Supplemental materials for:

Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance

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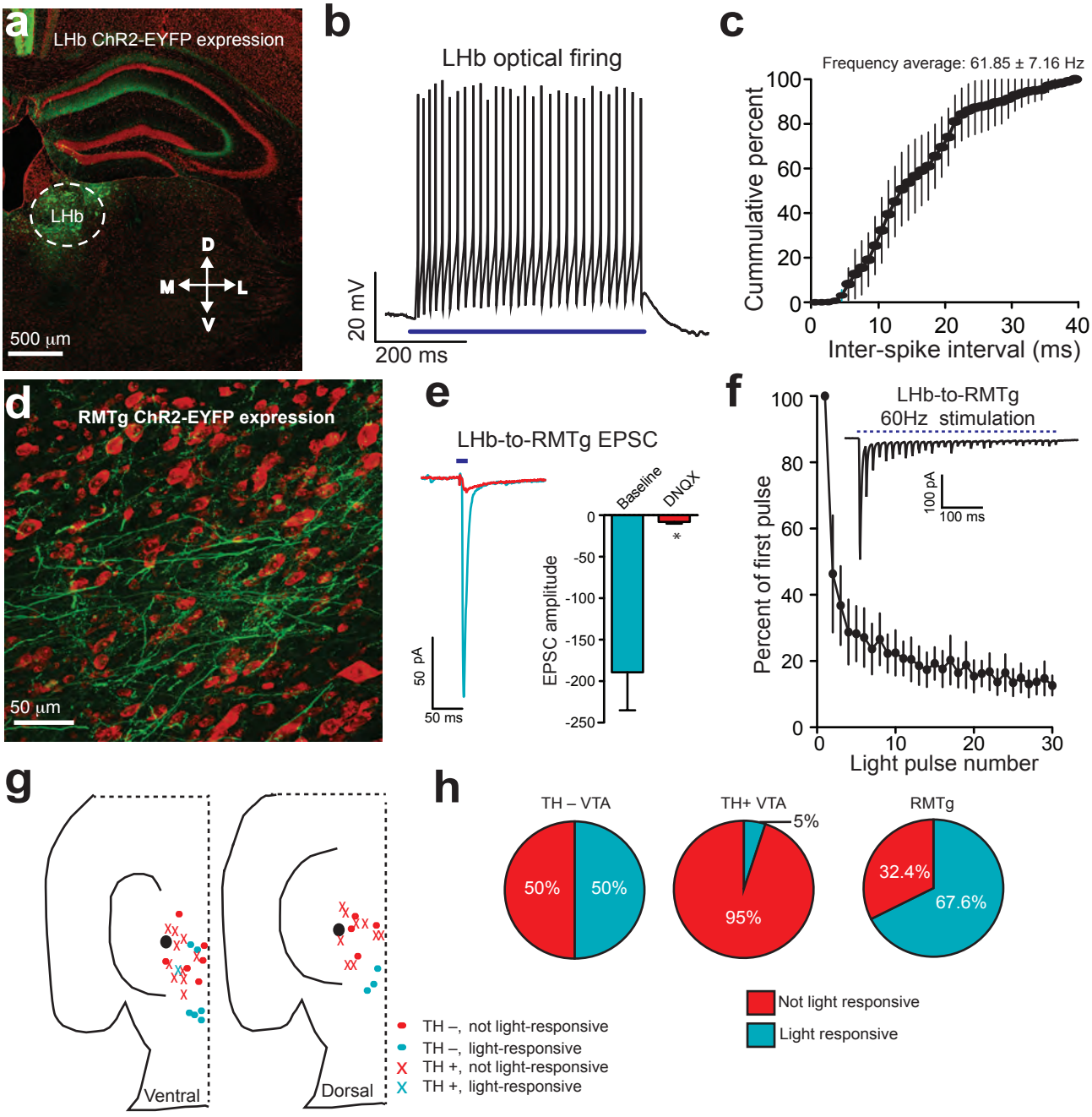
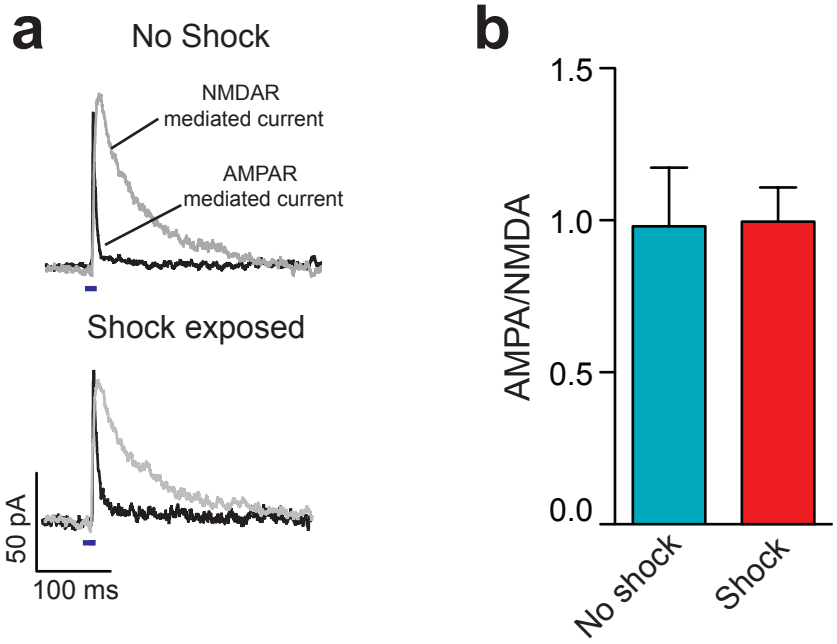
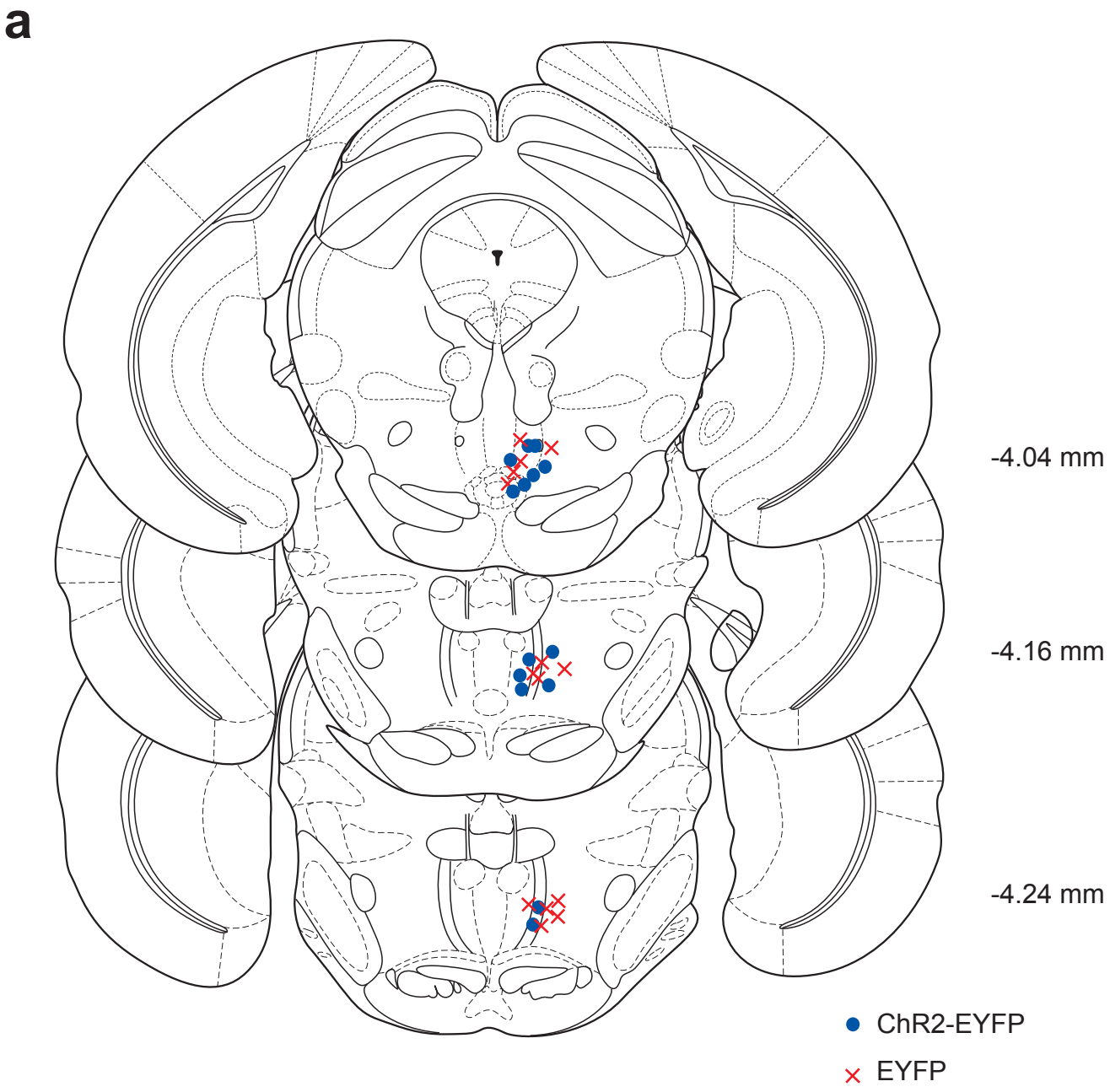


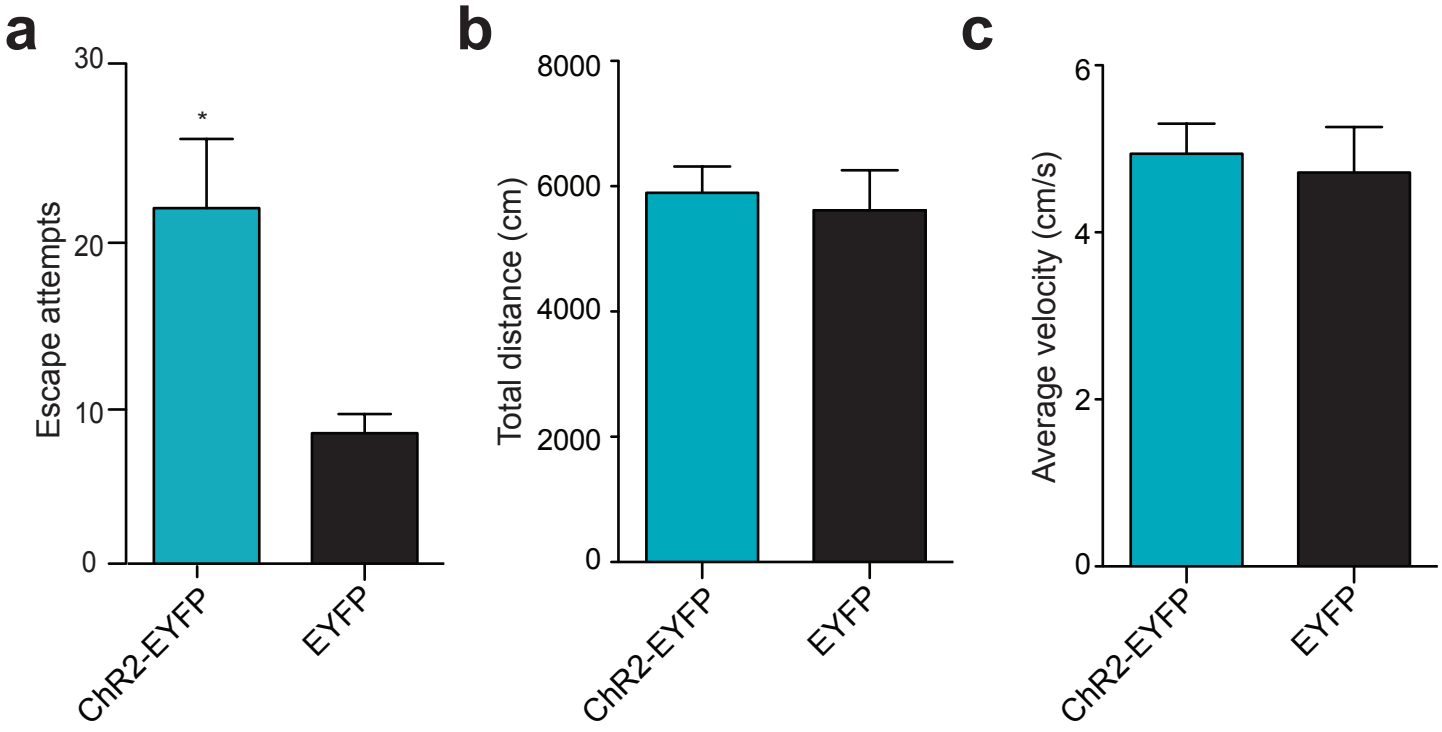
Supplementary Figure 1: Optogenetic stimulation of LHb terminals in the midbrain. (**a**) Expression of ChR2-EYFP (green) following injection of the viral construct into the LHb. Neurons were counter-stained using a red fluorescent Nissl stain. D, Dorsal; V, Ventral; M, Medial; L, Lateral. (**b,c**) Activation of ChR2 expressed in LHb cell bodies in brain slices resulted in sustained high frequency activation during the 500 ms stimulation (n = 7 cells). (**d**) Confocal compressed z-stack showing that ChR2-EYFP is expressed in LHb projection fibers in the RMTg after virus injection into the LHb. (**e**) Postsynaptic optically-evoked EPSCs recorded from RMTg neurons were significantly attenuated following bath application of 10 mM DNQX ($t(6) = 3.94$, $p = 0.07$, n = 4 cells). (**f**) LHb efferents to the RMTg were stimulated at 60 Hz for all behavioral tasks. Optically-evoked EPSCs at this frequency for 500 ms show a significant reduction in amplitude across the pulse train stimulation ($F_{2,29} = 60.21$, $p < 0.001$, n = 5 cells) (**g**) Location of TH+ and TH– light-responsive and non-light-responsive neurons in horizontal midbrain brain slices. (**h**) Percentage of light-responsive TH+ midbrain neurons, TH– midbrain neurons, and RMTg neurons. 5% (1/20) of TH+ neurons in the VTA were light responsive. 50% (10/20) of VTA TH– neurons were light responsive. 67.6% (75/111) of RMTg neurons were light responsive in a sagittal slice preparation.



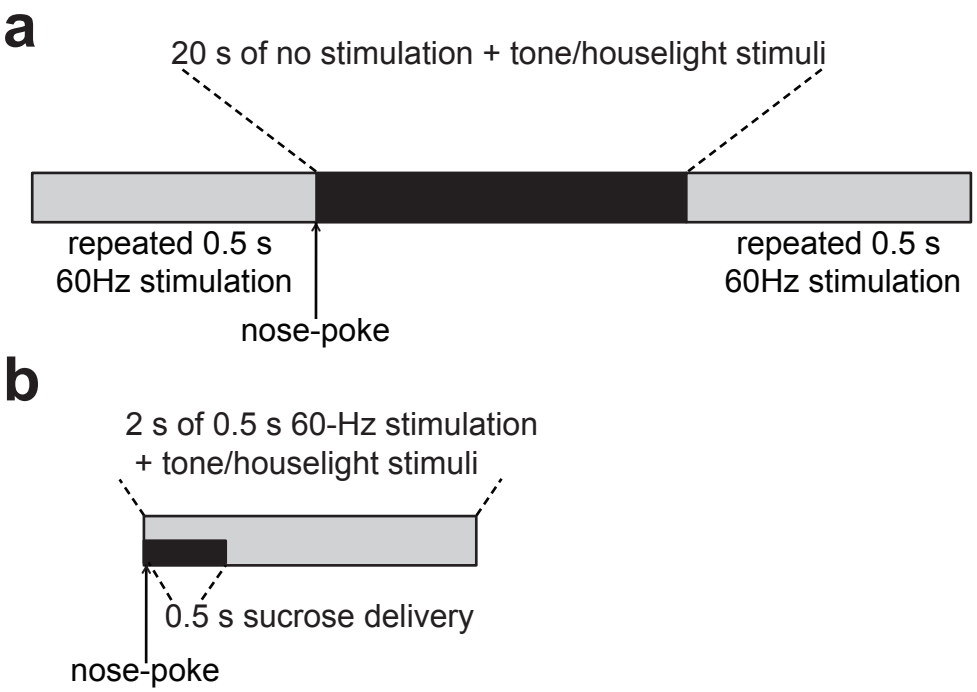
Supplementary Figure 2: Acute unpredictable shock does not alter AMPA/NMDA ratio (**a**) Representative optically evoked AMPA/NMDA ratios at LHb-to-RMTg synapses following 0 or 19 foot shocks. (**b**) Optically evoked AMPA/NMDA ratios were not significantly different between the groups ($t(14) = 0.36$, $p = 0.86$). n = 8 cells/group.



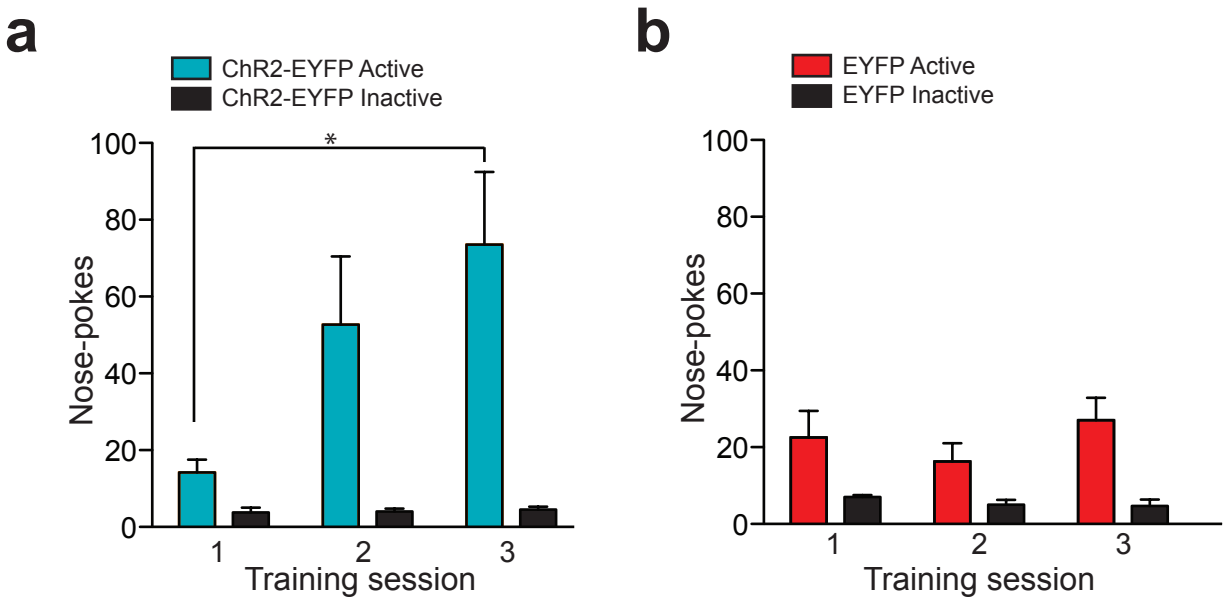
Supplementary Figure 3: Optical fiber placements (**a**) LHb-to-RMTg optical stimulation sites for behavioral experiments. Symbols represent where the fiber tract terminated.



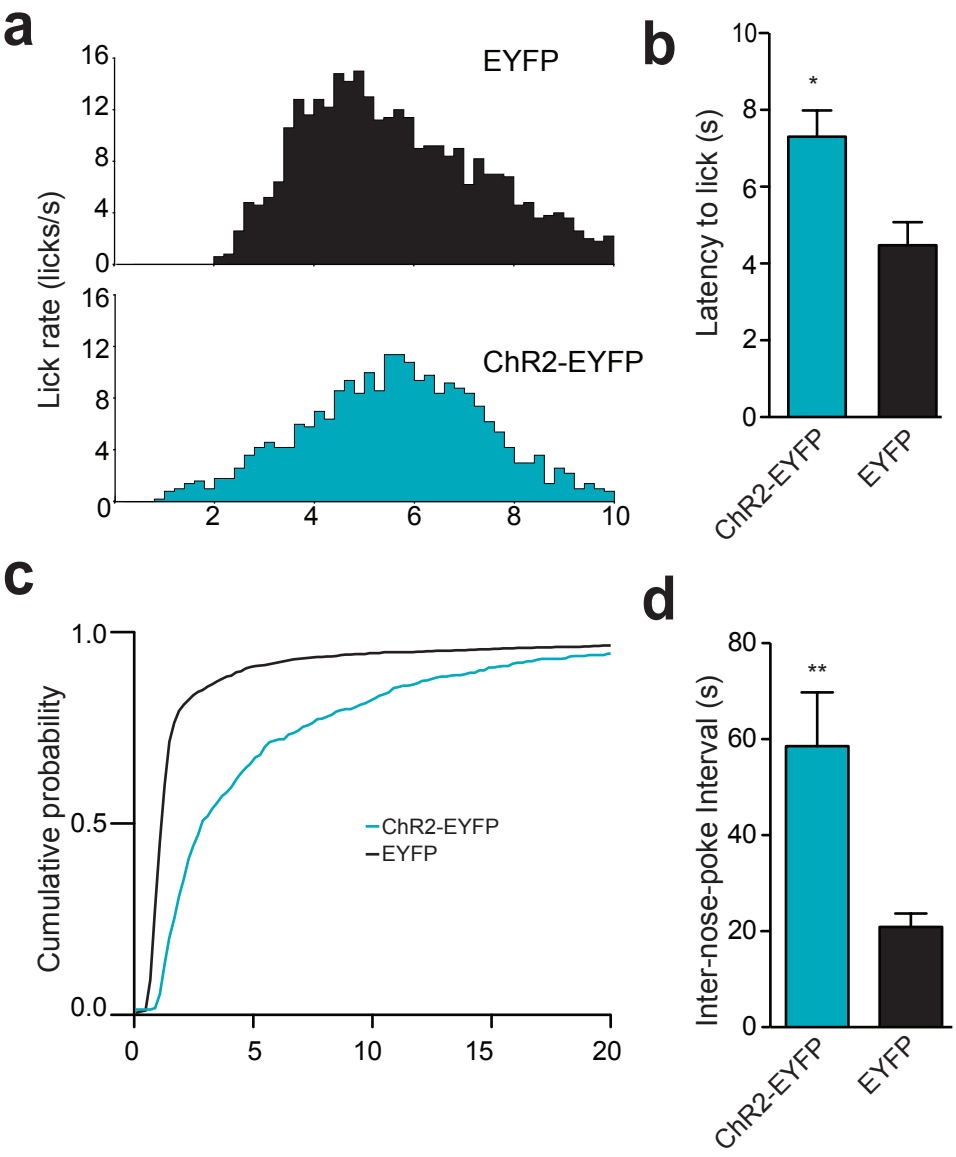
Supplementary Figure 4: Real-time place preference (a) ChR2-EYFP-expresssing mice made significnatly more escape attempts during the real-time place preference session than EYFP-expressing mice ($t(10) = 2.82$, $p = 0.018$) (**b**) Total distance (cm) during the real time place preference experiment across the entire arena was not significnatly different between groups ($t(10) = 0.37$, $p = 0.72$) (**c**) Average velocity across the entire 20-min session across the entire arena was not significantly different between groups ($t(10) = 0.34$, $p = 0.74$). n= 6 mice per group.



Supplementary Figure 5: Schematic of negative and positive reinforcement tasks. (**a**) Behavioral schematic for the 1-hr negative reinforcement session. (**b**) Behavioral schematic for the 1-hr positive reinforcement session.



Supplementary Figure 6: Acquisition of nose-poking behavior in negative reinforcement task. (**a**) Active and inactive nose-poke responses from ChR2-EYFP-expressing mice over the first 3 days of training. There was a significant interaction between active lever presses and days ($F_{2,10} = 3.86$, $p = 0.03$). (**b**) Active and inactive nose-poke responses from EYFP-expressing mice over the first 3 days of training. There was no significant interaction between active lever presses and days ($F_{2,10} = 0.84$, $p = 0.44$). n = 6 mice/group.



Supplementary Figure 7: Activation of LHb inputs to the RMTg decreases nose-pokes for sucrose and increases latency to lick during a positive reinforcement task (**a**) Example histograms of licks time-locked to active nose-poke for EYFP-expressing mouse (top) and ChR2-EYFP-expressing mouse (bottom). (**b**) Average latency to lick following stimulation for ChR2-EYFP and EYFP-expressing mice ($t(2032) = 2.5$, $p = 0.01$). (**c**) Cumulative probability of the latency to lick following stimulation for ChR2-EYFP and EYFP-expressing mice. (**d**) The inter nose-poke interval (time between each nose-poke averaged across the session) was significantly higher in ChR2-EYFP-expressing mice ($t(3577) = 10.8$, $p < 0.001$). n = 8 mice per group.

Supplemental video captions

Video S1: LHb-to-RMTg activation during real-time place preference. Video shows a representative ChR2-EYFP and EYFP-expressing mouse during the real-time place preference session.

Video S2: LHb-to-RMTg activation during positive reinforcement. Video shows a representative ChR2-EYFP and EYFP-expressing mouse during the positive reinforcement behavioral session, where each nose-poke is paired with a 60-Hz optical stimulation.