Supplementary Materials for

Chemogenetic stimulation of tonic locus coeruleus activity strengthens the default mode network

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Supplementary Methods

Axonal Labeling of Transgenic Mice
To label LC-NE axons, En1cre mice were crossed to mice expressing DbhFlpo and the dual recombinase reporter RC::FLTG (1) to generate En1cre; DbhFlpo; RC::FLTG mice that express GFP throughout LC-NE neuronal projections. Brains were post-fixed overnight by immersion in 4% PFA at 4°C. Following a rinse in PBS, brains were cryoprotected in 30% sucrose in PBS and embedded in Tissue Freezing Medium. 40 µm free-floating coronal cryosections were collected in PBS and incubated with primary antibodies for GFP (AB13970, Abcam) and NET (1447-NET, Phosphosolutions). Secondary antibodies were Alexa Fluor 488 and 633 (Invitrogen). After washing, the sections were stained with Neurotrace 435/455 blue fluorescent Nissl stain (1:50, N21479, Thermo Fisher) and mounted using Prolong Diamond anti-fade mountant (Thermo Fisher, Scientific, Waltham, MA, USA). Sections were imaged on a Zeiss LSM 880 inverted confocal microscope with a 40x objective. Zen Black Software (Carl Zeiss) was used to convert z-stacks to maximum intensity projections. Images were modified only by adjusting brightness and contrast across the entire image to optimize the fluorescence signal.

fMRI Acquisition Preparations
Clozapine-n-oxide (CNO) was prepared fresh and protected from light-induced degradation immediately before each scan. Once prepared, the setup from putting a mouse down to starting the acquisition took approximately 27-45 minutes. Mice were anesthetized using ~2-3% isoflurane and maintained at 1%, where an i.v. catheter line was implanted into the tail vein for the injection of home-synthesized carboxymethyl-dextran coated iron oxide nanoparticle contrast agent (CION; 30 mg/kg) for CBV measurements. We observed no difference in the anesthetic recovery rate between experimental groups. The scalp and belly were shaved before the mice were transferred to a heated MRI cradle. Prior to sending the mice into the MRI bore, mice received proper head fixation, physiological probe installation/verification, and an i.p. catheter placement. The i.p. needle was inserted and secured with tape (#1538S-1, 3M Durapore silk tape 1") for CNO administration. Both i.v. and i.p. catheters used a shortened 30-gauge needle (cut to ~7 mm) to reduce magnetic pulling force on the needle inside the scanner. These needles were connected and glued to a syringe via a ~1 m length of flexible PE-10 tubing (#14-170-12P, BD Biosciences Intramedic™ PE (10) Tubing BD 427401).

fMRI Pre-processing
Slice-Time Correction: Slice-time correction interpolating the temporal offsets during interleaved acquisitions of slices to eliminate radio frequency pulse excitation leakage artifacts. Correction was done by adjusting slice timing to a reference time point using 3dTshift in AFNI.

Motion Correction: Motion correction applies rigid-body transformation to align all temporal volumes to a reference volume to correct for head motion during fMRI acquisitions that may produce spurious correlations if uncorrected. Our pipeline utilizes 3dvolreg in AFNI. The framewise displacement (FD) (2) was used to evaluate the head motion.
Brain Segmentation: CBV-fMRI time series were averaged to single volume and corrected for image intensity non-uniformities using N4BiasFieldCorrection in Advanced Normalization Tools (ANTS). Brain segmentation was performed on averaged, bias-corrected imaged using an established U-Net deep-learning skull stripping tool (3) and applied across the time series.

Spatial Normalization: We first generated an averaged interim CBV-fMRI mouse atlas which was co-registered and placed through linear and non-linear warping using antsRegistrationSyN.sh in ANTs into the template space of the Allen Mouse Brain Atlas (v3) (4). The Allen Mouse Brain Atlas utilizes the Mouse Common Coordinate Framework allowing researchers to overlap their data into the standardize template space. We then spatially normalized all individual subject (n=21) to our interim CBV-fMRI mouse atlas on ANTs. Linear affine transformation matrices and warp field maps were applied across the entire CBV-fMRI time series using WarpTimeSeriesImageMultiTransform in ANTs. A final CBV-fMRI mouse atlas was generated for future studies using all spatially normalized subjects into the Allen Mouse Brain Atlas template space using the deformation template generator antsMultivariateTemplateConstruction2.sh in ANTs to form averaged atlases that are openly available in the posted study data.

Cleaning and De-noising fcMRI data: Spatially normalized fcMRI datasets were first de-spiked using 3dDespike in AFNI to eliminate large signal intensity spikes that surpassed ±2.5 standard deviations above the local median absolute deviation calculated at every time frame against ±4 adjacent time frames. Next, the data underwent nuisance variable regression based on a General Linear Model (GLM) using 3dDeconvolve in AFNI to eliminate the contribution of measurable noise from non-meaningful sources such as head motion and periodic physiological pulsations that include cardiac, respiratory, vascular and CSF oscillations. The GLM included six motion regressors (accounting for motion correction in the X, Y and Z translation directions and pitch, yaw, and roll in rotation directions) and CSF oscillations extracted from a mask layer of the ventricles of the mouse brain to routinely account for cardiac pulsation noise. We did not regress the global signal since it has been demonstrated to introduce spurious anti-correlations throughout the brain in some cases (5). The data then underwent spatial smoothing using 0.6 mm full width at half maximum (FWHM) Gaussian kernel to improve signal to noise ratio (SNR). The data was then de-trended to remove drifting likely associated with MRI gradient heating during acquisitions. Lastly, the data underwent temporal filtering using a high pass filter (>0.01 Hz) to extract meaningful oscillations while eliminating ultralow frequency noises/artifacts. We also used independent component analysis (ICA) to identify and remove remaining physiological, movement and thermal (machine) noise components (6,7).

fMRI Analysis
Before settling on the following method, we attempted an ROI analysis on the LC and failed to extract meaningful responses for this study. One major limitation is that the LC in mice is only ~0.3 mm in size (8), which is about the size of a single echo planar images (EPI) voxel acquired in this study. We considered the LC signal to be extremely difficult to isolate since it could easily suffer from partial volume effect due to its proximity to the fourth ventricle.

CBV Calculations: We calculated the percentage change in CBV using the following equation:
\[
\% \text{CBV} = \frac{-1}{TE} \ln \left( \frac{S_{\text{CNO}}}{S_{\text{preCNO}}} \right) - \frac{-1}{TE} \ln \left( \frac{S_{\text{preCNO}}}{S_0} \right)
\]

where \(S_0\) represents MR signal intensity before iron oxide administration, \(S_{\text{preCNO}}\) represents MR signal intensity after iron oxide administration but before CNO administration, and \(S_{\text{CNO}}\) represents MR signal intensities after CNO administration.

**Regional Homogeneity (ReHo) and Amplitude of Low-Frequency Fluctuation (ALFF) analyses of DMN modules:** ReHo (9) is a voxelwise analysis to determine local synchronization of fMRI signal among all brain regions. Higher ReHo scores typically represent greater neuronal coherence and centrality within nodes but does not necessarily equate to higher neuronal activity. ALFF (10) is a voxelwise technique to calculate the total power of regional neuronal activity within 0.01-0.1 Hz. Specifically, the filtered time series of each voxel was transformed into the frequency domain with a Fast Fourier Transform and the power spectrum was then obtained. The ALFF was measured by obtaining the square root of the signal across 0.01–0.1 Hz for each voxel. Overlapping regions that share high ALFF and ReHo scores are thought to have enhanced synchronization of low frequency neuronal activity.

**ICA analysis:** We utilized group-level ICA (FSL MELODIC) (11) with high-order component numbers (ICs = 100) (12–15) to achieve more refined functional brain segmentation (16–19). MRI data were decomposed into 100 FCs using baseline data from all subjects via a group-level ICA. ICA spatial maps were back-reconstructed for each subject to estimate subject-specific temporal components and associated spatial maps using dual-regression (DR) analysis (20), and a one-sample two-sided t-test was performed to generate group component maps. Individual time courses from 17 pre-selected DMN nodes were extracted from the first stage of DR and the Fisher z-transformed Pearson correlation were computed among all node pairs to form a correlation matrix. To keep the strongest connections without isolated nodes, the z-threshold = 0.26 (connection density = 48%) was set in the mean FC matrix across all subjects. Functional modules of the DMN were parcellated using the Louvain community detection algorithm (21).

To evaluate the selected ICs are associated with the conventional DMN, we estimated the spatial similarity between seed based DMN and selected 17 DMN components from ICA (Fig. S4 C and D). Specifically, the conventional DMN map was conducted by a seed-based analysis in RSC and a one-sample t-test. We then computed the spatial correlation of the functional connectivity map between the seed-based DMN and dual regression results of all 17 DMN ICs. (Fig. S4 C and D).

**Modularity Analysis:** Modules refer to groups of nodes that are highly connected with each other but less connected with other nodes in a network. First, to examine community metrics at different resolutions, we used a sweep of the modularity resolution parameter gamma from 0.6 to 1.4 in increments of 0.2. The modularity analysis was conducted on each resolution. Partitions of the DMN nodes were compared within each resolution. Similarity between partitions was
estimated using normalized mutual information (NMI)(22), an information-theoretic measure which measures the similarity between two sets of partitions. The highest averaged similarity among each resolution was obtained when gamma = 1 (Fig. S4 F and G). To identify the most robust partition, we repeated the modularity analysis 100 times. The NMI among each repetition is 1 indicated the robustness of identified partitions. Finally, the modularity Q quantifies the efficacy of partitioning a network into modules by evaluating the difference between the actual number of intramodule connections and the expected number for the same modules in a randomized network. The objective of a module detection procedure is to find a specific partition that maximizes the modularity Q. Newman's spectral algorithm was used for modular detection. To examine whether a network had significantly higher modularity than the random graphs, we randomized the original network with preserved strength distribution 1,000 times and calculated the mean \((\mu)\) and standard deviation \((\sigma)\) of those modularity values. We compared the modularity \(Q\) of the real network to those values:

\[
Z = \frac{Q - \mu}{\sigma}
\]

which measures how many standard deviations the real modularity is above the mean for the random graph.

**FC analysis within and between DMN modules:** Within- and between-module connectivity was defined as the average of FC across node pairs within or between the identified DMN modules. We conducted network-based statistics (NBS) (23) to investigate the significant connection changes. For each comparison, a primary component-forming threshold \((P < 0.05, \text{uncorrected})\) was applied to form a set of supra-threshold edges and all the remaining connected subnetworks in the matrix were then evaluated under the null hypothesis of random group membership (5,000 permutations).

**DCM analysis:** We specified a DCM model (24) with full connectivity consisting of three modules (Frontal, RSC-HIPP, and Association module) from DMN. Specifically, for each DMN module, the fMRI time series was averaged from the DR timeseries of nodes. We then used spectral DCM in SPM12 (25) to estimate pairwise EC among the DMN modules and constructed a directed and weighted graph (representing an EC network) for each subject. The maximal number of iterations was set to be 128 and all DCMs were converged within 128 iterations.

**Mediation analysis:** We applied serial-multiple mediation analysis model using the structural equation modeling method (26) in AMOS 17.0 (SPSS Inc., Chicago, IL, USA) to uncover underlying functional pathways within DMN. Specifically, we first estimated the direct relationships between the dependent variable (EC from RSC-HIPP module to Association module) and the independent variable (FC within Frontal module). Then, in a mediation model, the FC between the Association and Frontal modules was added as a mediator. The mediation model was estimated using the following equation:

\[
Y = \alpha_1 + aX + bM + \text{error}
\]
where $Y$ is the FC change within *Frontal* module, $\alpha$ is the intercept term, $X$ is the reduced causal control from *RSC-HIPP* to *Association* modules, and $M$ is the FC change between *Association* and *Frontal* module. In this context, full mediation occurs when the relationship between the independent variable ($X$) and the dependent variable ($Y$) is no longer significant with the inclusion of a mediator variable (27). For each pathway, we utilized the bootstrapping test 1,000 times to test the significance. Finally, the mediation effect was evaluated using the Sobel test (28).

**Sobel Test Calculations:** The mediation effect of each significant path was evaluated using the Sobel test whereby the $z$-value was estimated using the following equation:

$$ z-value = \frac{a \times b}{\sqrt{b^{2} \times S_{a}^{2} + a^{2} \times S_{b}^{2}}} $$

where $a$ is the mean regression coefficient for the association between independent variable and mediator, $b$ is the mean regression coefficient for the association between mediator and dependent variable, $S_{a}$ is the standard error of $a$, and $S_{b}$ is the standard error of $b$.

**Co-activation pattern (CAP) analysis:** While we found the significant FC changes before and after CNO administration in LC-NE/hM3Dq group, we then conducted the CAP analysis (29,30) to evaluate the dynamic state change in the LC-NE group (800 volumes $\times$ subjects). Each time frame contained 17 selected DMN components from the DR timeseries. Temporal clustering was performed using k-means. Distance measurements in k-means were 1–cc, where cc is the spatial correlation coefficient. The number of clusters (k) was determined by ratio of within- and between-cluster distance and elbow criteria.

**Specificity of Functional Connectivity:** To benchmark the overall FC specificity of this dataset, we evaluated an individual specificity metric similar to those described by others (31–33). As shown in our recent work (33), we selected bilateral seeds in the primary somatosensory cortices (right and left S1) and retrosplenial cortex (RSC) to extract FC values (Fig. S3E). We first calculated a connectivity map using the left S1 seed (Fig. S3F), then estimated the $z$-scores by Fisher transformation. To determine the threshold of specific FC between left S1 and right S1 and also between left S1 and RSC, we calculated the averaged FC from randomized individual FC maps for 10,000 iterations. The threshold for a significant right S1 FC was $z$-value = 0.19 ($P < 0.05$ against randomized distribution of right S1 FC map) and an RSC FC was $z$-value = 0.28 ($P < 0.05$ against randomized distribution of RSC FC map). A higher FC between matching bilateral sensory cortices (left and right S1) and a lower FC between left S1 and RSC within the same subject was considered specific. Our approach leveraged the parcellated spatiotemporal features for determining seed coordinates. Using the thresholds of $z$-value, we identified 64.7% specificity from this dataset (Fig. S3G). This is comparable to the 73.6% reported in our rat database (33), but slightly lower, which may be attributed to the use of isoflurane – a limitation of this study.
Fiber Photometry

We measure calcium-related neuronal activity across Cg1 neurons. Expression was achieved by the pan-neuronal hSyn promoter, which has been shown to have high transduction efficiency in the mouse cerebral cortex (34). This would allow expression on both cortical excitatory and inhibitory interneurons and reflect overall calcium-dependent output activity. Although we expect the responses to be dominated by excitatory principal neurons, caution should be taken when interpreting data presented herein.

The fiber photometry setup for this study required triple-excitation continuous wave lasers at 488 nm (OBIS Galaxy 488nm LX 100mW, 1236444, Coherent Inc., Santa Clara, CA, USA), 561 nm (OBIS Galaxy 488nm LS 80mW, 1275608, Coherent Inc., Santa Clara, CA, USA) and a 644 nm (OBIS Galaxy 640nm LX 75mW, 1236445, Coherent Inc., Santa Clara, CA, USA) housed in an OBIS LX/LS Laser Box (1228877, Coherent, Inc.). The lasers were aligned and combined using OBIS Galaxy Laser Beam Combiner (1253556, Coherent Inc., Santa Clara, CA, USA) and the beam was sent through a neutral density filter (NEK01, Thorlabs, Newton, NJ) and a dichroic mirror (ZT405/488/561/640rpcv2, Chroma Technology Corp., Bellow Falls, VA, USA) within a fluorescence cube (DFM1, Thorlabs, Newton, NJ, USA). The combined laser beam was cast through an achromatic fiber port (PAFA-X-4-A, Thorlabs, Newton, NJ, USA) into a multimode optical fiber patch cable with a 105 μm core (M61L01, Thorlabs, Newton, NJ, USA), terminating into a 1.25 mm outer diameter ceramic ferrule connected to the surgically implanted optical fiber cannula via a ceramic spilt sleeve (SM-CS12SS, Precision Fiber Products Inc., San Diego, CA, USA). Emitted fluorescent signals travel back along the patch cable, through the emission filters of the dichroic mirror and emission filter (ZET405/488/561/640mv2, Chroma Technology Corp., Bellow Falls, VA, USA), then launch through an aspheric fiber port (PAF-SMA-11-A, Thorlabs, Newton, NJ, USA) into the core of an AR-coated 200/230 mm core/cladding multi-mode patch cable (M200L02S-A, Thorlabs, Newton, NJ, USA). The AR-coated multi-mode patch cable was connected to a spectrometer (QE Pro-FL, Ocean Optics, Largo, FL, USA) for spectral data acquisition, which was operated by a UI software OceanView (Ocean Optics, Largo, FL, USA).

In order to perform spectral linear unmixing of the spectroscopy data, we used the following linear regression algorithm:

\[ Y(t) = A + C_{oNE2.1} \cdot S_{NE2.1} + C_{oJRGECO1a} \cdot S_{JRGECO1a} + C_{oCYS-Dextran} \cdot S_{CYS-Dextran} + \varepsilon(t) \]

whereby \( Y(t) \) is the observed mixed spectrum at any given time and \( S_{NE2.1} \), \( S_{JRGECO1a} \) and \( S_{CYS-Dextran} \) are the normalized reference emission spectra for NE2.1, JRGECO1a and CYS-conjugated Dextran fluorescent dye, respectively. \( A \) is an unknown constant while \( C_{oNE2.1} \), \( C_{oJRGECO1a} \), and \( C_{oCYS-Dextran} \) are unknown regression coefficients that correspond to the green, red, and far-red channels, respectively. Lastly, \( \varepsilon(t) \) denotes the random error associated with the model. The model allows us to estimate \( A \), \( C_{oNE2.1} \), \( C_{oJRGECO1a} \), and \( C_{oCYS-Dextran} \) at each time point. The \( C_{oNE2.1} \), \( C_{oJRGECO1a} \), and \( C_{oCYS-Dextran} \) were further detrended according to their baseline period using a homemade Matlab script. The spike SNR during the first 10 min baseline and the last 10 min post-CNO recording were calculated following high-pass filtering (cut at 0.1 Hz) on z-transformed time segments. Any local maximum with z-score > 1.96 was considered a spike.
Fig. S1. LC-NE projections to DMN brain regions. To label LC-NE axons, En1<sup>cre</sup> mice were crossed to mice expressing Dbh<sup>Flpo</sup> and the Flp/Cre-responsive recombinase reporter RC::FLTG. Recombination of RC::FLTG by En1<sup>cre</sup> and Dbh<sup>Flpo</sup> results in GFP expression in LC-NE neurons. Coronal sections show innervation of LC-NE axons (green) co-localized with the noradrenergic transporter, NET (white) in the Cg1 and RSC of the DMN. Scale bar indicates 50 µm.
Fig. S2. Representative T2*-weighted, single-shot gradient EPI functional scans. A single subject in (A) BOLD-weighted images and (B) CBV-weighted images using intravenous CION contrast. (C) Slice-dependent motion artifacts were estimated in the raw EPI datasets by comparing framewise displacement (FD) in the forebrain/midbrain (green) to the region composed of the cerebellum/hindbrain (blue) known to be susceptible to physiological motion artifacts. Our
results show the averaged FD was 0.13 mm for both brain regions, which is smaller than our 0.3 mm voxel size. Error bars represent ± SEM. (D) Single subject EPI scans (n=21) were spatially warped to the Allen Mouse Common Coordinate Framework (CCF) and Reference Atlas (v3) using ANTS to generate symmetric diffeomorphic EPI mouse brain atlases for BOLD, CBV and ReHo images.
Fig. S3. Detailed IC maps from baseline fMRI scans. (A) Group IC maps from 100-component ICA derived from baseline fMRI data of all subjects (n=21) prior to CNO administration. ICs were grouped to illustrate the DMN ICs and other functionally parcellated clusters. Regions with clear contralateral counterparts are identified. (B) To assure the reproducibility of components, ICA was conducted again using randomly ordered components. Highly similar components could be found in the randomized data. T-value threshold = 10 (one-sample t-test, P_{3dClustSim-corrected}<0.001). (C) Group ICA maps of 20 ICs depicted bilateral connectivity of various brain regions as commonly seen in the literature. Data were derived from baseline fMRI of all subjects (n=21) prior to CNO administration (one-sample t-test, P_{3dClustSim-corrected}<0.01). (D) ICs representing Sensorimotor network (left: from 20-component ICA; right: from 100-component ICA). Four ICs from the 100-component ICA showed significant correlation with the Sensorimotor network (r = 0.84, 0.80, 0.77, 0.68).
component ICA exhibit high spatial similarity ($r>0.6$) to those derived by the 20-component ICA. (E) Functional connectivity specificity of the dataset was assessed using seed-based ROIs on the left and right Primary Somatosensory Cortices (S1) and the RSC. (F) Group-level statistics of voxel-wise connectivity from the left S1 Cortex found bilateral connectivity with the right S1 Cortex ($P_{FDR\text{-corrected}} < 0.01$) but not the RSC. (G) The distribution of individual data within a scatter plot comparing the averaged FCs between left S1 and right S1 against left S1 and RSC. A specificity of 64.7% was found in this dataset.
Fig. S4. Detailed evaluation of ICs associated with DMN. (A) Selected DMN ICs and their corresponding DMN brain regions. (B) Masks of dual regression results of the 17 DMN ICs and RSC seed-based connectivity maps (one-sample t-test, $P < 0.01$). Note that only a single solid color
was used and any color difference is the result of 3D rendered shading. (C) Spatial similarity estimates the spatial correlation between mean RSC seed-based and dual regression IC01 resultant functional connectivity maps. (D) Spatial similarity between the RSC seed-based connectivity map and dual regression of all 17 DMN ICs. One-sample t-test, \( P < 0.01 \). (E) To access the baseline variability, ANOVA was performed among the baseline scans of LC-NE and control groups. No significant FC change was identified between subject groups across 17 ICs. (F) Similarity between partitions was estimated using the information-theoretic measure, normalized mutual information (NMI). (G) Highest averaged NMI was found when gamma = 1 compared to other resolutions.
Fig. S5. ALFF changes following LC-NE activation. (A) Periodogram of ALFF spectral signals in the Frontal module shows significant increase in power between 0.01-0.05 Hz following LC-NE activation. (B) Area-under-the-curve of ALFF changes following LC-NE activation were restricted to frequency bands ranging from 0.01-0.05 Hz. *P < 0.05, *** P < 0.001, error bars represent ± SEM.
Fig. S6. Fiber photometry CBV measurement. (A) Wash-out of CY5-Dextran dye in the bloodstream. High goodness-of-fit ($R^2=0.9937$) to a two-phase exponential decay curve was observed, likely due to rapid clearance of unconjugated dye during the fast phase and a slower wash-out phase of conjugated dye. All photometry data reported in this study were collected during the second wash-out phase where the trend is closer to linear. The trend was further removed by fitting a linear slope during pre-CNO baseline period. (B) Effects of CNO on average percent CBV changes from baseline in Cg1 after CNO administration. * $P < 0.05$, ** $P < 0.01$, horizontal lines represent means, and error bars represent ± SD.
Fig. S7. Comparison of FDG uptake and changes in CBV. (A) A representative 3D rendered brain mask for PET analysis. Skull-stripping was guided by the CT scan of the same subject. (B) CBV and FDG uptake changes in the Frontal DMN module among LC-NE/hM3Dq and control subjects that underwent both modalities. A robust correlation was observed ($r=0.9218$, $P < 0.05$), suggesting a relationship between FDG and CBV observations following the activation of LC-NE neurons.
Fig. S8. Graph properties of the mouse DMN nodes and anti-correlations between DMN and anterior insula following LC-NE activation. (A) Participation coefficient and within-module degree were estimated to identify hubs and connectors in the DMN, respectively. (B) Hubs and their respective FCs to other nodes. (C) Comparisons of FC changes within Frontal module against FC changes between the Frontal module and anterior insula in LC-NE mice. The observed negative correlation suggests the existence of antagonizing relationship ($r = -0.86$, $P < 0.005$). (D) Post-hoc analyses of the relationships between FC changes within the Frontal module and FC changes between the anterior insula and each Frontal module node in LC-NE/hM3Dq mice. *$P_{FDR}$-corrected $< 0.05$. 
**Fig. S9.** Dynamic causal modeling (DCM) and co-activation pattern (CAP) analysis (A) Dynamic causal modeling (DCM) analysis among DMN modules of the littermate control groups. No causal associations were detected following CNO injection. Horizontal lines represent means, and error bars represent ± SD. (B) K-means clustering identified six CAP states among the DMN nodes (red arrow) from only the LC-NE group. (C) LC-NE activation significantly increased the occurrence of
CAP State 5 and significantly decreased the occurrence of CAP State 6. No changes were found in the occurrence the remaining CAP states. State 5 represents isolated HIPP activation that is asynchronous with the rest of the RSC-HIPP module nodes, whereas State 6 represents synchronous RSC-HIPP and Association modules with opposing polarities. While CAP data do not infer causality, the high occurrence of State 6 suggests a robust state recapitulating that RSC-HIPP and Association activity are opposed during resting state, but become less polarized following CNO administration. \( P_{FDR\text{-corrected}} < 0.05 \), error bars represent ± SD.
Fig. S10. Mediation analysis and FC changes among DMN modules. (A) No mediation effect was observed from the dependent variable (enhanced FC within Frontal module) to the independent variable (decreased causal control from RSC-HIPP to Association) via mediator (enhanced FC between Association and Frontal modules). (B) FC changes within and between DMN modules using 10-30 and 15-30 min post-CNO time blocks. *P < 0.05, error bars represent ± SD.
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