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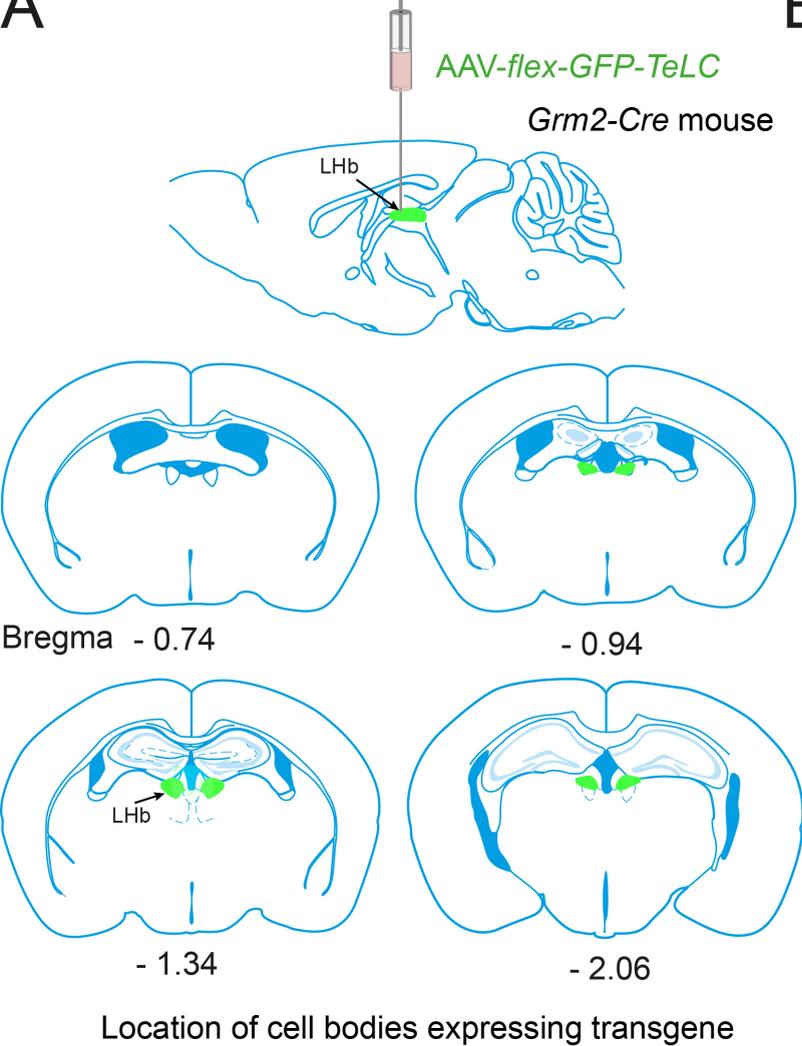
Supplemental Information

Excitatory Pathways from the Lateral

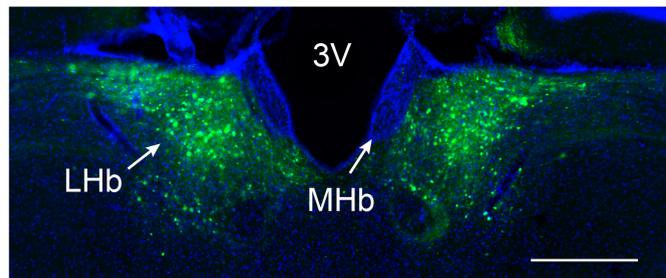
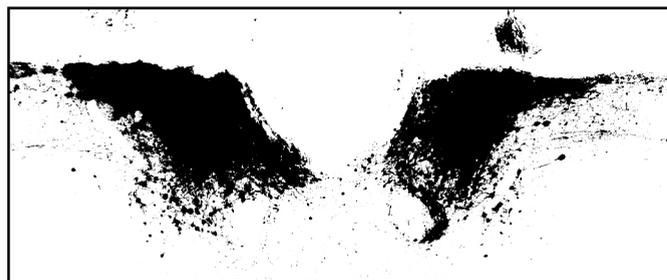
Habenula Enable Propofol-Induced Sedation

Cigdem Gelegen, Giulia Miracca, Mingzi Z. Ran, Edward C. Harding, Zhiwen Ye, Xiao Yu, Kyoko Tossell, Catriona M. Houston, Raquel Yustos, Edwin D. Hawkins, Alexei L. Vyssotski, Hailong L. Dong, William Wisden, and Nicholas P. Franks

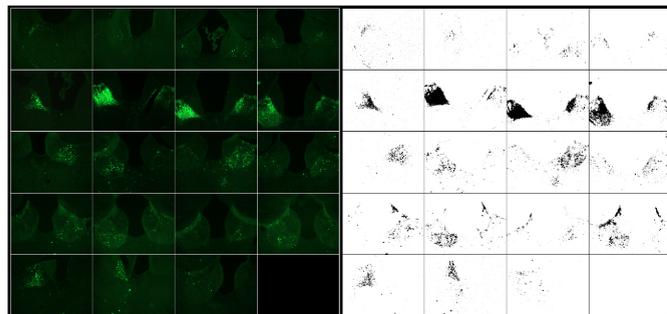
A



B

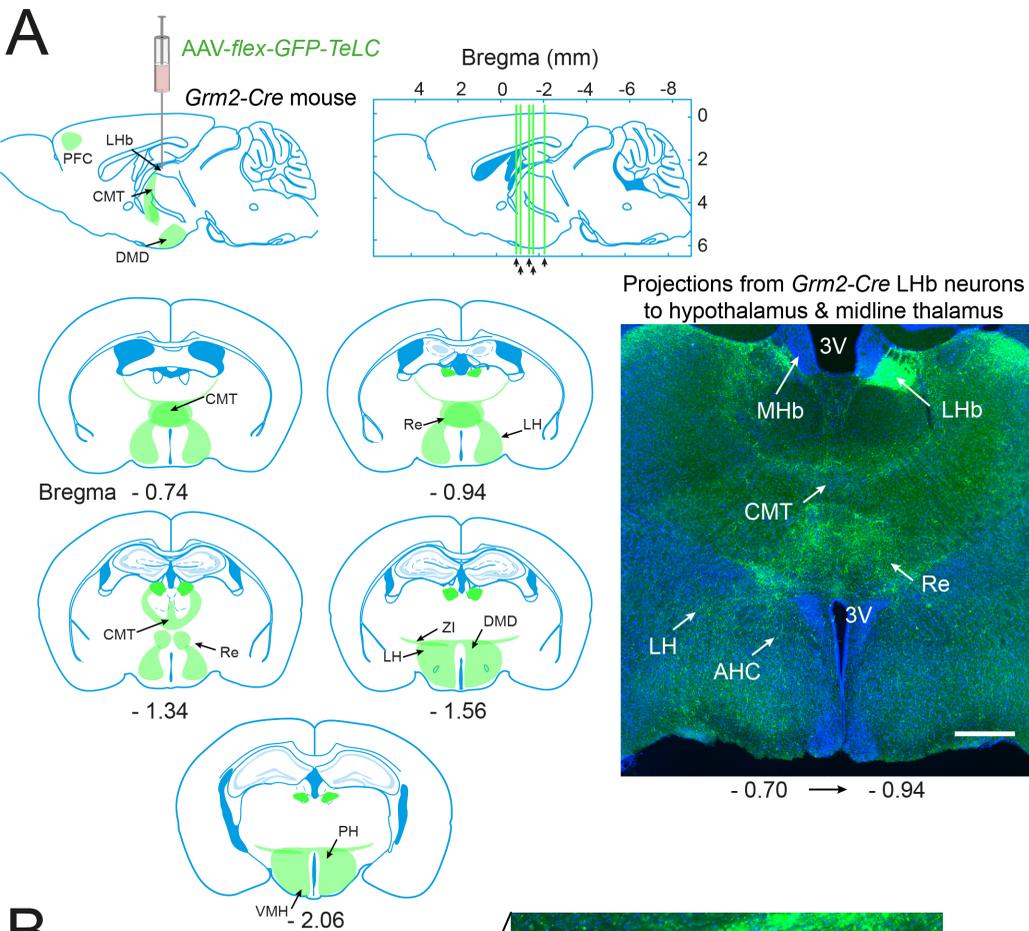
GFP-TeLC in LHb of *Grm2-Cre* mouse

Quantification threshold



Example mouse

Figure S1. Related to Figure 1. The extents of bilateral injections of AAV-*flex-GFP-TeLC* into the LHb of *Grm2-Cre* mice were determined by measuring integrated fluorescence intensity along the AP axis. (A) Example sections from Bregma -0.74 to -2.06 mm showing the location of *GFP-TeLC* expression (green). (B) Top: A typical coronal section showing GFP fluorescence in the LHb (note that this is the same image shown in **Figure 1A**); scale bar is 500 μm . Middle: All pixels above a chosen threshold are shown using the triangle algorithm and a custom macro in ImageJ (version 1.50e; <http://imagej.nih.gov/ij/>). Bottom: The threshold was applied to a tiled montage of a single mouse brain.

A**B**

Projections from *Grm2-Cre* LHb neurons to hypothalamus & zona incerta

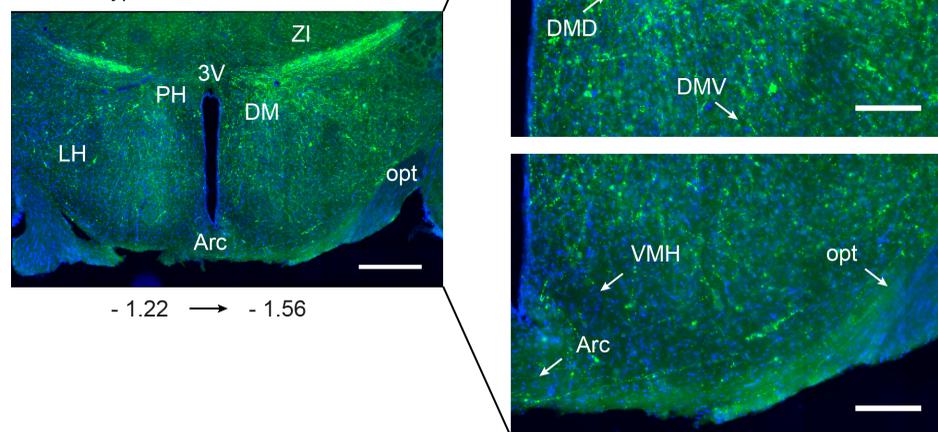
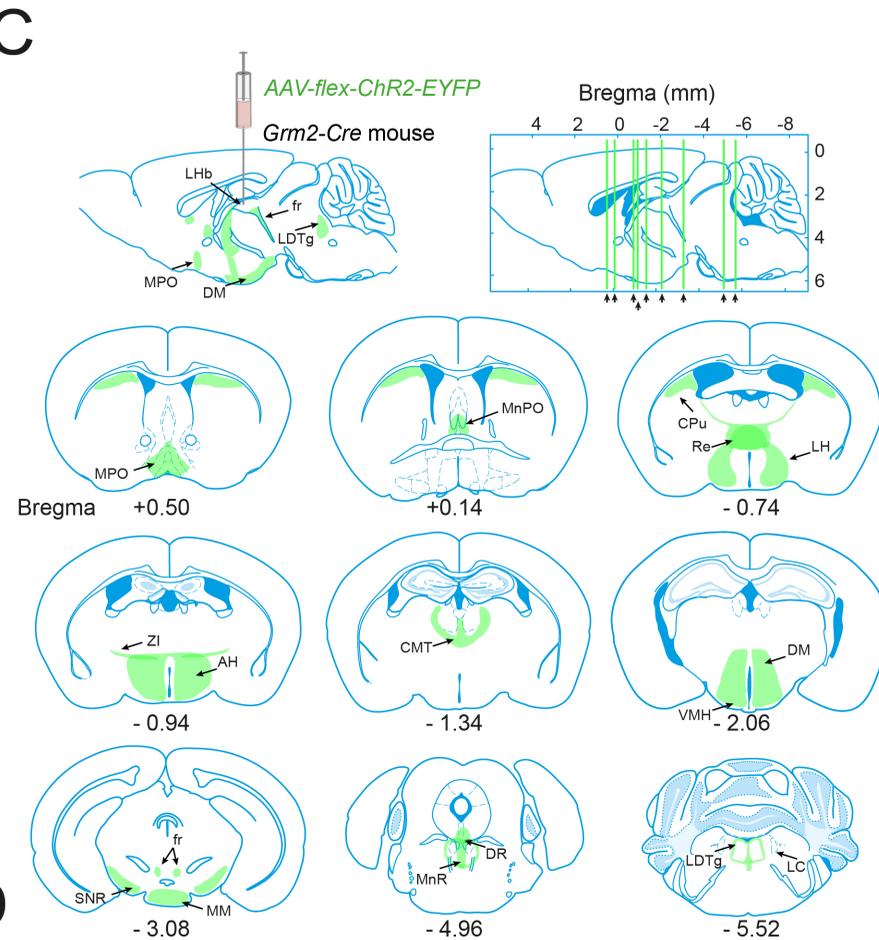
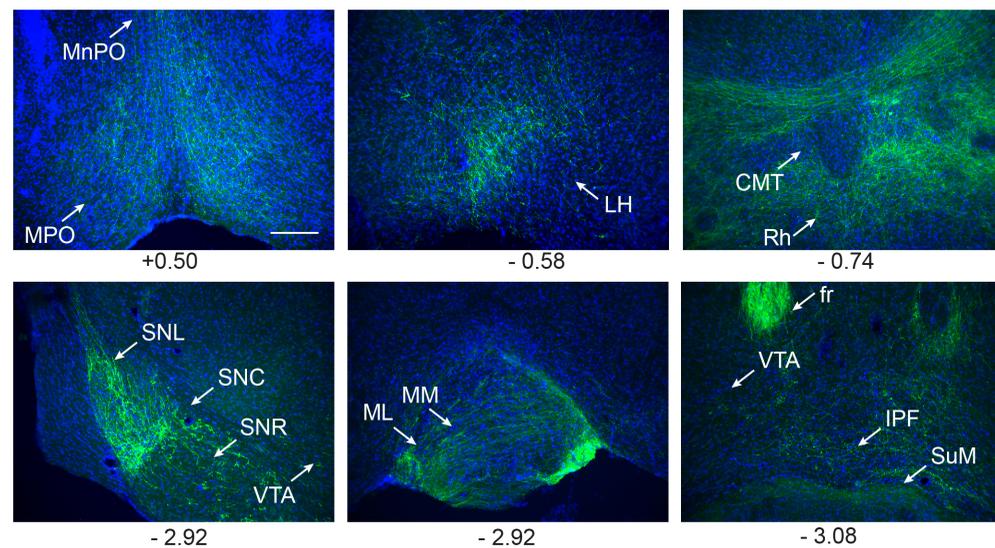
**C****D**

Figure S2. Related to Figure 1. (A) Projections resulting from bilateral injections of *AAV-flex-GFP-TeLC* into the LHb of *Grm2-Cre* mice were mapped across the brain from Bregma -0.76 to -2.06 mm. The approximate distribution of projection targets is shown in the coronal schematics together with a picture of thalamic projections (scale bar 500 μ m). (B) Hypothalamic projections (scale bar 500 μ m) together with two expanded sections on the right (scale bars 200 μ m). (C) Injections of *AAV-flex-ChR2-EYFP* were also used to map projections between Bregma +0.5 to -5.52 mm from the *Grm2-Cre* neurons in the LHb. Coronal schematics illustrate the approximate distribution of the projections. Where the fibre tract followed the same path as *GFP-TeLC* projections the same colour pattern was used for both figures. (B) Example images of distinct projection sites and local nuclei (scale bar 200 μ m). AH, anterior hypothalamus; AHC, anterior hypothalamus central part; Arc, arcuate hypothalamic nucleus; CMT, central medial thalamus; CPu, caudate putamen; DM, dorsal medial hypothalamus; DMD, dorsal medial hypothalamus, dorsal part; DMV, dorsal medial hypothalamus, ventral part; DR, dorsal raphe nucleus; fr, fasciculus retroflexus; LC, locus coeruleus; LDTg, laterodorsal tegmental nucleus; LHb, lateral habenula; LH, lateral hypothalamus; MHb, medial habenula; MPO, medial preoptic nucleus; MnPO, median preoptic nucleus; MM, medial mammillary nucleus, medial part; ML, medial mammillary nucleus, lateral part; MnR, median raphe nucleus; opt, optic tract; PFC, pre-frontal cortex; PH, posterior hypothalamus; PO, preoptic area; Re, reuniens thalamic nucleus; SNR, substantia nigra, reticular part; SNC, substantia nigra, compact part; ZI, zona incerta; 3V, third ventricle; VMH, ventromedial hypothalamic nucleus.

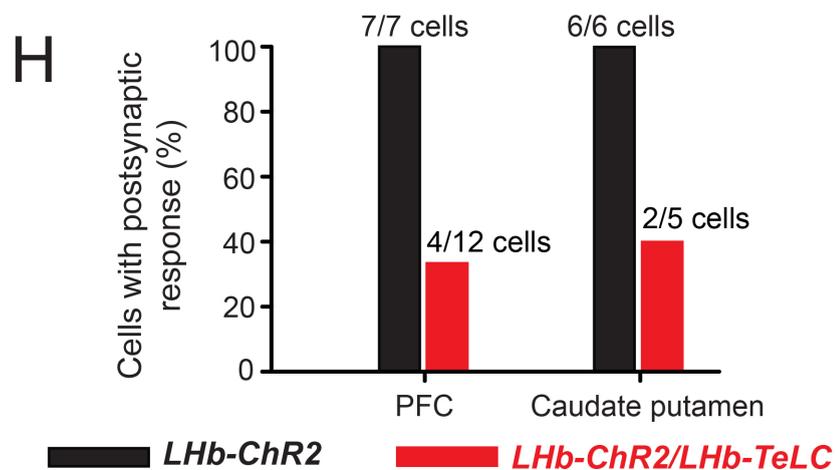
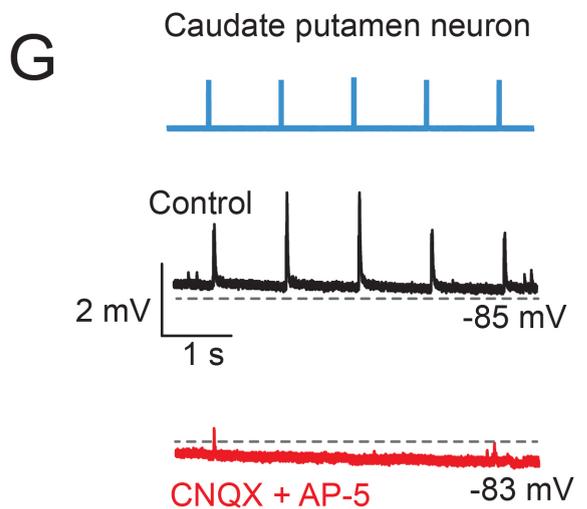
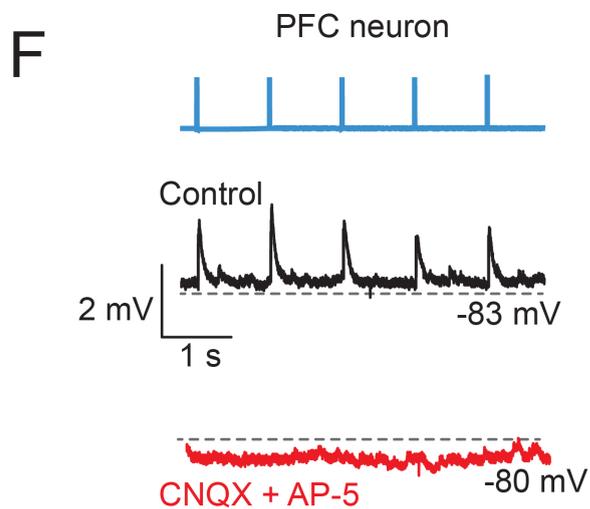
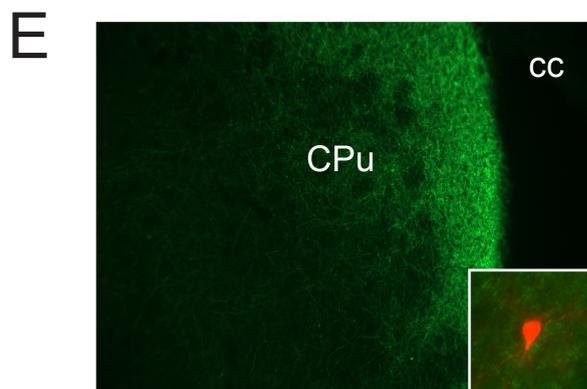
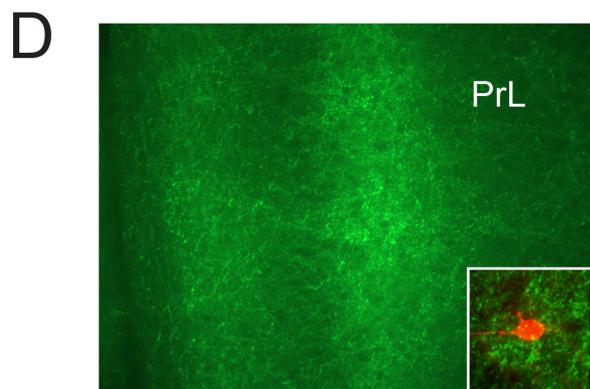
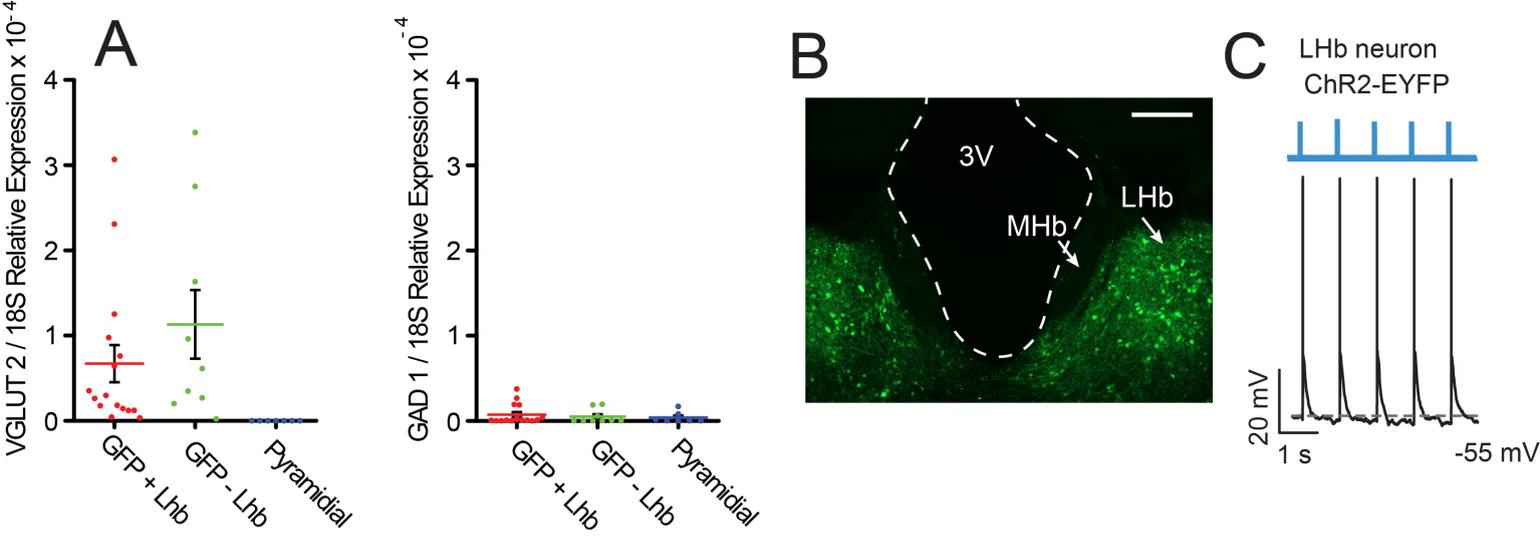


Figure S3. Related to Figure 1. The output of *Grm2-Cre* LHb neurons is glutamatergic, and is blocked by TeLC. (A) Single-cell-patch RT-PCR analysis of transmitter phenotype of neurons ($n = 26$) in the LHb in acute brain slices from *LHb-ChR* mice. Both *Grm2-Cre* GFP-positive ($n = 17$) and GFP-negative ($n = 17$) neurons predominantly express the *Vglut2* gene, but with low levels of *Gad1* transcripts as well. As a control for specificity of the PCR, neocortical pyramidal neurons ($n = 7$) patched from the same slices, and run in parallel assays, do not contain *Vglut2* or *Gad1* transcripts. The horizontal lines represent the means and the error bars represent the SEMs. (B) Bilateral injection of *AAV-flex-ChR2-EYFP* into the LHb region of *Grm2-Cre* mice; scale bar is 200 μm . (C) An example whole-cell current-clamp recording from a LHb neuron expressing ChR2-EYFP. Brief (2-ms) light pulses (blue lines) consistently produced action potentials ($n = 18$ neurons, 3 mice). The *Grm2-Cre* LHb axons project to the infralimbic (PrL) prefrontal cortex (D) and dorsal caudate-putamen (CPu). Cc, corpus callosum. (E) The insets show examples of biocytin-filled neurons post recording. Light stimulation of ChR2-EYFP positive LHb axon terminals evoked EPSPs (black traces) in postsynaptic prefrontal cortex neurons (F) and dorsal-lateral caudate-putamen neurons (G), which were blocked by CNQX and AP-5 (red traces). (H) Percentage of cells with detected post-synaptic EPSPs in *LHb-ChR2* mice (black) and *LHb-ChR2/LHb-TeLC* mice (red) in prefrontal cortex and caudate-putamen respectively.

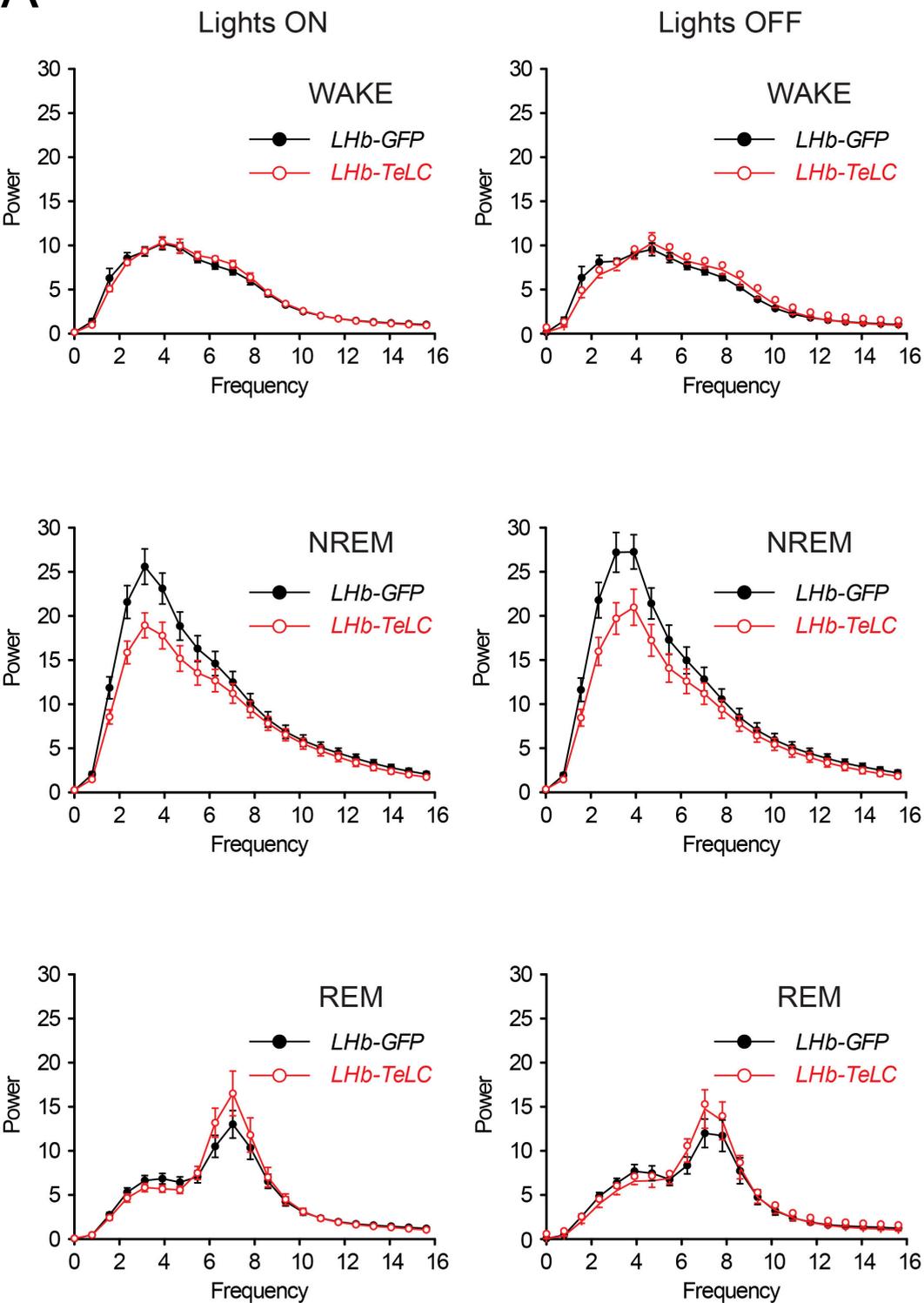
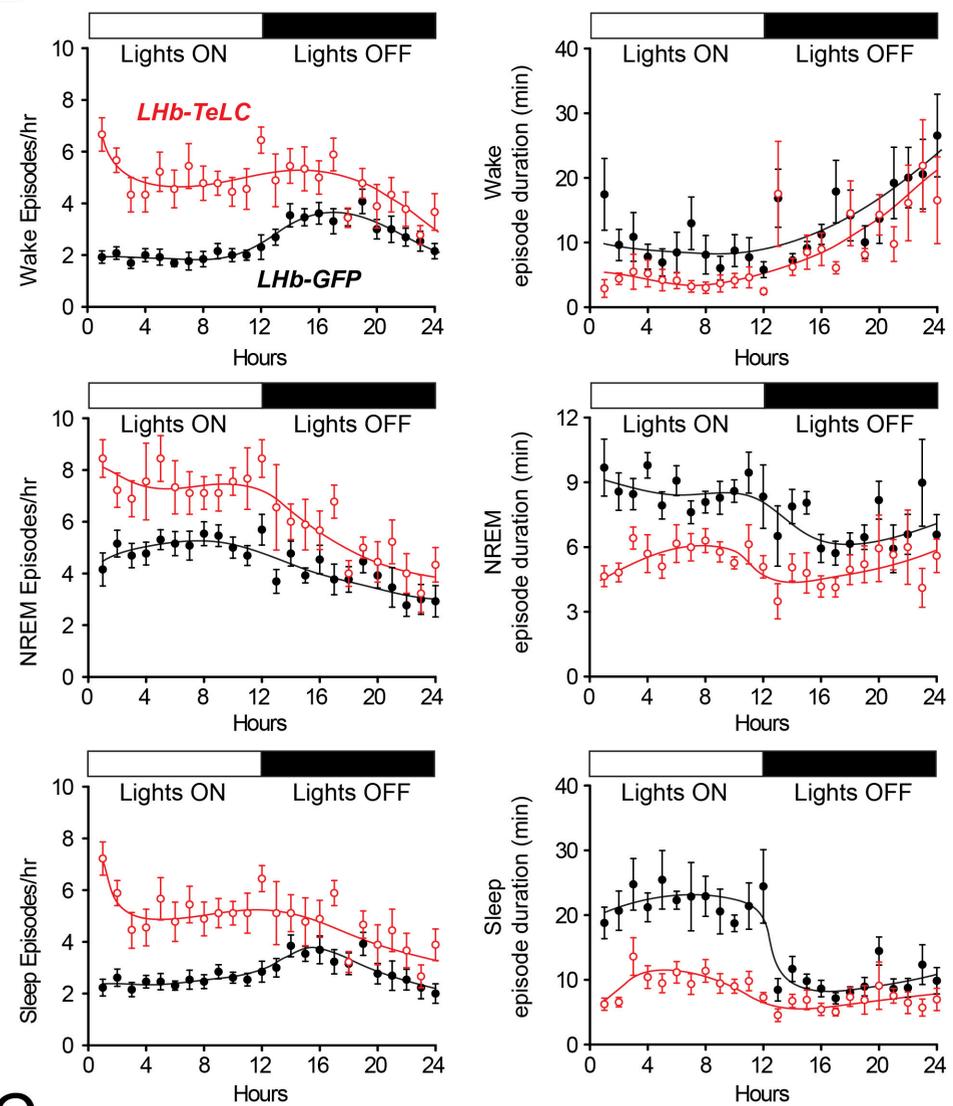
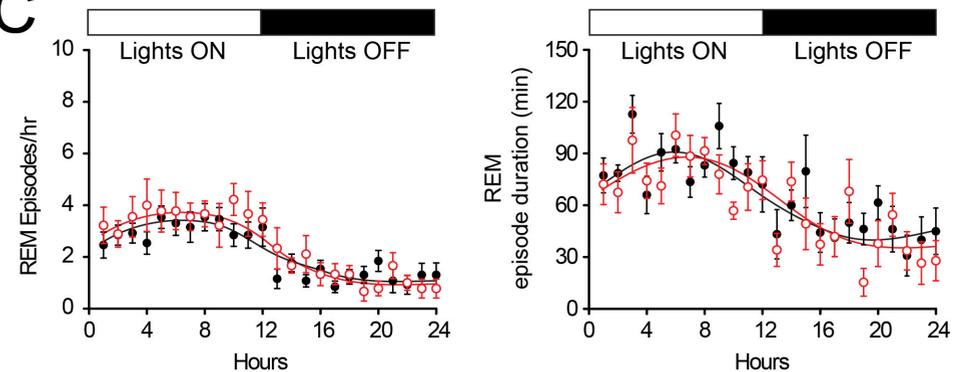
A**B****C**

Figure S4. Related to Figure 4. Sleep in *LHb-TeLC* mice compared with *LHb-GFP* mice. (A) Power spectra of vigilance states for the *LHb-GFP* and *LHb-TeLC* mice. The Fourier transform power spectra for the *LHb-GFP* and *LHb-TeLC* mice were no different during Wake and REM states, but did show slightly lower power over delta frequencies (1-4Hz) during NREM. This was true during both “Lights ON” ($P = 0.022$, $t = 2.45$, $df = 21$, $n = 13$ *LHb-GFP* mice and 10 *LHb-TeLC* mice; unpaired two-tailed t -test) and “Lights OFF” ($P = 0.020$, $t = 2.51$, $df = 21$, $n = 13$ *LHb-GFP* mice and 10 *LHb-TeLC* mice; unpaired two-tailed t -test). (B) For Wake, NREM and Sleep (defined as consolidated bouts of NREM and REM) the number of episodes per hour (left-hand panels) are shown over a 24-hour period while the duration of episodes is shown on the right hand panels. (C) The number of REM episodes per hour (left) and durations of episodes over a 24-hour period ($n = 13$ *LHb-GFP* mice and $n = 9$ *LHb-TeLC* mice). Symbols in panels A, B and C represent means \pm SEMs.