Supporting Information

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SI Materials and Methods

Animals. Thirty-two male Long–Evans rats (weight 300–400 g at surgery; Charles River Laboratories) were used. Rats were housed in temperature- and humidity-controlled conditions with ad libitum food and water in a 12-h light/dark cycle (06:00 lights off). All experiments were undertaken during the animals' active cycles. All efforts were made to minimize the number of animals used and their suffering. All procedures were in strict compliance with Medical University of South Carolina Institutional Animal Care and Use Committee protocols and in accordance with the guidelines described in the US National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (1).

Viral Vectors. Viral vectors were custom-subcloned and packaged by the University of Pennsylvania Viral Vector Core. In an AAV2/9 vector, the synthetic PRSx8 promoter (2) was used to restrict expression of the hM3Dq DREADD gene with an HA tag (3) to noradrenergic neurons in the locus coeruleus (LC) area. The control vector contained the PRSx8 promoter driving an mCherry reporter protein only.

Surgery. All surgeries were performed under isoflurane anesthesia. Viral vectors were delivered to the LC from a glass pipette (40-μm tip) at –3.8 anteroposterior (behind lambda), ±1.3 mediolateral, and 6.5–7.0 dorsoventral (nose tilted down 15°). Virus (1.4 μL per side) was delivered via brief pneumatic pulses (Picospritzer III; Parker Instruments) over ∼5 min. Some subjects received bilateral EEG leads implanted over frontal and occipital cortices or a 26-gauge guide cannula into a lateral ventricle. Implants were secured to the skull with C&B Metabond (Parkell). Animals were allowed a recovery period of at least 2 wk before use in experiments.

Drugs. All systemic drugs were dissolved at a volume of 1 mL/kg. S(−)propranolol hydrochloride (Tocris) was dissolved in sterile saline, and prazosin hydrochloride (Tocris) was dissolved in sterile water. The S(−)propranolol-prazosin mixture was dissolved in 1% DMSO and sterile water. Clozapine-*N*-oxide (CNO; 10 mg/mL stock; National Cancer Institute, and National Institute of Mental Health Chemical Synthesis and Drug Supply Program) was dissolved in 2.5% DMSO and diluted with sterile saline. Intraparenchymal CNO (5 μM) was dissolved in artificial cerebrospinal fluid. The i.c.v. S(−)propranolol-prazosin (0.10 mg/μL + 0.0042 mg/μL) mixture was dissolved in 10% DMSO and sterile water and administered at 2 μL/kg. Isoflurane was supplied by Medical University of South Carolina Pharmacy Services.

Electrophysiology. LC recording and microinjection via double-barrel pipettes were carried out as described previously (4, 5). Rats were stabilized at 2% isoflurane for least 2 h before recordings. LC- norepinephrine (NE) units were identified by standard criteria, including wide action potentials, slow baseline discharge rates, phasic burst inhibition after foot pinch, as well as location relative to adjacent structures, such as the mesenphalic trigeminal nucleus. An injection pipette (20-μm tip) glued <150 μm above the recording tip was used to deliver 30–60 nL of 5 μM CNO to LC neurons via brief pneumatic injections (Picospritzer; Parker). Multiple microinjections were delivered to each animal, with each microinjection delivered at least 30 min after the previous one, and only one microinjection delivered per cell. Throughout recording, rectal temperature was monitored and maintained (36.0–37.5 °C) with a thermistor-controlled heating pad (FHC). Bipolar surface to depth twisted stainless steel wire electrodes (250-μm diameter) provided ipsilateral frontal EEG during single-unit recordings and CNO microinjection. During emergence, cortical EEG from bilateral frontal cortex screws (3 mm apart) was referenced to screws over the occipital cortex. EEG signals were recorded differentially, filtered (0.1–50 Hz) through a bioamplifier (BMA-831/C; CWE), and digitized at 1,000 Hz through CED Spike 2.

Emergence. All emergence experiments were undertaken between 10:00 and 17:00 h, with at least a 3-d rest between experiments. After induction, rats were immediately moved to a facemask delivering 2% isoflurane (∼1.5 minimum alveolar concentration) (6) in medical air for 40 min while body temperature was stabilized using isothermal pads (Braintree Scientific). After 20 min, rats received an i.p. injection of vehicle or CNO 0.1, 1.0, or 10 mg/kg in a Latin square design. Isoflurane was continued for an additional 20 min, after which the animal was placed supine in room air (total 40 min exposure to 2% isoflurane). Emergence was defined as the time from isoflurane termination to return of righting reflex (RORR) with all four paws on the floor. The antagonist [10 mg/kg S(−)propranolol, 0.42 mg/kg prazosin i.p., or a mixture of propranolol-prazosin at either 10/0.42 mg/kg or 0.20/0.0084 mg/kg i.p.] was given 30 min before induction. An i.c.v. propranolol-prazosin mixture (0.20 mg/kg + 0.0084 mg/kg) was delivered immediately before isoflurane induction at a volume of 2 μL/kg over 3 min.

Loss of Righting Reflex. CNO (1 mg/kg) was given 20 min before continuous 2% isoflurane induction. The induction chamber was rotated every 30 s. Loss of righting reflex (the timepoint at which a subject failed to right itself within 30 s) served as a proxy for isoflurane biophase.

Locomotor Activity. Spontaneous locomotion in awake subjects was evaluated for 60 min immediately after CNO injection. Locomotion was tested between subjects in unfamiliar clear acrylic chambers (40 × 40 × 30 cm) equipped with Digiscan monitors (AccuScan Instruments) that monitored horizontal (16 × 16 photobeam array) and vertical (16 photobeams) activity.

Histology. Animals were terminally anesthetized and perfused transcardially with 0.9% saline and 4% paraformaldehyde, after which brains were extracted. Tissue was postfixed overnight before cryoprotection in 20% sucrose azide, and 40 μm-thick serial coronal sections were collected with a cryostat (Leica; 160 μm between sections). Tissues from all animals were examined for transgene expression as follows. Sections were blocked in PBS with 0.1% Triton X-100 for 1 h with 3% normal donkey serum (immunobuffer). Primary antibodies—mouse anti-tyrosine hydroxylase (TH) (1:1,000; Immunostar), rabbit anti-HA (1:500; Cell Signaling Technologies), and rabbit anti-DsRed (1:500; Clontech)—were incubated overnight. Secondary antibodies—donkey anti-mouse Alexa Fluor 488 (Invitrogen) and donkey anti-rabbit Alexa Fluor 594 (Invitrogen) conjugated secondary antibodies (1:500)—were incubated for 3 h. All immunohistochemistry incubations were performed at room temperature with shaking. Tissue was counterstained using TOPRO-3 (Invitrogen) and coverslipped with Citifluor AF1. All imaging was performed with a confocal laser-scanning microscope (Leica TCS SP5) equipped with argon, argon/krypton, and helium/neon lasers. Channels were captured serially to avoid cross-excitation and bleed-through.

Data Analysis. Spectrograms and power spectral densities were produced in NeuroExplorer (Nex Technologies). Unit activity was analyzed for 100 s before and 100 s after CNO microinjection. Individual units were normalized to baseline before microinjection; rates are presented as change relative to baseline (0%), excitation >10% increase above the basal firing rate. LC-NE activity after systemic CNO administration is based on 300 s of activity for each recording. EEG changes from 60-s epochs within 100 s before and after CNO microinjections were analyzed. EEG frequency bands (low δ, 0.4–2.7 Hz; θ, 2.7–7.0 Hz; α, 7.0–12.9 Hz; β, 12.9–20 Hz) were based on visual inspection of anesthetic-sensitive frequencies, as demonstrated previously (7). EEG frequency bands were expressed relative to total power owing to CNO related changes in total EEG power seen in LC-hM3Dq subjects. Burst suppression was calculated when EEG amplitude was within ±20 μV for at least 100 ms (8). Onset of the final EEG signature before purposeful movement during emergence was identified by visual inspection. We compared 15 s of the final EEG signature after CNO administration with the same time point after vehicle administration.

Parametric and/or nonparametric statistics (described in *Results* and figure legends) and graph composition were done with Prism for Windows version 5.0 (GraphPad Software; alpha $P < 0.05$). Unless stated otherwise, data in graphs are presented as mean ± SE. Colocalization of mCherry or HA cells with TH was quantified with ImageJ (National Institutes of Health). Histological images were minimally processed for image-wide brightness and/or contrast, and all figures were compiled in Abobe Illustrator CS3.

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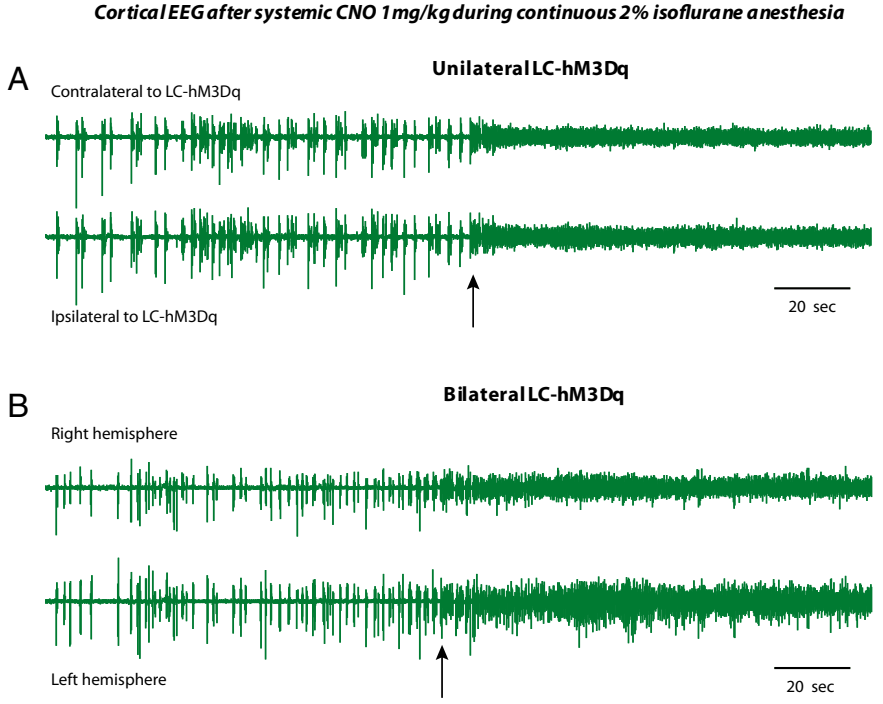


Fig. S1. Cortical EEG is highly sensitive to CNO-mediated activation of unilateral ($n = 4$) or bilateral ($n = 6$) LC-hM3Dq. (*A*) In unilateral LC-hM3Dq animals, there was no temporal difference in the onset of cortical EEG changes ipsilateral vs. contralateral to LC-hM3Dq expression after CNO delivery (1 mg/kg i.p.). Shown are excerpts from left (ipsilateral) and right (contralateral) raw EEG traces from a unilateral LC-hM3Dq subject after CNO delivery (1 mg/kg i.p.). (*B*) Similar EEG traces from a bilateral LC-hM3Dq subject. Arrows in *A* and *B* denote the onset of cortical EEG activation by visual inspection at 551 s after CNO injection in unilateral example and at 453 s after CNO injection in bilateral example during continuous isoflurane anesthesia. There was no significant difference in time to EEG activation after CNO between unilateral and bilateral subjects ($P = 0.81$, unpaired t test). In addition, there was no significant difference in CNO-mediated emergence times between unilateral and bilateral LC-hM3Dq subjects ($P = 0.14$, unpaired t test). Although direct cortical projections from LC are primarily ipsilateral, LC-NE projects bilaterally to many subcortical regions that are also nodes in arousal networks, and may contribute to the temporal confluence seen in cortical EEG arousal.

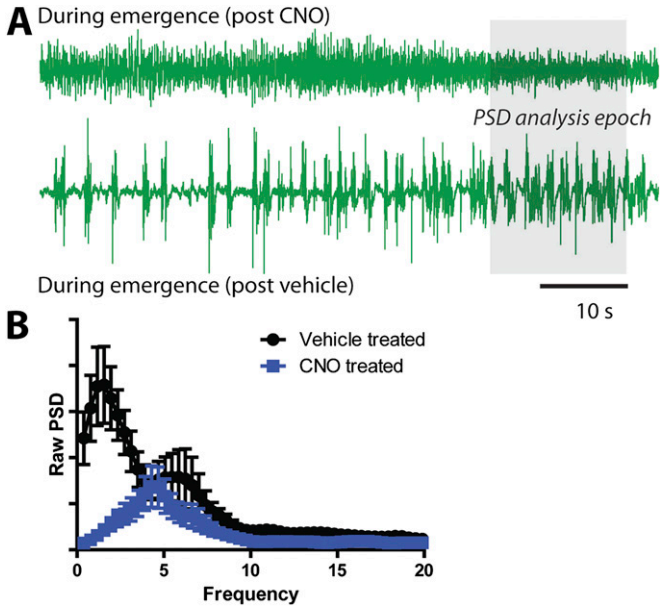


Fig. S2. LC-hM3Dq stimulation modulates cortical arousal during emergence in room air. (*A*) Raw EEG traces from the same subject beginning 1 min after removal of isoflurane, 21 min after administration of CNO (*Upper*) or vehicle (*Lower*). (*B*) Power spectral density (PSD) comparison during emergence in room air (shaded epoch in *A*). During emergence, at the time point at which CNO-treated subjects (blue) showed cortical arousal in EEG, vehicle-treated rats (black) did not ($P < 0.0001$, paired t test; $n = 10$ rats).

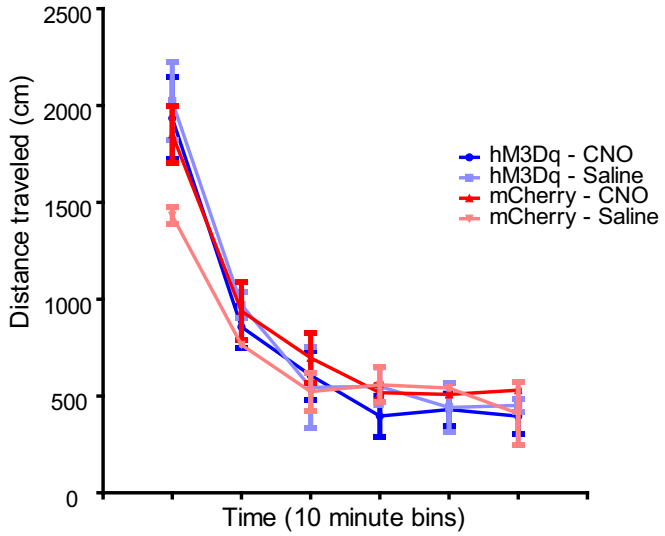
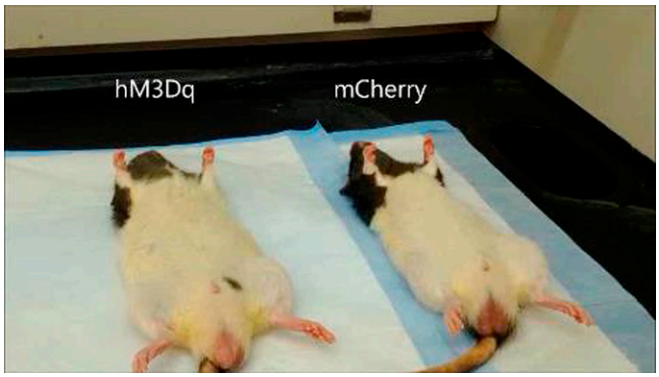


Fig. S3. No significant differences in total locomotor activity were seen between LC-hM3Dq and LC-mCherry rats given saline or 1 mg/kg CNO for 60 min after administration of CNO ($P = 0.65$, two-way ANOVA; $n = 14$).



Movie S1. CNO (1 mg/kg, i.p.) administered 20 min before discontinuation of isoflurane facilitated behavioral emergence (RORR) in LC-hM3Dq rat (*Left*), but not in LC-mCherry rat (*Right*), after discontinuation of 40-min 2% isoflurane exposure. Video recording began at 3 min after removal of isoflurane and is played back at 2× speed.

Movie S1