Supplementary Material

Hyperactivation of distinct thalamic nuclei differentially impairs sleep physiology in rats

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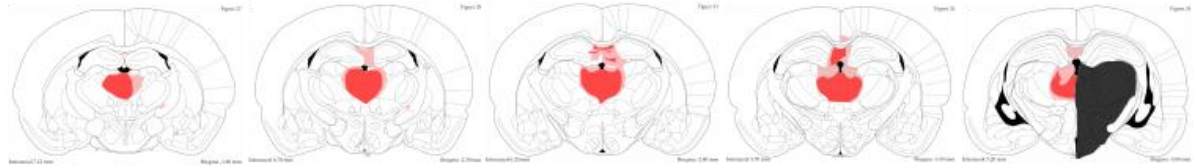
A

MDT-hM3Dq

Rat 1



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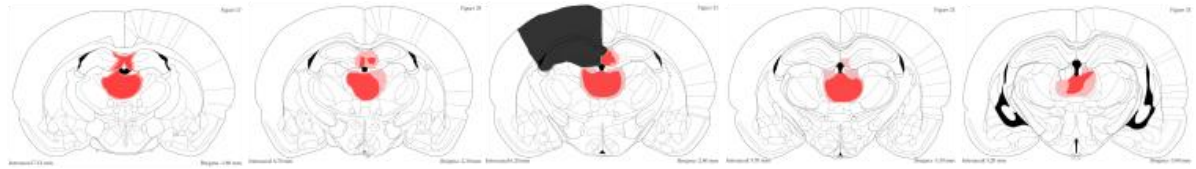

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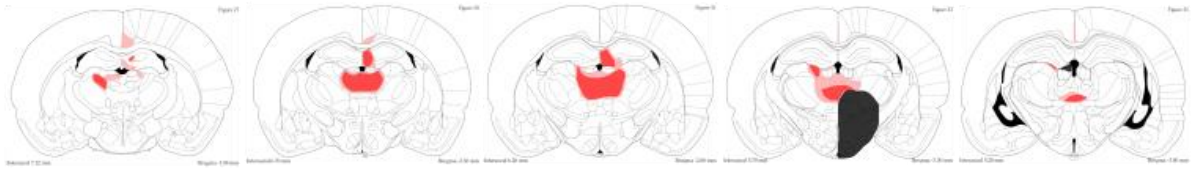

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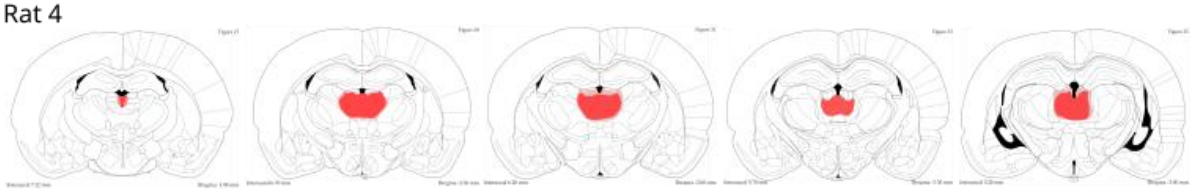

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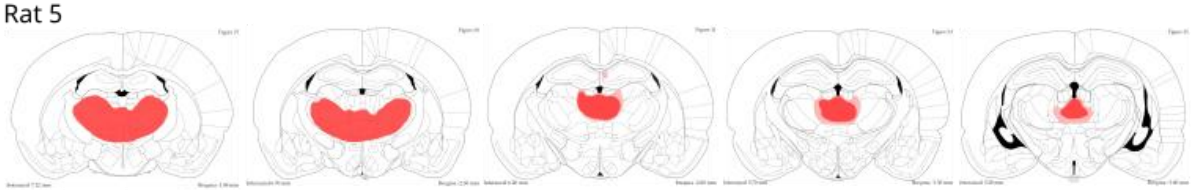

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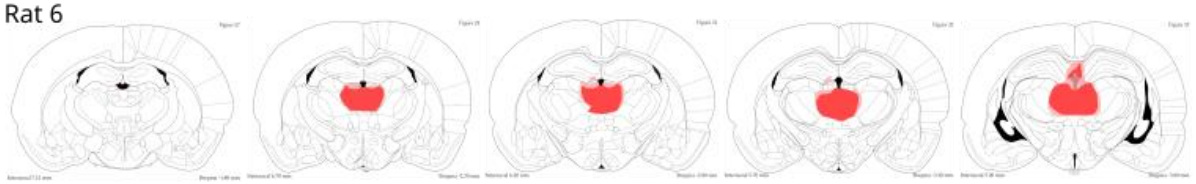

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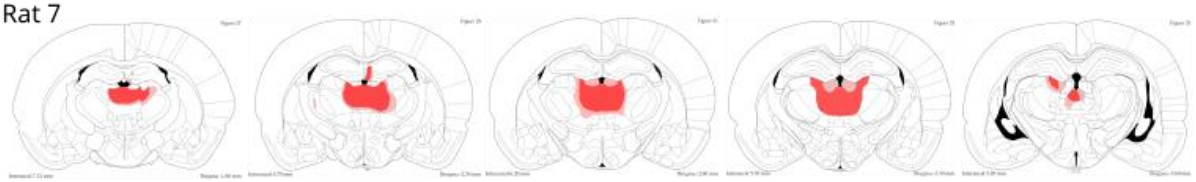

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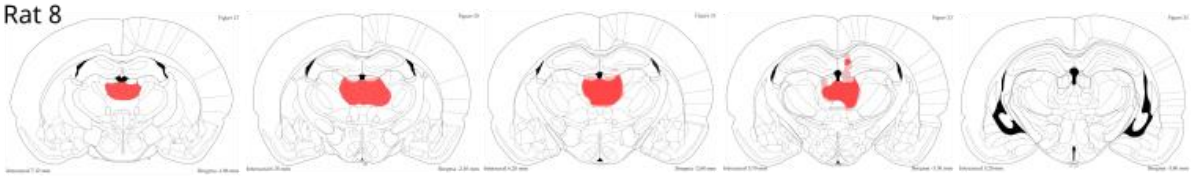
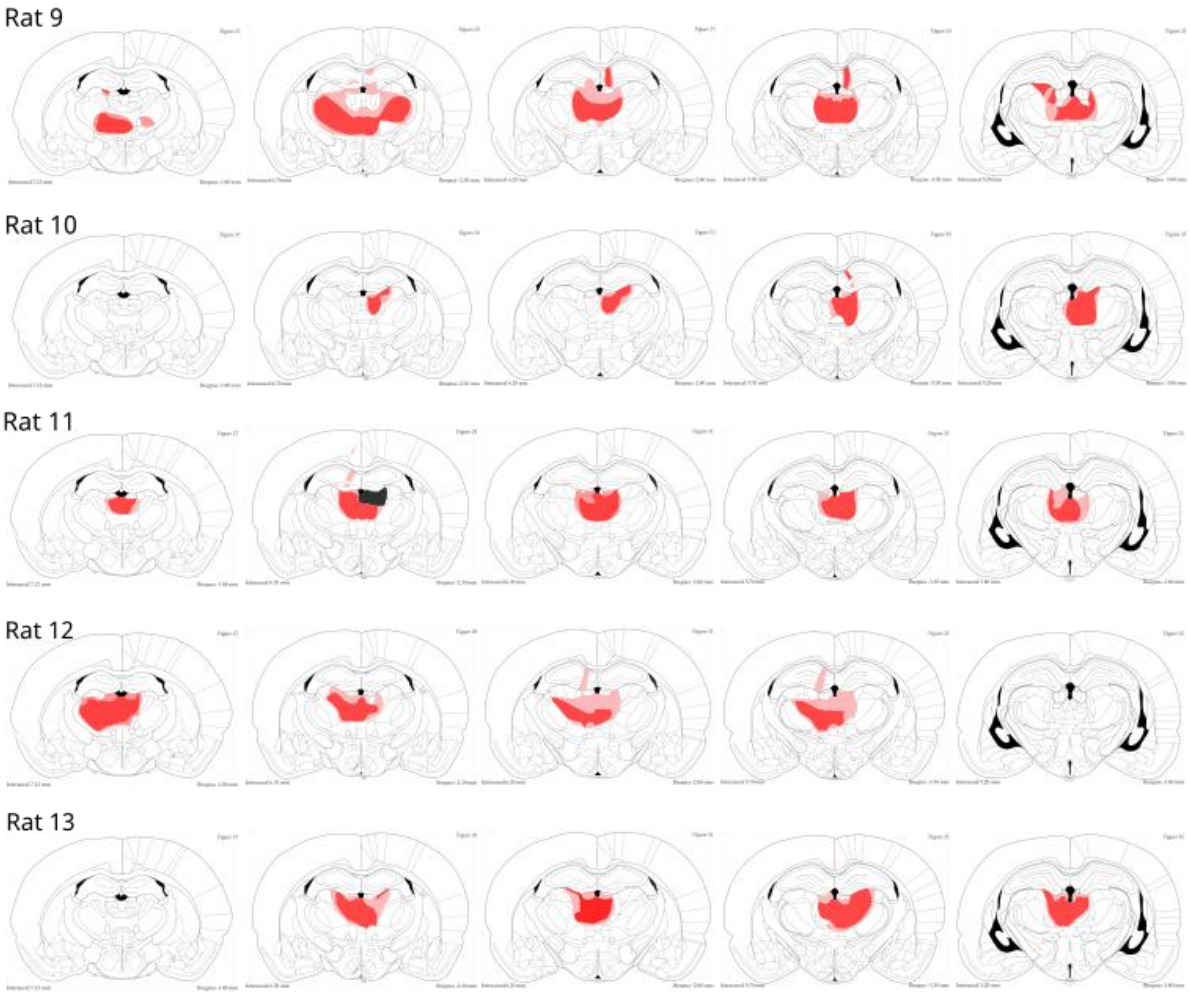
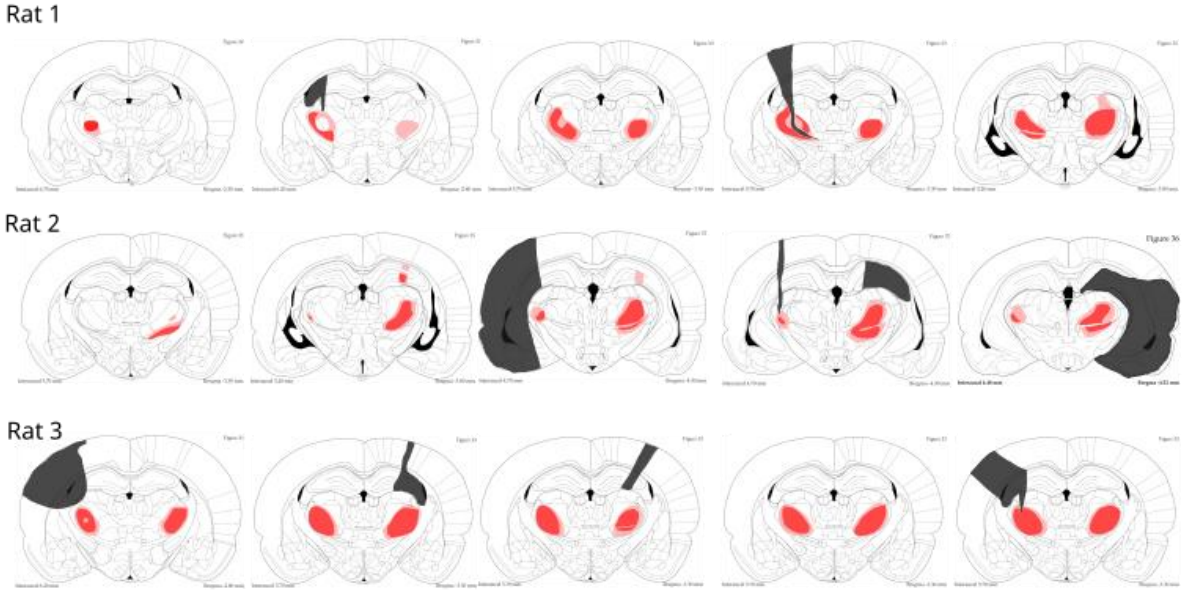
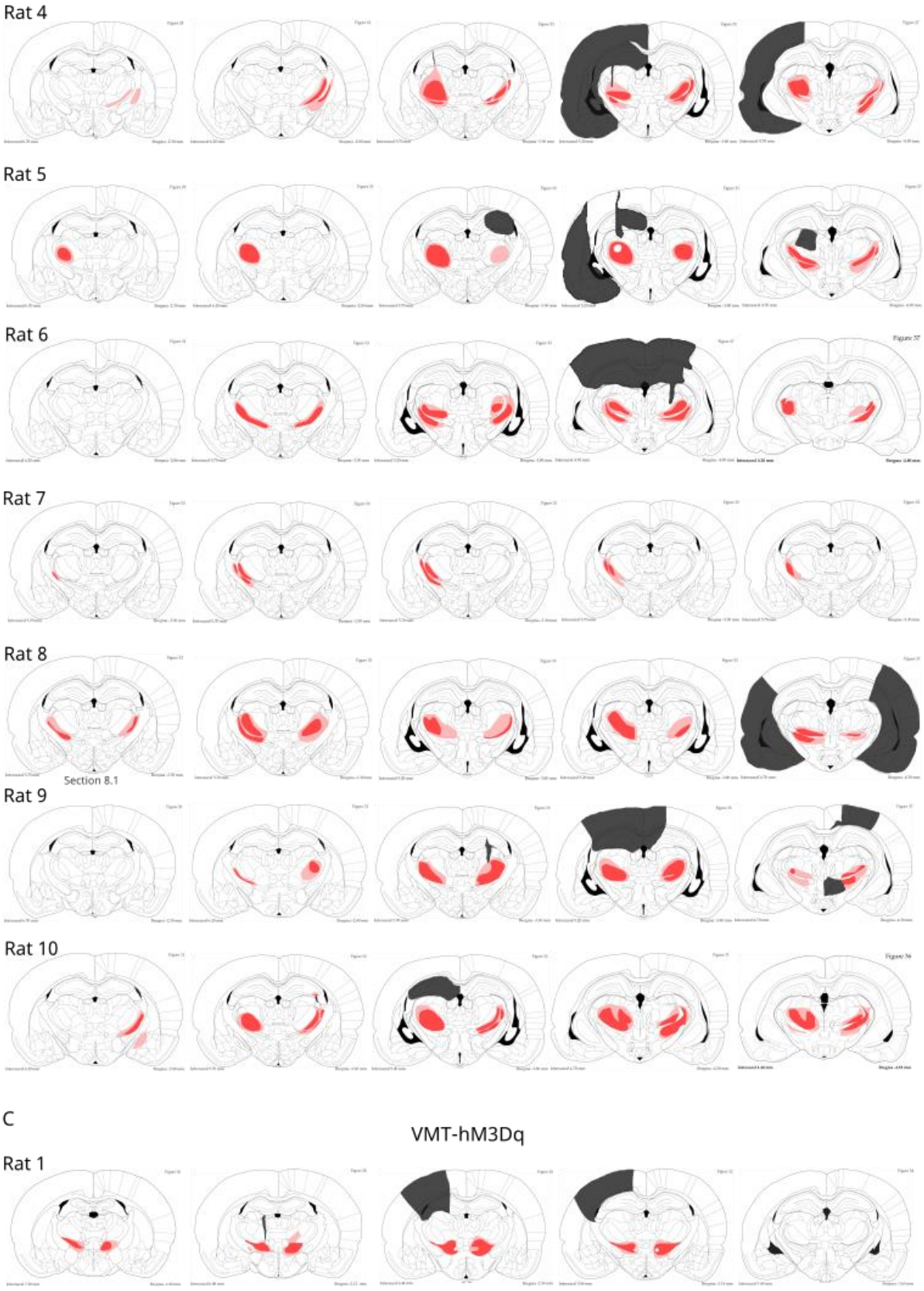
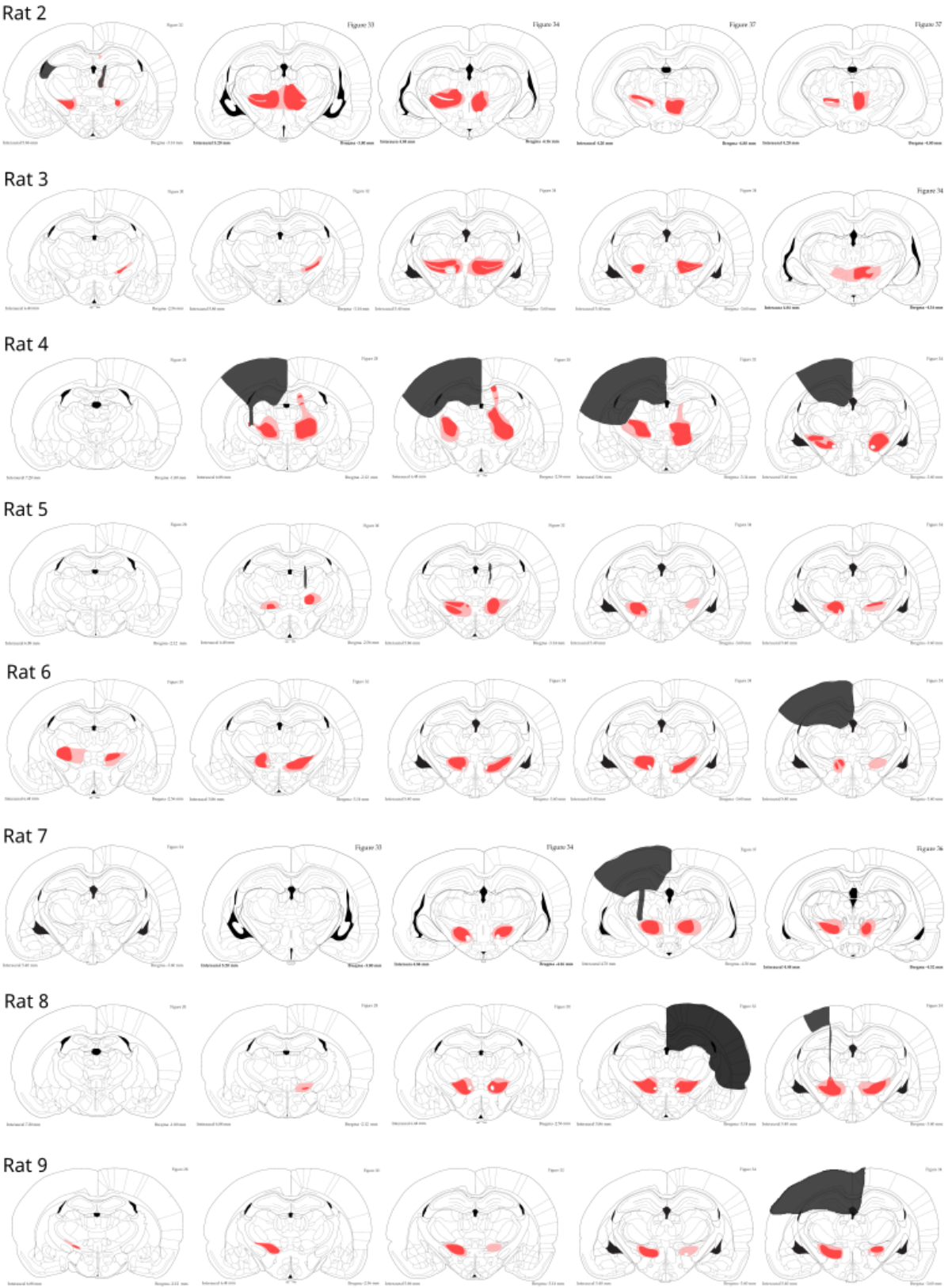




B VPT-hM3Dq

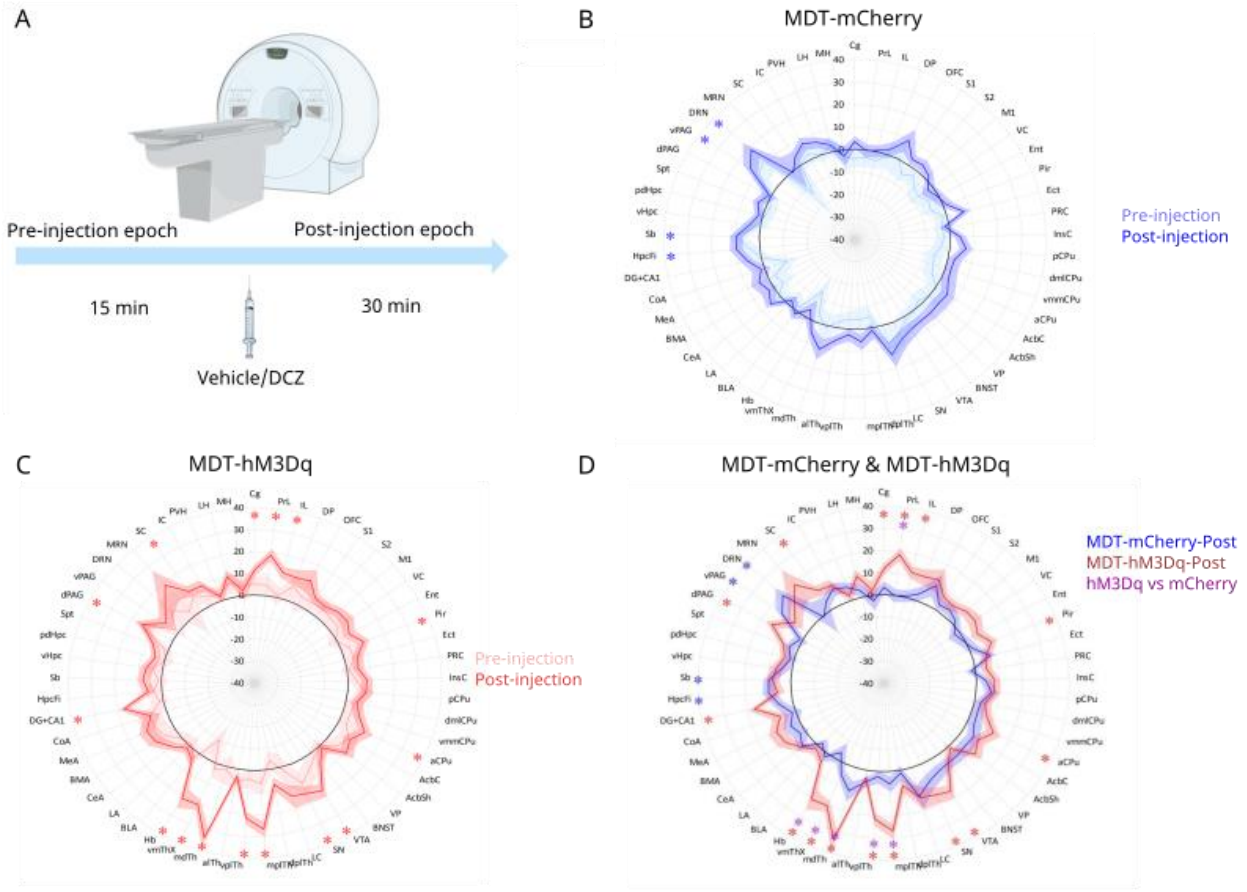



Rat 4
Rat 5
Rat 6
Rat 7
Rat 8
Section 8.1
Rat 9
Rat 10
C
VMT-hM3Dq
Rat 1


Rat 2
Rat 3
Rat 4
Rat 5
Rat 6
Rat 7
Rat 8
Rat 9

Supplementary Figure 1. Stereotactic targeting of MDT, VPT and VMT with AAV9-CaMKIIα-hM3D(Gq)_mCherry virus.

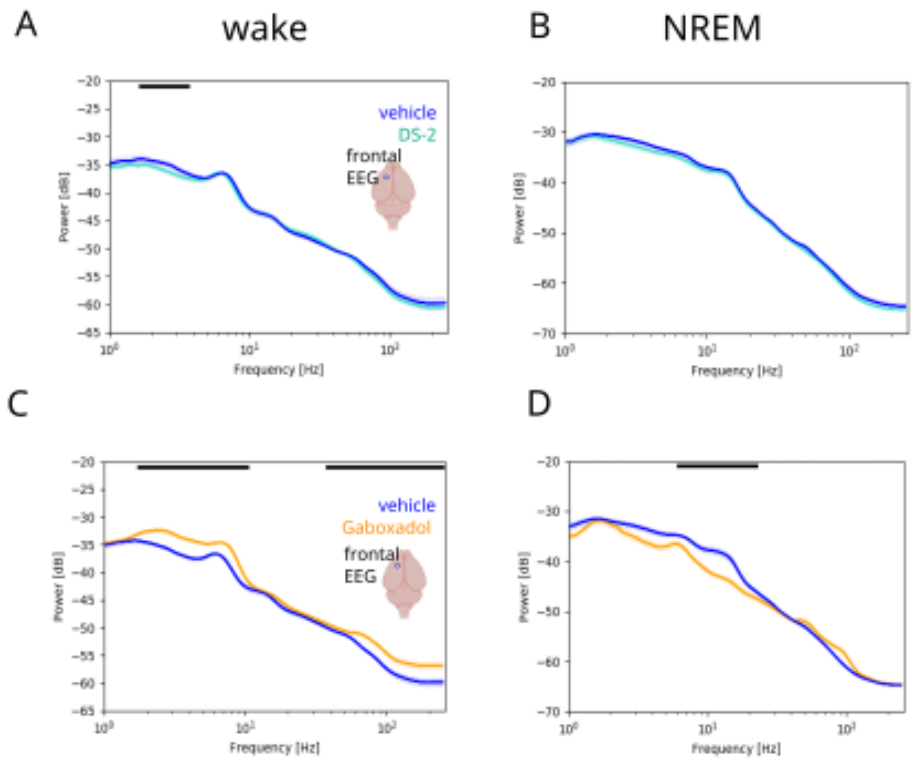
(A) Corresponding coronal rat brain atlas images from Bregma level -2.30 to -4.30. MDT is highlighted in red. (B) Images corresponding to the VPT-hM3Dq animals. (C)Images corresponding to the VMThM3Dq animals.



Supplementary Figure 2. Perfusion-based MRI reveals distinct neuronal activation profiles associated with MDT activation. (A) Schematic of the experimental design and dosing scheme for the chemogenetic manipulation. (B–D) Radar plots showing ROI-wise perfusion responses to DCZ treatment in MDT-mCherry (blue) and MDT-hM3Dq (red) animals ($n = 10$ per group) during the pre-injection (pale colors) and the post-injection (dark colors) epochs, respectively. Response profiles (colored lines) are shown as group-mean differences of the DCZ condition versus the vehicle condition (black circles at zero), along with standard errors (shaded baNDDs/NPDs). Asterisks indicate modulations that are significant in the sense of passing control of the false discovery rate (FDR) at 1 %; blue and red asterisks refer to the respective perfusion responses within each group, while purple asterisks highlight significant differences between the responses of the two groups (interaction contrasts).

ROI abbreviations, in clockwise order: Cg: cingulate cortex, PrL: prelimbic cortex, IL: infralimbic cortex, DP: dorsal peduncular cortex, OFC: orbitofrontal cortex, S1: primary SSC, S2: secondary SSC, M1: primary motor cortex, VC: visual cortex, Ent: entorhinal cortex, Pir: piriform cortex, Ect: ectorhinal cortex, PRC: perirhinal cortex, InsC: insula, pCPu: posterior CPu, dmlCPu: dorsomediolateral CPu, vmmCPu: ventromediomedial CPu, aCPu: anterior CPu, AcbC: nucleus accumbens core, AcbSh: nucleus accumbens shell, VP: ventral pallidum, BNST: bed nucleus of the stria terminalis, VTA: ventral tegmental area, SN: substantia nigra, LC: locus coeruleus, dplTh: dorsal posterolateral thalamus, mplTh: medial posterolateral

thalamus, vplTh: ventral posterolateral thalamus, alTh: anterolateral thalamus, mdTh: mediodorsal thalamus, vmThX: other ventral nuclei of the mTh, Hb: habenula, BLA: basolateral amygdala, LA: lateral amygdala, CeA: central amygdala, BMA: basomedial amygdala, MeA: medial amygdala, CoA: cortical amygdala, DG+CA1: dentate gyrus & CA, HpcFi: fimbria hippocampi, Sb: subiculum, vHpc: ventral hippocampus, pdHpc: posterior dorsal hippocampus, Spt: septal region, dPAG: dorsal PAG, vPAG: ventral PAG, DRN: dorsal raphe nucleus, MRN: median raphe nucleus, SC: superior colliculus, IC: inferior colliculus, PVH: paraventricular hypothalamic nucleus, LH: lateral hypothalamus, MH: median hypothalamus



Supplementary Figure 3. Effects of Gaboxald and DS-2 in the absence of VMT activation.

(A-B) Power spectral density plots during wake and NREM. for VMT-hM3Dq animals upon DS-2 or vehicle administration (C-D) Power spectral density plots during wake and NREM. for VMT-hM3Dq animals upon gaboxadol or vehicle administration.
Gaboxadol (15mg/kg) (orange); DS-2 (100 mg/kg) (cyan). N = 10 VMT-hM3Dq rats. Statistically significant clusters ($p < 0.05$) are indicated as bars on top based on a paired cluster-based permutation test.

Supplementary Methods

Pharmacological magnetic resonance imaging (phMRI).

Experimental design and procedure

A total of 20 adult male Sprague-Dawley rats were used in this study, divided into two experimental groups: control (*n*=10) and MDT-hM3Dq (*n*=10). Anesthesia of animals was induced with isoflurane and maintained with medetomidine. Specifically, animals were initially anesthetized with 4% isoflurane in oxygen-enriched air (40% O_2), delivered in an inhalation chamber. Delivery was then continued at 1.2 l/min via a face mask at a lower concentration of 2% for approx. 5 min and eventually at a maintenance concentration of 0.5% for the rest of the MRI session. In parallel to isoflurane reduction, subcutaneous medetomidine (Dormilan®) administration was started with a bolus of 0.2 mg/kg (1 ml/kg), followed by continuous infusion at 0.1 mg/kg/h (1 ml/kg/h). During the 5-minute anesthesia transition phase, animals were positioned on a custom-made cradle, their heads were immobilized in a stereotaxic frame with ear and bite bars, and an ophthalmologic ointment was applied to their eyes to prevent drying. Anesthesia depth was monitored through breathing rate (target: 60 breaths/minute) and O_2 and CO_2 levels

in inhaled and exhaled breathing gases using a PowerLab system (ADInstruments, Spechnach, Germany). The body temperature was maintained at 37.5 °C with an electric heating blanket in a feedback loop with a rectal thermometer. The total time under anesthesia was approximately 90 min.

MRI was performed on a Biospec 7T / 20 cm horizontal-bore, small-animal MRI scanner (Bruker BioSpin, Ettlingen, Germany) equipped with an actively decoupled 2-coil system consisting of a body resonator for signal excitation and a head surface coil for reception. Image acquisition was based on a standardized scan procedure. Briefly, following localization of the most rostral extension of the corpus callosum as a landmark on scout images, 8 coronal image planes were selected at +2.3, +1.0, –0.3, –1.6, –2.9, –5.3, –7.8, and –10 mm from the bregma. All subsequent images were acquired in these planes, with a field of view of 40 mm x 40 mm and a slice thickness of 1 mm. Firstly, a set of T2-weighted fast spin-echo images (repetition time [TR]/effective echo time [TE_{eff}] = 2000 ms/34 ms, acceleration factor 8, matrix 256 x 256 data points) was obtained as an anatomical reference. Next, a T1-weighted image series required to quantitate perfusion was obtained using an inversion-recovery gradient-echo sequence with 8 inversion times (inversion time [TI] = 21.7–1475.7 ms, TR/echo time [TE] = 3750 ms/1.4 ms, matrix 128 x 64 data points). Finally, cerebral blood perfusion (as a proxy of neuralactivity) was assessed by continuous arterial spin-labeling (CASL) with centered-RARE readout (TR/TE_{eff} = 3750 ms/3.5 ms, acceleration factor = 64, matrix 128 x 128 data points, labeling pulse 2.5 s, postlabeling delay 0.4 s). 3 such CASL volumes were acquired over a 12-minute epoch starting approx. 30 min after induction of anesthesia (“pre-injection epoch”). After intraperitoneal injection of either vehicle or DCZ (0.1 mg/kg) at approx. 50 min, a second and a third epoch with 3 CASL volumes each were acquired, starting at approx. 55 min and 70 min, respectively (“post-injection epochs”).

Data processing and analysis

MRI images were processed and analyzed using a Roche-developed software pipeline implemented in MATLAB (The Mathworks; Natick, MA). In brief, structural (T2-weighted) image volumes of each individual animal were spatially normalized to a Roche rat-brain

template using the open-source software SPM5 (Wellcome Trust Centre for Neuroimaging, London, UK). Spatial normalization comprised a 12-parameter affine as well as a nonlinear transform. The template was in alignment with a Roche digital atlas delineating 53 predefined anatomical regions of interest (ROIs). T1-weighted and CASL images were subjected to the spatial normalization transforms estimated from the respective structural images and were then jointly processed to obtain maps of regional blood perfusion, which was taken as a quantitative proxy for local neural activity. Perfusion was averaged within each of the predefined ROIs and within each of the 3 12-minute CASL epochs (across the 3 successive scans), yielding one scalar perfusion value per animal (20), visit (2), ROI (53) and epoch (1 pre-, 2 post-injection). All image reconstruction and processing steps prior to the statistical analysis were blind to treatment. The statistical analysis as a final step did not include any further manipulation of the data and thus excluded any expectation bias.

Statistical analysis

The statistical analysis of MRI data was performed in JMP Pro 17.2 (SAS Institute, Cary, NC, USA). For each ROI, perfusion values were entered into a mixed model with *group* (mCherry, hM3Dq) and *treatment sequence* (vehicle→DCZ, DCZ→vehicle) as fixed between-subject effects, *treatment* (vehicle, DCZ) and *epoch* (pre-, post-injection) as fixed within-subject effects and *subject* (animal) as random effect, plus all (also higher-order) interactions among the fixed effects. While the *sequence* effect per se was not of primary interest to us, it was important to factor it out (as it did actually interact with *treatment* and to a lesser extent also with *group* $*$ *treatment*), thereby increasing the statistical sensitivity on the other effects. Within the framework of this model (i.e., based on joint variance estimates), we formally tested the treatment effect (i.e., DCZ–vehicle, 2-tailed) at pre-injection and at post-injection (both epochs jointly), in each group and as interaction with group (hM3Dq–mCherry). Multiple testing was accounted for by controlling the false discovery rate (FDR) at 1 % across the 6 contrasts and 53 ROIs, using the Benjamini-Hochberg approach (Benjamini & Hochberg 1995). The specific statistical tests applied to each analysis are detailed within the figure legends.

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