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Supplemental Information

Topological Organization of Ventral Tegmental Area Connectivity Revealed by Viral-Genetic Dissection of Input-Output Relations

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Supplemental Information:

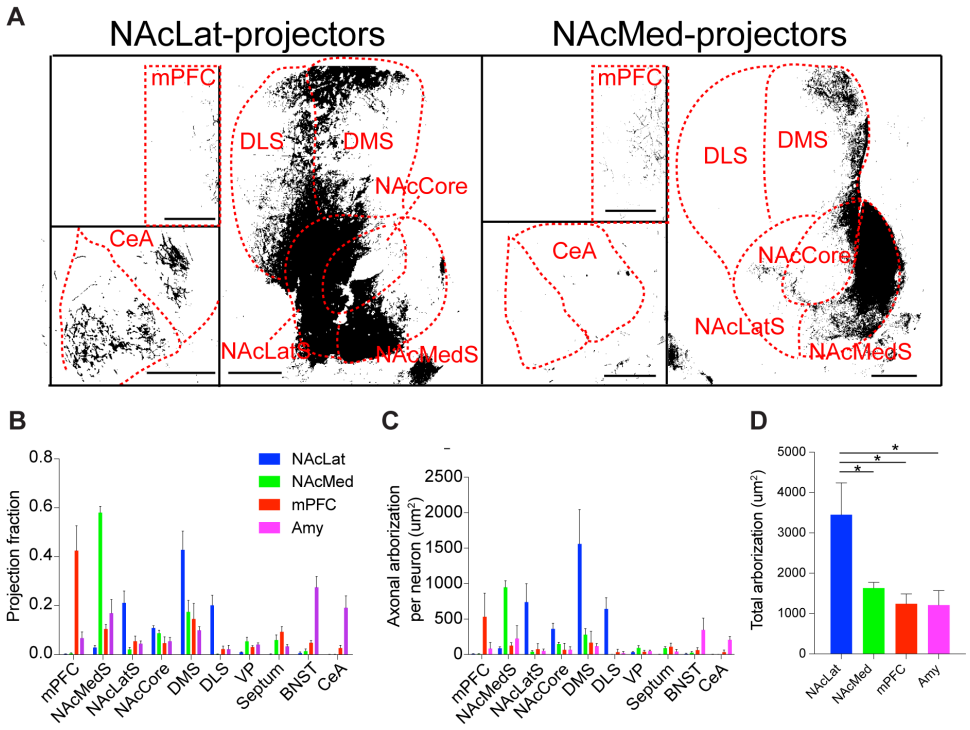

Figure S1: VTA-DA axon collateralization, including previously published data from NAcLat-projecting and NAcMed-projecting VTA-DA neurons (Beier et al., 2015), shown for comparison purposes.

(A) Sample images of projections from VTA-DA subpopulations targeted by *CAV-FLEx^{loxP}-Flp* injections into the NAcLat and NAcMed. Scale, 500 μm.

(B) Projection fraction of each subtype to ten different brain regions.

(C) Average axonal arborization per labeled VTA-DA neuron in each brain region.

(D) Total quantified arborization per each VTA-DA neuron.

Related to Figure 1. B-D, n=4 for NAcLat and Amy; n=5 for NAcMed and mPFC. Error bars, SEM. *p < 0.05.

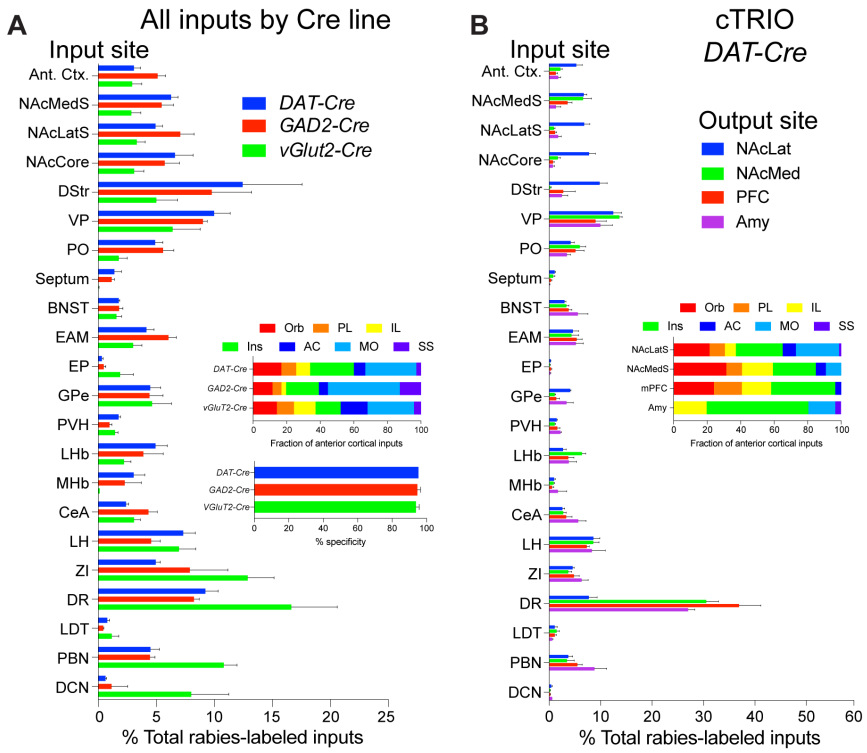

Figure S2: Input tracing and cTRIO data for *DAT-Cre* mice.
(A) Quantified percentage of rabies-labeled inputs from one of 22 different input sites throughout the brain, for *DAT-Cre*, *GAD2-Cre*, and *vGluT2-Cre* mouse lines. n = 4 for each condition. The data for *DAT-Cre* and *GAD2-Cre* mice were published previously (Beier et al., 2015) and are shown for comparison purposes. Top inset shows the fraction of anterior cortical inputs from the seven quantified cortical subregions. Bottom inset shows the fidelity of the Cre lines used. *DAT-Cre* fidelity was tested using an anti-TH antibody, whereas *GAD2-Cre* and *vGluT2-Cre* fidelity were tested using in situ hybridization.
(B) cTRIO data for *DAT-Cre* mice, as published previously (Beier et al., 2015), shown here for comparison purposes.
Related to Figure 2. n=4 for each condition. Error bars, SEM.

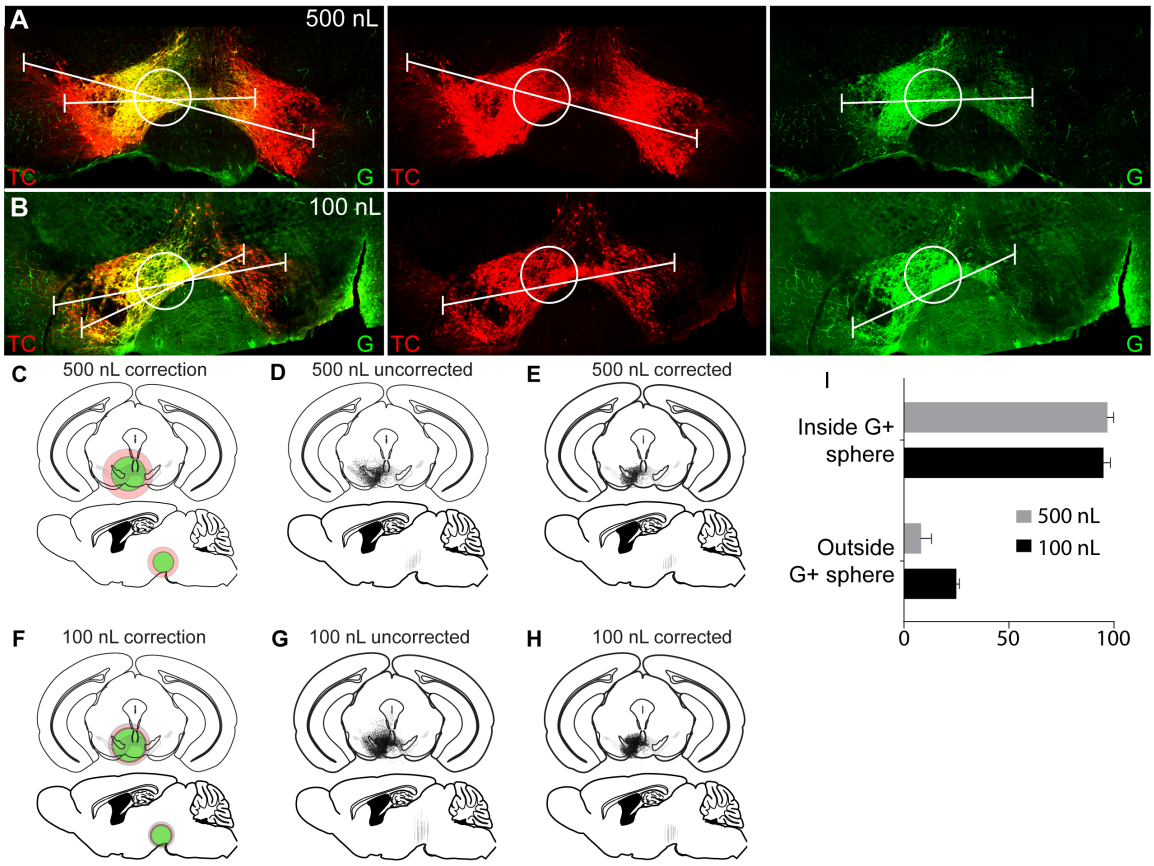

Figure S3: Measurement of viral spread for calculation of corrected starter cell center of mass (COM).
(A and B) Injections of 500 nL (A) or 100 nL (B) of a mixture of *AAV$_5$-FLEx^{loxP}-TC* and *AAV$_8$-FLEx^{loxP}-G* into the VTA. G was visualized using an anti-G antibody. The white circle shows the injection site, and the bar covers the spread of the virus, encompassing the maximal distance through the injection site. Images in panels A and B were created by stitching tiled confocal z-stacks and taking the maximum intensity projection using Zen software.
(C-E) Sections showing the starter cell corrections for brains where 500 nL of TC/G were injected. "True" starter cells were considered those within the TC+/G+ region. Any TC+ cells throughout the 76-brain data set that were outside of this radius were eliminated from analysis. The location of cells is shown in coronal and parasagittal section before (D) and after (E) correction.
(F-H) Same as C-E, but for brains with 100 nL injections.
(I) Percentage of cells inside or outside the sphere where G was expressed (green circle in C, F) that co-expressed TC and G.
Related to Figure 3. Error bars, SEM.

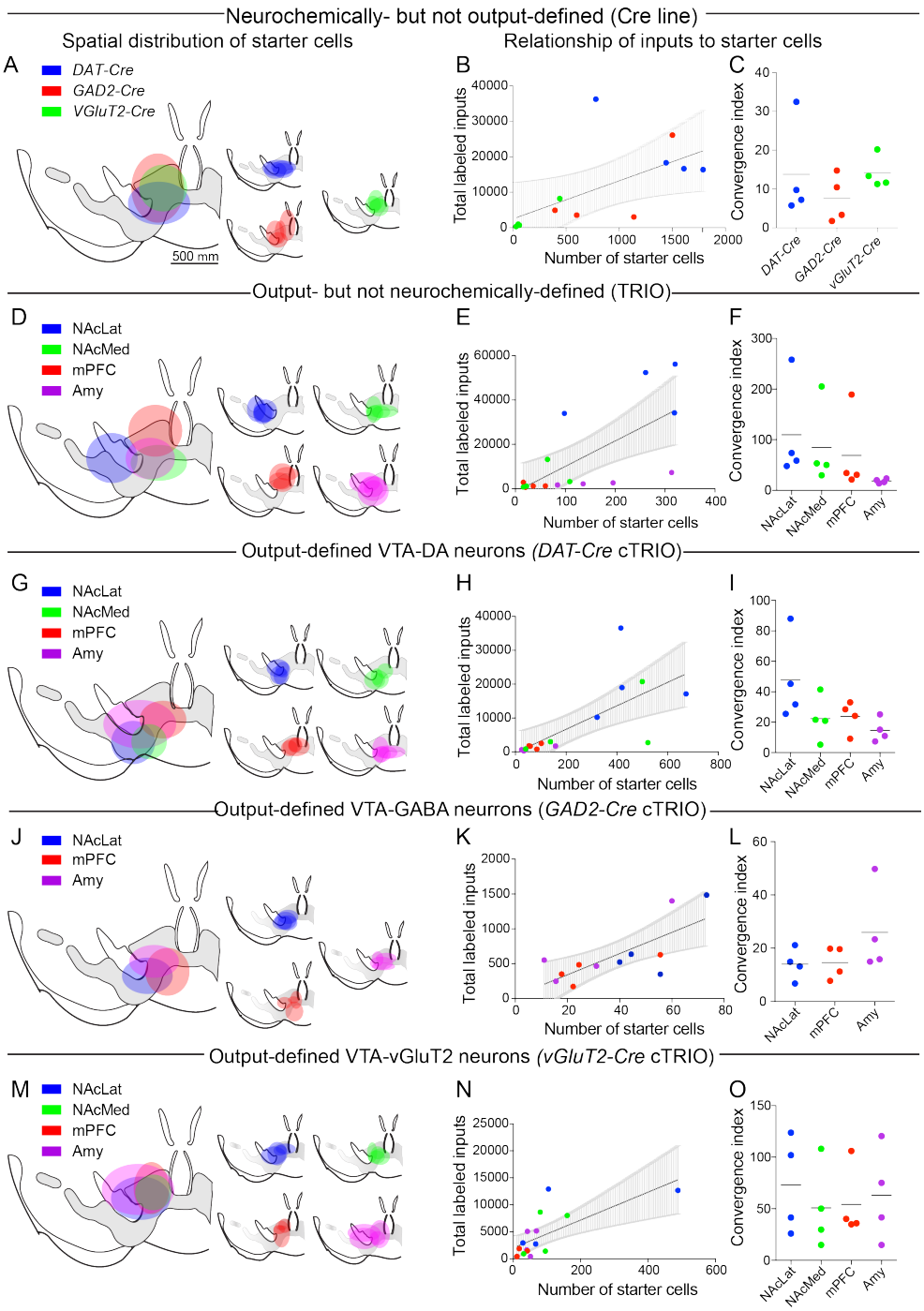

Figure S4: Starter cell distributions and convergence indices for each brain in the dataset.
(A) Starter cell distribution in the VTA for neuronal populations defined by Cre driver line. The distribution of starter cells, representing one standard deviation in both X and Y axes, is shown for each experiment on the small midbrain sections on the right. The aggregated data for each condition is shown on the large section to the left.
(B) Total quantified rabies-labeled inputs as a function of starter cells in the VTA. The 95% confidence interval is shown in gray.
(C) The convergence index (inputs per starter cell) for each condition. Similar data are presented for TRIO experiments (D-F), *DAT-Cre* cTRIO (G-I), *GAD2-Cre* cTRIO (J-L), and *vGluT2-Cre* cTRIO (M-O). Related to Figure 3. n=4 for all conditions.

Table S1: Statistical parameters of regression analysis. Related to Figure 3.

Table S2: Relative axonal projection and input values obtained using the Allen Mouse Brain Connectivity Atlas and cTRIO for all 20 quantified inputs, as described in Figure 4. Related to Figure 4.

Table S3: URLs for all Allen Mouse Brain Connectivity Atlas images analyzed. Related to Figure 4.