

MnPO - hM3Dq/mCherry Negative

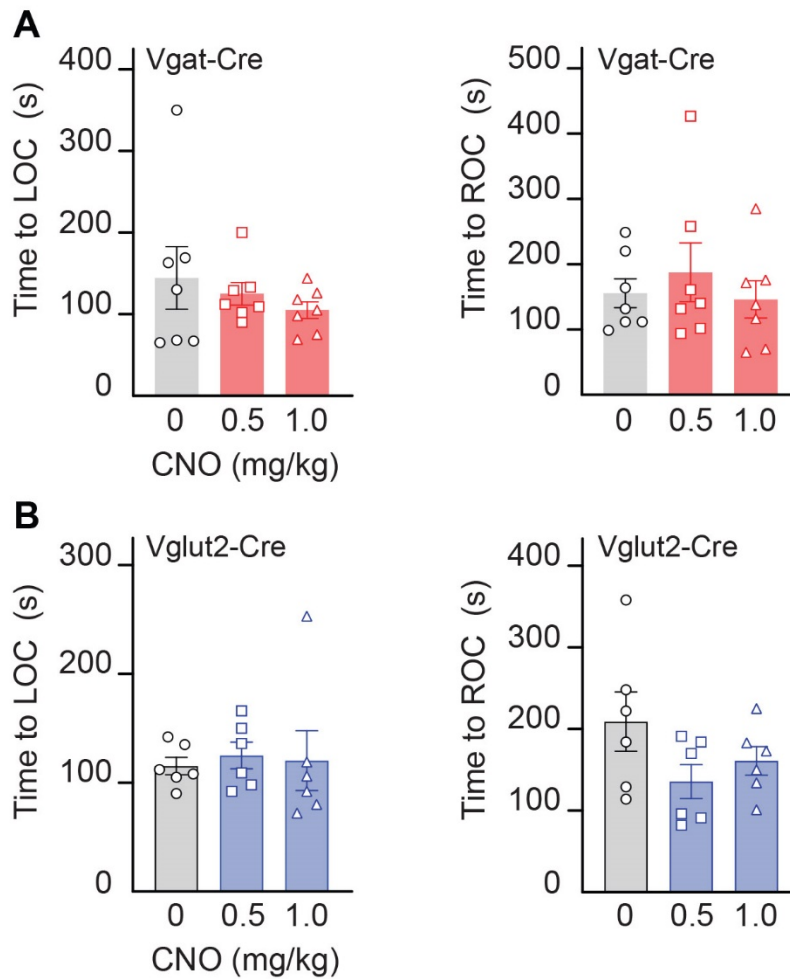


Figure S1. CNO administration to control mice without designer receptor expression did not alter anesthetic state transitions, Related to Figure 1

Time to loss and resumption of consciousness (left and right panels, respectively) in Vgat-Cre (panels in **A**) and Vglut2-Cre (panels in **B**) mice that received vector injections into the MnPO but did not express hM3Dq receptors (negative controls). Statistical comparisons were conducted using a Friedman test. Data are shown as mean \pm standard error of the mean.

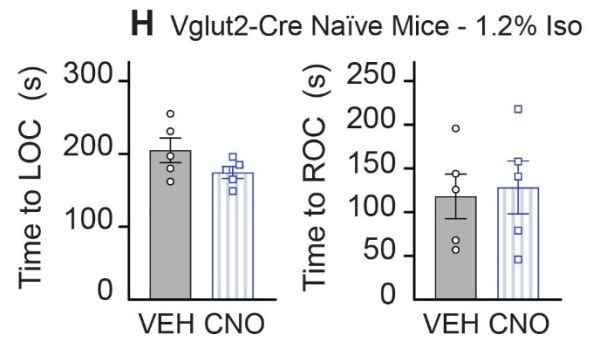
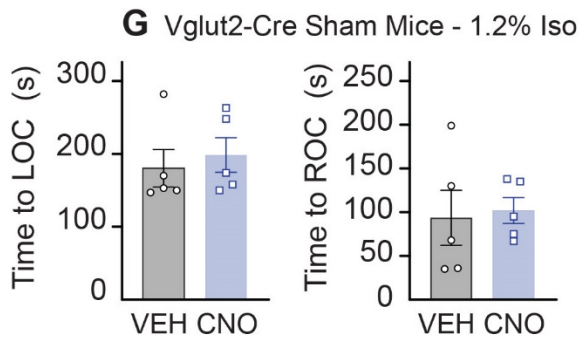
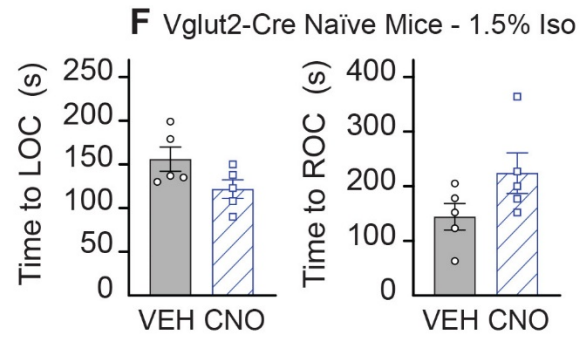
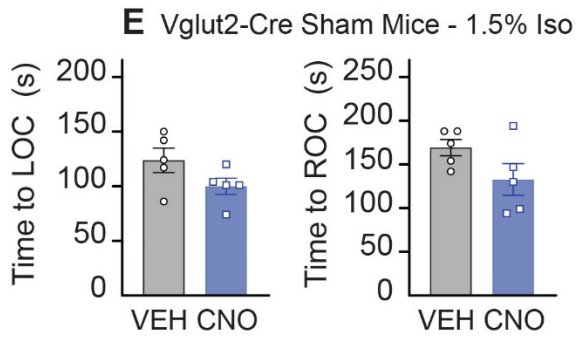
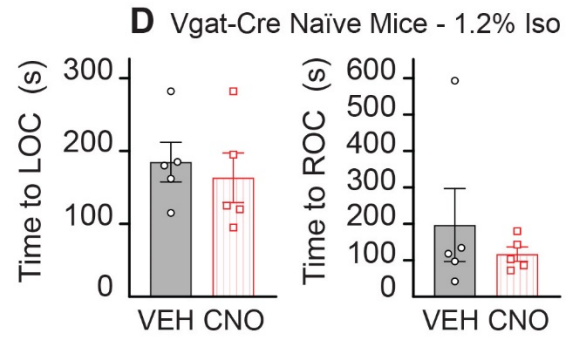
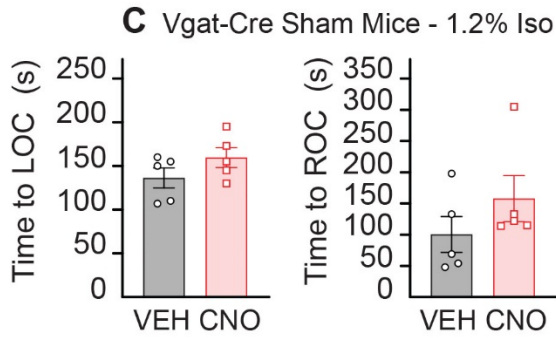
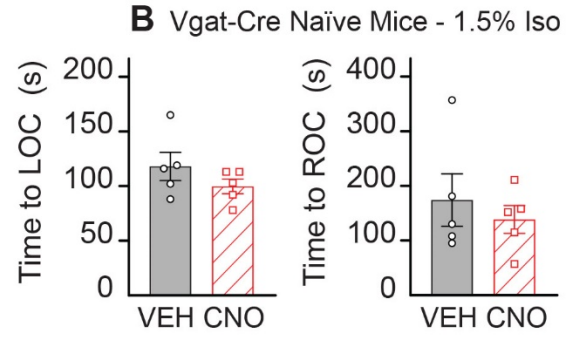
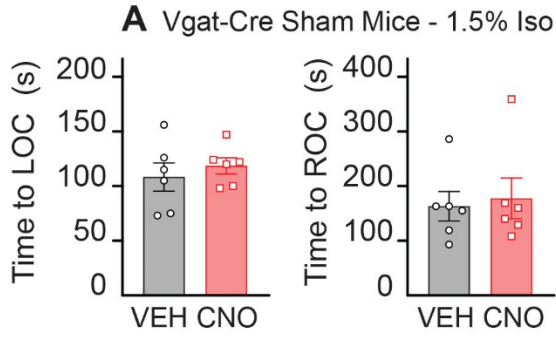


Figure S2. CNO administration to sham and naïve control mice did not alter anesthetic state transitions, Related to Figures 1 and 5

Time to loss and resumption of consciousness (left and right panels, respectively) in sham (**A**) and naïve (**B**) Vgat-Cre mice exposed to 1.5% isoflurane. **C** and **D** show the time to loss and resumption of consciousness in sham and naïve Vgat-Cre mice exposed to 1.2% isoflurane.

Time to loss and resumption of consciousness (left and right panels, respectively) in sham (**E**) and naïve (**F**) Vglut2-Cre mice exposed to 1.5% isoflurane. **G** and **H** show the time to loss and resumption of consciousness in sham and naïve Vglut2-Cre mice exposed to 1.2% isoflurane.

Statistical comparisons were conducted using a two-tailed Wilcoxon test. Data are shown as mean \pm standard error of the mean.

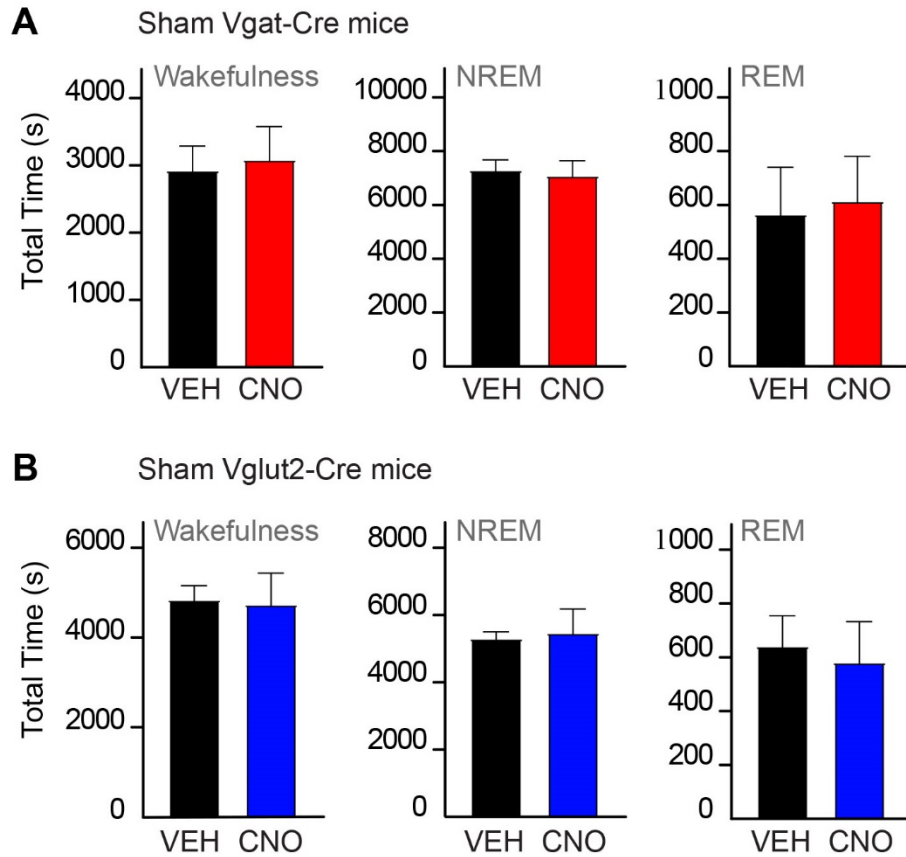


Figure S3. CNO administration to sham control mice did not alter sleep architecture, Related to Figures 2 and 6

Total time in wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep in sham-operated (without AAV injection) Vgat-Cre (panels in **A**) and Vglut2-Cre mice (panels in **B**) after injection of vehicle control (VEH) or clozapine-N-oxide (CNO; 1.0 mg/kg). Data are shown as mean \pm standard error of the mean.

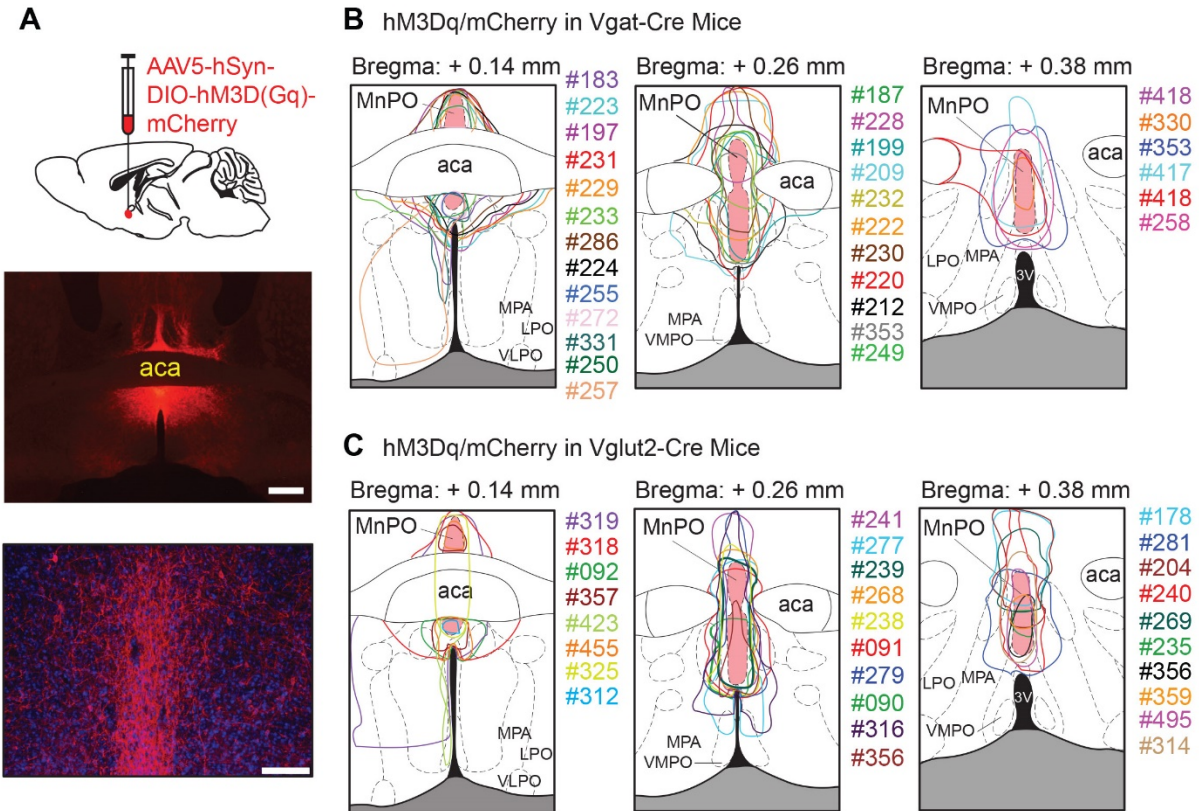
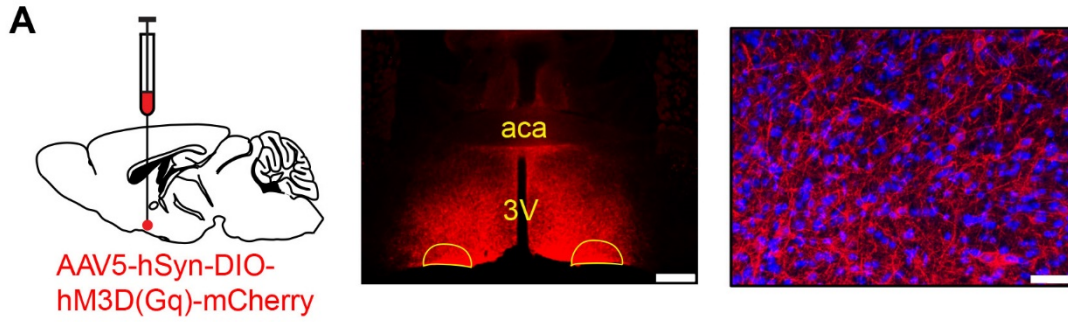


Figure S4. Vector injection sites in the MnPO, Related to Figures 1, 2 and 3

A. Schematic representation of AAV injection into the median preoptic nucleus (MnPO). In the top panel (low magnification photograph), mCherry immunohistochemical staining (red) indicates the expression of the excitatory designer receptor hM3Dq within the MnPO of a Vglut2-Cre mouse. The bottom panel is a representative high magnification photograph demonstrating mCherry immunohistochemical staining (red) and DAPI nuclear staining (blue) in the MnPO. **B.** Color-coded vector injection sites (i.e., area of hM3Dq receptor expression) within the MnPO of Vgat-Cre mice used for anesthesia, sleep and temperature experiments are represented on coronal schematics of the preoptic area modified from a mouse brain atlas [S1]. Color-matched identification numbers for each mouse are listed on the right side of each panel.

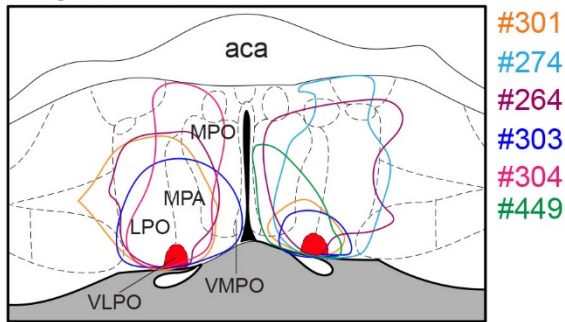
C. Vector injection sites within the MnPO of Vglut2-Cre mice used for anesthesia experiments.

Abbreviations: aca, anterior commissure; LPO, lateral preoptic area; MnPO, median preoptic nucleus; MPA, medial preoptic area; 3V, third ventricle; VLPO, ventrolateral preoptic nucleus; VMPO, ventromedial preoptic nucleus. Calibration bars in A, 200 μm (top) and 100 μm (bottom).

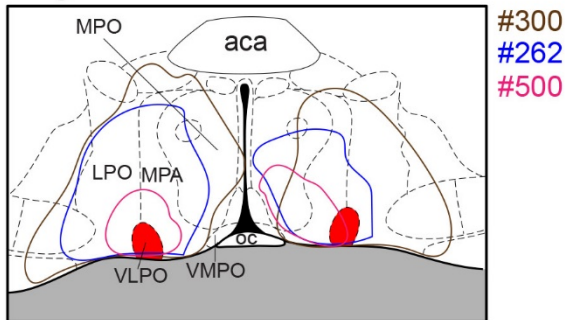


B hM3Dq/mCherry in Vgat-Cre Mice

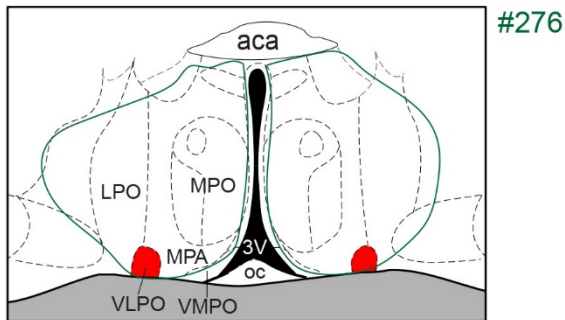
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Bregma: + 0.02 mm

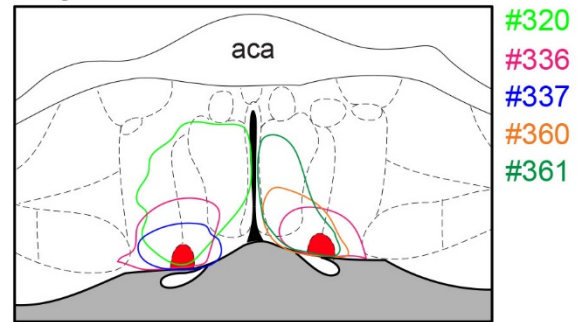


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C hM3Dq/mCherry in Vglut2-Cre Mice

Bregma: + 0.14 mm



Bregma: + 0.02 mm

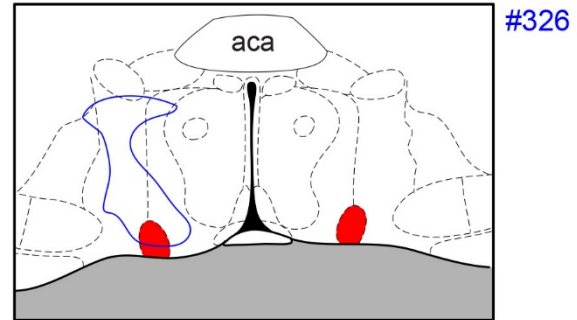


Figure S5. Vector injection sites in the VLPO, Related to Figures 5 and 6

A. The first panel is a schematic representation of AAV injection into the ventrolateral preoptic nucleus (VLPO). In the center panel (low magnification photograph), mCherry immunohistochemical staining (red) indicates the expression of the excitatory designer receptor hM3Dq within the VLPO of a Vglut2-Cre mouse. The third panel is a representative high magnification photograph demonstrating mCherry immunohistochemical staining (red) and DAPI nuclear staining (blue) in the VLPO. **B.** Color-coded vector injection sites (i.e., area of hM3Dq receptor expression) within the VLPO of Vgat-Cre mice used for anesthesia and sleep experiments are represented on coronal schematics of the preoptic area modified from a mouse brain atlas [S1]. Color-matched identification numbers for each mouse are listed on the right side of each panel. **C.** Vector injection sites within the VLPO of Vglut2-Cre mice used for anesthesia and sleep experiments. Abbreviations: aca, anterior commissure; LPO, lateral preoptic area; MnPO, median preoptic nucleus; MPA, medial preoptic area; 3V, third ventricle; VLPO, ventrolateral preoptic nucleus; VMPO, ventromedial preoptic nucleus. Calibration bars in A, 200 μm (center panel) and 20 μm (right side panel).

Supplemental Reference

S1. Paxinos, G. and Franklin, K.B.J. (2001). *The Mouse Brain in Stereotaxic Coordinates*, Second Edition (San Diego: Academic).