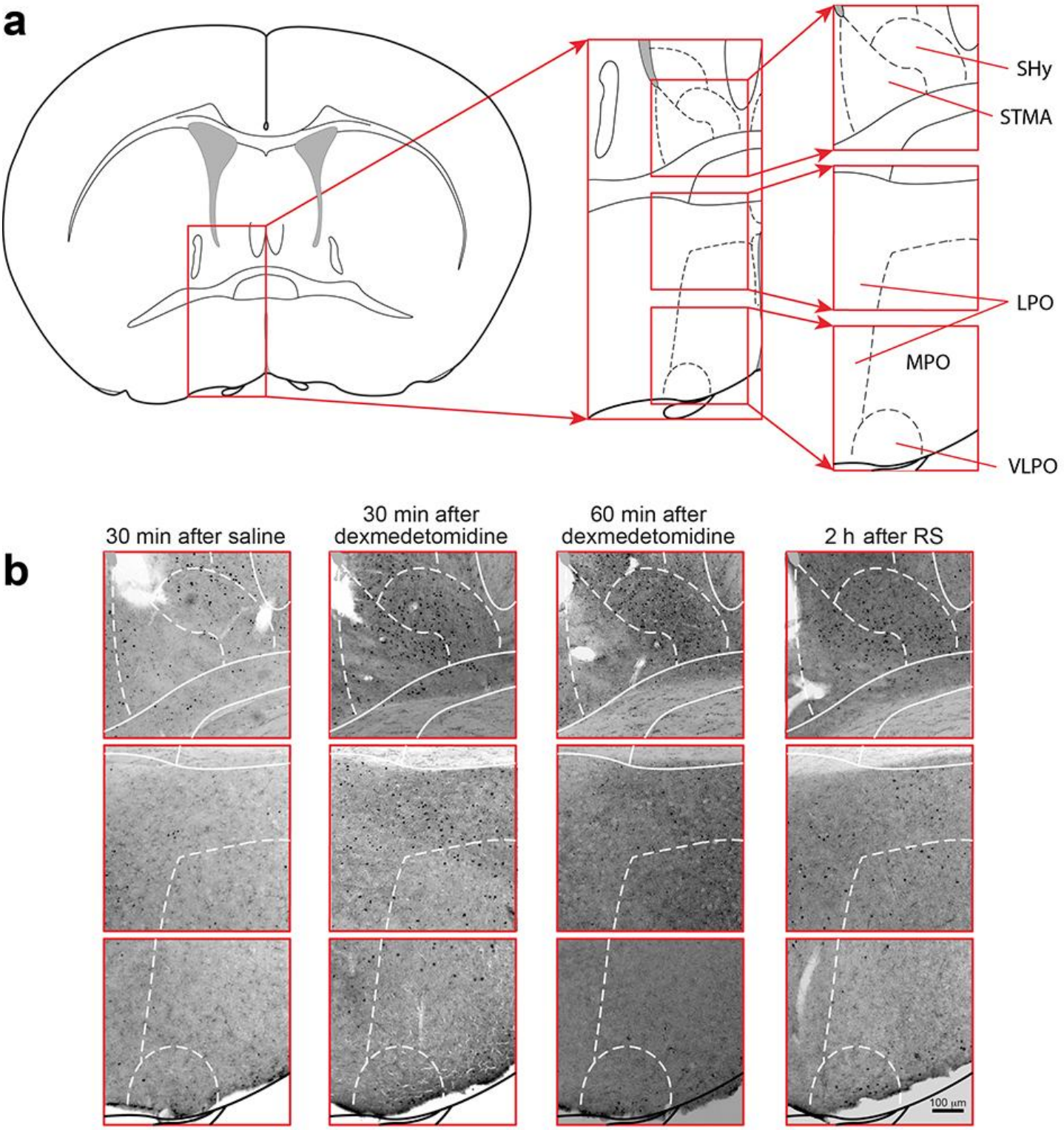
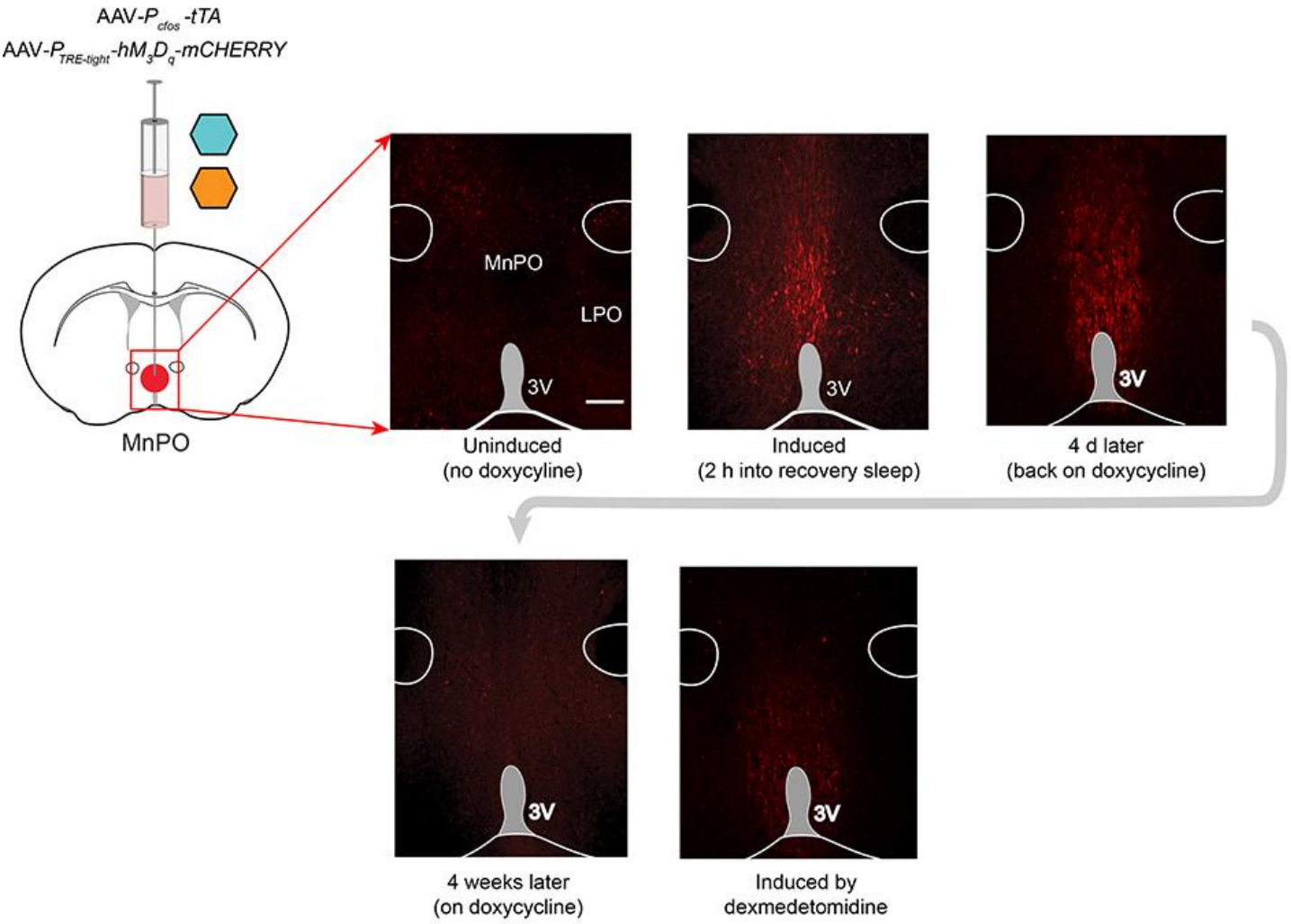


Supplementary Figure 1

Dexmedetomidine-induced sedation and recovery sleep induce *cfos* expression in overlapping regions of the mouse hypothalamic preoptic area and septum.

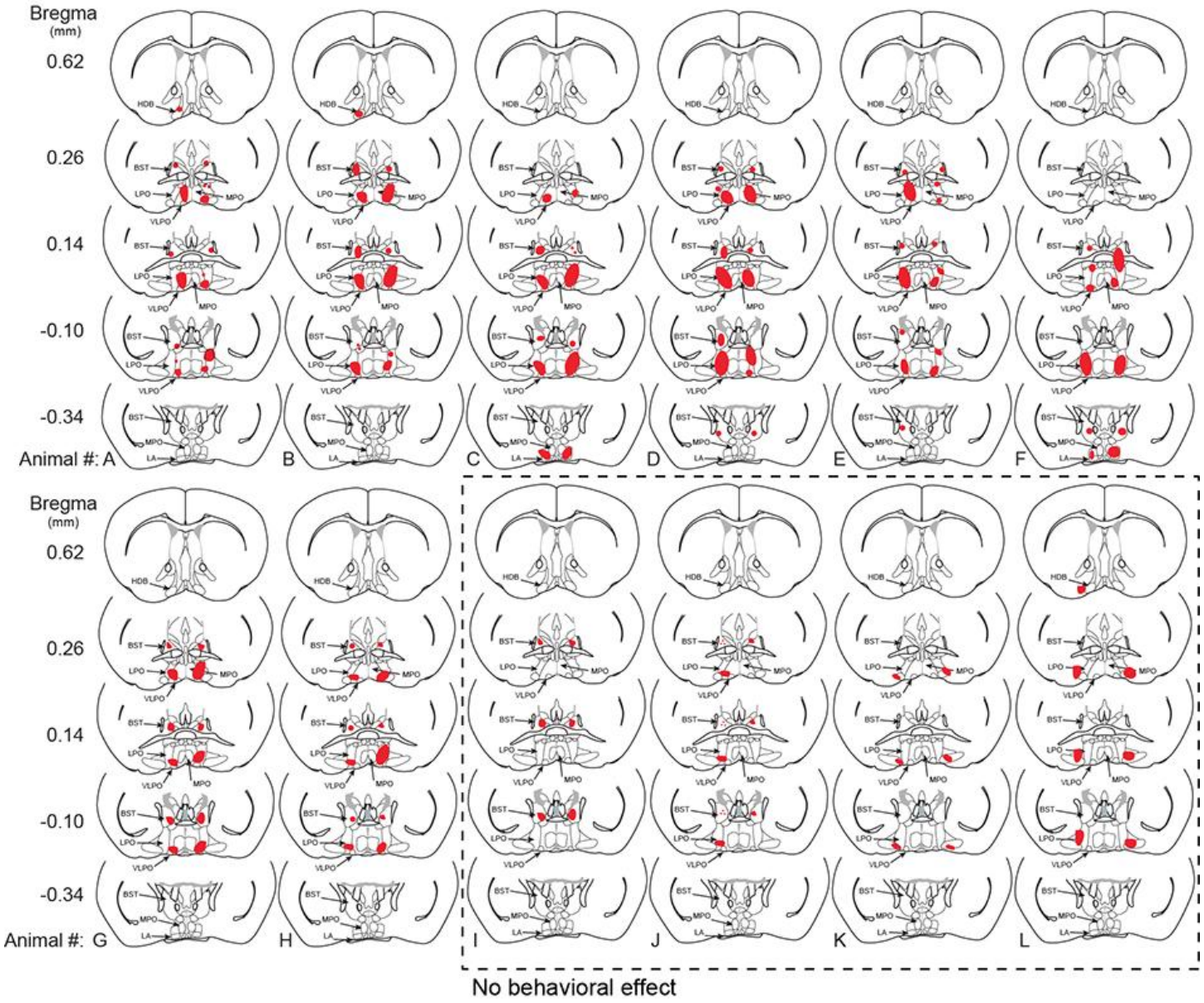
(**a**) Schematic of the preoptic hypothalamic and septal area: left-hand drawing, coronal section, boxed areas, magnified on the right, show the relevant anatomical sites corresponding to the photographs below; (**b**) Photographs of nuclear *cfos* protein (DAB staining method) 30 minutes after saline, 30 minutes and 1 hour after dexmedetomidine (100 μg kg^{-1}) injections or 2 hours into recovery sleep. Abbreviations, LPO, lateral preoptic area; LSV, lateral septum, ventral; MPO, medial preoptic area SHy, septo-hypothalamic nucleus; STLD, stria terminalis lateral dorsal; STMA, stria terminalis medial anterior; VLPO, ventral lateral preoptic area.



Supplementary Figure 2

Induction of *hM_3D_q–mCHERRY* transgene during recovery sleep in MnPO-*TetTag-hM_3D_q* mice.

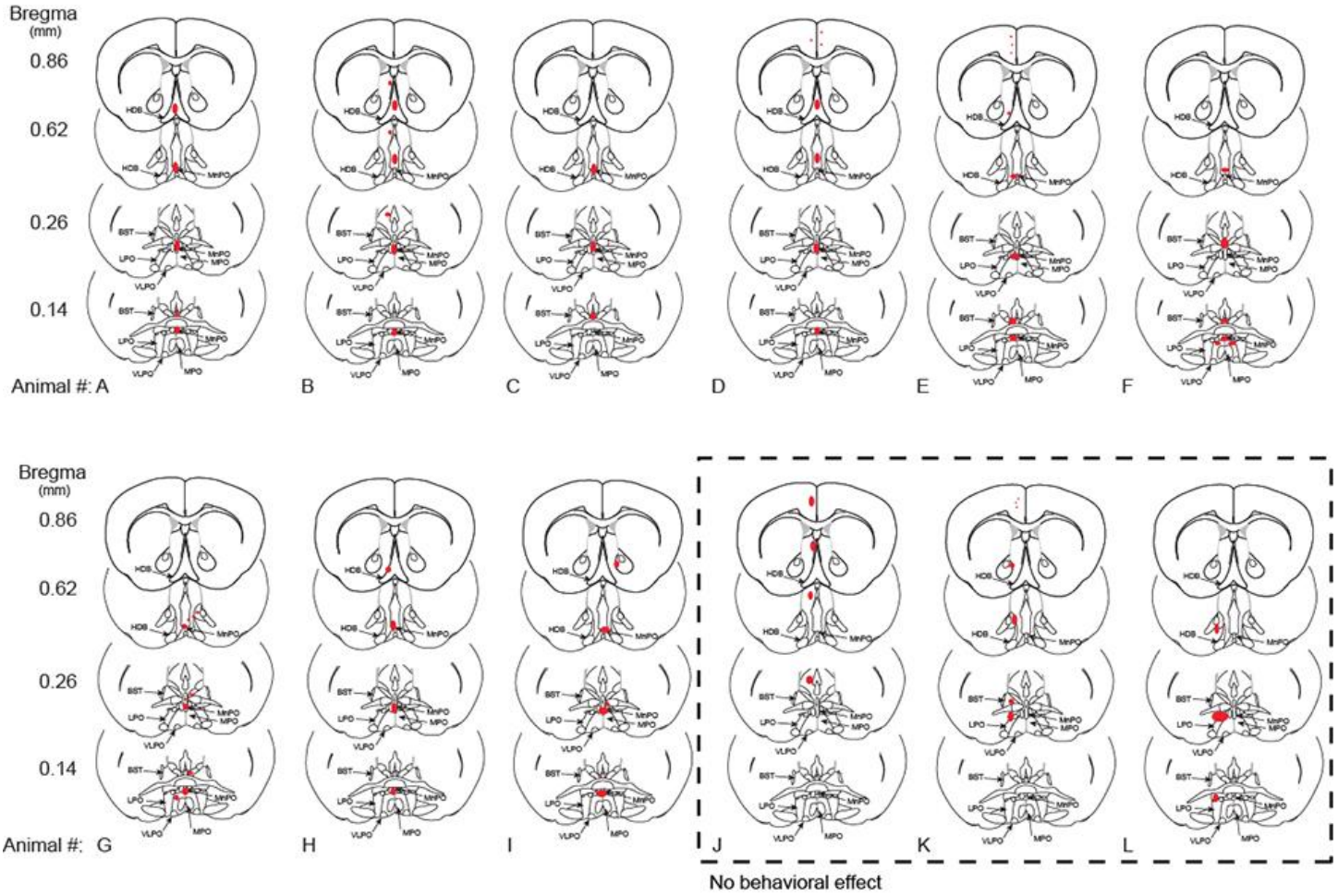
The TetTag AAVs were injected at the midline into the MnPO area. The photographs show coronal sections stained for *hM_3D_q–mCHERRY* expression (red). The left-hand photograph in the top row shows basal transgene expression with no doxycycline; the middle picture shows induced *hM_3D_q–mCHERRY* transgene expression in the MnPO area 2 hours into recovery sleep; the third figure shows expression 4 days later with the mice back on doxycycline. The images below show the low expression 4 weeks later and the figure to the right shows the relatively low *hM_3D_q–mCHERRY* transgene induction following dexmedetomidine sedation. Scale bar, 50 μm; Abbreviations: LPO, lateral preoptic area; MnPO, median preoptic area; 3V, third ventricle.



Supplementary Figure 3

LPO TetTag expression patterns: Induction of *hM$_3$D$_q$-mCHERRY* expression 2 hours following dexmedetomidine-induced sedation where AAVs were bilaterally injected into the LPO and surrounding areas.

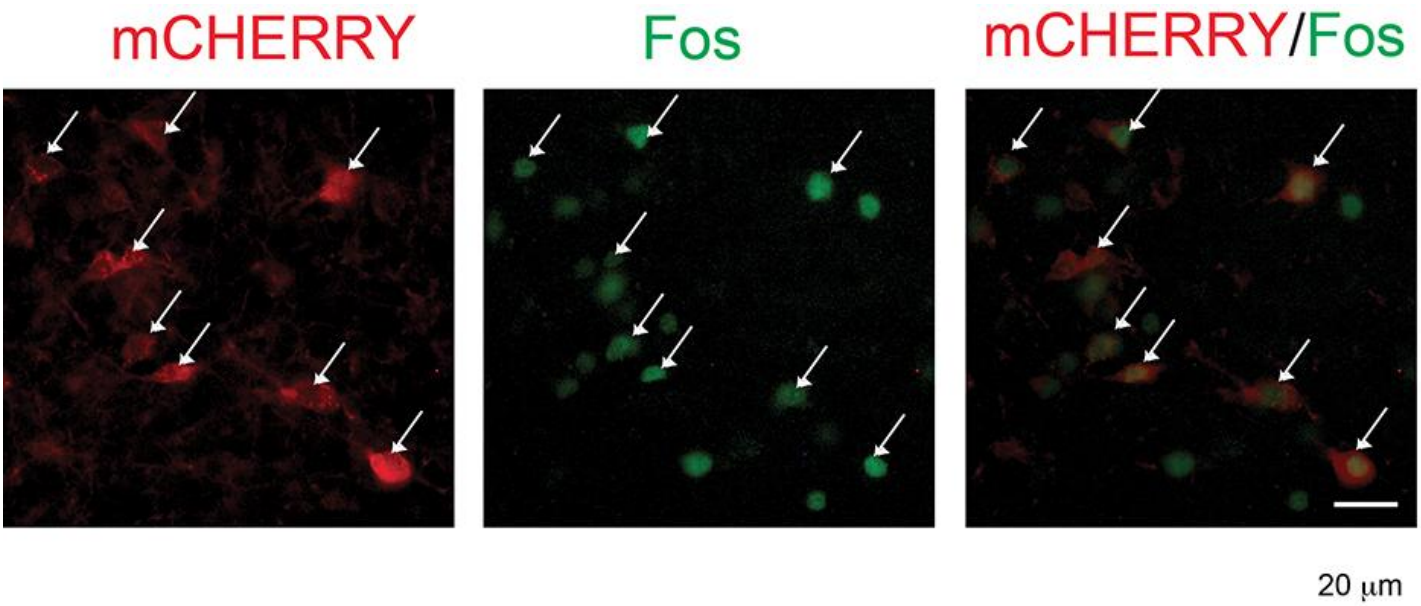
Each letter, A through to L, summarizes the expression in individual mice as seen by serial coronal sectioning through the AAV injection sites. Red indicates *hM$_3$D$_q$-mCHERRY* expression. The boxed sections in animals I-L were mice where *hM$_3$D$_q$-mCHERRY* transgene was induced but there was no behavioral or EEG signs of sedation. Some LPO-*TetTag-hM$_3$D$_q$* mice that had been sedated with dexmedetomidine did not show any subsequent CNO-induced behavior at either the EEG or behavioral levels relative to that observed with saline injection. Although the *hM$_3$D$_q$-mCHERRY* gene was clearly induced by dexmedetomidine treatment in these animals, the transgene expression sites were on the lateral margin of the LPO area or even further out laterally (animals I, J, K and L). Thus activating these lateral TetTagged neurons with CNO was not sufficient to induce sleep. One animal, (mouse I), had induced hM$_3$D$_q$ receptor only in the BST areas, but also exhibited no CNO-induced behavior, so BST stimulation alone was not sufficient to recapitulate dexmedetomidine-induced sedation. Abbreviations: BST, bed nucleus stria terminalis; LPO, lateral preoptic area; MPO, medial preoptic area; VLPO, ventral lateral preoptic area.



Supplementary Figure 4

MnPO TetTag expression patterns: Induction of *hM$_3$D$_q$-mCHERRY* expression 2 hours into recovery sleep after sleep deprivation (animals A-F) or two hours following dexmedetomidine-induced sedation (animals G-I) where AAVs were midline-injected into MnPO and surrounding areas.

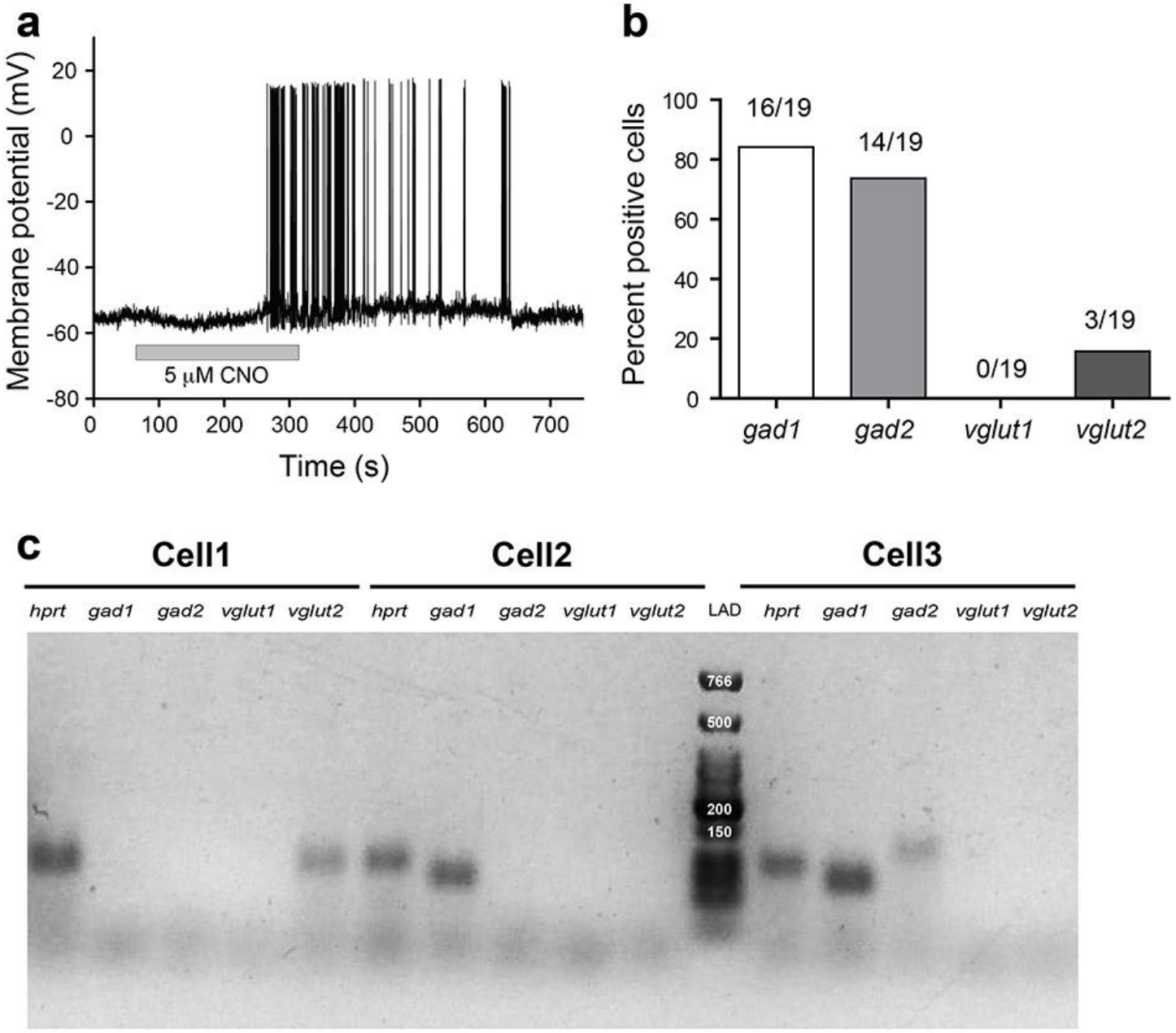
Each letter, A through to I, summarizes the expression in individual mice as seen by serial coronal sectioning through the AAV injection site. Red indicates induced *hM$_3$D$_q$-mCHERRY* expression. The boxed sections, J-L, were from mice where the *hM$_3$D$_q$-mCHERRY* transgene was induced following sleep-deprivation and recovery sleep, but there was no behavioral or EEG signs of NREM sleep following CNO administration. In animals K & L, for example, the intended midline injection of AAV into MnPO missed, and resulted in unilateral *hM$_3$D$_q$-mCHERRY* induction in the LPO area; but activating these receptors with CNO was insufficient to trigger sleep behavior. Abbreviations: BST, bed nucleus stria terminalis; LPO, lateral preoptic area; MPO, medial preoptic area; VLPO, ventral lateral preoptic area.



Supplementary Figure 5

CNO induces nuclear *cfos* expression (green) in *hM_3D_q-mCHERRY* (red) expressing neurons in the LPO area of LPO-TetTag-*hM_3D_q* mice.

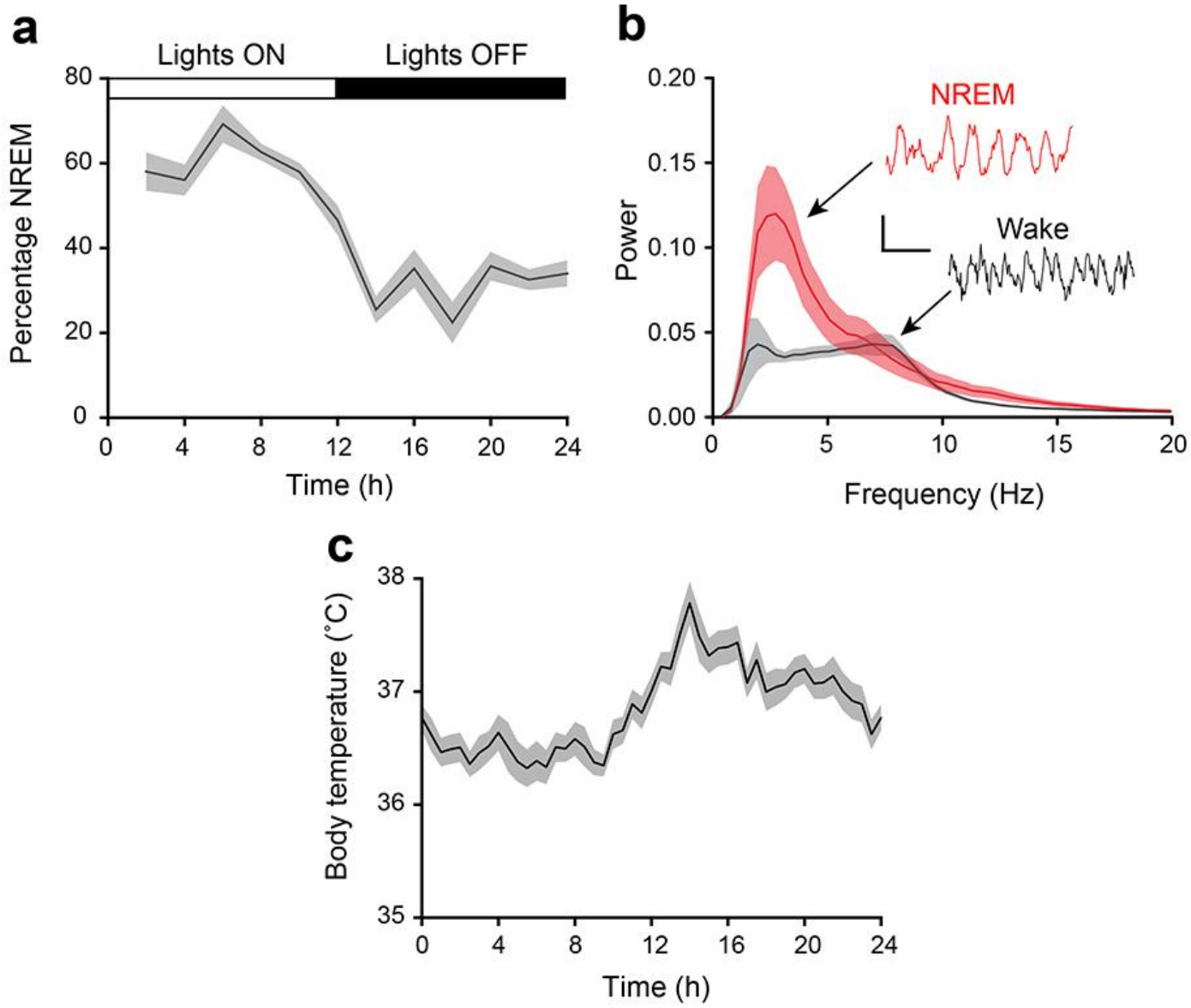
Double-label immunocytochemistry with antisera to *mCHERRY* and *cfos*. The *hM_3D_q-mCHERRY* expression was induced by a sedative dose (100 μg kg^{-1}) of dexmedetomidine and then mice were injected with CNO and their brains taken 2 hours afterwards. Arrows indicate examples of co-labeled cells.



Supplementary Figure 6

TetTagged neurons are excited by CNO and are predominantly GABAergic.

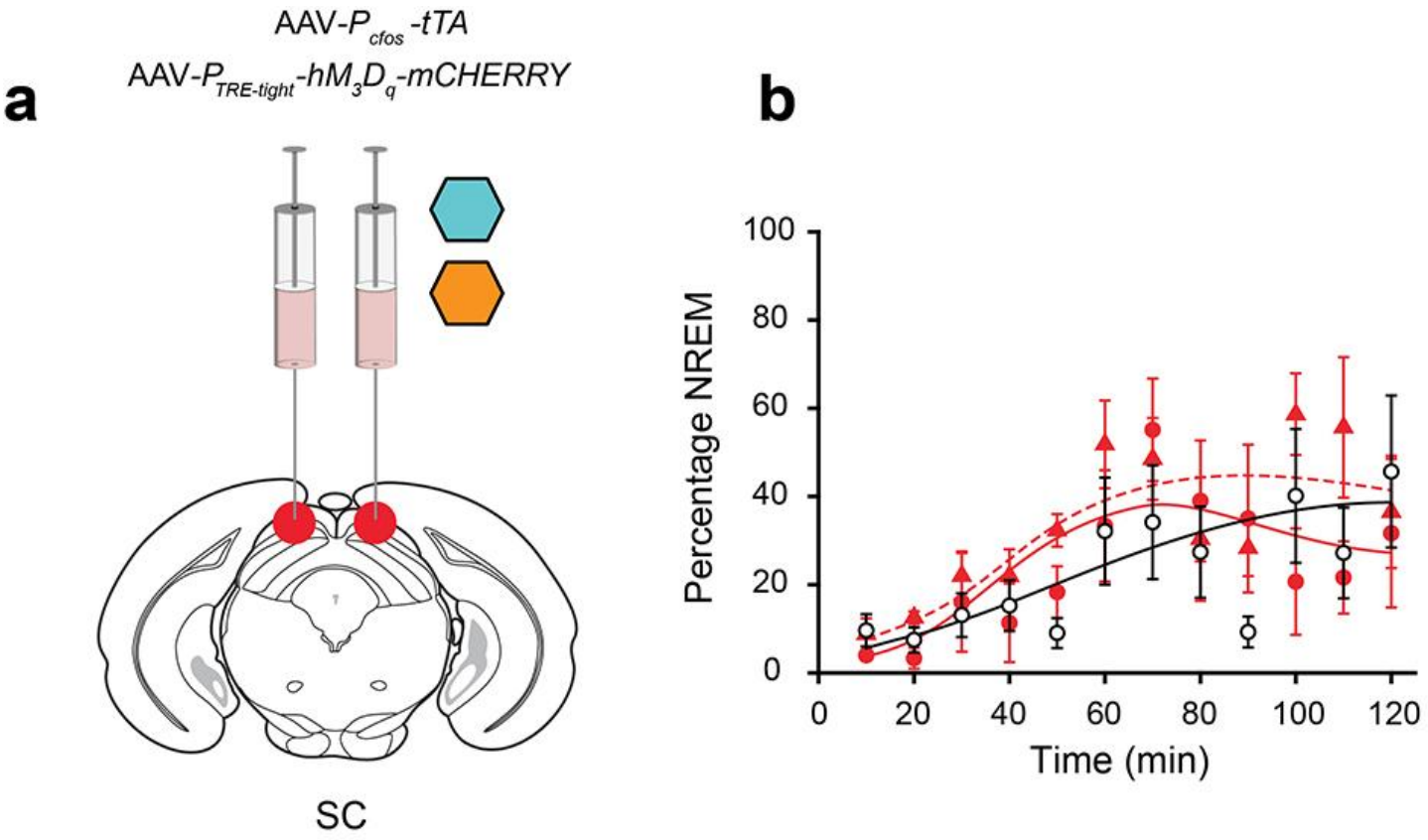
(**a**) A representative example of the effects of 5 μM CNO on a *mCHERRY*-positive neuron in LPO-*TetTag-hM$_3$D$_q$* mice. On average, neurons (*n*=8; 3 mice) were depolarized by 10.2 ± 2.1 mV. In the example shown, action potential firing was triggered. (**b**) These neurons were predominantly GABAergic. 84% were *gad1 and*/or *gad2* positive, as assayed by single-cell qPCR. The remainder were glutamatergic (*vglut2*-positive). (**c**) A representative example of the qPCR assay run out on a gel from three of the neurons. LAD, sizes shown are base pairs.



Supplementary Figure 7

Characteristics of natural NREM sleep and circadian body temperature of virally-injected C57BL/6 mice, the strain used for the TetTagging experiments.

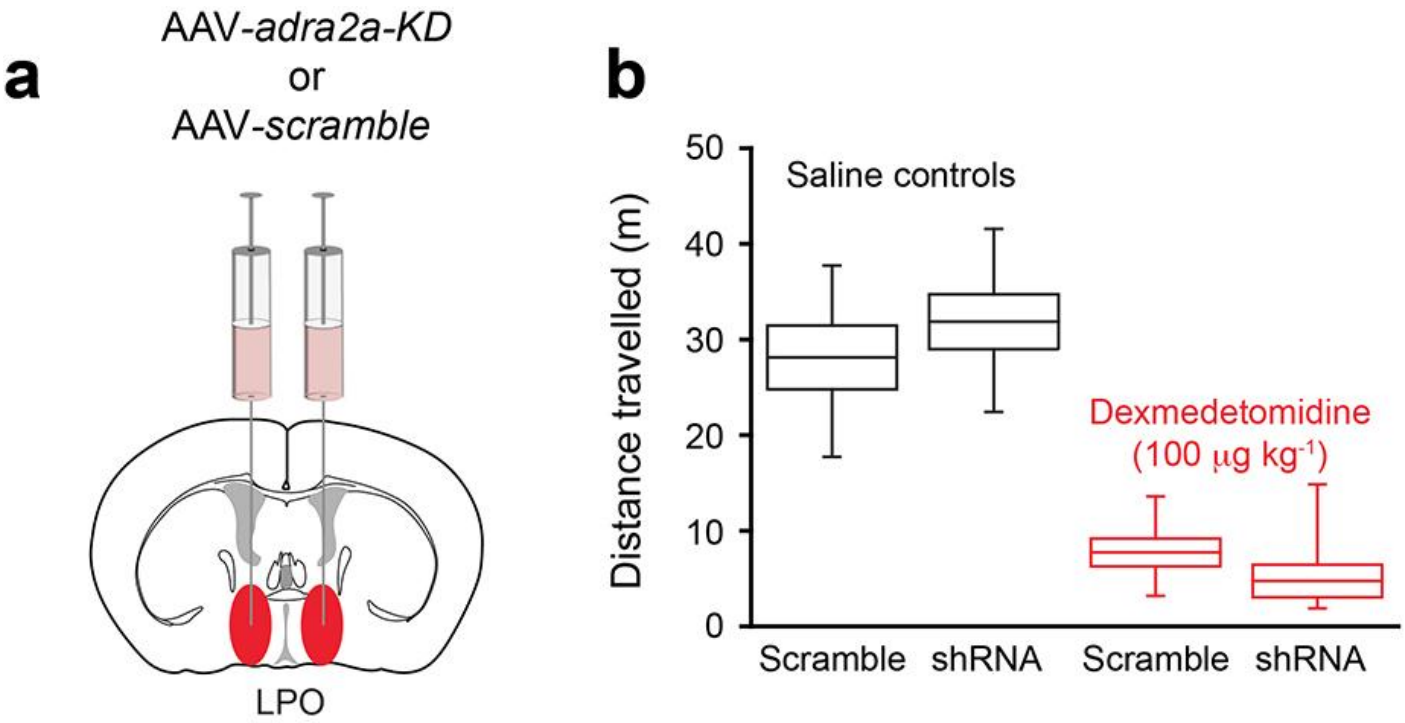
(**a**) Percentage of NREM sleep throughout the 24-hour sleep-wake cycle (*n*=10). (**b**) Fourier Transform power spectra when the EEG and EMG signals from the natural sleep-wake cycle were scored as either sleep (red) or wake (black). Each spectrum is calculated by combining EEG segments totally 20 minutes. The envelopes represent the s.e.m. The inserts show representative EEG traces, and the accompanying calibration bars represent 100 μV and 500 msec (*n*=10). (**c**) Body temperature throughout the normal 24-hour sleep-wake cycle. The envelopes represent the s.e.m. All of these data are for LPO-*TetTag-hM$_3$D$_q$* (*n*=10) and MnPO-*TetTag-hM$_3$D$_q$* mice (*n*=10) combined, because these were indistinguishable. All data are from 12 hours light: 12 hours dark cycles.



Supplementary Figure 8

SC-*TetTag-hM$_3$D$_q$* (negative control) mice

(**a**), schematic illustrating bilateral injection of the two *TetTag-DREADD* AAVs (AAV-P_{cfos}-*tTA* and AAV-$P_{TRE-tight}$-hM_3D_q-*mCHERRY*) into the superior colliculi and the experimental procedures with drug administration and time plan following that outlined in **Fig. 3b**. (**b**) Four days following either 100 μg kg^{-1} dexmedetomidine-induced sedation or after 2 hours into recovery sleep following sleep deprivation, CNO was administered to SC-*TetTag-hM$_3$D$_q$* mice. Open circles (*n*=6): control CNO injection without prior sedation or recovery sleep. Filled red circles (*n*=5): CNO injection after prior dexmedetomidine sedation. Filled red triangles (*n*=5): CNO injection after prior recovery sleep. The mice injected with CNO following dexmedetomidine sedation (two-way ANOVA, *P*=0.79) or recovery sleep (two-way ANOVA, *P*=0.71) were indistinguishable from controls.



Supplementary Figure 9

Knock down of adrenergic α2A receptor transcripts in the preoptic area (LPO) of the hypothalamus had no effect on dexmedetomidine-induced sedation.

(**a**) Schematic illustrating bilateral injection of AAVs expressing either *dsRED-mir30-shadra2a* or *dsRED-mir30-shscramble* transgenes into the LPO of adult mice. (**b**) The total distance travelled in 15 minutes by saline-injected (white bars) or dexmedetomidine-injected (100 μg kg^{-1}) (red bars) mice was not significantly different (*n*=6 scramble, *n*=7 shRNA; *P*=0.19) for mice with AAVs expressing either the knock-down or scramble transgenes. The boxes represent the s.e.m, and the bars show the range of the data.