

Cell Reports, Volume 40

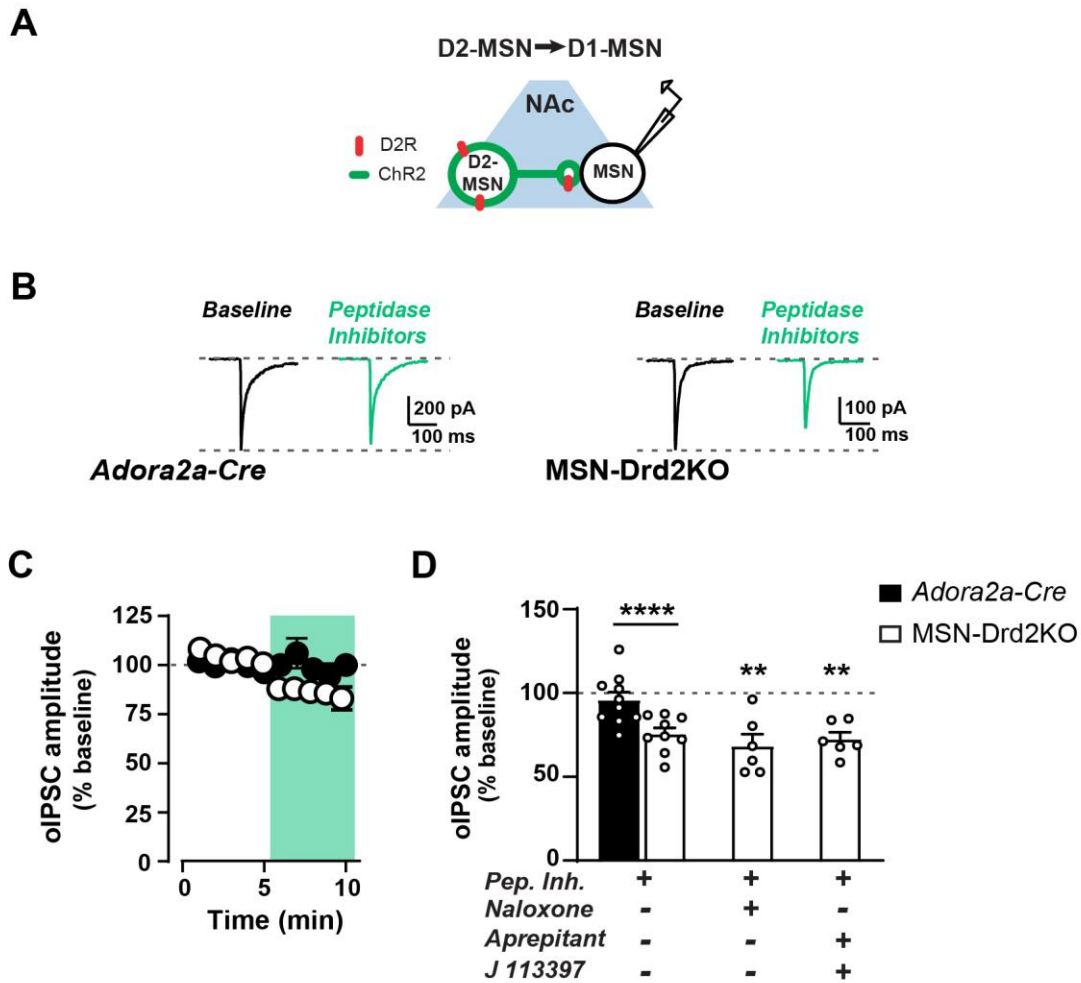
Supplemental information

Dopamine D2 receptors bidirectionally

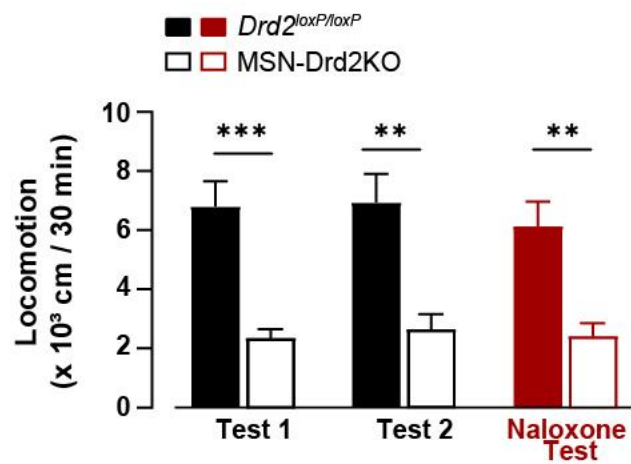
regulate striatal enkephalin

expression: Implications for cocaine reward

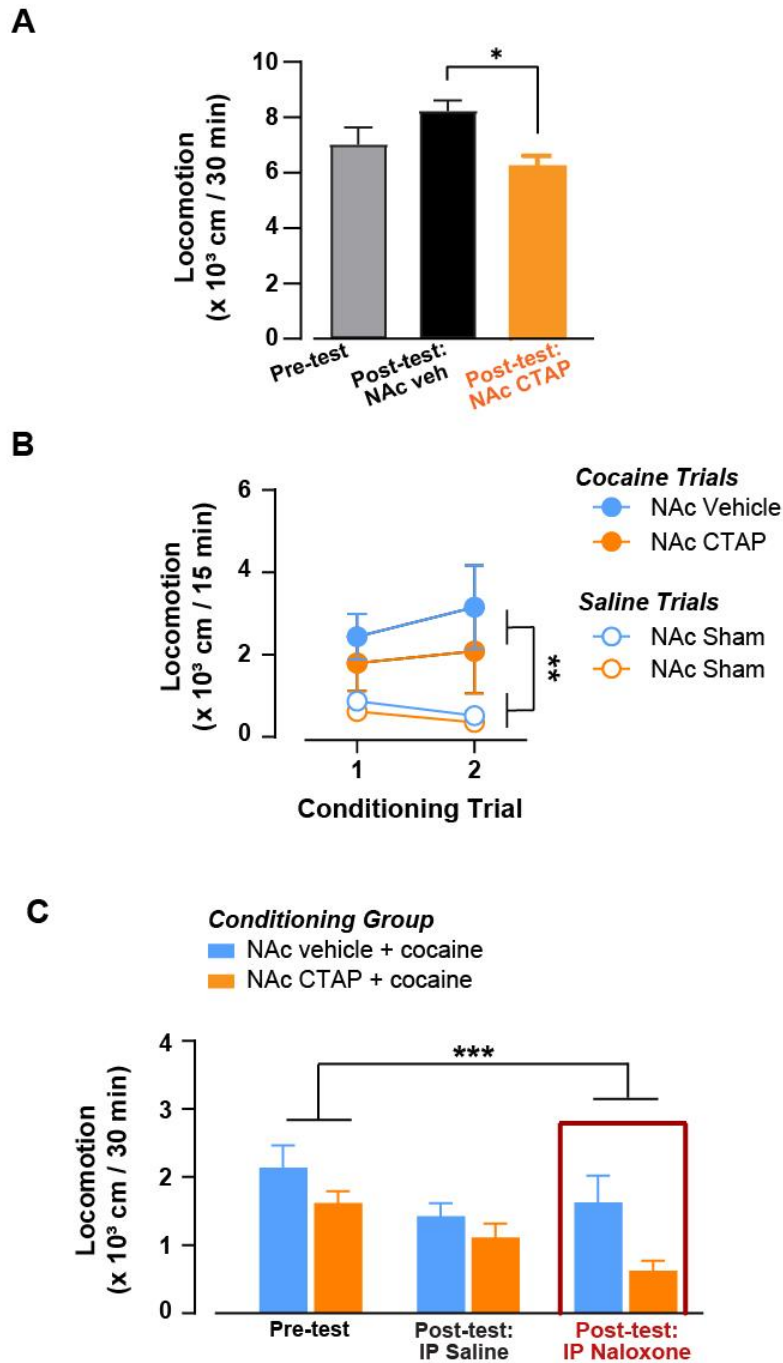
Kathy Z. Dai, In Bae Choi, Ryan Levitt, Mariah B. Blegen, Alanna R. Kaplan, Aya Matsui, J. Hoon Shin, Miriam E. Bocarsly, Eleanor H. Simpson, Christoph Kellendonk, Veronica A. Alvarez, and Lauren K. Dobbs



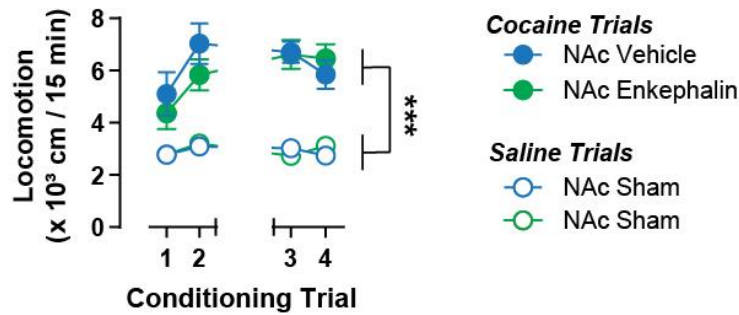
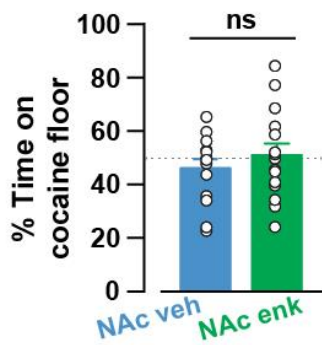
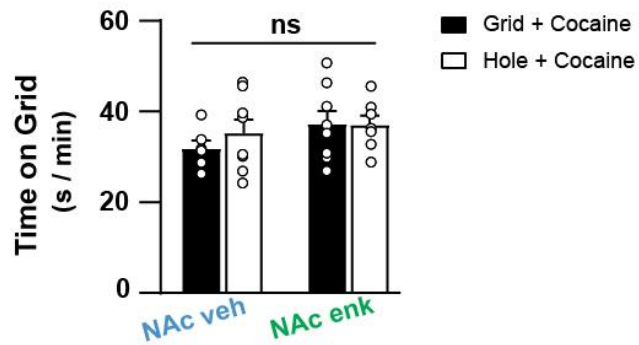
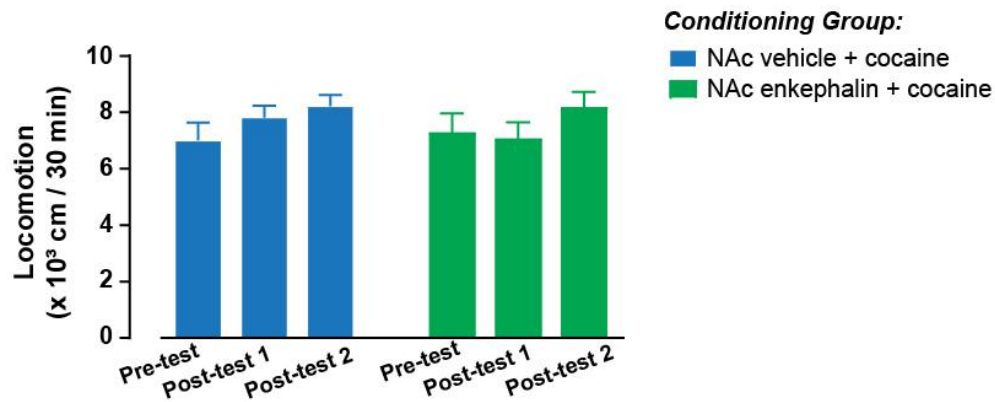
Supplemental Figure 1. Peptidase inhibitors suppress optically-evoked GABA transmission in the striatum. (A) Schematic showing ChR2-expressing D2-MSN (green) and whole-cell recording from a ChR2-negative NAc MSN. D2R: dopamine D2 receptor. (B) Representative oIPSC traces recorded from MSNs in Adora2a-Cre (left) or MSN-Drd2KO (right) in the presence of a peptidase inhibitor cocktail (2 μ M thiorphan, 10 μ M bestatin; green). (C) Time course of oIPSC amplitude expressed as a percent of baseline recorded from MSNs from Adora2a-Cre (B, filled, n = 10 cells / 3 mice) and MSN-Drd2KO mice (C, open, n = 10 cells / 3 mice). (D) Summary bar graph of oIPSC amplitude collapsed over drug wash for Adora2a-Cre (filled) or MSN-Drd2KO (open). Application of peptidase inhibitors (Pep. Inh.) suppressed oIPSC amplitude in MSN-Drd2KO compared to controls (2W-Mixed Model). This suppression was not blocked by pre-incubation in antagonists for opioid receptors (Naloxone, 5 μ M), neurokinin receptor (Aprepitant, 1 μ M), or the nociceptin receptor (J 113397, 1 μ M) (1-sample t-tests). ** p < 0.01 (vs. 100% baseline), **** p < 0.01 (Adora2aCre vs MSN-Drd2KO) Mean \pm SEM and/or individual values/traces shown. Related to Figure 3.



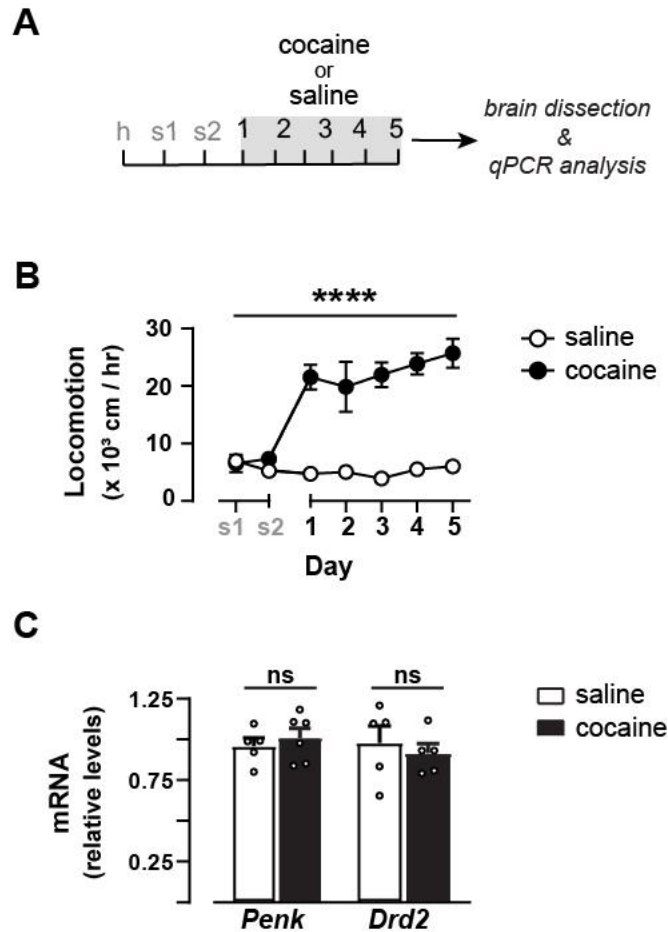
Supplemental Figure 2. MSN-Drd2KO mice show suppressed locomotor activity during naloxone place aversion testing. Locomotor activity is shown for each conditioning test following naloxone place aversion conditioning (t-tests). ** p < 0.01; *** p < 0.001. Mean ± SEM. Related to Figure 4.



Supplemental Figure 3. Systemic, but not intra-NAc, opioid antagonists suppress locomotor activity during place preference testing. (A) Locomotor activity in *Drd2^{loxP/loxP}* mice is lower following intra-NAc CTAP during preference testing. (RMANOVA) (B) In MSN-Drd2KO mice, locomotor activity was higher during cocaine conditioning trials (filled) than saline trials (open). Intra-NAc CTAP (orange) had no effect on cocaine-induced locomotion compared to intra-NAc vehicle controls (blue). (3W-RMANOVA) (C) Locomotor activity during preference testing was suppressed by IP naloxone (red box) for both conditioning groups (2W-RMANOVA). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Mean \pm SEM. Related to Figure 5.

A**B****C****D**

Supplemental Figure 4. Locomotor activity during cocaine place conditioning was not affected by intra-NAc enkephalin administration. (A) Locomotor activity was higher on cocaine conditioning trials (filled circles) than saline trials (open circles) for both the NAc vehicle group (blue) and the NAc enkephalin group (green; 3W-RMANOVA). (B-C) Neither group showed a significant pre-conditioning place preference when expressed as percent time on the cocaine floor (B) or time spent on the grid floor for the conditioning subgroups (C). (D) Locomotor activity during preference testing was not different between conditioning groups or across tests. *** $p < 0.001$, ns = not significant; Mean \pm SEM. Related to Figure 6.



Supplemental Figure 5. Short-term cocaine exposure increases locomotor activity but does not alter *Penk* or *Drd2* expression. (A) Timeline of behavioral experiment (h, habituation, s, saline). After 5 days of saline or cocaine treatment (15 mg/kg), mice were immediately euthanized for qPCR. (B) Cocaine (filled, $n = 5-6$) increased horizontal locomotor activity compared to saline controls (open, $n = 5$; 2W-RMANOVA). (C) Relative expression levels of pre-proenkephalin (*Penk*) and the D2 receptor (*Drd2*) are shown for the ventral striatum from mice previously treated for 5 days with saline or cocaine (open bars, saline, $n = 5$; filled bars, cocaine: $n = 6$) (t-tests). ns, not significant; **** $p < 0.0001$; Mean \pm SEM and/or individual values shown. Related to Figure 6.