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Supplemental Information

Prefrontal Pathways Provide Top-Down Control of Memory for Sequences of Events

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Supplemental Information

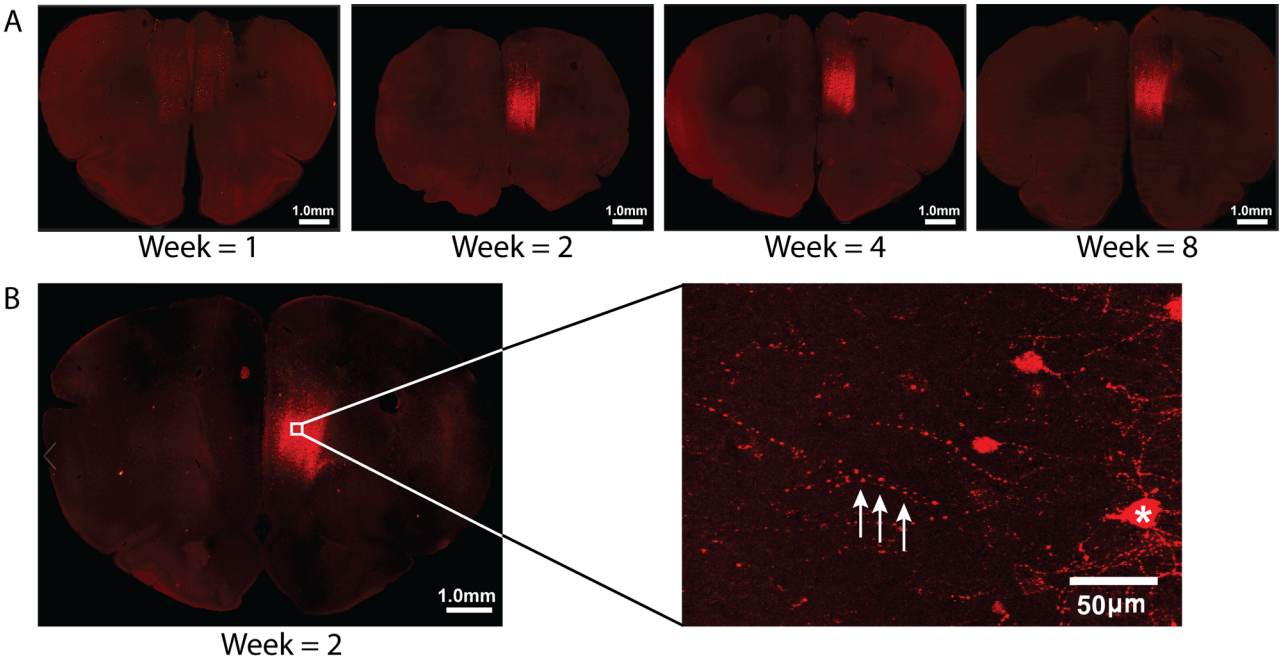

Figure S1. Incubation time and AAV9.hM4Di expression for these experiments., Related to Figure 1.

AAV9.hM4Di was fully expressed within 2 weeks within the axons and soma of neurons

(A) The images show that by the 2-week mark, the virus was well expressed, and thus behavioral experiments began after this incubation time.

(B) AAV9.hM4Di expressed along the axons of mPFC neurons, as expected. The magnified fluorescent image shows an example of hM4Di expression in an axon (arrows) and soma (asterisk).

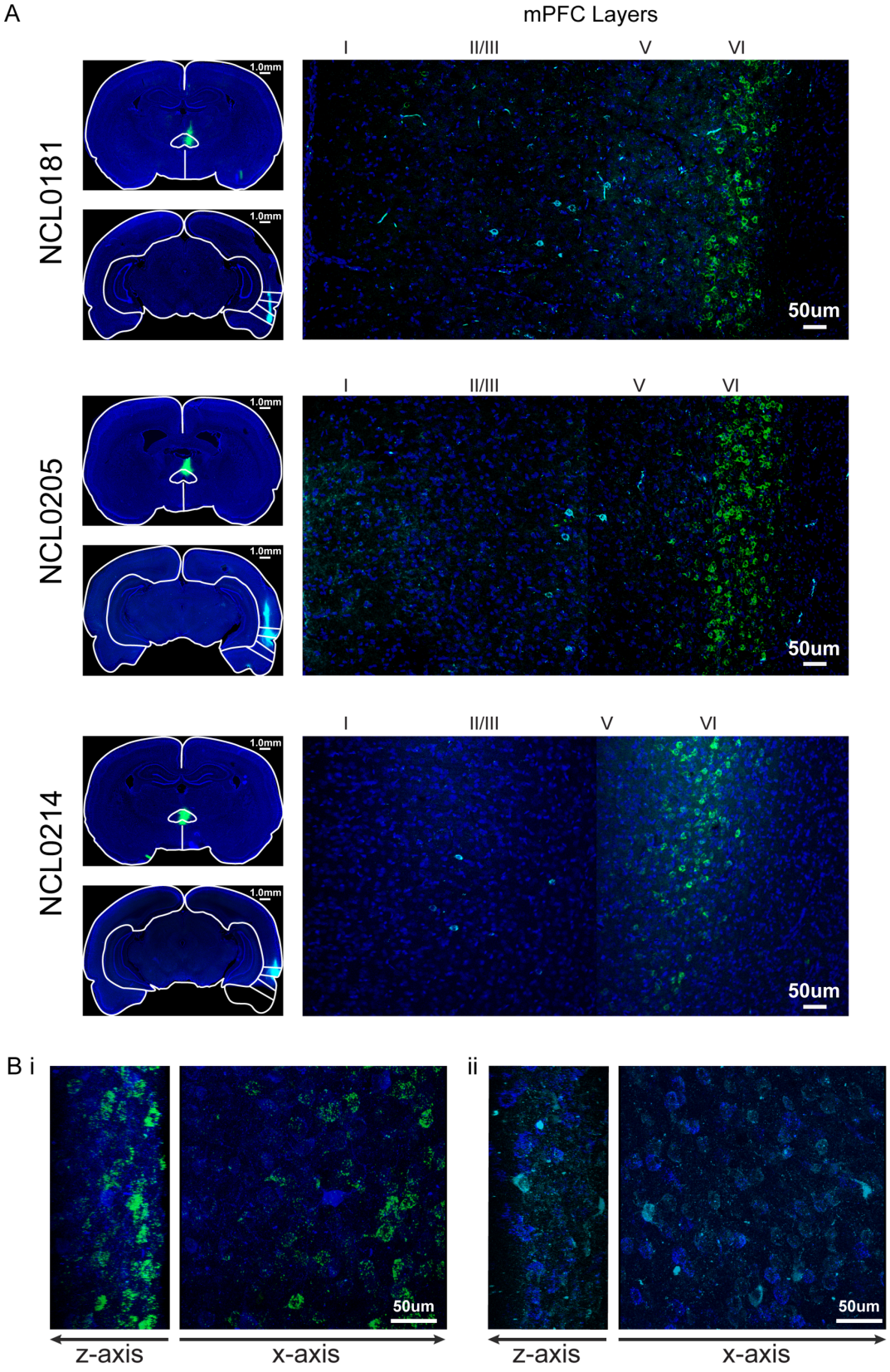

Figure S2. Dual retrograde labeling and immunoflourescence using GAD67, Related to Figure 1.

(A) Each rats confocal photomicrograph of the RE (green) and PER (cyan; color was altered for consistency purposes) injection sites with CTB-488 or CTB-594 and dapi (blue). mPFC layers are displayed on the right that show retrograde labeled cells from RE and PER. Note these layer sections are from ventral prelimbic cortex (vPL).

(B) Representative confocal image of dual labeled GAD67 (blue), CTB-488 (i; mPFC→RE, green), and CTB-594 (ii; mPFC→PER, cyan).

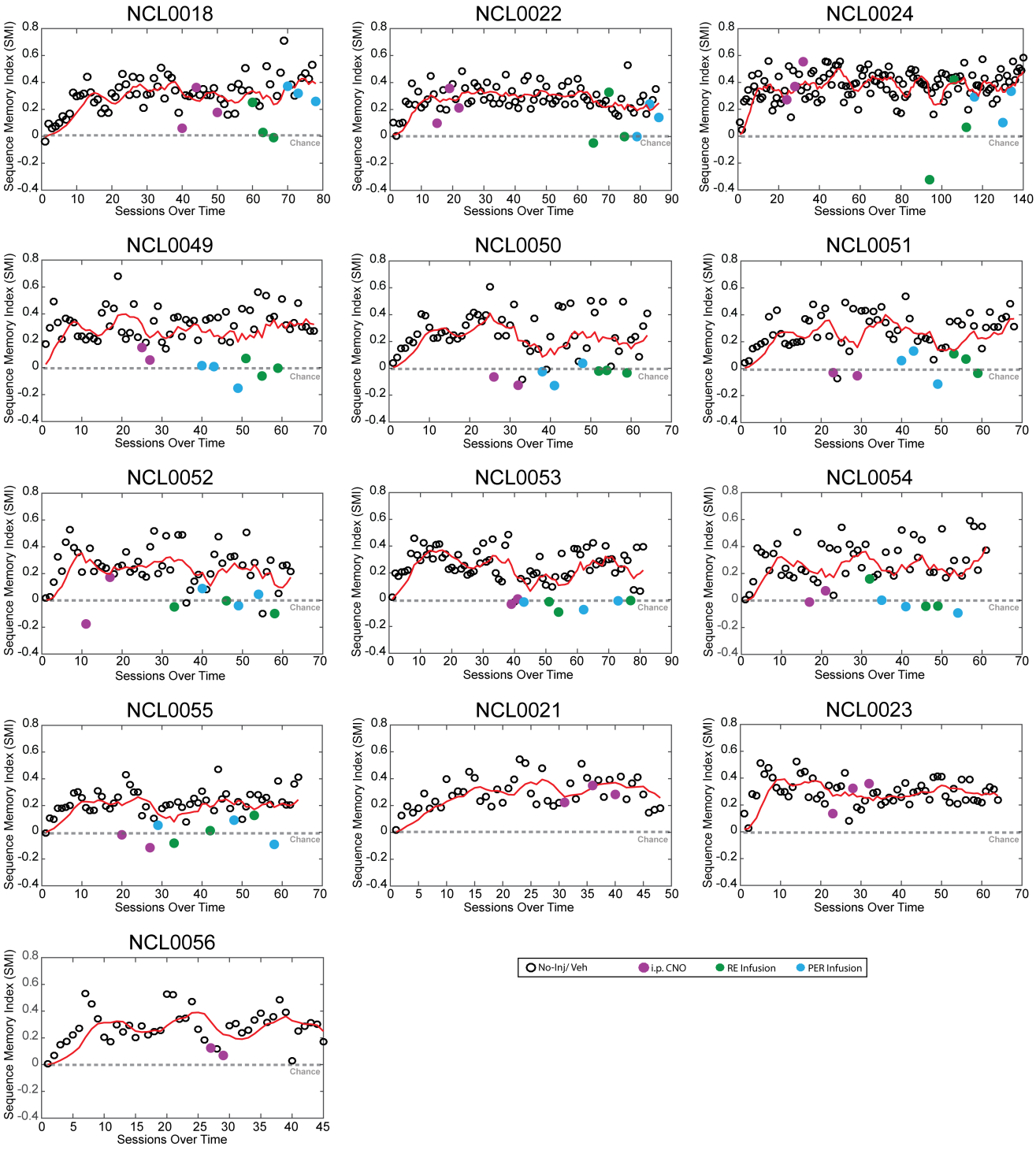

Figure S3. Individual behavioral performance, Related to Figure 2.

Each rat's behavioral data was collapsed into a single normalized measure (SMI) for each session. The identification of each rat is indicated at the top of each graph (NCLXXXX). The red line represents the mean SMI as the rat learned the task. All rats reached steady state and were able to maintain their SMI except for when infusions/injections occurred. With each infusion/injection, there was a clear decrease (to chance levels) in SMI.

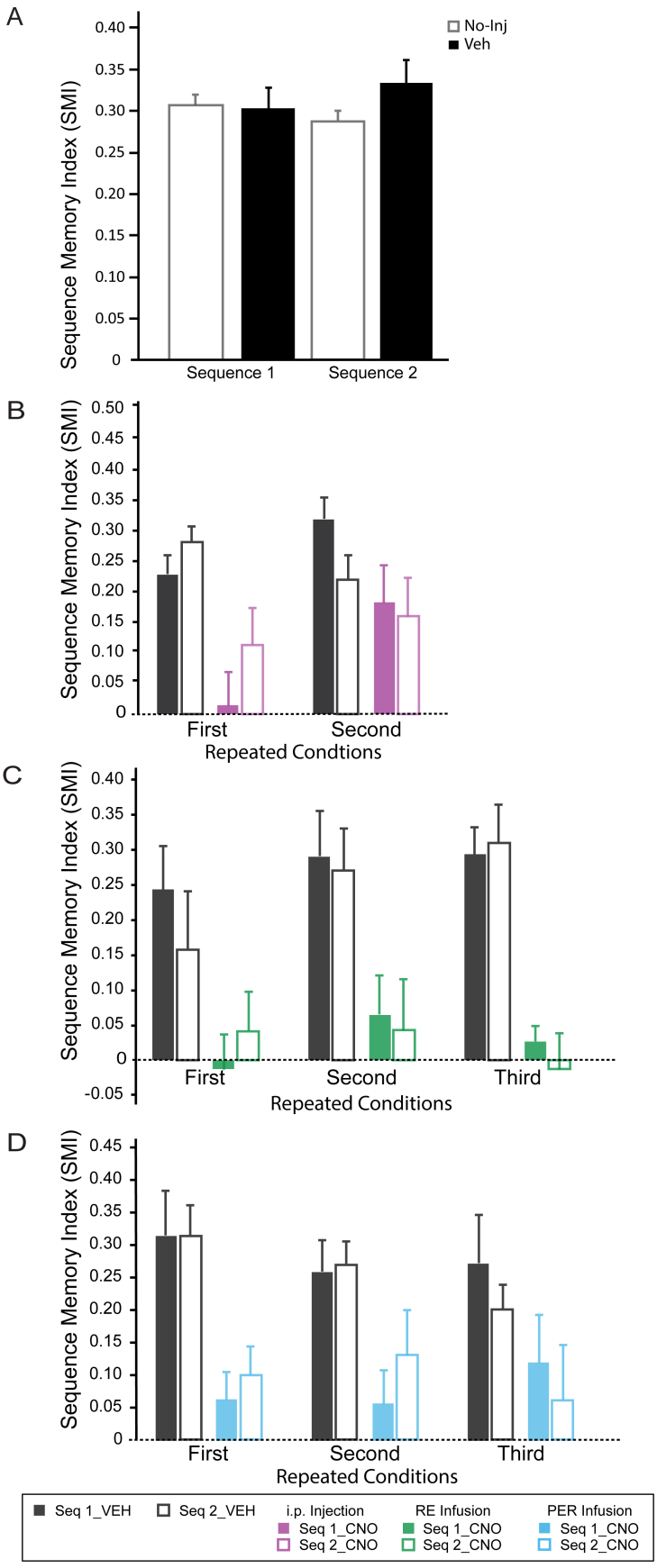

Figure S4. Sequence 1 vs. sequence 2, Related to Figure 1, 3, 4, and 5.

Comparison of sequence 1 vs. sequence 2 revealed that the rats performed at comparable levels on both sequences.

(A) Comparing No-Inj vs Veh days in sequence 1 and sequence 2 indicated nonsignificant differences. Moreover, there was no significant difference between sequences for the No-Inj days and Veh days.

(B) There was no significant difference between sequences across repeated conditions for i.p. CNO administration.

(C) SMI was not significantly different between sequences across repeated conditions for mPFC→RE infusions.

(D) Similarly, SMI was not significantly different between the sequences across repeated conditions for mPFC→PER infusions.

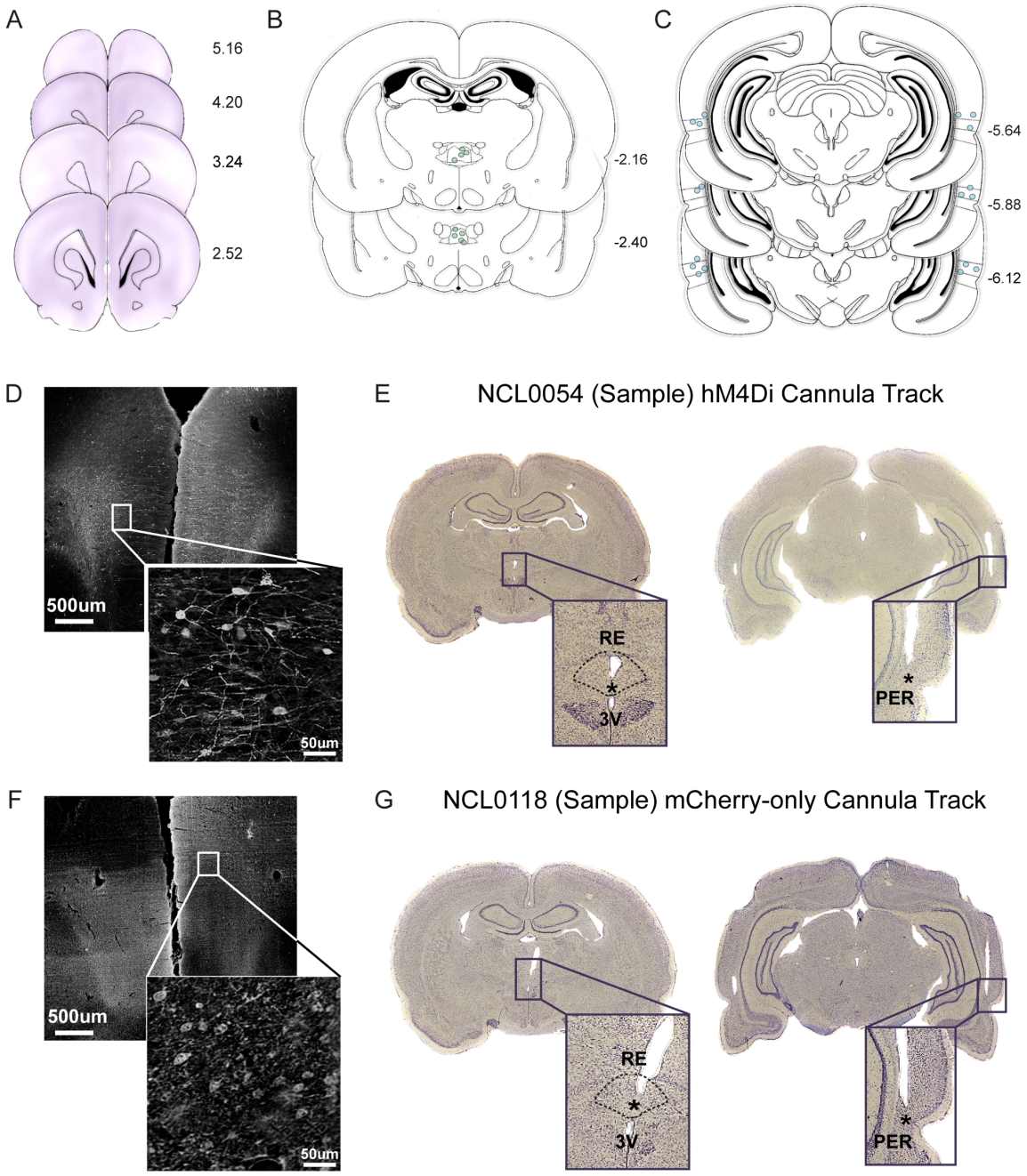

Figure S5. Viral expression and cannula placements in experimental groups, Related to Figure 3, 4, and 5.

(A) Schematic representation AAV9.mCherry maximal viral spread in the mPFC for all rats. While infected cells in mCherry-only rats were visualized outside of the medial wall of the PFC, extending to the orbital and motor cortices, the null behavioral effects of these rats confirmed that our findings were not associated with nonspecific effects related to the viral construct or CNO. Numbers to the right of each section indicate distance (mm) anterior to bregma.

(B) Microinfusion injector tip location in the RE for all AAV9.mCherry rats. Numbers to the right of each section indicate distance (mm) anterior to bregma.

(C) Microinfusion injector tip location for all AAV9.mCherry rats in the PER. Numbers to the right of each section indicate distance (mm) anterior to bregma.

(D) Representative image of AAV9.hM4D$_{i}^{nrxn}$ expression in the mPFC.

(E) Representative Nissl image of RE and PER (bilateral) for hM4di+. The asterisk indicates the infusion cannula tip location.

(F) AAV9.mCherry expression in the mPFC from a representative rat.

(G) Representative Nissl image of the RE and PER (bilateral) for mCherry-only. Asterisk indicates infusion cannula tip location.

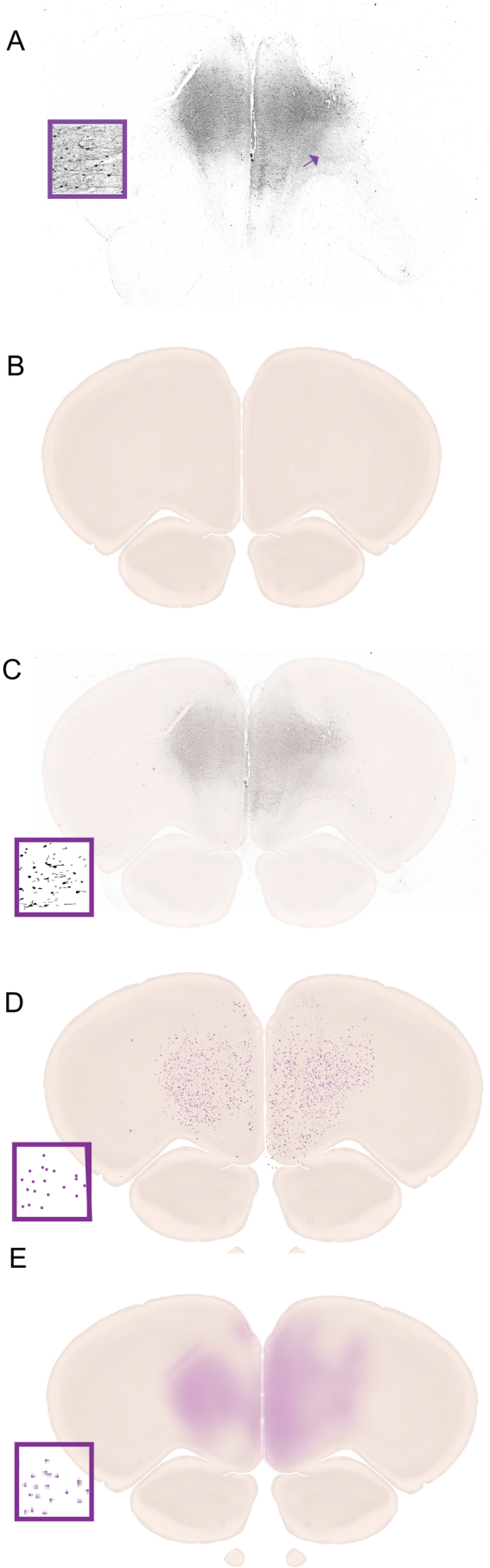

Figure S6. Schematic of viral spread in h4MDi$^+$ rats, Related to Figure 3 and STAR Methods.

Illustration of how the spread of the viral injection was analyzed. A representative sample of a schematic rendering of the viral spread at one level for one rat is shown.

(A) Coronal micrographs were obtained at 100x of whole slices across the frontal cortex from immunostained tissue (antisera for mCherry) for each h4MDi$^+$ rat.

(B) The micrographs were transposed over rendered plates drawn from Nissl sections and modified schematic plates (Swanson, 2004) in Procreate (Savage Software Group).

(C) These images were imported into Adobe Illustrator (Adobe Inc.) and using the 'Image Trace' function, only labeled cell bodies were identified and used to pixelate the image

(D) This produced a shaded image of the injection spread, such that the shading intensity (pixilation) corresponded to cell expression density.

(E) The opacity of each shaded area was reduced to 40% and all rats were superimposed onto one another to create a final schematic for five anterior posterior levels.