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Supplemental Information

The Projection Targets of Medium Spiny Neurons Govern Cocaine-Evoked Synaptic Plasticity in the Nucleus Accumbens

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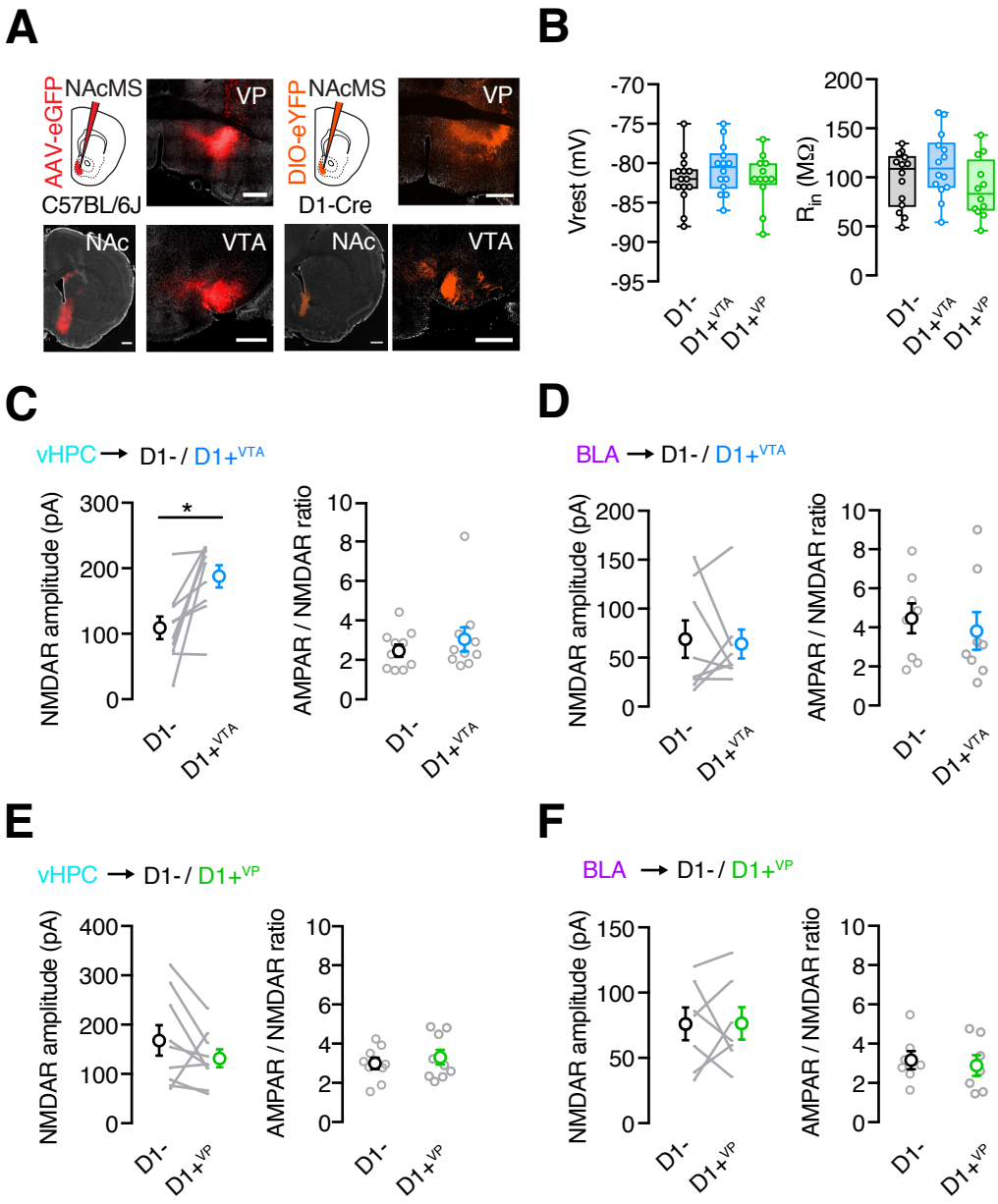
A
AAV-eGFP
NAcMS
VP
C57BL/6J
DIO-eYFP
D1-Cre
NAc
VTA
B
Vrest (mV)
R_{in} (MΩ)
D1-
D1+VTA
D1+VP
C
vHPC → D1- / D1+VTA
NMDAR amplitude (pA)
AMPAR / NMDAR ratio
*
D
BLA → D1- / D1+VTA
E
vHPC → D1- / D1+VP
F
BLA → D1- / D1+VP

Figure S1

Figure S1: Intrinsic properties of NAcMS neurons and baseline vHPC and BLA inputs onto D1+^{VTA} and D1+^{VP} MSNs. Related to Figure 1 and 2. A) Schematics and representative images of AAV-eGFP or AAV-DIO-eYFP injections into the NAcMS of C57BL/6J (left, red) or D1-CRE (right, orange) mice (scale bar = 500 μm). D1+ MSNs project to both the VP and VTA (scale bar = 500 μm). **B)** Summary of resting membrane potential (left) and input resistance (right) of D1- (black), D1+^{VTA} (blue), and D1+^{VP} (green) MSNs. There were no differences for resting membrane potential (D1- = -82 ± 0.9 mV, D1+^{VTA} = -81 ± 0.8 mV, D1+^{VP} = -82 ± 1.0 mV; Kruskal-Wallis: H(2) = 1.3, p = 0.5) or input resistance (D1- = 98 ± 8 MΩ, D1+^{VTA} = 112 ± 9 MΩ, D1+^{VP} = 89 ± 9 MΩ; Kruskal-Wallis: H(2) = 3.2, p = 0.2) for all 3 cell types (n = 12-14 cells, 8-10 mice per group). **C)** Left: summary of the absolute amplitude of vHPC-evoked NMDAR EPSCs at D1- and D1+^{VTA} MSNs, where lines indicate pairs of recorded neurons. NMDAR EPSCs were larger at D1+^{VTA} MSNs (NMDAR: D1- = 109 ± 17 pA, D1+^{VTA} = 188 ± 17 pA; Wilcoxon test: W = 53, p = 0.04; n = 10 pairs, 7 mice). Right: summary of the AMPAR / NMDAR ratio for vHPC-evoked EPSCs at D1- and D1+^{VTA} MSNs. There was no difference in the AMPAR / NMDAR ratio between cell types (D1- = 2.5 ± 0.3, D1+^{VTA} = 3.0 ± 0.6; Wilcoxon test: W = 5, p = 0.8). **D)** As in (C), for vHPC-evoked EPSCs at D1- and D1+^{VP} MSNs. NMDAR EPSCs were similar at both cell types (NMDAR: D1- = 168 ± 31 pA, D1+^{VP} 132 ± 18 pA; Wilcoxon test: W = -29, p = 0.1; n = 9 pairs, 8 mice). There was no difference in the AMPAR / NMDAR ratio between cell types (D1- = 3.0 ± 0.3, D1+^{VP} = 3.3 ± 0.4; Wilcoxon test: W = 17, p = 0.4). **E)** As in (C), for BLA-evoked EPSCs at D1- and D1+^{VTA} MSNs. NMDAR EPSCs were similar at both cell types (NMDAR: D1- = 69 ± 19 pA, D1+^{VTA} = 64 ± 15 pA; Wilcoxon test: W = 0, p > 0.9; n = 8 pairs, 6 mice). There was no difference in the AMPAR / NMDAR ratio between cell types (D1- = 4.5 ± 0.8, D1+^{VTA} = 3.8 ± 1.0; Wilcoxon test: W = -14, p = 0.4). **F)** As in (A), for BLA-evoked EPSCs at D1- and D1+^{VP} MSNs. NMDAR EPSCs were similar at both cell types (NMDAR: D1- = 76 ± 13 pA, D1+^{VP} = 77 ± 12 pA; Wilcoxon test: W = 0, p > 0.9; n = 7 mice, 6 mice). There was no difference in the AMPAR / NMDAR ratio between cell types (D1- = 3.2 ± 0.5, D1+^{VP} = 2.9 ± 0.5; Wilcoxon test: W = -4, p = 0.8).

Box and whisker plots represent median and minimum to maximum.
Values are represented as mean ± SEM, * $p < 0.05$.

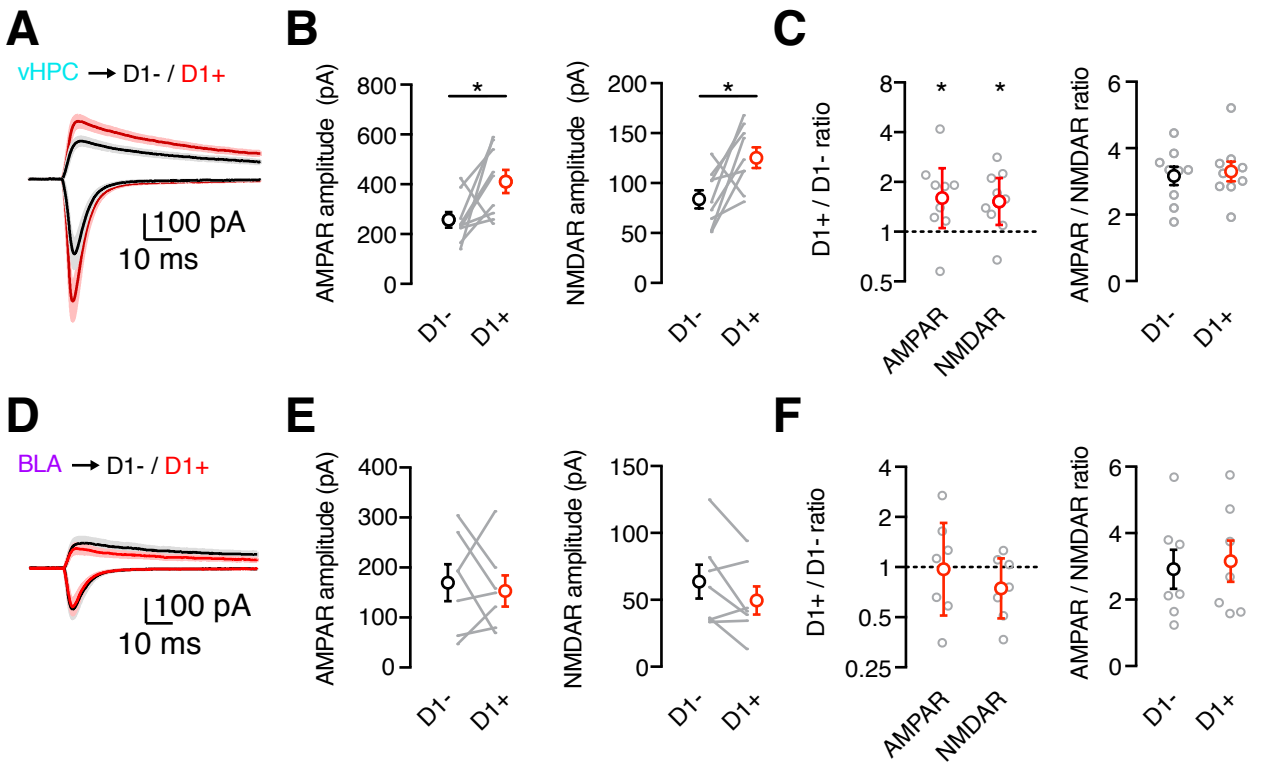

Figure S2: Baseline connectivity onto D1- and D1+ MSNs. Related to Figure 2. A) vHPC-evoked EPSCs at D1- MSNs (mean in black, SEM in grey) and D1+ MSNs (mean in red, SEM in pink) in naïve mice (n = 9 pairs, 5 mice). **B)** Summary of the absolute amplitude of vHPC-evoked AMPAR (left) and NMDAR (right) EPSCs at D1- and D1+ MSNs, where lines indicate pairs of recorded neurons. AMPAR and NMDAR EPSCs were larger at D1+ MSNs (AMPAR: D1- = 258 ± 31 pA, D1+= 411 ± 46 pA, W = 35, p = 0.04; NMDAR: D1- = 84 ± 9 pA, D1+ = 125 ± 10 pA; Wilcoxon test: W = 37, p = 0.03). **C)** Left: summary of the D1+ / D1- amplitude ratio. vHPC input was biased onto D1+ MSNs for both AMPAR EPSCs (1.6, 95% CI = 1.1 – 2.4; One-sample t test: t(8) = 2.6, p = 0.03) and NMDAR EPSCs (1.5 , 95% CI = 1.1 – 2.1; One-sample t test: t(8) = 3.0, p = 0.02). Right: there was no difference in the AMPAR / NMDAR ratio of vHPC input onto D1- and D1+ MSNs (D1- = 3.2 ± 0.3; D1+ = 3.3 ± 0.3; Wilcoxon test: W = -7, p = 0.7). **D)** As in (A), for BLA-evoked EPSCs. This data was collected with 1-8 ms LED durations (n = 7 pairs, 5 mice). **E)** As in (B), for BLA-evoked EPSCs. EPSC amplitudes were similar onto both cell types (AMPAR: D1- = 170 ± 37 pA, D1+= 153 ± 31 pA, W = -4, p = 0.8; NMDAR: D1- = 64 ± 13 pA, D1+ = 50 ± 11 pA; Wilcoxon test: W = -16, p = 0.2). **F)** As in (C), for BLA-evoked EPSCs. There was no bias onto either cell type (AMPAR: 1.0, 95% CI = 0.5 – 1.8; One-sample t test: t(6) = 0.6, p = 0.6; NMDAR: 0.7, 95% CI = 0.5 – 1.1; One-sample t test: t(6) = 1.6, p = 0.2) and no difference in the AMPAR / NMDAR ratio (D1- = 2.9 ± 06; D1+ = 3.2 ± 0.6; Wilcoxon test: W = 4, p = 0.8).

Values are represented as mean ± SEM (A, B, $C_{(right)}$ D, E, $F_{(right)}$) or geometric mean ± 95% CI ($C_{(left)}$, $F_{(left)}$).
* $p < 0.05$.

Figure S2

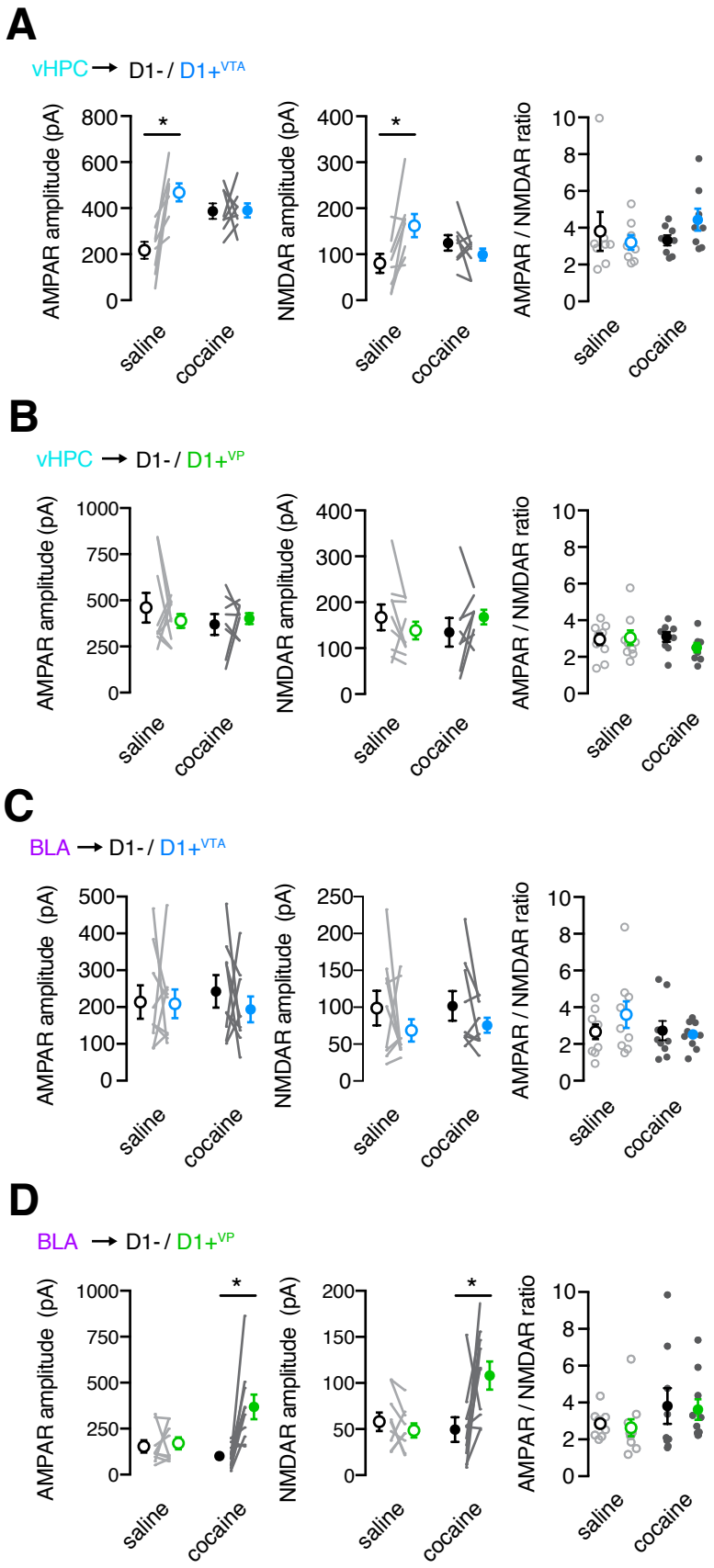
A
vHPC → D1- / D1+VTA
AMPAR amplitude (pA)
NMDAR amplitude (pA)
AMPAR / NMDAR ratio
saline
cocaine
B
vHPC → D1- / D1+VP
C
BLA → D1- / D1+VTA
D
BLA → D1- / D1+VP

Figure S3

Figure S3: vHPC and BLA EPSCs in saline- and cocaine-treated mice. Related to Figure 3. A) Summary of the absolute amplitude of vHPC-evoked AMPAR EPSCs (left) and NMDAR EPSCs (middle) at D1- and D1+^{VTA} MSNs, where lines indicate pairs of neurons. In saline mice, AMPAR and NMDAR EPSCs were larger at D1+^{VTA} MSNs (AMPAR: D1- = 217 ± 37 pA, D1+^{VTA} = 469 ± 39.0 pA, p = 0.0006; NMDAR: D1- = 81 ± 21 pA, D1+^{VTA} = 162 ± 25 pA, p = 0.003; n = 8 pairs, 6 mice), but were equalized in cocaine mice (AMPAR: D1- = 387 ± 33 pA, D1+^{VTA} = 390 ± 31 pA, p = 1.0; NMDAR: D1- = 125 ± 17 pA, D1+^{VTA} = 99 ± 13 pA, p = 0.4; n = 8 pairs, 5 mice) (AMPAR: Two-way ANOVA drug treatment x cell type interaction, $F(1,14) = 11.1$, p = 0.005; NMDAR: Two-way ANOVA drug treatment x cell type interaction, $F(1,14) = 13.2$, p = 0.003). Right: summary of the AMPAR / NMDAR ratio in saline and cocaine mice. There was no difference in the AMPAR / NMDAR ratio of vHPC-evoked EPSCs for D1- and D1+^{VTA} MSNs (saline: D1- = 3.8 ± 1.1, D1+^{VTA} = 3.2 ± 0.4, p = 0.8; cocaine: D1- = 3.3 ± 0.3, D1+^{VTA} = 4.4 ± 0.6, p = 0.4; Two-way ANOVA drug treatment x cell type interaction, $F(1,14) = 1.9$, p = 0.2). **B)** Same for vHPC-evoked EPSCs at D1- and D1+^{VP} MSNs. AMPAR and NMDAR EPSC amplitudes were unbiased at D1- and D1+^{VP} MSNs in saline mice (AMPAR: D1- = 461 ± 81 pA, D1+^{VP} = 389 ± 38 pA, p = 0.6; NMDAR: D1- = 167 ± 28 pA, D1+^{VP} = 139 ± 19 pA, p = 0.4; n = 9 pairs, 9 mice), this was unchanged in cocaine mice (AMPAR: D1- = 370 ± 56 pA, D1+^{VP} = 401 ± 30 pA, p = 0.9; NMDAR: D1- = 135 ± 31 pA, D1+^{VP} = 168 ± 16 pA, p = 0.3; n = 8 pairs, 7 mice) (AMPAR: Two-way ANOVA drug treatment x cell type interaction, $F(1,15) = 0.90$, p = 0.4; NMDAR: Two-way ANOVA drug treatment x cell type interaction, $F(1,15) = 3.5$, p = 0.08). There was no difference in the AMPAR / NMDAR ratio of vHPC-evoked EPSCs for D1- and D1+^{VP} MSNs (saline: D1- = 3.0 ± 0.3, D1+^{VP} = 3.0 ± 0.4, p = 1.0; cocaine: D1- = 3.1 ± 0.3, D1+^{VP} = 2.5 ± 0.3, p = 0.2; Two-way ANOVA drug treatment x cell type interaction, $F(1,15) = 1.8$, p = 0.2). **C)** As in (A), for BLA-evoked AMPAR and NMDAR EPSCs at D1- and D1+^{VTA} MSNs. AMPAR and NMDAR EPSC amplitudes were similar at D1- and D1+^{VTA} MSNs in saline mice (AMPAR: D1- = 214 ± 45 pA, D1+^{VTA} = 209 ± 39 pA, p = 1.0; NMDAR: D1- = 99 ± 23 pA, D1+^{VTA} = 69 ± 15 pA, p = 0.4; n = 9 pairs, 7 mice), this was unchanged in cocaine mice (AMPAR: D1- = 243 ± 44 pA, D1+^{VTA} = 194 ± 35 pA, p = 0.7; NMDAR: D1- = 102 ± 21 pA, D1+^{VTA} = 76 ± 10 pA, p = 0.5; n = 9 pairs, 6 mice) (AMPAR: Two-way ANOVA drug treatment x cell type interaction, $F(1,16) = 0.2$, p = 0.6; NMDAR: Two-way ANOVA drug treatment x cell type interaction, $F(1,16) = 0.01$, p = 0.9). There was no difference in the AMPAR / NMDAR ratio of BLA-evoked EPSCs for D1- and D1+^{VTA} MSNs (saline: D1- = 2.7 ± 0.4, D1+^{VTA} = 3.6 ± 0.7, p = 0.4; cocaine: D1- = 2.7 ± 0.5, D1+^{VTA} = 2.5 ± 0.2, p = 1.0; Two-way ANOVA drug treatment x cell type interaction, $F(1,16) = 1.3$, p = 0.3). **D)** As in (A) for BLA-evoked AMPAR and NMDAR EPSCs at D1- and D1+^{VP} MSNs. AMPAR and NMDAR EPSC amplitudes were similar at both cell types in saline mice (AMPAR: D1- = 154 ± 33 pA, D1+^{VP} = 171 ± 32 pA, p = 1.0; NMDAR: D1- = 58 ± 10 pA, D1+^{VP} = 49 ± 8 pA, p = 0.9; n = 9 pairs, 7 mice), but were biased onto D1+^{VP} neurons in cocaine mice (AMPAR: D1- = 101 ± 19 pA, D1+^{VP} = 368 ± 67 pA, p = 0.0005; NMDAR: D1- = 50 ± 13 pA, D1+^{VP} = 108 ± 15 pA, p = 0.01; n = 10 pairs, 10 mice) (AMPAR: Two-way ANOVA drug treatment x cell type interaction, $F(1,17) = 8.1$, p = 0.01; NMDAR: Two-way ANOVA drug treatment x cell type interaction, $F(1,17) = 7.2$, p = 0.02). There was no difference in the AMPAR / NMDAR ratio of BLA-evoked EPSCs for D1- and D1+^{VP} MSNs (saline: D1- = 2.7 ± 0.3, D1+^{VP} = 4.1 ± 0.9, p = 0.5; cocaine: D1- = 2.7 ± 0.5, D1+^{VP} = 3.6 ± 0.6, p = 0.5; Two-way ANOVA drug treatment x cell type interaction, $F(1,17) = 0.00003$, p = 0.9).

Values are represented as mean ± SEM, * $p < 0.05$.

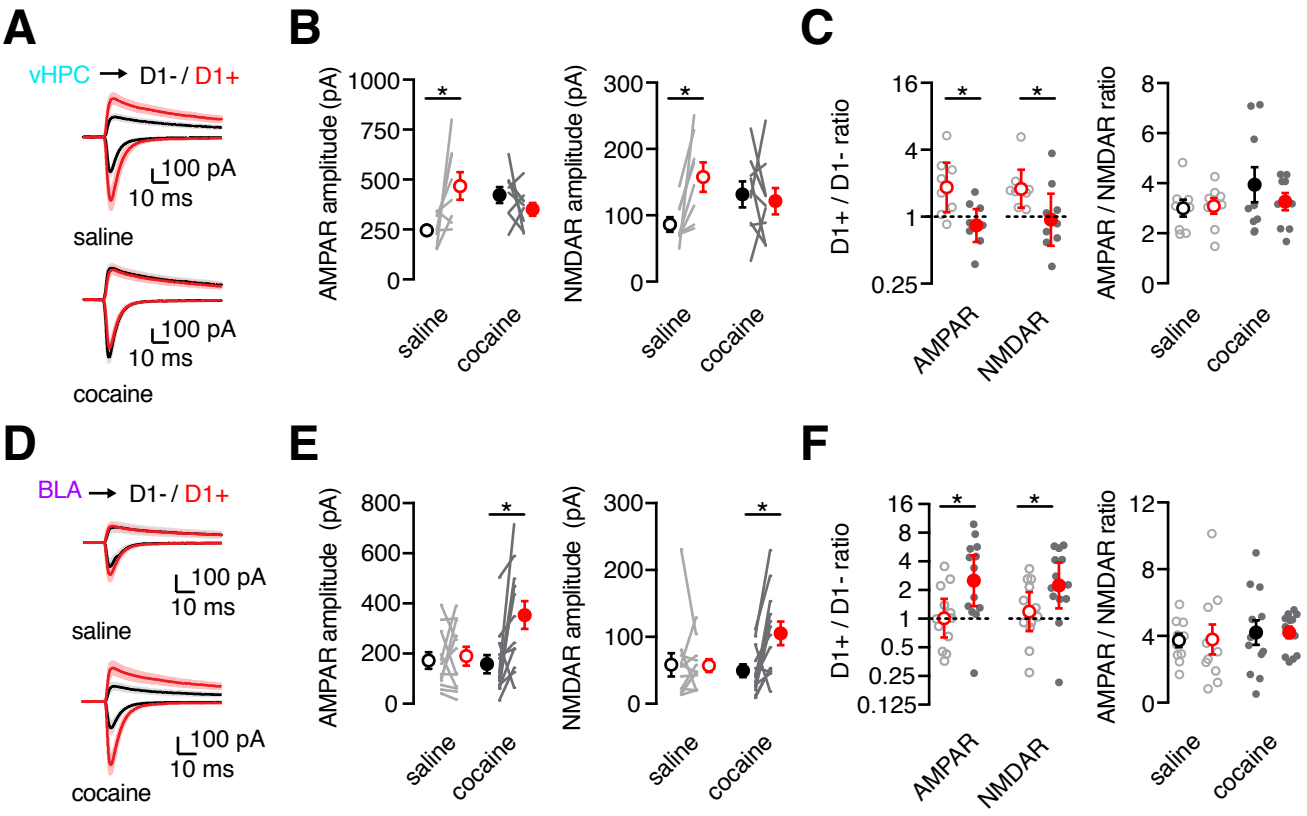

Figure S4: Cocaine-evoked vHPC and BLA plasticity at D1- and D1+ MSNs. Related to Figure 3. A) vHPC-evoked EPSCs at D1- MSNs (mean in black, SEM in grey) and D1+ MSNs (mean in red, SEM in pink) on day 6, from mice injected with saline (top) or cocaine (bottom). **B)** Summary of the absolute amplitude of vHPC-evoked AMPAR EPSCs (left) and NMDAR EPSCs (right) at D1- and D1+ MSNs, in saline (open circles) and cocaine (filled circles) mice. Lines indicate pairs of neurons (saline in light grey, cocaine in dark grey). AMPAR and NMDAR EPSCs were larger at D1+ MSNs in saline mice (AMPAR: D1- = 246 ± 27 pA, D1+= 468 ± 69 pA, p = 0.01; NMDAR: D1- = 86 ± 11 pA , D1+ = 158 ± 22 pA, p = 0.02; n = 8 pairs, 6 mice), but amplitudes were equalized in cocaine mice (AMPAR: D1- = 423 ± 40 pA, D1+= 351 ± 31 pA, p = 0.5; NMDAR: D1- = 132 ± 20 pA, D1+ = 122 ± 20 pA, p = 0.9; n = 9 pairs, 5 mice) (AMPAR: Two-way ANOVA drug treatment x cell type interaction, F (1,15) = 9.1, p = 0.009; NMDAR: Two-way ANOVA drug treatment x cell type interaction, F (1,15) = 6.1, p = 0.03). **C)** Left: summary of the D1+ / D1- amplitude ratios for each pair of recorded neurons in saline (open circles) and cocaine (filled circles) mice. Cocaine normalized input onto D1+ and D1- MSNs for both AMPAR (left) and NMDAR (right) EPSCs (AMPAR: saline D1+ / D1- ratio = 1.84, 95% CI = 1.1 - 3.1; cocaine D1+ / D1- ratio = 0.8, 95% CI = 0.6 - 1.2; Mann-Whitney: U = 10, p = 0.01) (NMDAR: saline D1+ / D1- ratio = 1.8 , 95% CI = 1.2 – 2.6; cocaine D1+ / D1- ratio = 0.9, 95% CI = 0.5 – 1.6; Mann-Whitney: U = 13, p = 0.03). Right: summary of the AMPAR / NMDAR ratio for D1- and D1+ MSNs in saline (open circles) and cocaine (closed circles) mice. Cocaine exposure did not alter the AMPAR / NMDAR ratio in D1- or D1+ MSNs (saline: D1- = 3.0 ± 0.3, D1+ = 3.1 ± 0.3, p=1.0; cocaine: D1- = 4.0 ± 0.7, D1+ = 3.3 ± 0.3, p = 0.4) (Two-way ANOVA drug treatment x cell type interaction, F(1,15) = 0.9, p = 0.4). **D)** As in (A), for BLA-evoked EPSCs at D1- and D1+ MSNs. This data was collected with 18 ms LED durations. **E)** As in (B), for BLA-evoked EPSCs at D1- and D1+ MSNs. AMPAR and NMDAR EPSCs were similar in saline-treated mice (AMPAR: D1- = 180 ± 39 pA, D1+= 187 ± 44 pA, p = 1.0; NMDAR: D1- = 61 ± 20 pA , D1+ = 57 ± 12 pA, p = 0.9; n = 12 pairs, 9 mice), but were larger at D1+ MSNs in cocaine-treated mice (AMPAR: D1- = 164 ± 38 pA, D1+= 372 ± 57 pA, p = 0.001; NMDAR: D1- = 47 ± 10 pA , D1+ = 102 ± 19 pA, p = 0.005; n = 13 pairs, 9 mice) (AMPAR: Two-way ANOVA drug treatment x cell type interaction, F (1,23) = 6.1, p = 0.02; NMDAR: Two-way ANOVA drug treatment x cell type interaction, F (1,23) = 7.0, p = 0.01). **F)** Left: as in (C), for the ratio of BLA-evoked EPSCs. Cocaine increased BLA input onto D1+ MSNs for both AMPAR and NMDAR EPSCs (AMPAR: saline D1+ / D1- ratio = 0.9, 95% CI = 0.5 –1.6; cocaine D1+ / D1- ratio = 2.6, 95% CI = 1.3 – 5.2; Mann-Whitney: U = 25, p = 0.02) (NMDAR: saline D1+ / D1- ratio = 1.1, 95% CI = 0.6 – 2.0; cocaine D1+ / D1- ratio = 2.4, 95% CI = 1.2 – 4.5; Mann-Whitney: U = 29, p = 0.04). Right: there was no change in the AMPAR / NMDAR ratio after cocaine (saline: D1- = 3.7 ± 0.4, D1+ = 3.8 ± 0.9, p=1.0; cocaine: D1- = 4.2 ± 0.7, D1+ = 4.2 ± 0.4, p = 1.0) (Two-way ANOVA drug treatment x cell type interaction, F(1,20) = 0.001, p = 1.0).

Values are represented as mean ± SEM (A, B, $C_{(right)}$ D, E, $F_{(right)}$) or geometric mean ± 95% CI ($C_{(left)}$, $F_{(left)}$),
* $p < 0.05$.

Figure S4