

SUPPLEMENTARY INFORMATION

Dopamine and serotonin inversely modulate D2 medium spiny neurons to regulate cocaine reward

Daniel F. Cardozo Pinto^{1,5,6,*‡}, Michaela Y. Guo^{1,*}, Matthew B. Pomrenze¹, Wade Morishita¹, Mona X. Li¹, Larry S. Zweifel^{2,3}, Neir Eshel^{4,†,‡}, and Robert C. Malenka^{1,†,‡}

¹ Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305, USA

² Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98195, USA

³ Department of Pharmacology, University of Washington, Seattle, WA 98195, USA

⁴ Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305, USA

⁵ Current address: Society of Fellows, Harvard University, Cambridge, MA 02138, USA

⁶ Current address: Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA

* These authors contributed equally

† These authors jointly supervised this work

‡ Correspondence: dcardozopinto@fas.harvard.edu

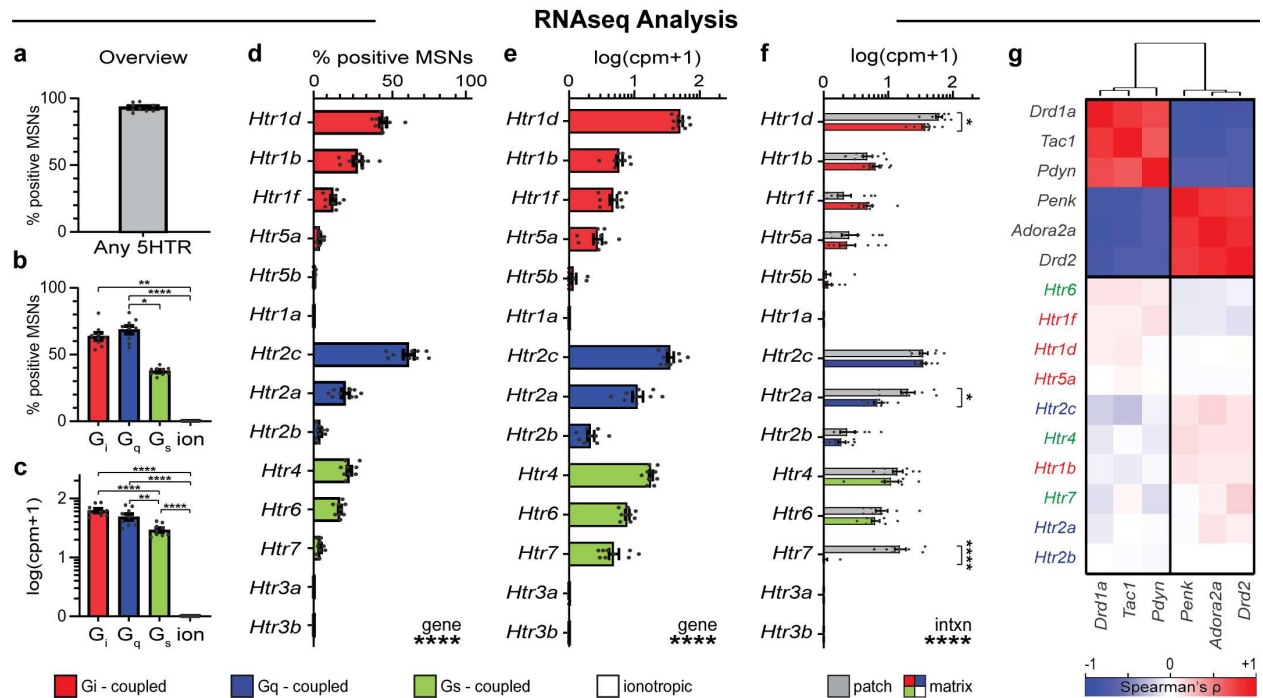
neshel@stanford.edu

malenka@stanford.edu

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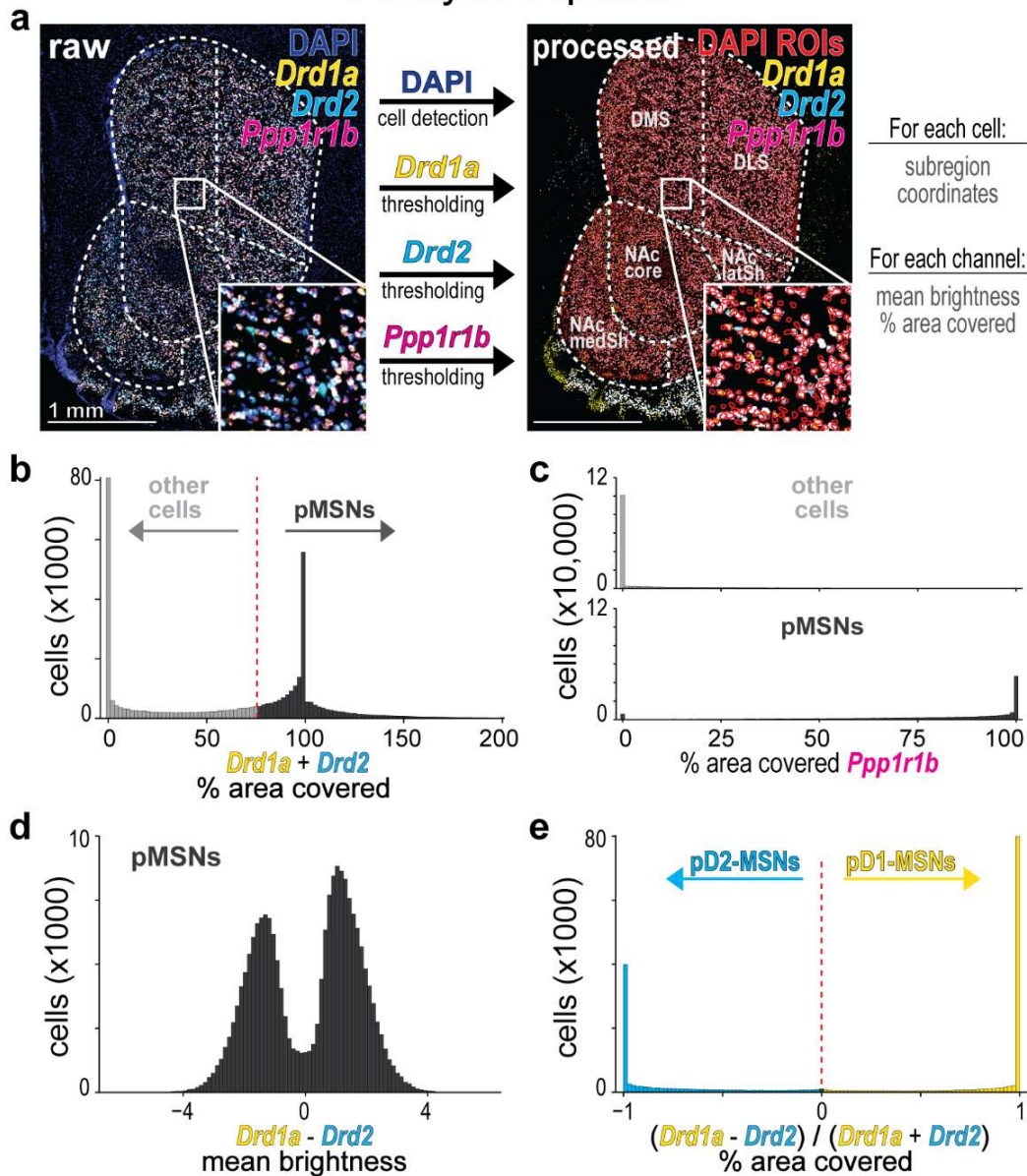
Supplementary Table 1



Supplementary Fig. 1: RNAseq analysis of 5HT receptor expression in striatal MSNs

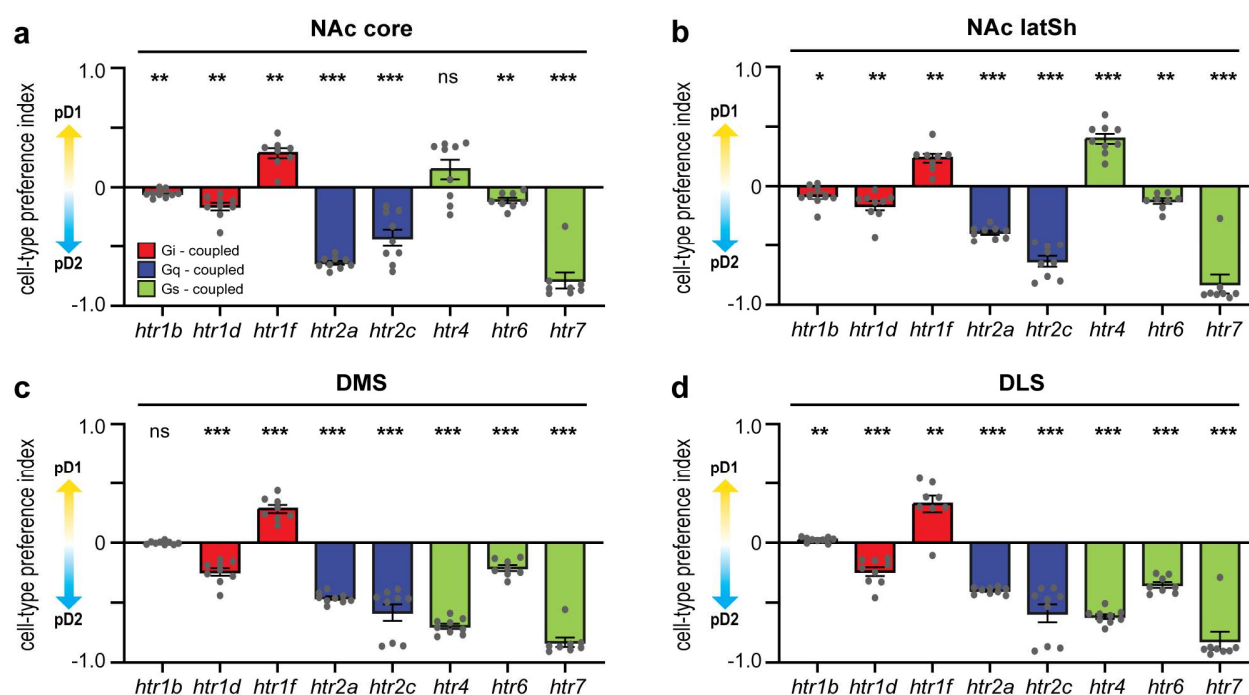
a, Percentage of MSNs expressing at least one copy of any 5HT receptor gene. **b**, Same as **a** but broken down by receptor type. **c**, Average expression level of 5HT receptor genes broken down by receptor type. **d**, Percentage of MSNs expressing at least one copy of each 5HT receptor gene. **e**, Average expression level of each 5HT receptor gene. **f**, Same as **e** but broken down by patch and matrix compartments. **g**, Heatmap showing correlation between expression levels of D1 and D2 MSN marker genes and a subset of 5HT receptor genes. Data in **a-g** are from ref 22 and patch and matrix identities were assigned in the original study on the basis of marker gene expression. In **a-f**, $n=9$ mice. In **g**, $n=1019$ MSNs. cpm, counts per million reads.

Analysis Pipeline



Supplementary Fig. 2: Analysis pipeline validation for fluorescence *in situ* hybridization experiments

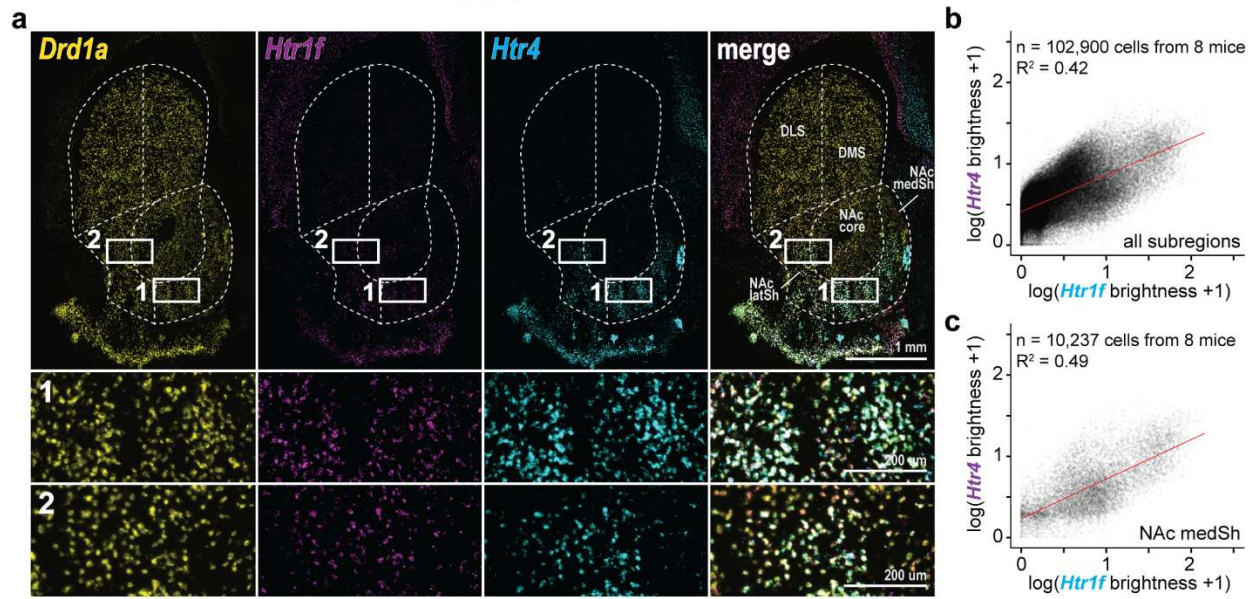
a, Depiction of image analysis pipeline for fluorescence *in situ* hybridization experiments. Anatomical regions were manually annotated, cell-detection was done based on DAPI signal, and for the remaining fluorescence channels the brightness (before thresholding) and fraction of area covered (after thresholding) were measured in each cell. See Methods for additional details. **b**, Histogram depicting percentage of area covered by *Drd1a* or *Drd2* signal in each cell. **c**, Histogram depicting percentage of area covered by signal from the MSN marker *Ppp1r1b* in putative non-MSNs (top) and pMSNs (bottom). In **b-c**, $n=376,311$ cells from 4 mice. **d**, Histogram depicting the difference in brightness between the scaled *Drd1a* and *Drd2* signals for all pMSNs. **e**, Histogram depicting the cell-type index score for all pMSNs, defined on the basis of their *Drd1a* and *Drd2* signals. In **d-e**, $n=199,127$ pMSNs from 4 mice.



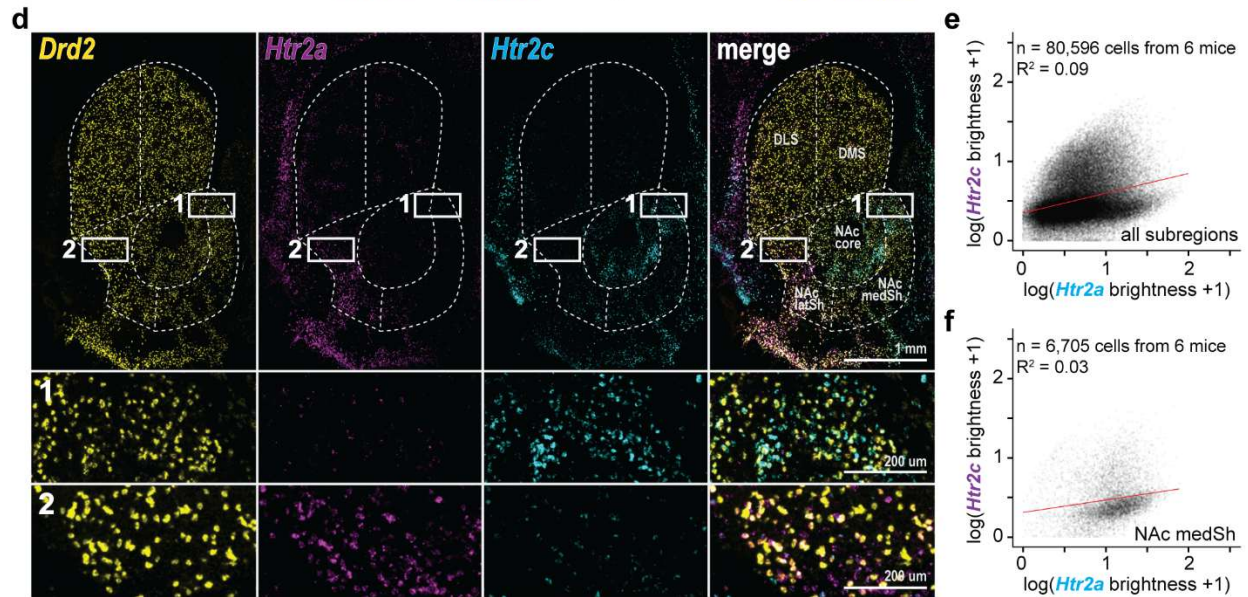
Supplementary Fig. 3: Cell-type preference analysis for 5HT receptor expression across striatal regions

a, Cell-type preference analysis for 5HT receptor expression in the NAc^{core}. **b**, Same as **a** but for the NAc^{latSh}. **c**, Same as **a** but for the DMS. **d**, Same as **a** but for the DLS. In **a-d**, n=8-9 mice.

Htr1f & *Htr4* Coexpression in D1-MSNs

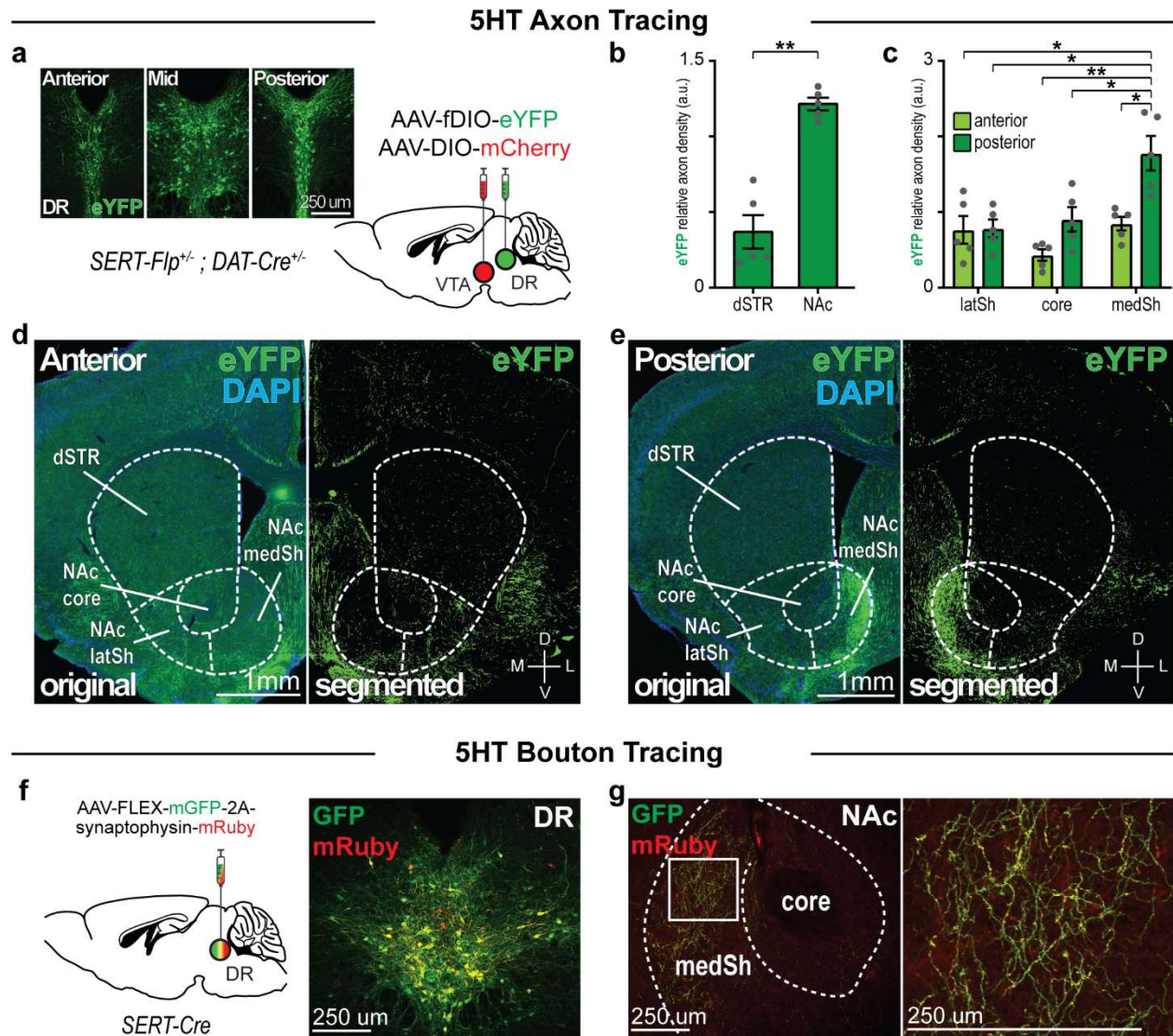


Htr2a & *Htr2c* Coexpression in D2-MSNs



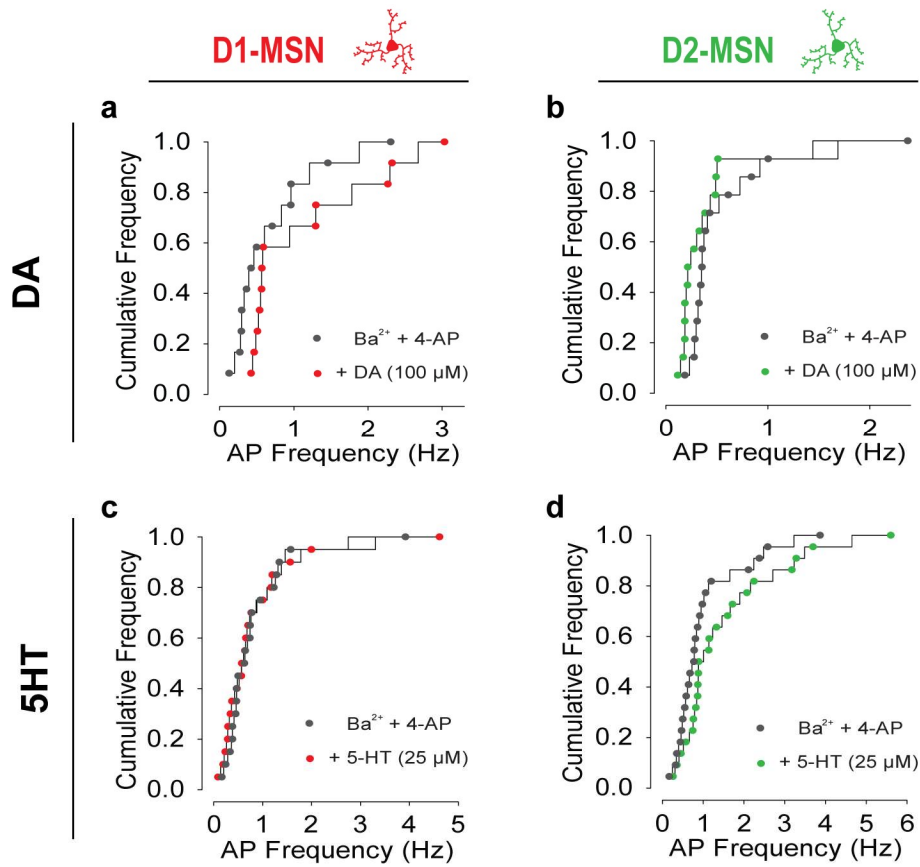
Supplementary Fig. 4: Co-expression analysis for 5HT receptors enriched on D1- or D2-MSNs

a, Example images of *Drd1a*, *Htr1f*, and *Htr4* co-expression in the striatum. **b**, Correlation between *Htr1f* and *Htr4* expression in pD1-MSNs across all striatal subregions. **c**, Correlation between *Htr1f* and *Htr4* expression in pD1-MSNs in the NAc^{medSh}. **d**, Example images of *Drd2*, *Htr2a*, and *Htr2c* co-expression in the striatum. **e**, Correlation between *Htr2a* and *Htr2c* expression in pD2-MSNs across all striatal subregions. **f**, Correlation between *Htr2a* and *Htr2c* expression in pD2-MSNs in the NAc^{medSh}.



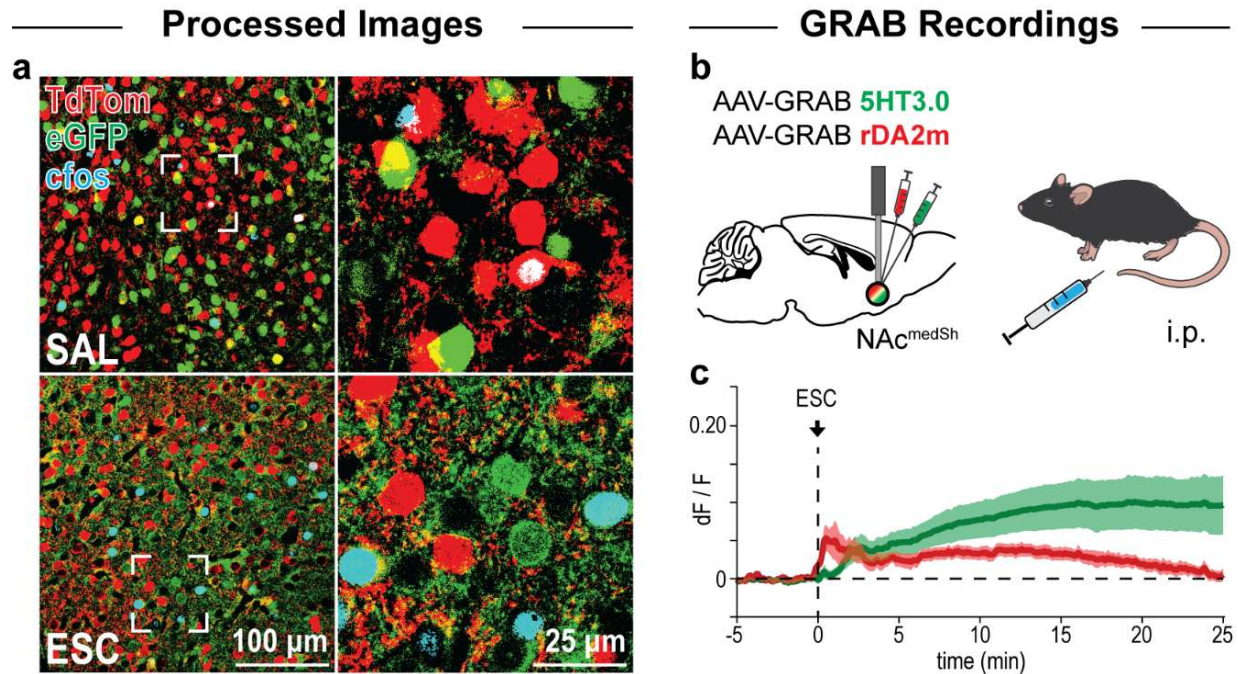
Supplementary Fig. 5: 5HT inputs to striatum are topographically organized

a, Surgical strategy (right) for labeling DR^{5HT} neurons and example images of the injection site (left). **b**, Quantification of axon density in the dSTR and NAc (left). **c**, Quantification of axon density across subregions of the NAc. In **b-c**, $n=5$ mice. **d-e**, Example images of labeled DR^{5HT} in the anterior (**d**) and posterior (**e**) striatum. **f**, Left, surgical strategy for labeling axons and presynaptic boutons in DR^{5HT} neurons. Right, example image of the injection site. **g**, Left, example images of labeled DR^{5HT} axons and presynaptic boutons in the NAc. Right, inset of boxed region on the left. Credits: mouse brain schematic, adapted from ref.⁹¹ (<https://creativecommons.org/licenses/by/4.0/>).



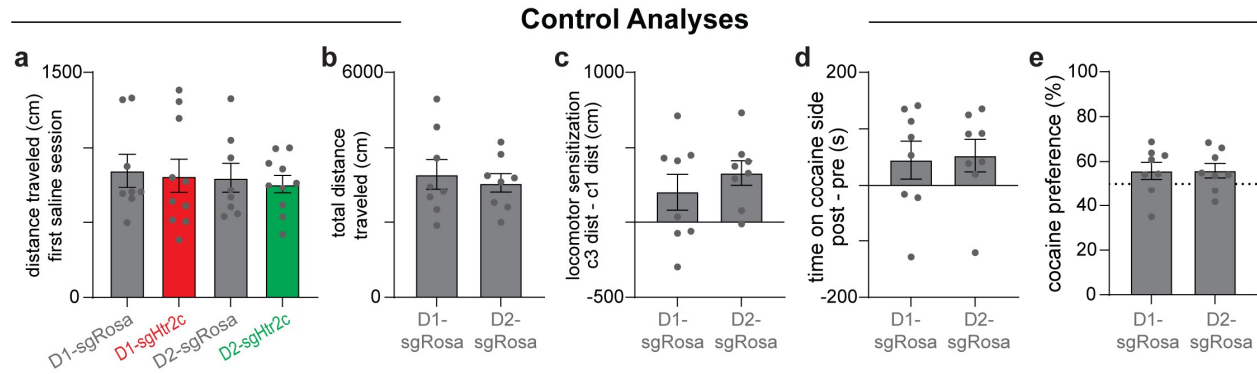
Supplementary Fig. 6: Cumulative frequency plots for cell-attached recording experiments

a-b, Cumulative frequency plots for cell-attached recordings from D1- (**a**, $n=12$ cells from 4 mice) and D2- (**b**, $n=14$ cells from 4 mice) MSNs before and after bath application of DA. **c** ($n=20$ cells from 6 mice), **d** ($n=22$ cells from 9 mice), Same as **a-b** but for bath application of 5HT.



Supplementary Fig. 7: Additional controls for intra-NAc drug infusion experiments

a, Examples of *cfos* expression in saline- and escitalopram- treated *Drd1a-TdTomato;Drd2-eGFP* mice after image pre-processing. **b**, Schematic of experimental strategy to measure striatal DA and 5HT release following high dose (50 mg/kg, i.p.) escitalopram. **c**, GRAB sensor recordings showing escitalopram evokes robust and sustained striatal 5HT release with modest, partially transient effects on striatal DA (n=6 mice). Credits: mouse, adapted from NIH BioArt (<https://bioart.niaid.nih.gov/bioart/279>); mouse brain schematic, adapted from ref.⁹¹ (<https://creativecommons.org/licenses/by/4.0/>).



Supplementary Fig. 8: Additional control analyses for cocaine CPP experiments

a, *Htr2c* loss-of-function in D1- or D2- MSNs has no effect on baseline locomotor behavior. **b-e**, Behavioral performance of D1-sgRosa and D2-sgRosa control groups was identical across measures of cocaine's locomotor (**b**), sensitization (**c**), and rewarding (**d-e**) effects. In **a**, $n=8$ D1-sgRosa mice, $n=8$ D2-sgRosa mice, $n=10$ D1-sgHtr2c mice, and $n=10$ D2-sgHtr2c mice. In **b-e**, $n=8$ D1-sgRosa mice, $n=8$ D2-sgRosa mice.

Supplementary Table 1: Details and results for all statistical tests in the manuscript

Figure	Group	n	units	Statistical Tests (all two-tailed)	Test Statistics	P values	Post-hoc Tests (all two-tailed)	P values	
1a	DLS	9	mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests			STRdl	pD1 vs pD2	0.0225
	DMS	9	mice	subregion	F (2.017, 16.13) = 114.9	P<0.0001	STRdm	pD1 vs pD2	0.9372
	Nac medSh	9	mice	cell-type	F (1.000, 8.000) = 1.414	P=0.2685	NACmed	pD1 vs pD2	0.0018
	Nac core	9	mice	subregion x cell-type	F (1.266, 10.13) = 19.71	P=0.0008	NACcor	pD1 vs pD2	0.0061
	Nac latSh	9	mice				NAClat	pD1 vs pD2	0.0395
1b	DLS	9	mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests			STRdl	pD1 vs pD2	0.0004
	DMS	9	mice	subregion	F (2.697, 21.57) = 24.41	P<0.0001	STRdm	pD1 vs pD2	0.0003
	Nac medSh	9	mice	cell-type	F (1.000, 8.000) = 39.54	P=0.0002	NACmed	pD1 vs pD2	0.0727
	Nac core	9	mice	subregion x cell-type	F (3.165, 25.32) = 27.00	P<0.0001	NACcor	pD1 vs pD2	0.0008
	Nac latSh	9	mice				NAClat	pD1 vs pD2	0.0008
1c	DLS	8	mice	Two-Way RM ANOVA				pD1 vs pD2	
	DMS	8	mice	subregion	F (1.781, 12.47) = 26.82	P<0.0001		pD1 vs pD2	
	Nac medSh	8	mice	cell-type	F (1.000, 7.000) = 32.87	P=0.0007		pD1 vs pD2	
	Nac core	8	mice	subregion x cell-type	F (1.618, 11.33) = 3.708	P=0.0648		pD1 vs pD2	
	Nac latSh	8	mice					pD1 vs pD2	
1d	DLS	9	mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests on Box-Cox transformed data (lambda=0.5)			STRdl	pD1 vs pD2	<0.0001
	DMS	9	mice	subregion	F (2.023, 16.19) = 93.05	P<0.0001	STRdm	pD1 vs pD2	<0.0001
	Nac medSh	9	mice	cell-type	F (1.000, 8.000) = 449.9	P<0.0001	NACmed	pD1 vs pD2	0.0003
	Nac core	9	mice	subregion x cell-type	F (1.820, 14.56) = 17.56	P=0.0002	NACcor	pD1 vs pD2	<0.0001
	Nac latSh	9	mice				NAClat	pD1 vs pD2	<0.0001
1e	DLS	9	mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests on Box-Cox transformed data (lambda=0)			STRdl	pD1 vs pD2	0.0015
	DMS	9	mice	subregion	F (1.416, 11.33) = 51.94	P<0.0001	STRdm	pD1 vs pD2	0.0015
	Nac medSh	9	mice	cell-type	F (1.000, 8.000) = 49.89	P=0.0001	NACmed	pD1 vs pD2	<0.0001
	Nac core	9	mice	subregion x cell-type	F (1.950, 15.60) = 5.228	P=0.0189	NACcor	pD1 vs pD2	0.0015
	Nac latSh	9	mice				NAClat	pD1 vs pD2	<0.0001
1f	DLS	9	mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests on Box-Cox transformed data (lambda=0)			STRdl	pD1 vs pD2	<0.0001
	DMS	9	mice	subregion	F (2.396, 19.16) = 259.4	P<0.0001	STRdm	pD1 vs pD2	<0.0001
	Nac medSh	9	mice	cell-type	F (1.000, 8.000) = 21.55	P=0.0017	NACmed	pD1 vs pD2	<0.0001
	Nac core	9	mice	subregion x cell-type	F (2.109, 16.87) = 163.2	P<0.0001	NACcor	pD1 vs pD2	0.1016
	Nac latSh	9	mice				NAClat	pD1 vs pD2	<0.0001
1g	DLS	8	mice	Two-Way RM ANOVA			STRdl	pD1 vs pD2	0.0003
	DMS	8	mice	subregion	F (2.747, 19.23) = 25.91	P<0.0001	STRdm	pD1 vs pD2	0.0009
	Nac medSh	8	mice	cell-type	F (1.000, 7.000) = 41.96	P=0.0003	NACmed	pD1 vs pD2	0.1456
	Nac core	8	mice	subregion x cell-type	F (2.180, 15.26) = 72.43	P<0.0001	NACcor	pD1 vs pD2	0.0047
	Nac latSh	8	mice				NAClat	pD1 vs pD2	0.0047
1h	DLS	8	mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests on Box-Cox transformed data (lambda=0)			STRdl	pD1 vs pD2	0.0002
	DMS	8	mice	subregion	F (2.339, 16.37) = 23.56	P<0.0001	STRdm	pD1 vs pD2	<0.0001
	Nac medSh	8	mice	cell-type	F (1.000, 7.000) = 92.50	P<0.0001	NACmed	pD1 vs pD2	0.0002
	Nac core	8	mice	subregion x cell-type	F (2.260, 15.82) = 11.53	P=0.0006	NACcor	pD1 vs pD2	0.0001
	Nac latSh	8	mice				NAClat	pD1 vs pD2	0.0002
1k	htr1b	9	mice	One-Sample T-tests (hypothetical mean = 0) with Holm-Sidak's multiplicity adjustment	t=5.072, df=8	0.004			
	htr1d	9	mice		t=1.731, df=8	0.2288			
	htr1f	8	mice		t=3.583, df=7	0.0265			
	htr2a	9	mice		t=5.577, df=8	0.0025			
	htr2c	9	mice		t=14.99, df=8	0.0008			
	htr4	9	mice		t=26.56, df=8	0.0008			
	htr6	8	mice		t=1.372, df=7	0.2288			
	htr7	8	mice		t=8.974, df=7	0.0008			
2c	pD1-MSNs	32 cells / 10 mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests				pD1-MSNs	ACSF vs BA/4AP	<0.0001
	pD2-MSNs	36 cells / 13 mice	cell-type x bath solution	F (1, 66) = 0.08219	P=0.7753	pD2-MSNs	ACSF vs BA/4AP	<0.0001	
			cell-type bath solution	F (1, 66) = 0.08219	P=0.7753				
2e	pD1-MSNs	12 cells / 4 mice	Wilcoxon matched-pairs signed rank test	F (1, 66) = 77.52	P<0.0001				
2g	pD2-MSNs	14 cells / 4 mice	Wilcoxon matched-pairs signed rank test		0.0015				
2i	pD1-MSNs	20 cells / 6 mice	Wilcoxon matched-pairs signed rank test		0.0256				
2k	pD2-MSNs	22 cells / 9 mice	Wilcoxon matched-pairs signed rank test		0.6477				
2l	pD1-MSN DA	12 cells / 4 mice	Two-Way ANOVA with Holm-Sidak post-hoc tests				DA	pD1 vs pD2	0.0056
	pD2-MSN DA	14 cells / 4 mice	cell-type x neuromodulator	F (1, 64) = 17.58	P<0.0001	5HT	pD1 vs pD2	0.0051	
	pD1-MSN 5HT	20 cells / 6 mice	neuromodulator	F (1, 64) = 0.9719	P=0.3279				
	pD2-MSN 5HT	22 cells / 9 mice	cell-type	F (1, 64) = 0.09852	P=0.7546				
2m	pD1-MSN DA	12 cells / 4 mice	Two-Way ANOVA with Holm-Sidak post-hoc tests				DA	pD1 vs pD2	<0.0001
	pD2-MSN DA	14 cells / 4 mice	cell-type x neuromodulator	F (1, 64) = 35.23	P<0.0001	5HT	pD1 vs pD2	0.0005	
	pD1-MSN 5HT	20 cells / 6 mice	neuromodulator	F (1, 64) = 0.0001742	P=0.9895				
	pD2-MSN 5HT	22 cells / 9 mice	cell-type	F (1, 64) = 1.947	P=0.1677				
2o	pD2-MSNs	18 cells / 6 mice	Paired t-test	t=2.595, df=17	0.0189				
2r	SAL	3 mice	Unpaired t-test	t=0.03859, df=6	0.9705				
2s	ESC	5 mice							
2t	SAL	3 mice	Unpaired t-test	t=3.293, df=6	0.0166				
	ESC	5 mice							
2t	SAL	3 mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests				SAL	pD1 vs pD2	0.0212
	ESC	5 mice	drug x cell-type	F (1, 6) = 28.72	P=0.0017	ESC	pD1 vs pD2	0.0063	
			drug	F (1, 6) = 0.000	P>0.9999	pD1-MSNs	SAL vs ESC	0.0003	
			cell-type	F (1, 6) = 0.2118	P=0.6616	pD2-MSNs	SAL vs ESC	0.0003	
3c	D1-sgRosa	8 mice	Two-Way RM ANOVA						
	D1-sgHtr2c	10 mice	Time x Group	F (1.726, 27.62) = 0.7288	P=0.4724				
			Time	F (1.726, 27.62) = 10.76	P=0.0006				
3d	D1-sgRosa	8 mice	Welch's unpaired t-test	F (1, 16) = 0.01354	P=0.9088				
	D1-sgHtr2c	10 mice		t=1.280, df=13.87	0.2217				
3e	D1-sgRosa	8 mice	Two-Way RM ANOVA						
	D1-sgHtr2c	10 mice	Time x Group	F (1, 16) = 2.169	P=0.1602				
			Time	F (1, 16) = 12.65	P=0.0026				
3f	D1-sgRosa	8 mice	Welch's unpaired t-test	F (1, 16) = 1.096	P=0.3108				
	D1-sgHtr2c	10 mice		t=1.444, df=14.54	0.1699				
3g	D2-sgRosa	8 mice	Two-Way RM ANOVA						
	D2-sgHtr2c	10 mice	Time x Group	F (1.414, 22.63) = 1.238	P=0.2954				
			Time	F (1.414, 22.63) = 9.871	P=0.0021				
3h	D2-sgRosa	8 mice	Welch's unpaired t-test	F (1, 16) = 1.736	P=0.2062				
	D2-sgHtr2c	10 mice		t=2.057, df=14.29	0.0584				
3i	D2-sgRosa	8 mice	Two-Way RM ANOVA with Holm-Sidak post-hoc tests				sgRosa	pre vs post	0.1988
	D2-sgHtr2c	10 mice	Time x Group	F (1, 16) = 8.695	P=0.0094	sgHtr2c	pre vs post	<0.0001	
			Time	F (1, 16) = 24.47	P=0.0001				
3j	D2-sgRosa	8 mice	Welch's unpaired t-test	F (1, 16) = 7.886	P=0.0126				
	D2-sgHtr2c	10 mice		t=3.603, df=15.97	0.0024				
3k	sgRosa	16 mice	Ordinary One-way ANOVA				sgRosa vs D1-Htr2c	0.1496	
	D1-sgHtr2c	10 mice		F (2, 33) = 7.831	P=0.0016	sgRosa vs D2-Htr2c		0.0011	
	D2-sgHtr2c	10 mice				D1-Htr2c vs D2-Htr2c		0.0632	
3l	sgRosa	16 mice	Ordinary One-way ANOVA				sgRosa vs D1-Htr2c	0.0915	
	D1-sgHtr2c	10 mice		F (2, 33) = 8.867	P=0.0008	sgRosa vs D2-Htr2c		0.0006	
	D2-sgHtr2c	10 mice				D1-Htr2c vs D2-Htr2c		0.0646	
3m	sgRosa	16 mice	Simple Linear Regression (Y = mX+b)			tests for non-zero slope	goodness of fit		
	D1-sgHtr2c	10 mice	sgRosa: Y = -0.003072*X + 65.66	F(1,14)=1.357	P=0.2635	R^2=0.0884			

	D1-sgHtr2c	10 mice				
	D2-sgRosa	8 mice				
	D2-Htr2c	10 mice				
S8b	D1-sgRosa	8 mice	Weich's unpaired t-test	t=0.4690, df=11.79	0.6476	
	D2-sgRosa	8 mice				
S8c	D1-sgRosa	8 mice	Weich's unpaired t-test	t=0.8956, df=12.13	0.3879	
	D2-sgRosa	8 mice				
S8d	D1-sgRosa	8 mice	Weich's unpaired t-test	t=0.1789, df=13.71	0.8606	
	D2-sgRosa	8 mice				
S8e	D1-sgRosa	8 mice	Weich's unpaired t-test	t=0.01220, df=13.63	0.9904	
	D2-sgRosa	8 mice				