LHb. a. Behavior of 52 animals after learned helplessness induction (see methods). Red symbols represent 26 animals displaying most severe learned helplessness that were chosen for electrode implantation (see methods for selection criteria). Behavioral measurements

included the number of successful lever presses (left) within 20 seconds of shock onset, and the test completion time (right). As lever presses lead to termination of the shock, low lever presses and long test completion times indicate learned helplessness behavior. b. Representative images of c-Fos expression in the coronal brain sections of animals received Sham stimulation or DBS at 150 or 300 μA current intensities in the LHb. The LHb is outlined by dotted lines. Upper: brain sections 200 μm anterior to where the stimulating electrodes were implanted. Both left and right LHb were shown. Stimulating electrodes were implanted in the left LHb. Lower: brain sections which contained the stimulating electrodes (arrows). Only the left LHb were shown. Note the prominent c-Fos expression in the 300 μA group, which was confined to the LHb and did not spread to the adjacent medial habenula and thalamic regions. The 150 μA group had less c-Fos expression, whereas the sham group had no c-Fos expression. c. Quantification of c-Fos expression. Left: the number of c-Fos positive cells as a function of distance from the tip of stimulating electrode in brain sections that contained the stimulating electrodes ($p = 0.0151$, $F = 4.916$ by One-way ANOVA; Sham vs. 300 μA: $p<0.05$). Right: the number of c-Fos positive cells within the LHb as a function of distance from the stimulating electrode along the anterior / posterior axis ($p = 0.0014$, $F = 9.078$ by One-way ANOVA; Sham vs. 300 μA: $p<0.01$; 150 μA vs. 300 μA: $p<0.05$). DBS at both 150 and 300 μA significantly induced c-Fos expression compared to the sham stimulation. DBS at 300 μA was most effective and affected a volume of about 1 mm^3, which is about the size of LHb. All error bars represent s.e.m. Scale bar: 200 μm.

Supplemental Figure 1

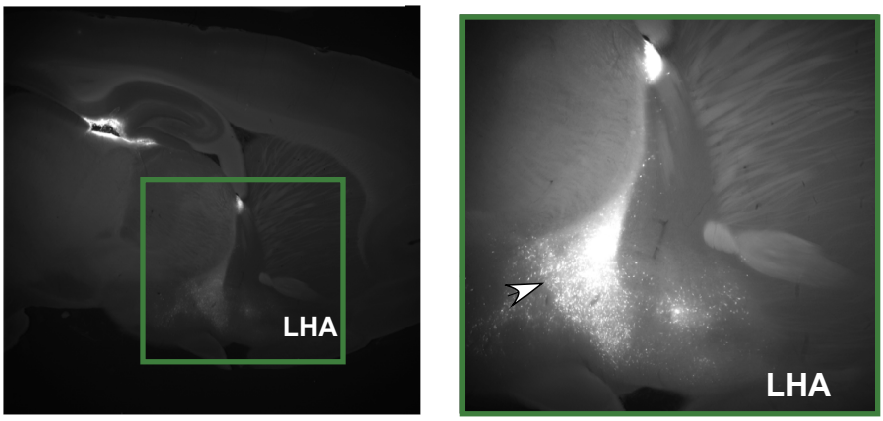
a

Lateral 1.0 mm from midline



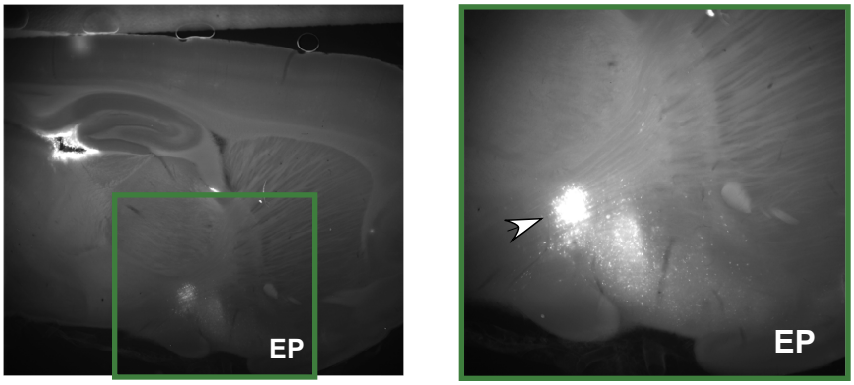
b

Lateral 2.1 mm from midline

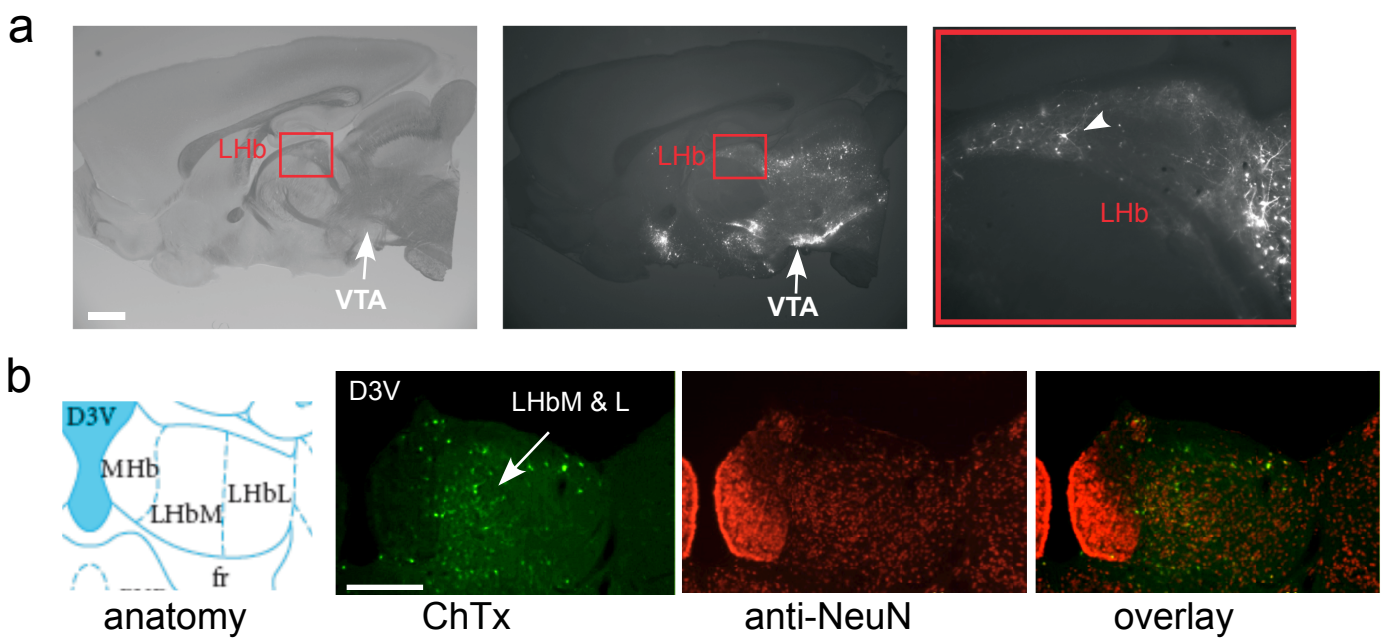


c

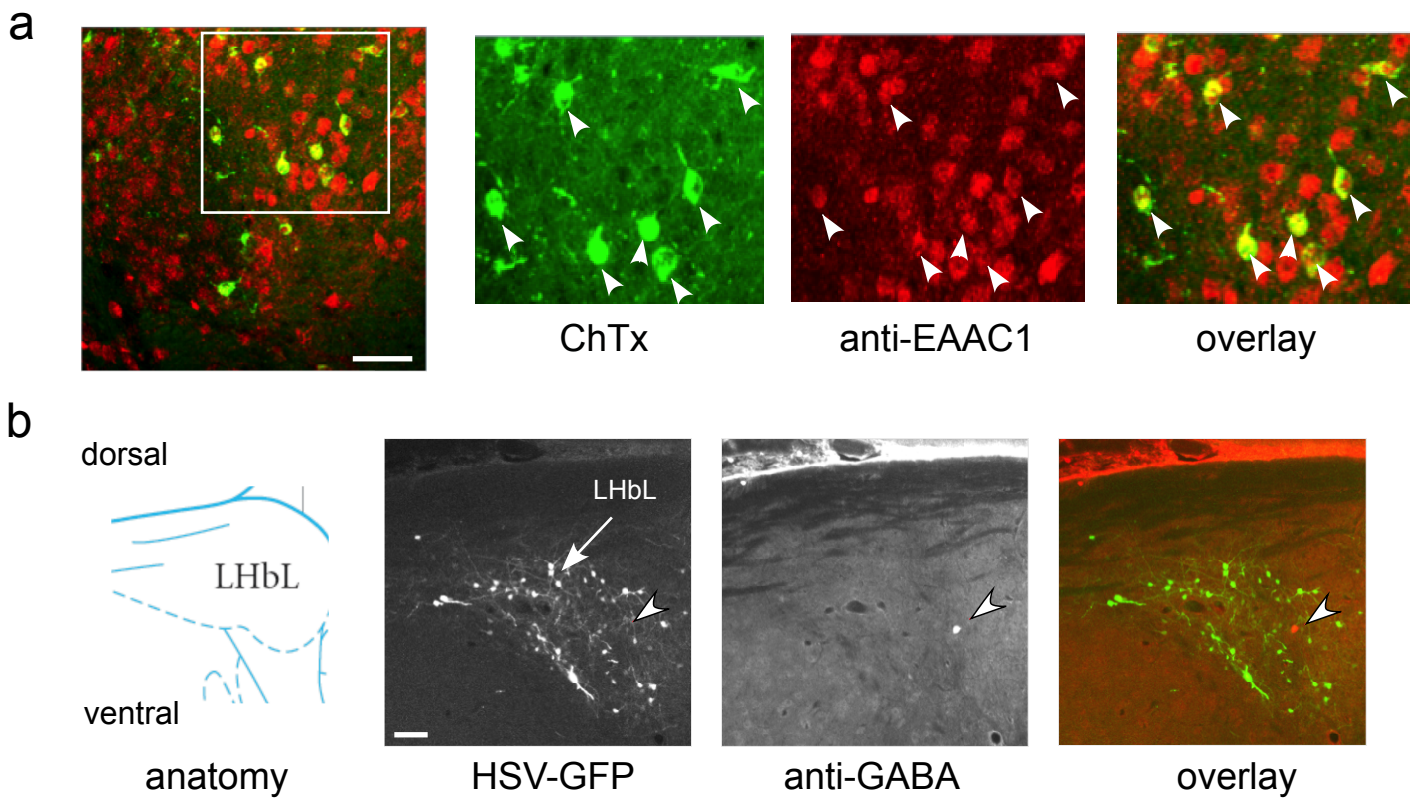
Lateral 2.7 mm from midline



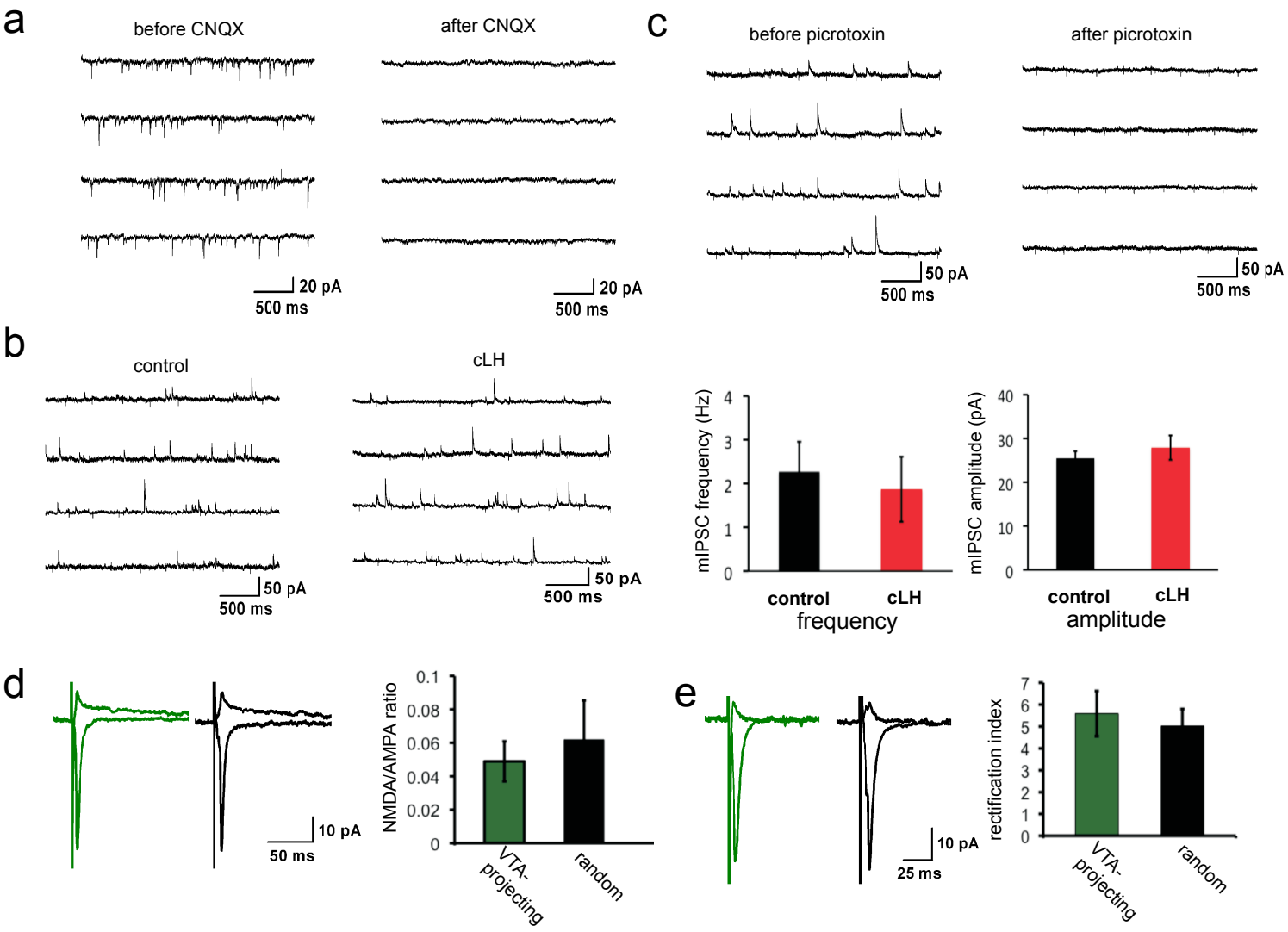
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5