Supplemental Online Material

Optogenetic Stimulation of Midbrain Dopamine Neurons Produces Striatal Serotonin Release

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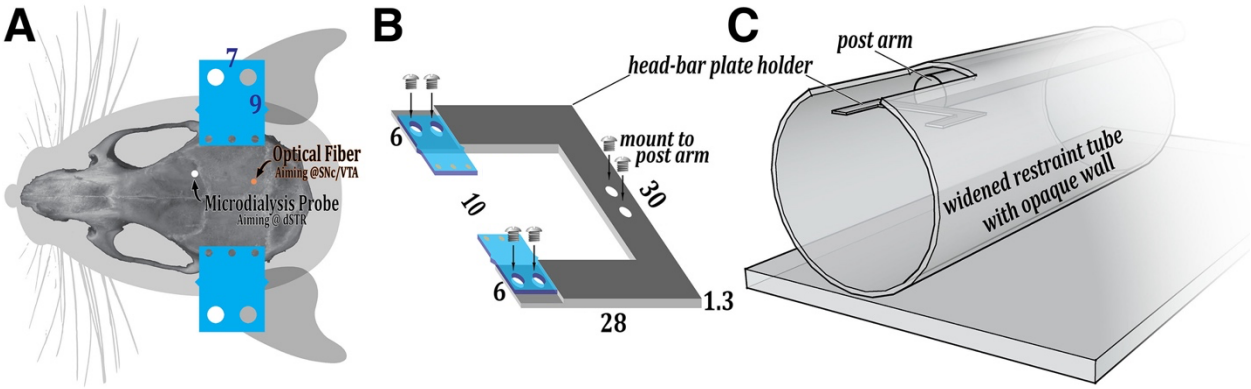

Figure S1: Head-fixed recording set-up. A. Schematic showing the locations of the head-bar implants (in blue and to scale) and the stimulation (stim) and recording (dSTR) site craniotomies relative to a mouse skull. **B.** Schematic of the head-bar plate holder (in gray and to scale). The head-bar plate holder was 30 mm long, 28 mm wide, and 1.3 mm thick. The mini-plates, which attach the holder to the head bars, were 9 mm long, 7 mm wide, and 0.65 mm thick, with a 10 mm gap between them. **C.** Schematic of the custom head-fixed tube used for fast microdialysis recordings with optical stimulation. The restraint tube (2" diameter), constructed of opaque (black) plexiglass, provided loose restraint to reduce spontaneous and stimulated physical movement, which can evoke movement-induced dopamine release artifacts in dorsal striatum.

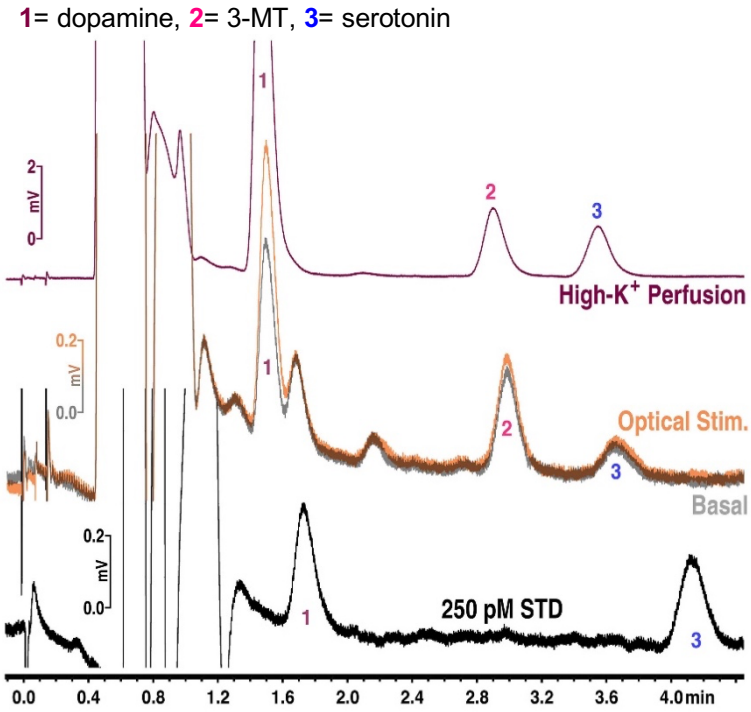

Figure S2: Optical stimulation of midbrain dopamine neurons increases in striatal serotonin and 3-methoxytyramine. Representative chromatograms from a Chrimson-transfected mouse under basal conditions (gray), and in response to optical stimulation (orange) or high-K^+ perfusion (red). Both optical stimulation and high-K^+ perfusion induced increases in neurochemicals (peaks 2 and 3), in addition to dopamine (peak 1). Chromatogram of a standard containing 250 pM dopamine (peak 1) and serotonin (peak 3) is shown in black. Peaks 2 and 3 in the dialysate samples could not be definitively identified based on comparison with retention times in the standard chromatogram.

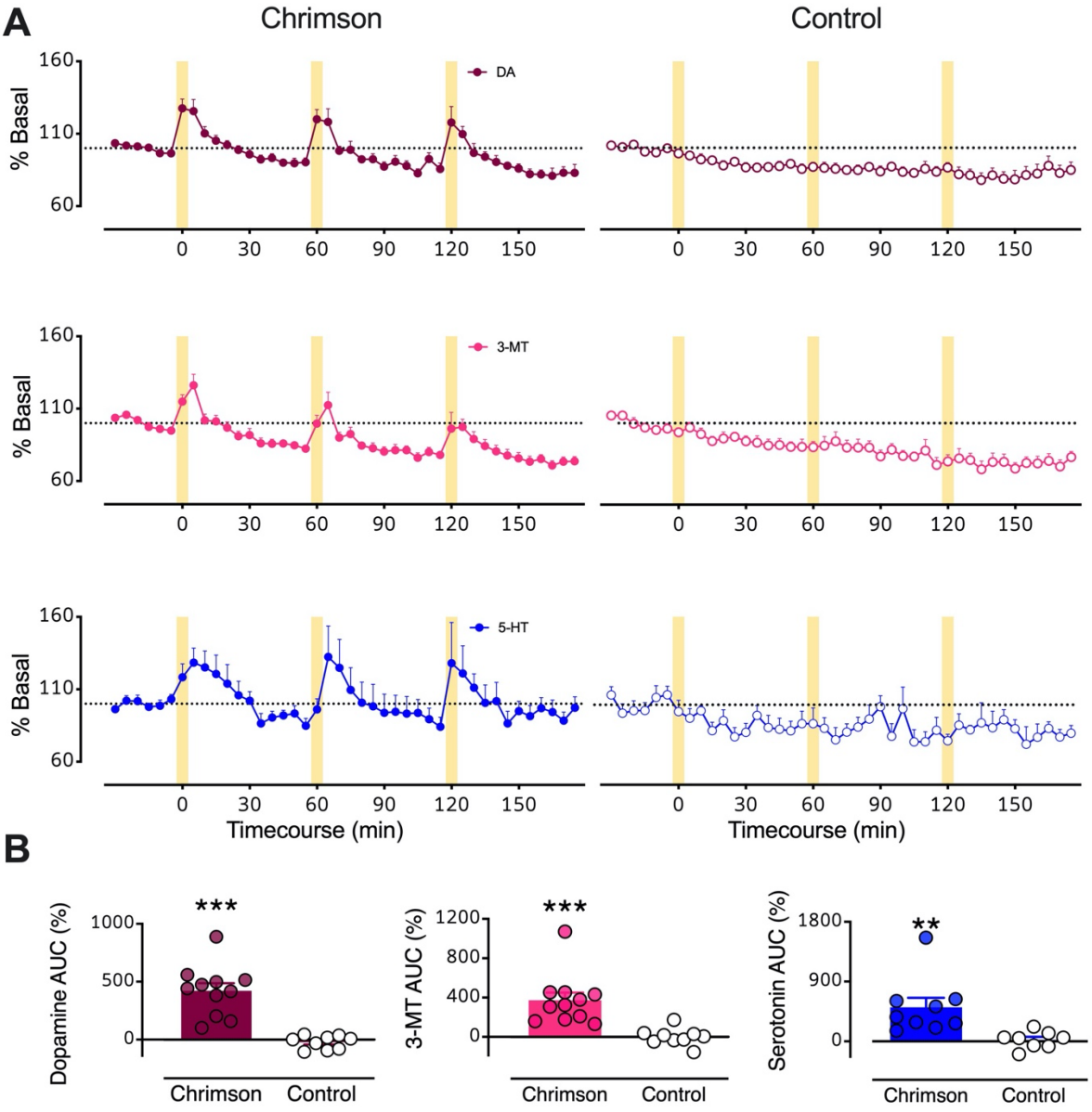

Figure S3: Normalized responses to optical stimulation. A. Time courses of %basal dialysate levels for DA (top, red), 3-MT (middle, pink), and serotonin (5-HT; bottom, blue) in mice expressing Chrimson (left) *vs.* mice transfected with a control protein (right). Optically induced overflow of dopamine, 3-MT, and serotonin were only detected in the Chrimson animals. **B.** The magnitudes of overflow are represented as areas under the curve percent (AUC (%)). Dialysate serotonin concentrations were below the detectable threshold in 2/11 Chrimson mice and 2/9 control mice. The yellow bars indicate optical stimulations (5 min). ***P*<0.01 and ****P*<0.001.

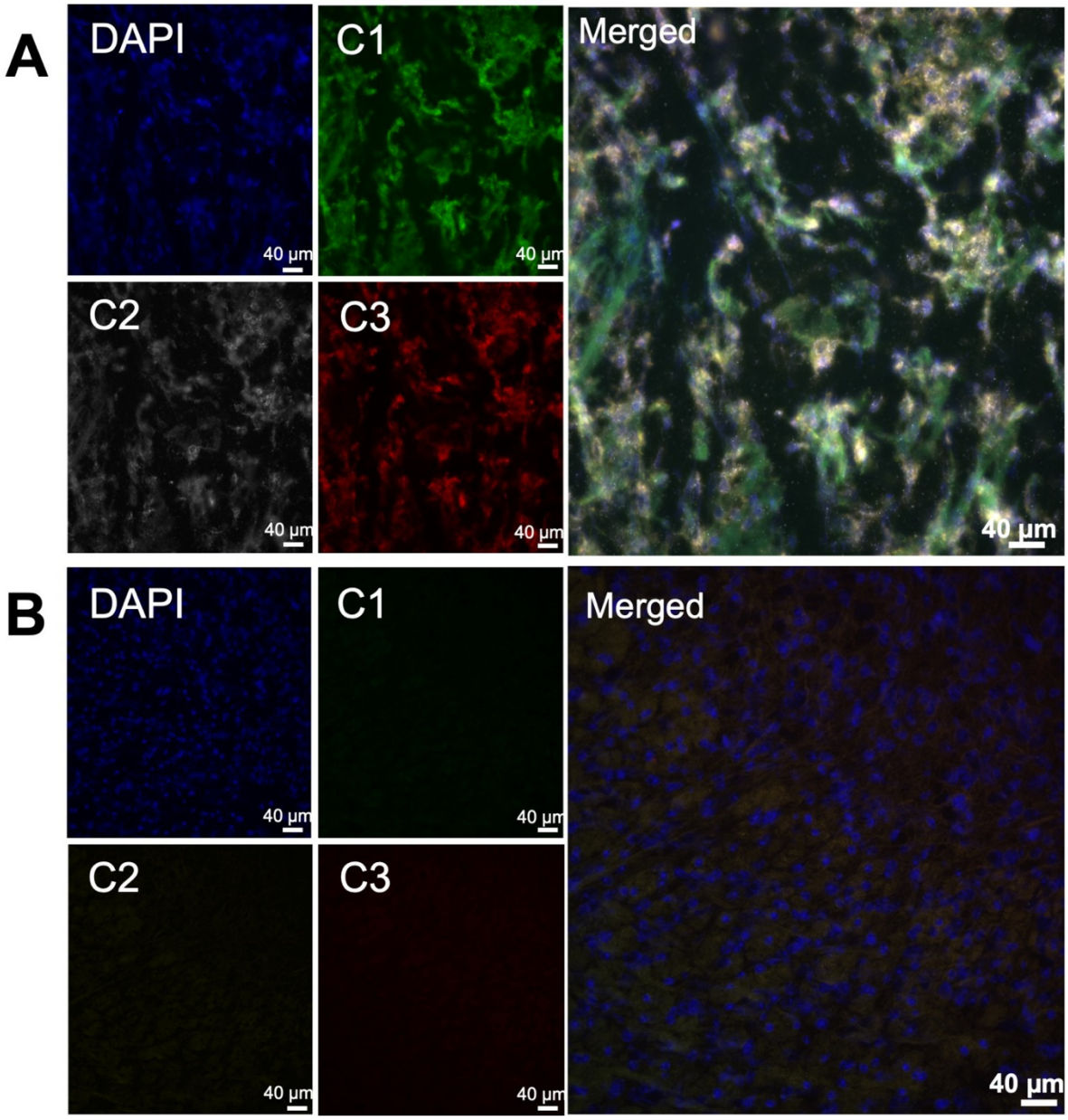

Figure S4: RNAscope *in situ* hybridization controls in dorsal raphe. A.The RNAscope® Multiplex Fluorescent Assay as a 3-plex positive control. The RNA polymerase II subunit RPB1 (Polr2a, C1 channel), cyclophilin B (PPIB, C2 channel), and ubiquitin C (UBC, C3 channel) are mRNAs found in all mouse cells. Cell nuclei stained by DAPI are shown in blue. The overlay is shown on the right **B.** The RNAscope® Multiplex Fluorescent Assay as a 3-plex negative control. A probe for DapB, an mRNA that codes for a reductase enzyme from *Bacillus subtilis*, was used in all three channels with each of the opal dyes to evaluate background staining.

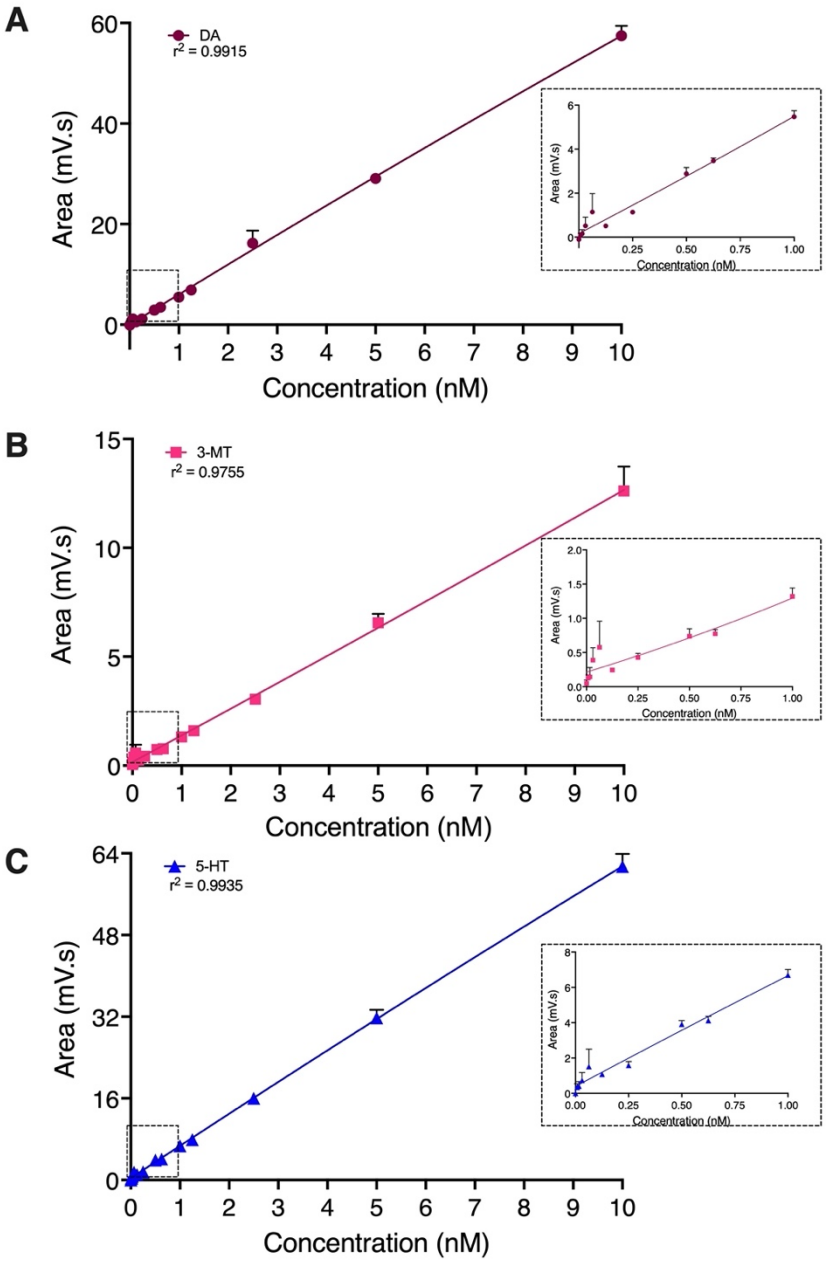

Figure S5: Standard curves for dopamine, 3-methyltyramine 3-MT), and serotonin. Fourteen standards (0 nM, 0.008 nM, 0.016 nM, 0.032 nM, 0.063 nM, 0.125 nM, 0.250 nM, 0.500 nM, 0.625 nM, 1 nM, 1.25 nM, 2.5 nM, 5 nM, and 10 nM) were injected into the HPLC (20 µL volumes) to create standard curves. Insets are zoomed in on the lower concentrations ranging from 0-1 nM. Quadratic curve-fits were applied to **A.** dopamine, **B.** 3-MT, and **C.** serotonin standards. Each point represents *N*=3 replicates measured on different days. Error bars (standard errors of the means) are too small to be visualized in some cases.

Table S1: Statistical summary

FIGURE	COMPARISON	TEST	RESULTS	SIGNIFICANT?
2C	Basal DA: control *vs.* Chrimson	Unpaired two-tailed t-test	t (18)=1.6; *P*>0.1	No
2D	**AUC DA: control *vs.* Chrimson**	**Unpaired two-tailed t-test**	**t (18)=3.0; *P*<0.01**	**
3A	DA: 5 mins pre- *vs.* 60 mins post-ESC	Paired two-tailed t-test	t (2)=0.92; *P*>0.4	No
3A	**5HT: 5 mins pre- *vs.* 60 mins post-ESC**	**Paired two-tailed t-test**	**t (2)=5.7; *P*<0.05**	*
3C	DA: 5 mins pre vs. 60 mins post TOL	Ratio paired two-tailed t-test	t (3) = 0.83; *P*>0.46	No
3C	**3MT: 5 mins pre vs. 60 mins post TOL**	**Ratio paired two-tailed t-test**	**t (3) = 9.6; *P*<0.01**	**
3C	5HT: 5 mins pre vs. 60 mins post TOL	Ratio paired two-tailed t-test	t (3) = 1.3; *P*>0.29	No
4A	Basal 3MT: control *vs.* Chrimson	Unpaired two-tailed t-test	t (18)=0.27; *P*>0.7	No
4A	Basal 5HT: control *vs.* Chrimson	Unpaired two-tailed t-test	t (18)=0.52; *P*>0.6	No
4C	**AUC 3MT: control vs. Chrimson**	**Unpaired two-tailed t-test**	**t (18)=3.1; *P*<0.01**	**
4C	**AUC 5HT: control vs. Chrimson**	**Unpaired two-tailed t-test**	**t (15)=4.4; *P*<0.001**	***
6A	**Basal DA: pre- *vs.* post-SCH**	**Ratio paired two-tailed t-test**	**t (3)=4.4; *P*<0.05**	*
6A	Basal 3MT: pre- *vs.* post-SCH	Ratio paired two-tailed t-test	t (3) = 0.17; *P*>0.87	No
6A	**Basal 5HT: pre- *vs.* post-SCH**	**Ratio paired two-tailed t-test**	**t (3) = 3.5; *P*<0.05**	*
6C	**AUC DA: pre- *vs.* post-SCH**	**Ratio paired two-tailed t-test**	**t (3) = 6.2; *P*<0.05**	**
6C	**AUC 3MT: pre- *vs.* post-SCH**	**Ratio paired two-tailed t-test**	**t (3) = 4.8; *P*<0.05**	*
6C	**AUC 5HT: pre- *vs.* post-SCH**	**Ratio paired two-tailed t-test**	**t (3) = 4.5; *P*<0.05**	*
7B	AUC (%) DA: pre vs. post SCH	Ratio paired two-tailed t-test	t (3) = 2.4; *P*<0.1	Trend
7B	**AUC (%) 3MT: pre vs. post SCH**	**Ratio paired two-tailed t-test**	**t (3) = 3.7; *P*<0.05**	*
7B	AUC (%) 5HT: pre vs. post SCH	Ratio paired two-tailed t-test	t (3) = 0.41; *P*>0.71	No
8A	Basal DA: pre vs. post ETC	Ratio paired two-tailed t-test	t (3) = 0.31; *P*>0.78	No
8A	Basal 3MT: pre vs. post ETC	Ratio paired two-tailed t-test	t (3) = 0.81; *P*>0.47	No
8A	Basal 5HT: pre vs. post ETC	Ratio paired two-tailed t-test	t (2) = 2.7; *P*>0.11	No

8C	AUC DA: pre vs. post ETC	Ratio paired two-tailed t-test	t (3) = 1.5; *P*<0.23	No
8C	AUC 3MT: pre vs. post ETC	Ratio paired two-tailed t-test	t (3) = 3.1; *P*<0.06	Trend
8C	AUC 5HT: pre vs. post ETC	Ratio paired two-tailed t-test	t (2) = 1.4; *P*>0.28	No
9B	AUC (%) DA: pre vs. post ETC	Ratio paired two-tailed t-test	t (3) = 2.6; *P*<0.08	Trend
9B	**AUC (%) 3MT: pre vs. post ETC**	**Ratio paired two-tailed t-test**	**t (3) = 4.4; *P*<0.05**	*
9B	AUC (%) 5HT: pre vs. post ETC	Ratio paired two-tailed t-test	t (2) = 1.8; *P*>0.21	No
S3B	**AUC (%) DA: control vs. chrimson**	**Unpaired two-tailed t-test**	**t (18) = 5.9; *P*<0.001**	***
S3B	**AUC (%) 3MT: control vs. chrimson**	**Unpaired two-tailed t-test**	**t (18) = 4.1; *P*<0.001**	***
S3B	**AUC (%) 5HT: control vs. chrimson**	**Unpaired two-tailed t-test**	**t (15) = 3.1; *P*<0.01**	**

*P < 0.05, **P < 0.01, ***P < 0.001