Science Translational Medicine
AAAS

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Supplementary Materials for

Distinct neural mechanisms for the prosocial and rewarding properties of MDMA

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Published 11 December 2019, *Sci. Transl. Med.* **11**, eaaw6435 (2019)
DOI: 10.1126/scitranslmed.aaw6435

The PDF file includes:

Fig. S1. Supplementary information supporting data shown in Fig. 1.
Fig. S2. Supplementary information supporting data shown in Fig. 2D.
Fig. S3. Supplementary information supporting data shown in Fig. 3.
Fig. S4. Low-dose MDMA effect on EPSCs in NAc brain slices is not blocked by an OxtR antagonist.
Table S1. Numerical and statistical data supporting Fig. 1 and fig. S1.
Table S2. Numerical and statistical data supporting Fig. 2.
Table S3. Numerical and statistical data supporting Fig. 3.
Table S4. Numerical and statistical data supporting Fig. 4 and fig. S4.

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/11/522/eaaw6435/DC1)

Data file S1 (Microsoft Excel format). Raw data.

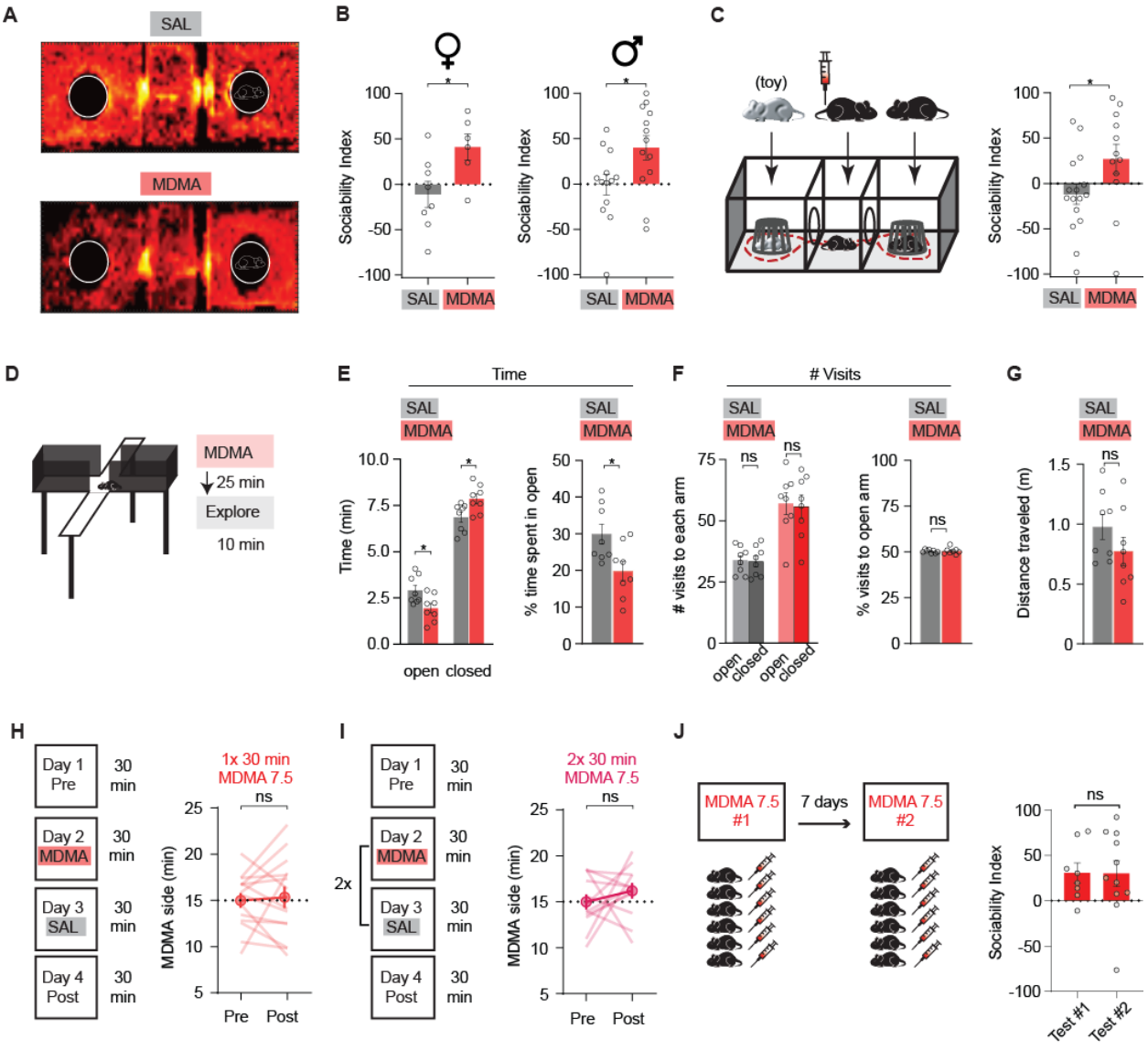

Fig. S1. Supplementary information supporting data shown in Fig. 1. (A) Sample heat map illustrating the relative amounts of time, which a saline- or MDMA-treated mouse spent within each position of the three-chamber testing apparatus over the 30-minute exploration period. Heat map construction: the sum total of visits to each square centimeter was calculated; for visualization, the map (M) was transformed by 1 + log(M); map was interpolated by a factor of 10; a gaussian smoothing function was applied. **(B)** Sociability after MDMA (7.5 mg/kg) in adult female mice (*left*; N=6-8) and adult male mice (*right*; N=13). **(C)** Sociability after MDMA (7.5 mg/kg) when an unfamiliar toy mouse (schematic at *left*) is placed in the empty cup (summary graph at *right*; N=12-16). **(D)** Schematic of elevated-plus maze and experimental time line. **(E)** Time spent in the open-arm areas after either MDMA (7.5 mg/kg) or saline treatment, as measured by total time and % of total time (N=8). **(F)** Same experiment as **(E)**, quantifying the

visits made to each arm as total number and % of total visits. **(G)** Same experiment as **(E)**, distance traveled after MDMA (7.5 mg/kg) compared to saline. **(H)** CPP data after a shorter duration of pairing MDMA 7.5 mg/kg with a unique context (30 min versus the 1 hr shown in **Fig. 1H**; N=14). **(I)** CPP data after two pairings of MDMA 7.5 mg/kg with a unique context (30 min each; N=12). **(J)** Sociability in the same cohort of mice given two separate doses of MDMA 7.5 mg/kg one week apart (N=12 mice in cohort; Day 0, N=8 and Day 7, N=11 mice meeting criteria for measurement). Data shown are means ± s.e.m. Significance was determined between groups using an unpaired t-test for (B), (C), (E), (F, *right*), (G), (J); within group (paired t-test) for (F, *left*), (H), (I). *P<0.05.

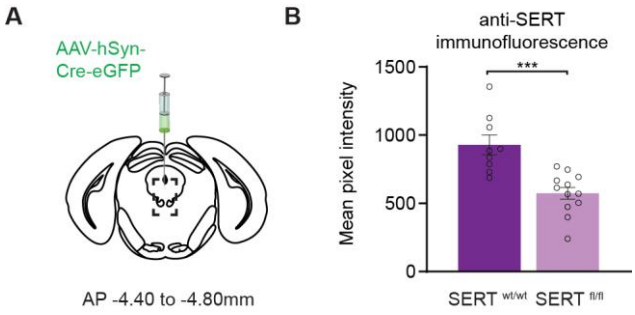

Fig. S2. Supplementary information supporting data shown in Fig. 2D. (A) Schematic drawing of DR injected with AAV-Cre, denoting approximate range of antero-posterior position for slices used to quantify anti-SERT immunofluorescence. **(B)** Anti-SERT immunofluorescence intensity quantification in DR of AAV-Cre injected $SERT^{wt/wt}$ and $SERT^{fl/fl}$ mice. Data shown are means ± s.e.m. Significance was determined between groups (unpaired t-test) for (B). ***P<0.001.

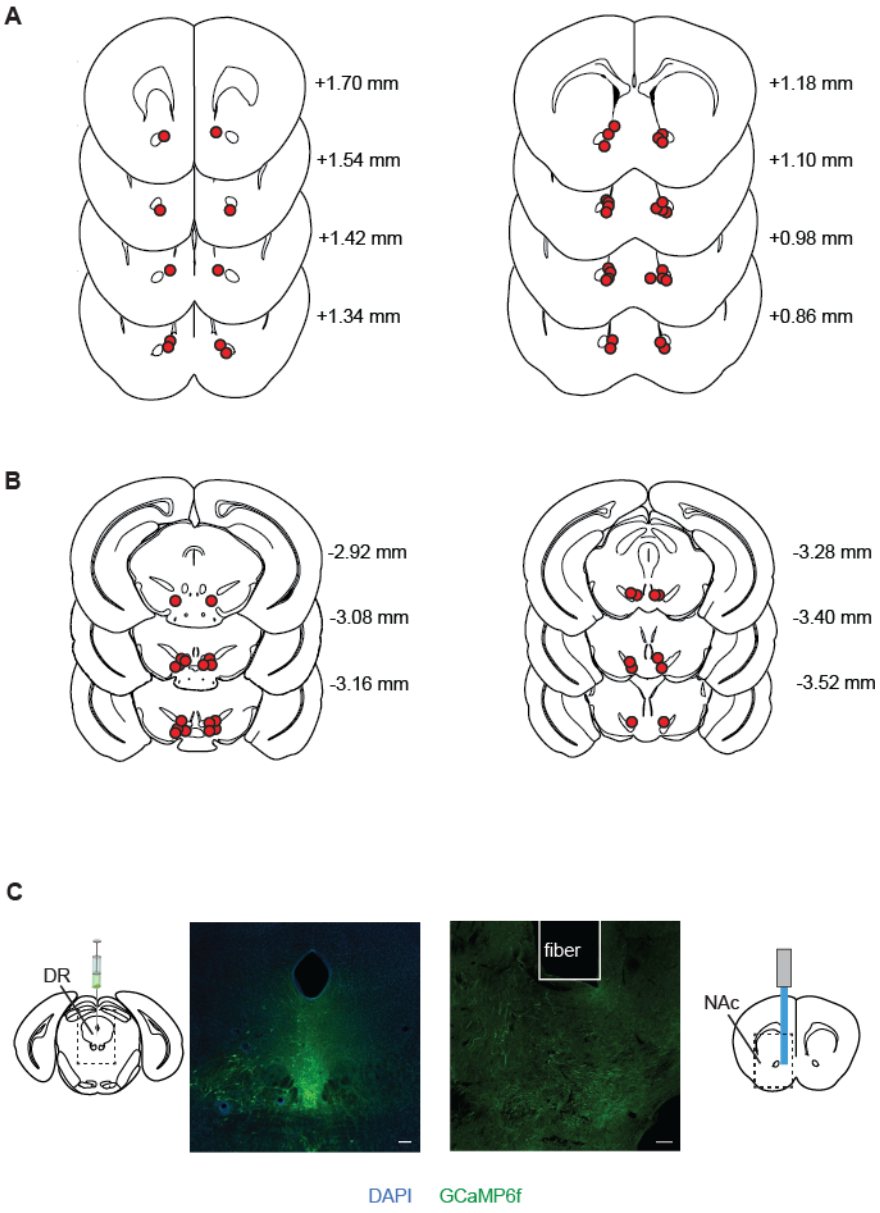

Fig. S3. Supplementary information supporting data shown in Fig. 3. (A and **B)** Location of infuser tips of cannulae implanted for intra-NAc drug delivery **(A; Fig. 3A, C, D)**, and intra-VTA drug delivery **(B; Fig. 3B)**. **(C)** Sample images showing virus injection (DJ-AAV-EF1a-DIO-GCaMP6f) into the DR of SERT-Cre mice *(left)* and optical fiber placement in the NAc *(right)*, for fiber photometry experiments **(Fig. 3E-L)**. GCaMP6f, green; DAPI, blue. Scale bar = 100 μm.

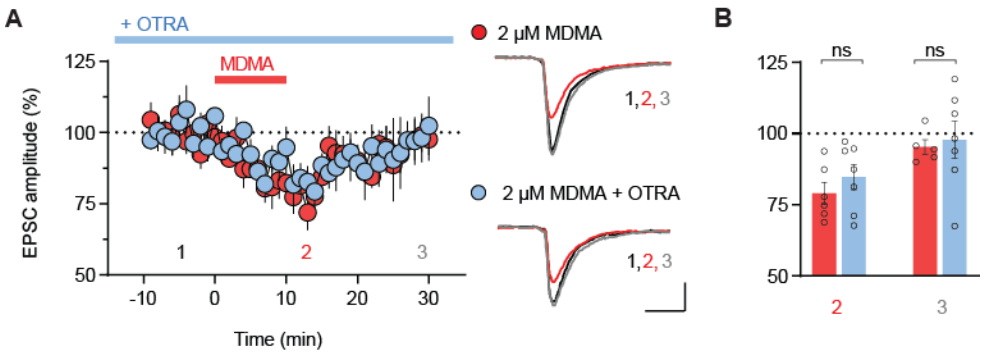

Fig. S4. Low-dose MDMA effect on EPSCs in NAc brain slices is not blocked by an OxtR antagonist. (A) *Left*, Summary timecourse graph showing the effect of a 10 minute bath application of MDMA (2 µM) on EPSC amplitude evoked in NAc MSNs, with and without incubation and continuous application of an OxtR antagonist, L-368,899 (5 µM;N=6-7 cells, 6 mice). *Right*, Sample EPSC traces taken from time points indicated: 1, baseline; 2, peak MDMA effect (10-15 min post infusion); 3, MDMA washout (25-30 min post infusion). Second stimulated EPSCs are omitted for clarity. Scale bars: 100 pA, 10 ms. **(B)** Summary graph of data in **A**, showing EPSC amplitudes at time points 2 and 3 for the 2µM MDMA versus the 2µM MDMA + OTRA groups. Data shown are means ± s.e.m. Significance was determined between groups (unpaired t-test) for (B). ns, P>0.05.

Table S1. Numerical and statistical data supporting Fig. 1 and fig. S1. [a] Planned *post hoc* comparisons performed using Sidak correction for multiple comparisons.

Panel	Group	N	Mean ± SEM (unit)	Primary Statistic	*Post hoc* test[a]
1B	SAL	13	empty, 11.92 ± 0.98 (min) mouse, 12.51 ± 1.10 (min)	One-way ANOVA, unmatched; $F_{7,82}$=6.20, P<0.0001	ns
	MDMA 3 mg/kg	9	empty, 7.76 ± 1.29 (min) mouse, 11.92 ± 1.67 (min)		ns
	MDMA 7.5 mg/kg	13	empty, 7.17 ± 1.13 (min) mouse, 16.21 ± 1.72 (min)		P<0.0001
	MDMA 15 mg/kg	10	empty, 7.94 ± 1.52 (min) mouse, 15.86 ± 1.98 (min)		P=0.0015
1C	SAL	16		Two-way ANOVA, ordinary; Treatment: $F_{1,163}$ = 8.42, P=0.0042 Time: ns	
	MDMA 7.5 mg/kg	14			
1D	SAL	16	-6.31 ± 7.69 (soc. ind.)	Unpaired t-test; P=0.022	
	MDMA 7.5 mg/kg	13	24.49 ± 10.42 (soc. ind.)		
1E	SAL, both mice	12	-2.49 ± 11.26 (soc. ind.)	One-way ANOVA, unmatched; $F_{3,61}$=5.87, P=0.0014	---
	MDMA,	18	14.75 ± 5.14 (soc. ind.)		*vs* SAL, ns
	MDMA, free mouse	20	26.55 ± 7.25 (soc. ind.)		*vs* SAL, P=0.023
	MDMA, both mice	15	42.30 ± 5.42 (soc. ind.)		*vs* SAL, P=0.0005
1F	SAL	10	2.12 ± .33 (m)	One-way ANOVA, unmatched; $F_{3,38}$=9.14, P=0.0001	---
	MDMA 7.5 mg/kg	10	2.18 ± .56 (m)		*vs* SAL, ns
	MDMA 15 mg/kg	11	5.79 ± .65 (m)		*vs* SAL, P=0.0002
1H	MDMA 7.5 mg/kg	10	pre, 15.96 ± 1.02 post, 15.75 ± 1.47	Paired t-test; ns	
	MDMA 15 mg/kg	11	pre 14.66 ± 1.45 post, 18.08 ± 1.43	Paired t-test; P=0.012	
S1B	Female SAL	8	-11.10 ± 14.38 (soc. ind.)	Unpaired t-test; P=0.027	
	Female MDMA 7.5 mg/kg	6	41.15 ± 14.36 (soc. ind.)		
	Male SAL	13	-0.70 ± 11.26 (soc. ind.)	Unpaired t-test; P=0.027	
	Male MDMA 7.5 mg/kg	13	40.14 ± 13.23 (soc. ind.)		
S1C	SAL	16	-11.96 ± 10.99 (soc. ind.)	Unpaired t-test; P=0.049	
	MDMA	12	27.01 ± 16.17 (soc. ind.)		
S1E	Open arm, SAL	8	2.93 ± 0.27 (min)	Unpaired t-test; P=0.020	
	Open arm, MDMA 7.5 mg/kg	8	1.95 ± 0.26 (min)		
	Closed arm, SAL		6.87 ± 0.27 (min)	Unpaired t-test; P=0.020	
	Closed arm, MDMA 7.5 mg/kg		7.86 ± 0.26 (min)		
	Open arm, SAL		29.89 ± 2.76 (% total time)	Unpaired t-test; P=0.020	
	Open arm, MDMA 7.5 mg/kg		19.88 ± 2.63 (% total time)		
S1F	Open arm, SAL	8	33.88 ± 1.95 (# visits)	Paired t-test; ns	
	Closed arm, SAL		33.38 ± 2.15 (# visits)		
	Open arm, MDMA 7.5 mg/kg	8	57.00 ± 4.46 (# visits)	Paired t-test; ns	
	Closed arm, MDMA 7.5 mg/kg		55.88 ± 4.60 (# visits)		
	Open arm, SAL		50.44 ± 0.32 (% total visits)	Unpaired t-test;	

	Open arm, MDMA 7.5 mg/kg		50.53 ± 0.61 (% total visits)	ns	
S1G	SAL	8	9.78 ± 1.04 (m)	Unpaired t-test;	
	MDMA 7.5 mg/kg	8	7.75 ± 1.18(m)	ns	
S1H	MDMA 7.5 mg/kg, 30 min x 1	14	pre, 14.98 ± 0.79 post, 15.33 ± 1.17	Paired t-test; ns	
S1I	MDMA 7.5 mg/kg, 30 min x 2	12	pre 14.91 ± 0.82 post, 16.11 ± 0.83	Paired t-test; ns	
S1J	Day 0 MDMA 7.5 mg/kg	8	30.53 ± 10.93 (soc. ind.)	Unpaired t-test;	
	Day 7 MDMA 7.5 mg/kg	11	29.92 ± 14.37 (soc. ind.)	ns	

Table S2. Numerical and statistical data supporting Fig. 2. [a] Planned *post hoc* comparisons performed using Sidak correction for multiple comparisons.

Panel	Group	N	Mean ± SEM (unit)	Primary Statistic	*Post hoc* test[a]
2A	SCIT + SAL	8	3.02 ± 12.78 (soc. ind.)	One-way ANOVA, unmatched; $F_{2,20}$=4.55, P=0.0235	*vs* SAL + MDMA 7.5 mg/kg, P=0.048
	SAL + MDMA 7.5 mg/kg	9	42.41 ± 10.70 (soc. ind.)		---
	SCIT + MDMA 7.5 mg/kg	6	-4.25 ± 12.59 (soc. ind.)		*vs* SAL + MDMA 7.5 mg/kg, P<0.030
2E	$SERT^{wt/wt}$ SAL	22	1.56 ± 9.78 (soc. ind.)	Unpaired t-test; P=0.0037	
	MDMA 7.5 mg/kg	14	54.80 ± 14.89 (soc. ind.)		
	$SERT^{wt/fl}$ SAL	17	13.24 ± 11.17 (soc. ind.)	Unpaired t-test; ns	
	MDMA 7.5 mg/kg	16	5.21 ± 17.37 (soc. ind.)		
	$SERT^{fl/fl}$ SAL	8	32.73 ±12.54 (soc. ind.)	Unpaired t-test; P=0.034	
	MDMA 7.5 mg/kg	14	-28.85 ± 19.00 (soc. ind.)		
2F	$SERT^{wt/wt}$ MDMA 15 mg/kg	10	Pre, 14.70 ± 0.73 (min) Post, 17.14 ± 1.09 (min)	Paired t-test; P=0.038	
	$SERT^{wt/fl}$ MDMA 15 mg/kg	22	Pre, 15.43 ± 0.36 (min) Post, 17.47 ± 0.93 (min)	Paired t-test; P=0.033	
	$SERT^{fl/fl}$ MDMA 15 mg/kg	11	Pre, 14.75 ± 0.70 (min) Post, 16.53 ± 1.02 (min)	Paired t-test; P=0.049	
2G	SAL + SAL	15	-16.11 ± 13.61 (soc. ind.)	One-way ANOVA, unmatched; $F_{2,30}$=6.32, P=0.0051	*vs* JHW + MDMA 7.5 mg/kg, P=0.0084
	JHW + MDMA 7.5 mg/kg	8	53.33 ± 15.06 (soc. ind.)		---
	JHW + SAL	10	-25.85 ± 17.33 (soc. ind.)		*vs* JHW + MDMA 7.5 mg/kg, P=0.0055
2H	SAL	10		Two-way ANOVA, ordinary; Treatment: $F_{1,163}$ = 8.42, P=0.029 Time: ns	
	METH 2 mg/kg	9			
2I	SAL, Day 0	8	2.96 ± 0.27 (m)	One-way ANOVA, repeated measures; $F_{3,21}$=50.96, P<0.0001	*vs* METH 2mg/kg Day 1, P=0.0010
	METH 2 mg/kg, Day 1		6.90 ± 0.71 (m)		---
	METH 2 mg/kg, Day 2		12.29 ± 1.06 (m)		---
	METH 2 mg/kg, Day 3		13.30 ± 1.06 (m)		*vs* METH 2mg/kg Day 1, P<0.00001
2J	METH 2 mg/kg	12	Pre, 14.08 ± 1.54 (min) Post, 16.80 ± 1.05 (min)	Paired t-test; P=0.041	
S2B	$SERT^{wt/wt}$ (4 mice)	9	929.6 ± 71.3 (intensity)	Unpaired t-test; P=0.0003	
	$SERT^{fl/fl}$ (6 mice)	12	574.2 ± 44.0 (intensity)		

Table S3. Numerical and statistical data supporting Fig. 3. [a] Planned *post hoc* comparisons performed using Sidak correction for multiple comparisons.

Panel	Group	N	Mean ± SEM (unit)	Primary Statistic	*Post hoc* test[a]
3A	Intra-NAc SCIT + SAL i.p.	20	11.54 ± 7.91 (soc. ind.)	One-way ANOVA, unmatched; $F_{2,51}$=3.36, P=0.043	*vs* Intra-NAc SAL + MDMA 7.5 mg/kg i.p., ns
	Intra-NAc SAL + MDMA 7.5 mg/kg i.p.	15	34.71 ± 13.94 (soc. ind.)		---
	Intra-NAc SCIT + MDMA 7.5 mg/kg i.p.	19	-6.52 ± 11.28 (soc. ind.)		*vs* Intra-NAc SAL + MDMA 7.5 mg/kg i.p., P=0.025
3B	Intra-VTA SCIT + SAL i.p.	18	-7.52 ± 9.01 (soc. ind.)	One-way ANOVA, unmatched; $F_{2,41}$=7.08, P=0.0023	*vs* Intra-VTA SAL + MDMA 7.5 mg/kg i.p., P=0.0042
	Intra-VTA SAL + MDMA 7.5 mg/kg i.p.	13	38.92 ± 10.38 (soc. ind.)		---
	Intra-VTA SCIT + MDMA 7.5 mg/kg i.p.	13	35.67 ± 11.42 (soc. ind.)		*vs* Intra-VTA SAL + MDMA 7.5 mg/kg i.p., ns
3C	Intra-NAc SAL + MDMA 7.5 mg/kg i.p.	13	40.14 ± 13.23 (soc. ind.)	One-way ANOVA, unmatched; $F_{2,33}$=7.89, P=0.0016	*vs* Intra-NAc SAL + SAL i.p., P=0.014
	Intra-NAc SAL + SAL i.p.	11	-1.27 ± 10.12 (soc. ind.)		---
	Intra-NAc MDMA + SAL i.p.	12	55.70 ± 4.56 (soc. ind.)		*vs* Intra-NAc SAL + SAL i.p., P=0.0010
3D	Intra-NAc SAL + MDMA 15 mg/kg i.p.	16	Pre, 14.82 ± 0.65 (min) Post, 17.17 ± 0.72 (min)	Paired t-test; P=0.012	
	Intra-NAc SCIT + MDMA 15 mg/kg i.p.	16	Pre, 15.00 ± 0.44 (min) Post, 18.45 ± 1.03 (min)	Paired t-test; P=0.0015	
	Intra-NAc RAC + MDMA 15 mg/kg i.p.	13	Pre, 14.86 ± 0.98 (min) Post, 15.13 ± 1.16 (min)	Paired –test; ns	
3G	SAL	3	94.21 ± 0.39 (% baseline std. dev.)	Unpaired t-test; P<0.0001	
	MDMA 7.5 mg/kg	3	21.74 ± 4.46 (% baseline std. dev.)		
3K	SERT-Cre, + GCaMP6f	5	Mouse, 2140.0 ± 445.3 (cum. z) Empty, 941.9 ± 263.4 (cum. z)	Unpaired t-test; P=0.049	
3L	SERT-Cre, + GCaMP6f	5	Slope = 0.22 ± 0.057; Y-intercept, 1.77 ± 0.41; X-intercept, -5.40	Linear regression; $F_{1,3}$=14.73, P=0.031	

Table S4. Numerical and statistical data supporting Fig. 4 and fig. S4. [a] Planned *post hoc* comparisons performed using Sidak correction for multiple comparisons.

Panel	Group	N	Mean ± SEM (unit)	Primary Statistic	*Post hoc* test[a]
4C	D1 MSN (7 mice)	8	72.78 ± 7.98 (% baseline EPSC)	Unpaired t-test, ns	
	D2 MSN (6 mice)	7	65.84 ± 5.84 (% baseline EPSC)		
4D	OTRA + SAL	8	-13.95 ± 9.84 (soc. ind.)	One-way ANOVA, unmatched; $F_{4,43}$=3.11, P=0.025	---
	SAL + MDMA 7.5 mg/kg	12	32.88 ± 11.10 (soc. ind.)		*vs* OTRA+ SAL, P=0.031
	OTRA + MDMA 7.5 mg/kg	12	30.70 ± 11.51 (soc. ind.)		*vs* OTRA + SAL, P=0.042
	OTRA high dose + MDMA 7.5 mg/kg	8	34.24 ± 10.61 (soc. ind.)		*vs* OTRA + SAL, P=0.046
	OTRA multiple dose + MDMA 7.5 mg/kg	8	46.45 ± 16.23 (soc. ind.)		*vs* OTRA + SAL, P=0.0086
4E	Intra-NAc SAL + SAL i.p.	11	-10.65 ± 11.69 (soc. ind.)	One-way ANOVA, unmatched; $F_{3,35}$=7.85, P=0.0004	---
	Intra-NAc SAL + MDMA 7.5 mg/kg i.p.	13	39.06 ± 11.23 (soc. ind.)		*vs* Intra-NAc SAL + SAL i.p., P=0.024
	Intra-NAc OTRA + MDMA 7.5 mg/kg i.p.	9	74.31 ± 12.36 (soc. ind.)		*vs* Intra-NAc SAL + SAL i.p., P=0.0003
4F	SAL	17	-2.39 ± 11.78 (soc. ind.)	Unpaired t-test; P=0.048	
	MDMA 7.5 mg/kg	14	33.29 ± 12.51 (soc. ind.)		
4G	Intra-NAc SAL + MDMA 7.5 mg/kg i.p.	12	32.84 ± 15.38 (soc. ind.)	Unpaired t-test, P=0.035	
	Intra-NAc NAS-181 + MDMA 7.5 mg/kg i.p.	11	-17.72 ± 16.43 (soc. ind.)		
4I	MDMA (5 mice)	8	70.10 ± 6.92 (% baseline EPSC)	Unpaired t-test, P=0.0087	
	NAS-181 (3 mice)	5	101.42 ± 5.44 (% baseline EPSC)		
4J	SAL	16	-2.69 ± 10.77 (soc. ind.)	One-way ANOVA, unmatched; $F_{3,46}$=3.43, P=0.025	---
	FEN 1 mg/kg	10	7.41 ± 4.01 (soc. ind.)		*vs* SAL, ns
	FEN 5 mg/kg	13	29.69 ± 8.27 (soc. ind.)		*vs* SAL, P=0.048
	FEN 10 mg/kg	11	34.42 ± 12.04 (soc. ind.)		*vs* SAL, P=0.027
4K	SAL	14		Two-way ANOVA, ordinary; Treatment: $F_{1,138}$=19.76, P<0.0001. Time: ns	
	FEN 10	11			
4L	FEN (3 mice)	5	69.35 ± 4.93 (% baseline EPSC)		
S4B	Time 2 MDMA (6 mice)	6	79.07 ± 3.92 (% baseline EPSC)	Unpaired t-test, ns	
	MDMA + OTRA (6 mice)	7	84.76 ± 4.45 (% baseline EPSC)		
	Time 3 MDMA (5 mice)	5	95.30 ± 2.52 (% baseline EPSC)	Unpaired t-test, ns	
	MDMA + OTRA (6 mice)	7	97.82 ± 6.50 (% baseline EPSC)		