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Supplemental Information

Allostatic Changes in the cAMP System

Drive Opioid-Induced Adaptation

in Striatal Dopamine Signaling

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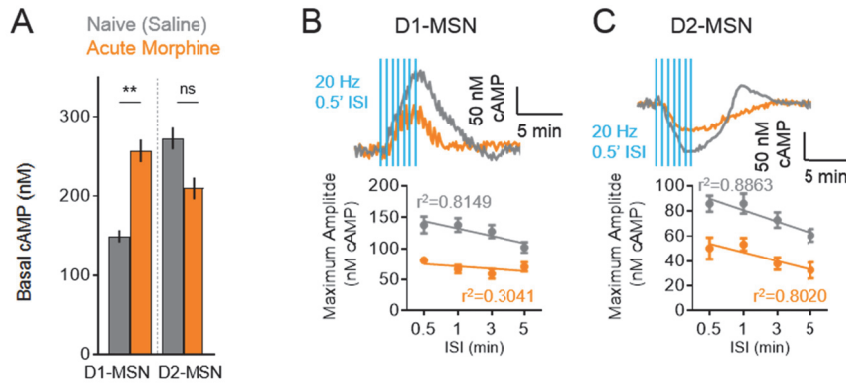


Figure S1. Acute morphine exposure alters kinetic dopamine-mediated signaling parameters in MSNs, Related to Figure 1. **A**, Baseline cAMP level prior to experimental manipulations in genetically defined striatal subpopulations. Naive D1-MSN 149.4 ± 7.8 nM cAMP (n=51 neurons) vs Acute D1-MSN 272.3 ± 13.9 nM cAMP (n=41 neurons); t-test $p < 0.0001$, Kolmogorov-Smirnov $D=0.6585$. Naive D2-MSN 256.6 ± 14.1 nM cAMP (n=47 neurons) vs Acute D2-MSN 210.7 ± 12.9 nM cAMP (n=40 neurons); t-test $p=0.1521$, Kolmogorov-Smirnov $D=0.2441$. **B**, cAMP responses in D1-MSN from 20 Hz stimulation presented at 0.5 minute inter-stimulation intervals (ISI). Maximum cAMP amplitude from varying ISI protocols were quantified. **C**, cAMP responses in D2-MSN from 20 Hz stimulation presented at 0.5 minute inter-stimulation intervals (ISI). Maximum cAMP amplitude from varying ISI protocols were quantified.

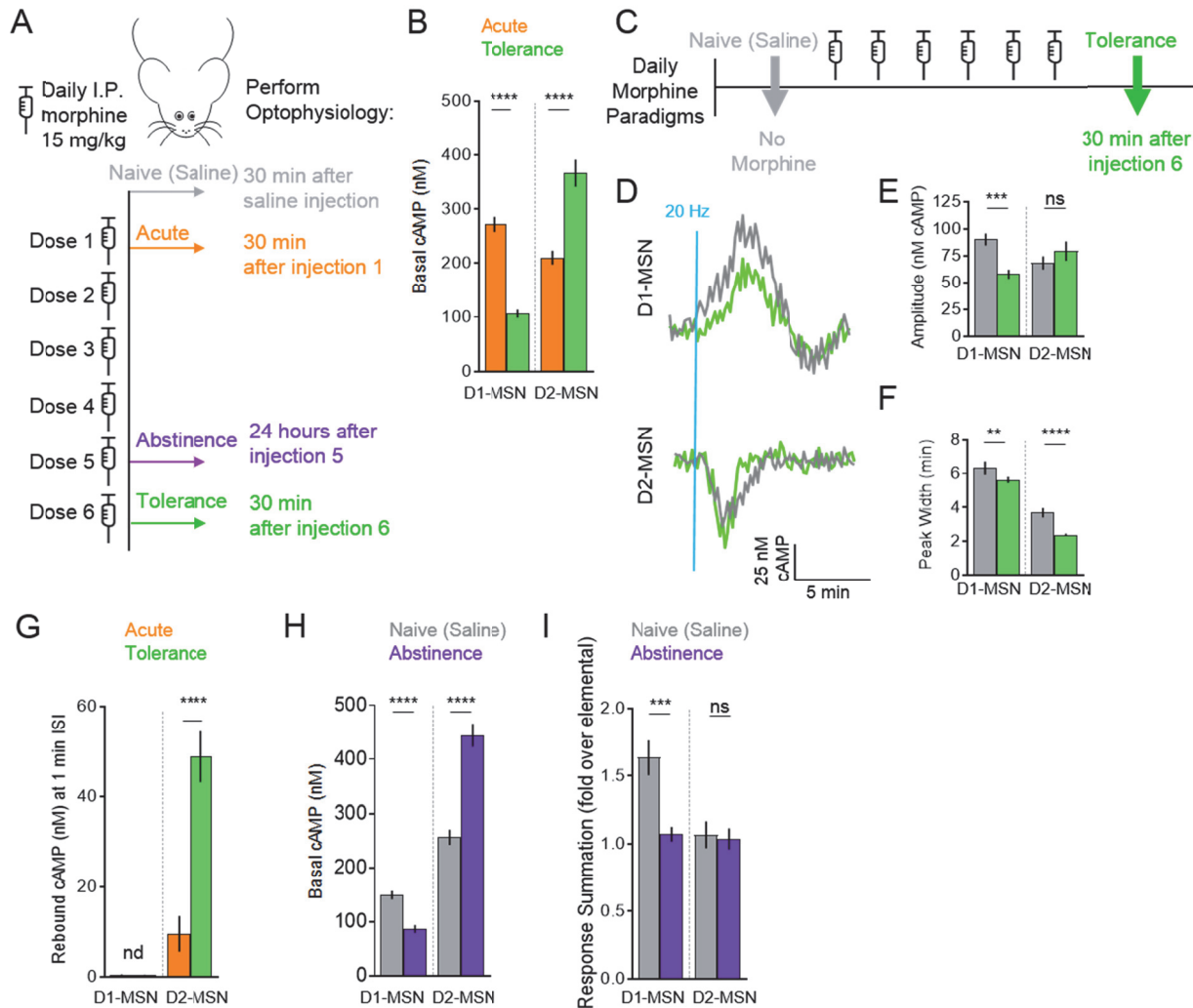


Figure S2. Intrinsic neuroadaptive changes in MSNs following exposure to opioids, Related to Figure 2. **A**, Schematic of four morphine paradigms utilized. **B**, Baseline cAMP level. Acute D1-MSN 272.3 ± 13.9 nM cAMP ($n=41$ neurons) vs Tolerance D1-MSN 106.4 ± 7.3 nM cAMP ($n=39$ neurons); t-test $p<0.0001$, KS $D=0.8049$. Acute D2-MSN 210.7 ± 12.9 nM cAMP ($n=40$ neurons) vs Tolerance D2-MSN 366.6 ± 25.3 nM cAMP ($n=46$ neurons); t-test $p<0.0001$, KS $D=0.5435$. **C**, Experimental design to compare six daily morphine injections (Tolerance) with saline treatment (Naive). **D**, cAMP response from optical stimulation. **E**, Max amplitude cAMP change. Naive D1-MSN 90.3 ± 5.5 nM cAMP ($n=51$ neurons; 7 mice) vs Tolerance D1-MSN 58 ± 4.5 nM cAMP ($n=39$ neurons; 4 mice); t-test $p=0.0004$, KS $D=0.4419$. Naive D2-MSN 68.5 ± 6.1 nM cAMP ($n=47$ neurons; 6 mice) vs Tolerance D2-MSN 80 ± 5.4 nM cAMP ($n=46$ neurons; 5 mice); t-test $p=0.1053$, KS $D=0.2516$. **F**, Response duration. Naive D1-MSN 6.3 ± 0.39 minutes ($n=51$ neurons; 7 mice) vs Tolerance D1-MSN 5.6 ± 0.19 minutes ($n=39$ neurons; 4 mice); t-test $p=0.0017$, KS $D=0.3997$. Naive D2-MSN 3.7 ± 0.27 minutes ($n=47$ neurons; 6 mice) vs Tolerance D2-MSN 2.4 ± 0.07 minutes ($n=46$ neurons; 5 mice); t-test $p<0.0001$, KS $D=0.5747$. **G**, Max amplitude cAMP change in opposite direction of the initial response before returning to baseline. Acute D1-MSN 0.35 ± 0.02 nM cAMP ($n=41$ neurons) vs Tolerance D1-MSN 0.39 ± 0.03 nM cAMP ($n=39$ neurons); t-test $p=0.0701$, KS $D=0.2896$. Acute D2-MSN 9.6 ± 3.9 nM cAMP ($n=40$ neurons) vs Tolerance D2-MSN 49.3 ± 5.6 nM cAMP ($n=46$ neurons); t-test $p<0.0001$, KS $D=0.9783$. **H**, Baseline cAMP level. Naive D1-MSN 149.4 ± 7.8 nM cAMP ($n=51$ neurons) vs Abstinence D1-MSN 86.6 ± 7.6 nM cAMP ($n=35$ neurons); t-test $p<0.0001$, KS $D=0.5272$. Naive D2-MSN 256.6 ± 14.1 nM cAMP ($n=47$ neurons) vs Abstinence D2-MSN 444.0 ± 21.2 nM cAMP ($n=38$ neurons); t-test $p<0.0001$, KS $D=0.692$. **I**, Summation of the cAMP response from 3' ISI data calculated by dividing max response by peak response to initial stimulation. Naive D1-MSN 1.63 ± 0.13 fold ($n=16$ neurons) vs Abstinence D1-MSN 1.07 ± 0.054 fold ($n=12$ neurons); t-test $p=0.0009$, KS $D=0.75$. Naive D2-MSN 1.07 ± 0.099 fold ($n=18$ neurons) vs Abstinence D2-MSN 1.04 ± 0.079 fold ($n=16$ neurons); t-test $p=0.8558$, KS $D=0.75$.

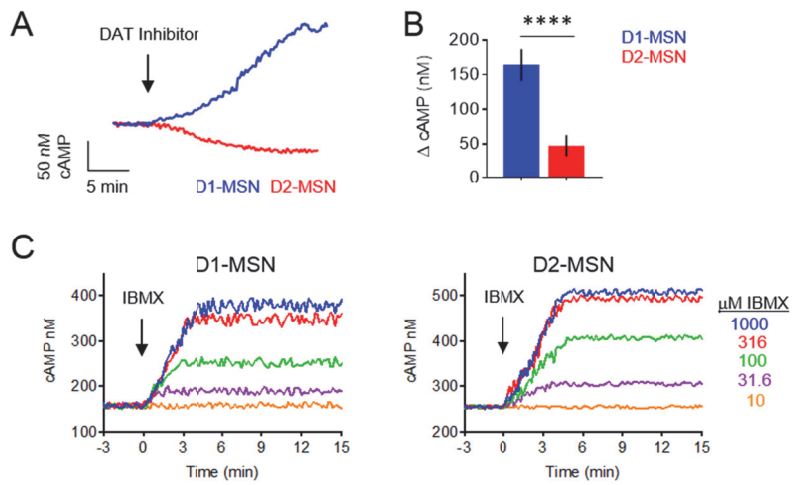


Figure S3. DAT and PDE inhibition influence basal cAMP level in MSNs, Related to Figure 3. **A**, cAMP response to bath application of DAT inhibitor (cocaine, 10 μ M). **B**, Maximum amplitude cAMP change induced by cocaine. D1-MSN 164 ± 23 nM cAMP (n=41 neurons) vs D2-MSN 47 ± 16 nM cAMP (n= 55 neurons); t-test $p < 0.0001$. **C**, cAMP response to bath application of nonspecific PDE inhibitor (IBMX).

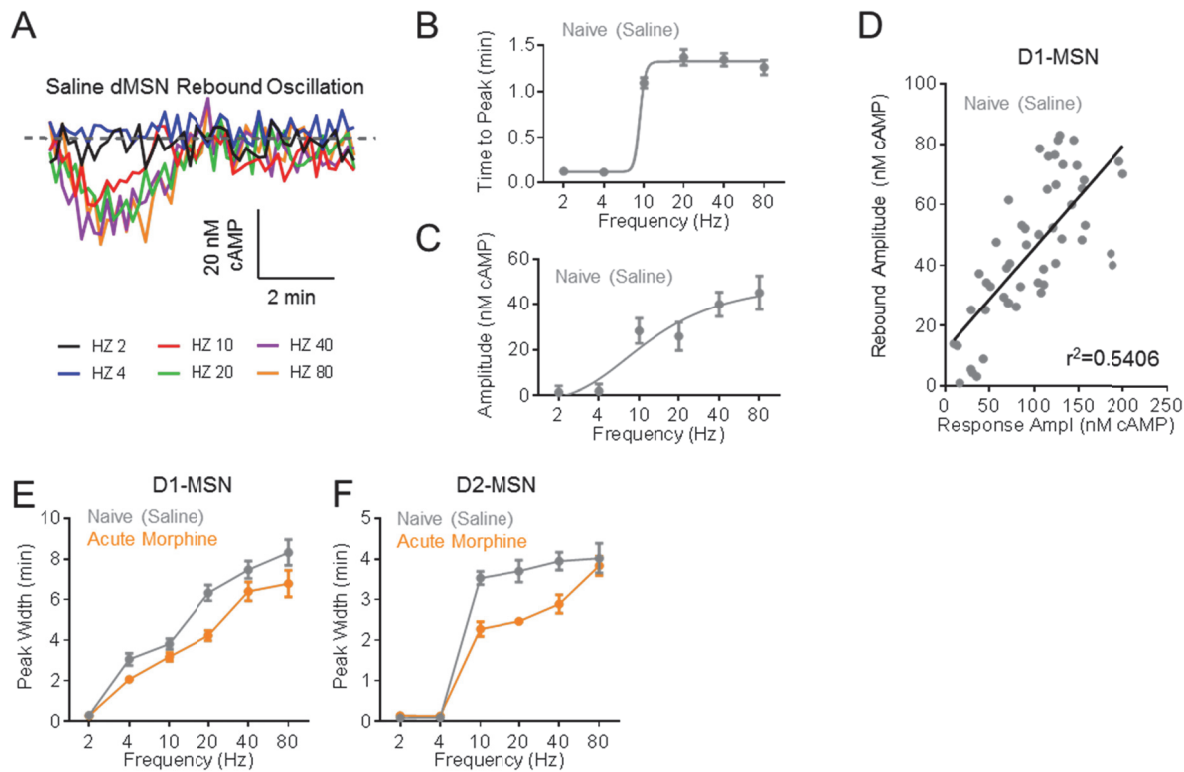


Figure S4. Stimulation frequency influence on cAMP rebound oscillation and response kinetics, Related to Figure 5. **A**, cAMP change in D1-MSN in the opposite direction of the initial response before returning to baseline. Traces highlight rebound cAMP from data from Fig. 5b. **B**, Time to peak for cAMP rebound in D1-MSN Saline from varying stimulation frequencies. **C**, Maximum cAMP rebound amplitude in D1-MSN Saline from varying stimulation frequencies. **D**, Correlation of cAMP rebound and initial response amplitude in D1-MSN Saline at 80 Hz stimulation. **E**, Peak width of cAMP response from varying stimulation frequencies in D1-MSN. **F**, Peak width of cAMP response from varying stimulation frequencies in D2-MSN.

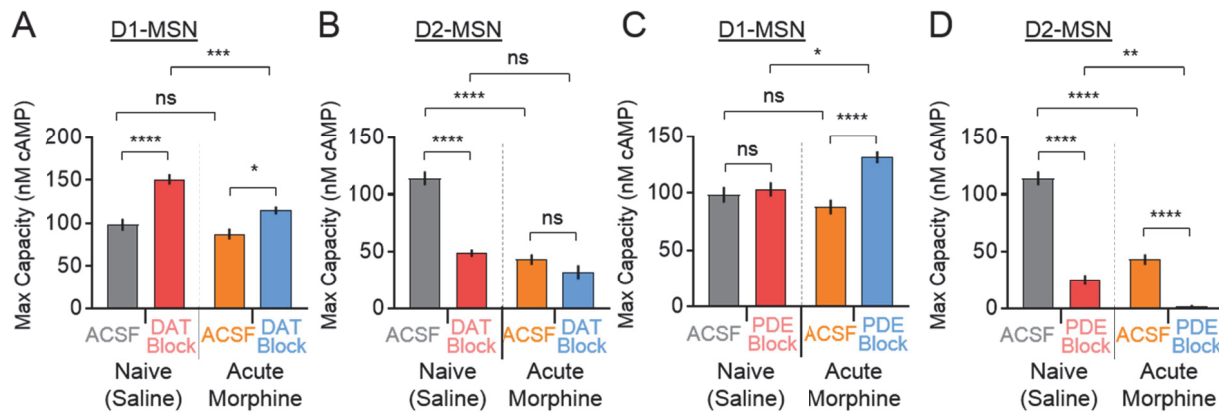


Figure S5. Regulation of maximum signaling capacity by DAT and PDE, Related to Figure 6. Maximum amplitude cAMP change at 80 Hz stimulation frequency. **A**, D1-MSN Naive ACSF 98.44 ± 6.90 nM cAMP, D1-MSN Naive DAT block 151.17 ± 5.85 nM cAMP, D1-MSN Opioid ACSF 87.90 ± 6.25 nM cAMP, D1-MSN Opioid DAT block 114.85 ± 4.37 nM cAMP. **B**, D2-MSN Naive ACSF 113.52 ± 6.15 nM cAMP, D2-MSN Naive DAT block 49.10 ± 3.25 nM cAMP, D2-MSN Opioid ACSF 43.31 ± 4.32 nM cAMP, D2-MSN Opioid DAT block 33.43 ± 5.27 nM cAMP. **C**, D1-MSN Naive ACSF 98.44 ± 6.90 nM cAMP, D1-MSN Naive PDE block 103.14 ± 6.05 nM cAMP, D1-MSN Opioid ACSF 87.90 ± 6.25 nM cAMP, D1-MSN Opioid PDE block 131.08 ± 5.52 nM cAMP. **D**, D2-MSN Naive ACSF 113.52 ± 6.15 nM cAMP, D2-MSN Naive PDE block 24.74 ± 3.50 nM cAMP, D2-MSN Opioid ACSF 43.31 ± 4.32 nM cAMP, D2-MSN Opioid PDE block 2.09 ± 0.15 nM cAMP. Two-way ANOVA; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

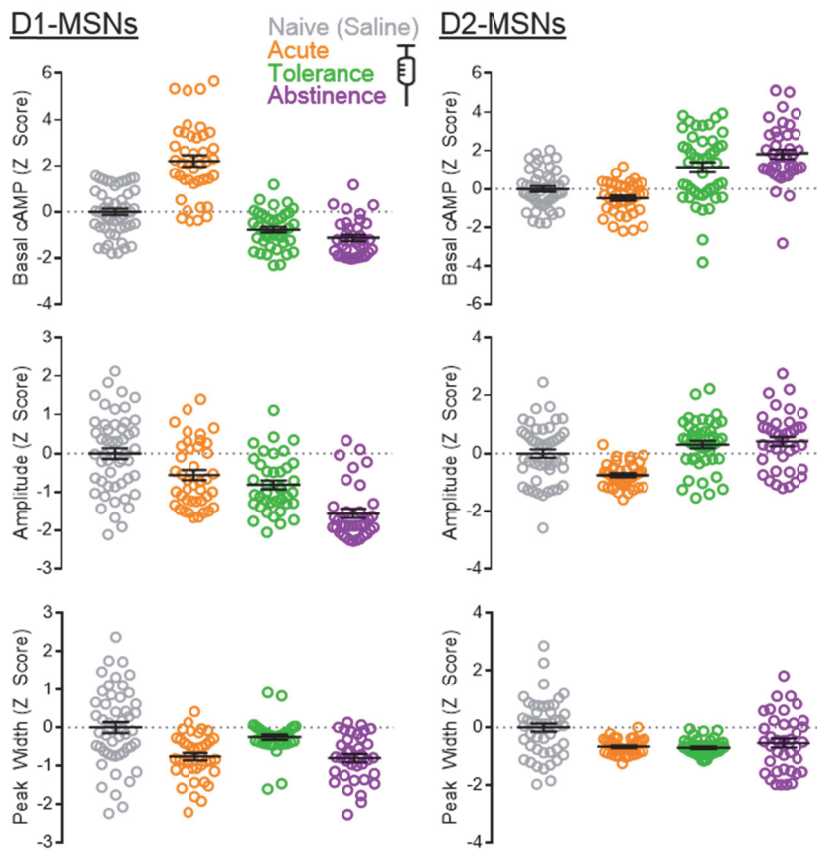


Figure S6. Z score MSN signaling parameters, Related to Figure 7. Individual Z score data normalized to Naive condition for basal cAMP, response amplitude, and peak width in both D1-MSN and D2-MSN.