Supplementary Information

Opioids induce dissociable forms of long-term depression of excitatory inputs to the dorsal striatum

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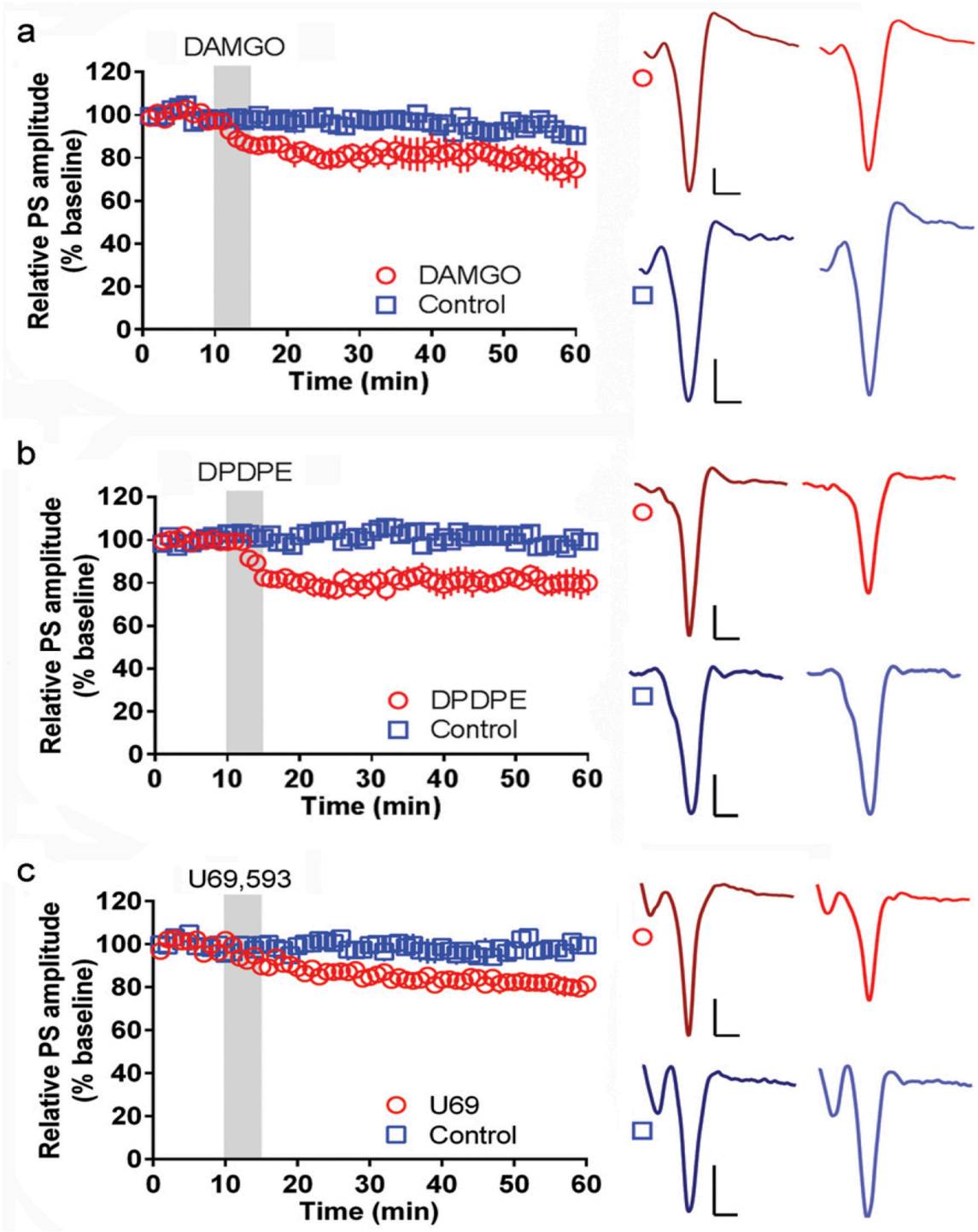
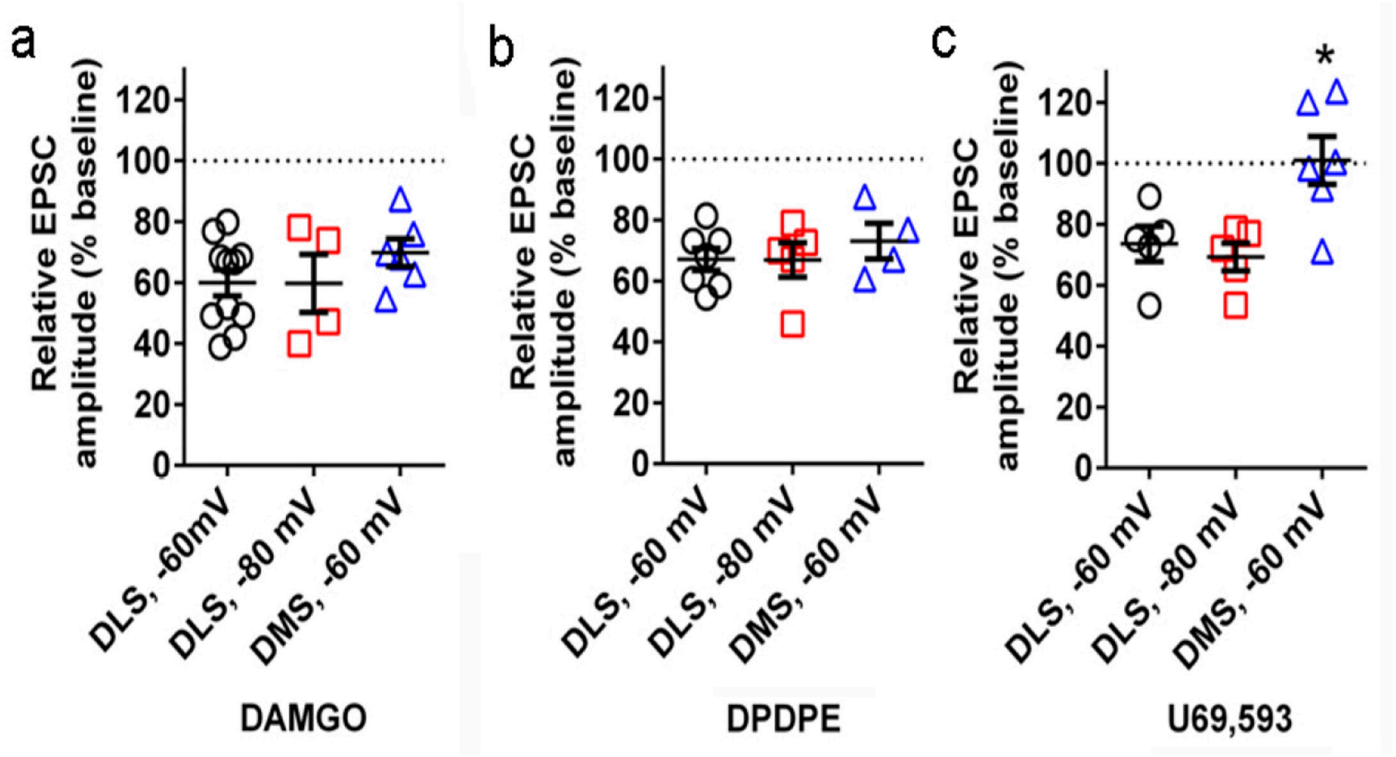


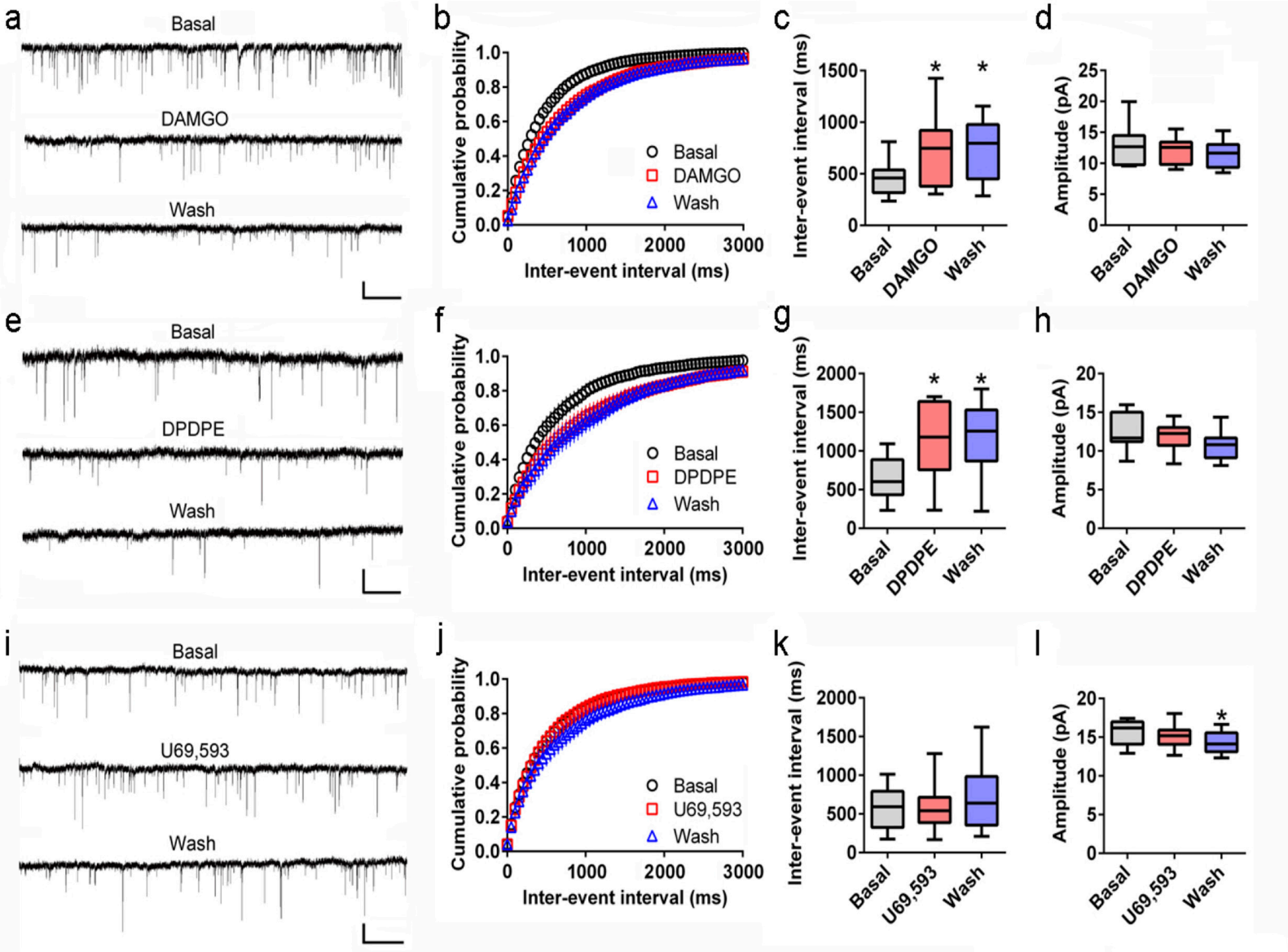
Supplementary Figure 1: Field recordings reveal OP-LTD of net striatal output.

(**a**) DAMGO (1 μM, 5 min) induced mOP-LTD of population spike amplitude in field recordings of the DLS (vs. control: P=0.0219, t(12)=2.631, n=14). (**b**) DPDPE (1 μM, 5 min) induced dOP-LTD of population spike amplitude in field recordings of the DLS (vs. control: P=0.0047, t(14)=3.360, n=16). (**c**) U69,593 (1 μM, 5 min) induced kOP-LTD of population spike amplitude in field recordings of the DLS (vs. control: P=0.0317, t(14)=2.386, n=16). Representative traces are the average of the first 5 min (first of each pair) and the final 5 min (second of each pair) of recording. Scale bars:0.2 mV, 2 ms. Data analyzed with unpaired Student's t-test.



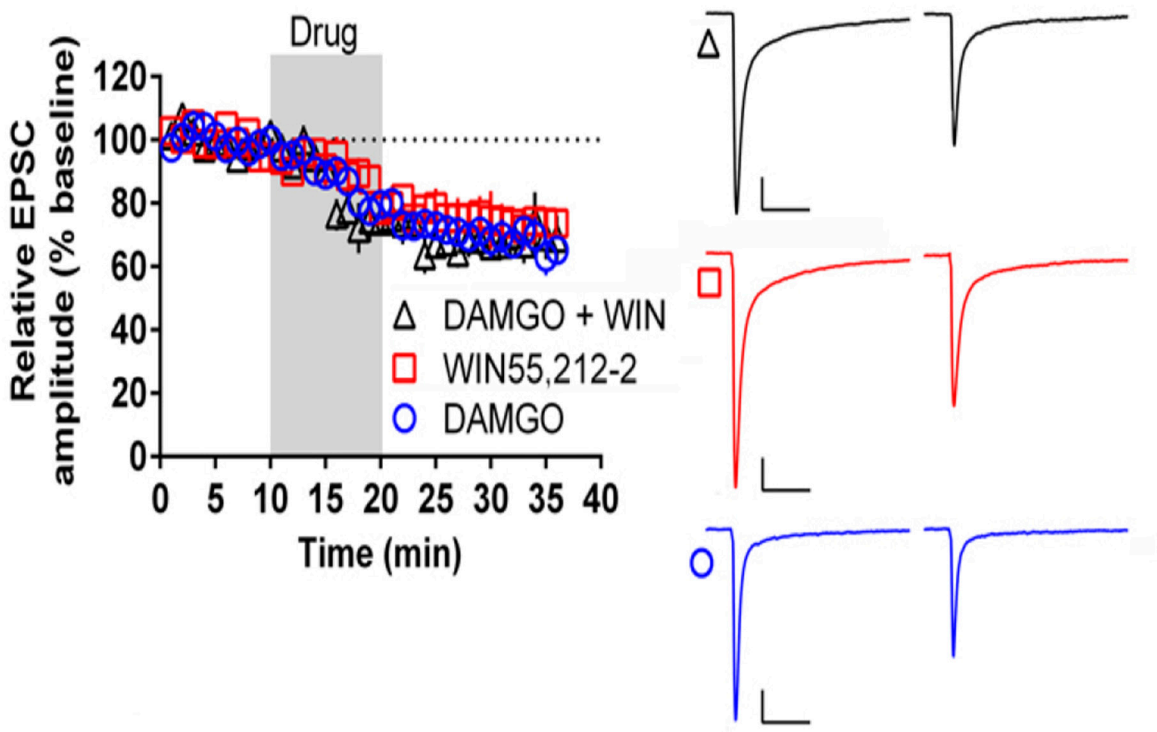
Supplementary Figure 2: OP-LTD is equivalent at -60 mV and -80 mV holding potentials in DLS, but only mOP- and dOP-LTD occur in DMS.

(**a**) DAMGO (1 μM, 5 min) induced comparable mOP-LTD in MSNs recorded at -60 mV (n=11) and -80 mV in DLS (n=4), and at -60 mV in DMS (n=6) (at 25 min: P=0.3741, $F_{(2,18)}$=1.039). (**b**) DPDPE (1 μM, 5 min) induced comparable dOP-LTD in MSNs recorded at -60 mV (n=7) and -80 mV in DLS (n=5), and at -60 mV in DMS (n=4) (at 25 min: P=0.6461, $F_{(2,13)}$=0.4518). (**c**) U69,593 (0.3 μM, 5 min) induced comparable kOP-LTD in MSNs recorded at -60 mV (n=5) and -80 mV in DLS (n=5). U69,593 failed to induce LTD in DMS (n=6)(at 25 min: P=0.0075, $F_{(2,13)}$=7.308). Data analyzed with a one-way ANOVA with Dunnett's multiple comparisons post-test: vs. DLS, -60 mV. *: P<0.05.



Supplementary Figure 3: mOP- and dOP-LTD are expressed presynaptically, whereas kOP-LTD has an unclear site of expression.

(**a**) Representative traces of sEPSCs recorded from a DLS MSN before, immediately after, and 20-25 min following DAMGO (0.3 μM, 5 min). DAMGO (0.3 μM, 5 min) induced a long-lasting increase in sEPSC inter-event interval (IEI) (**b,c**; P=0.0063, Friedman statistic=9.800, n=9) with no change in sEPSC amplitude (**d**; P=0.2223, Friedman statistic=3.200). (**e**) Representative sEPSC traces for DPDPE (0.3 μM, 5 min). DPDPE (0.3 μM, 5 min) produced a long-lasting increase in sEPSC IEI (**f,g**; P=0.0207, Friedman statistic=7.714, n=7) with no change in sEPSC amplitude (**h**; P=0.0854, Friedman statistic=5.429). (**i**) Representative sEPSC traces for U69,593 (0.3 μM, 5 min). U69,593 (0.3 μM, 5 min) produced no change in sEPSC IEI (**j,k**; P=0.0570, Friedman statistic=6.000, n=9), but produced a delayed decrease in sEPSC amplitude (**l**; P=0.0307, Friedman statistic=6.889). Scale bars: 20 pA, 2 s. Data in **c-d, g-h, and k-l** analyzed with Friedman test with Dunn's multiple comparisons post-test. *: P<0.05.



Supplementary Figure 4: The lack of an additive effect of a MOPr and a CB_1 agonist suggests a presynaptically localized occlusiveness of mOP-LTD and CB_1-LTD.

DAMGO (0.3 μM; n=5), WIN55,212-2 (1 μM, n=5), or their combination (n=6), each applied for 10 min, induced comparable magnitudes of depression (P=0.7797, $F_{(2,13)}$=0.2537). Representative traces are the average of the first 10 min (first of pair) and the final 10 min (second of pair) of recording. Scale bars: 50 pA, 50 ms. Data analyzed with one-way ANOVA with Tukey's post-test.

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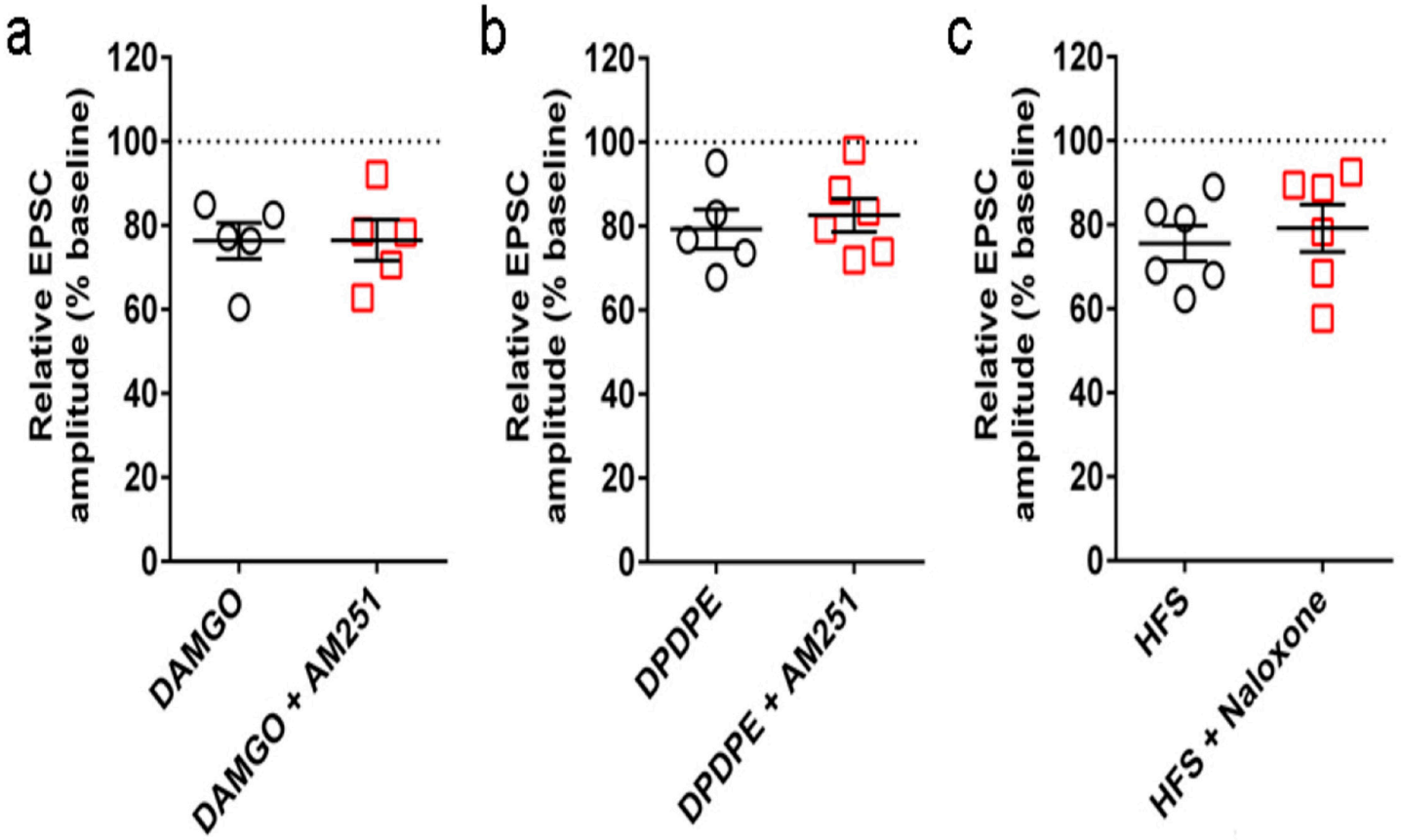

Supplementary Figure 5: eCB-LTD is not blocked by opioid receptor antagonists, and OP-LTD is not blocked by a CB_1 antagonist.

(**a**) Pre-application of the CB_1 receptor antagonist, AM251 (3 μM), did not alter mOP-LTD induced by DAMGO (0.3 μM to 1 μM, 5 min; at 30 min:P=0.9836, t(8)=0.02127, n=5 each). (**b**) Pre-application of AM251 (3 μM) did not alter dOP-LTD induced by DPDPE (0.3 μM to 1 μM, 5 min; at 30 min: P=0.5996; t(9)=0.5441, n=5 control, n=6 AM251). (**c**) Pre-application of the opioid receptor antagonist, naloxone (2 μM), did not alter eCB-LTD induced by HFS paired with postsynaptic depolarization (at 30 min: P=0.6162, t(10)=0.5173, n=6 each). Data analyzed with unpaired Student's t-test.

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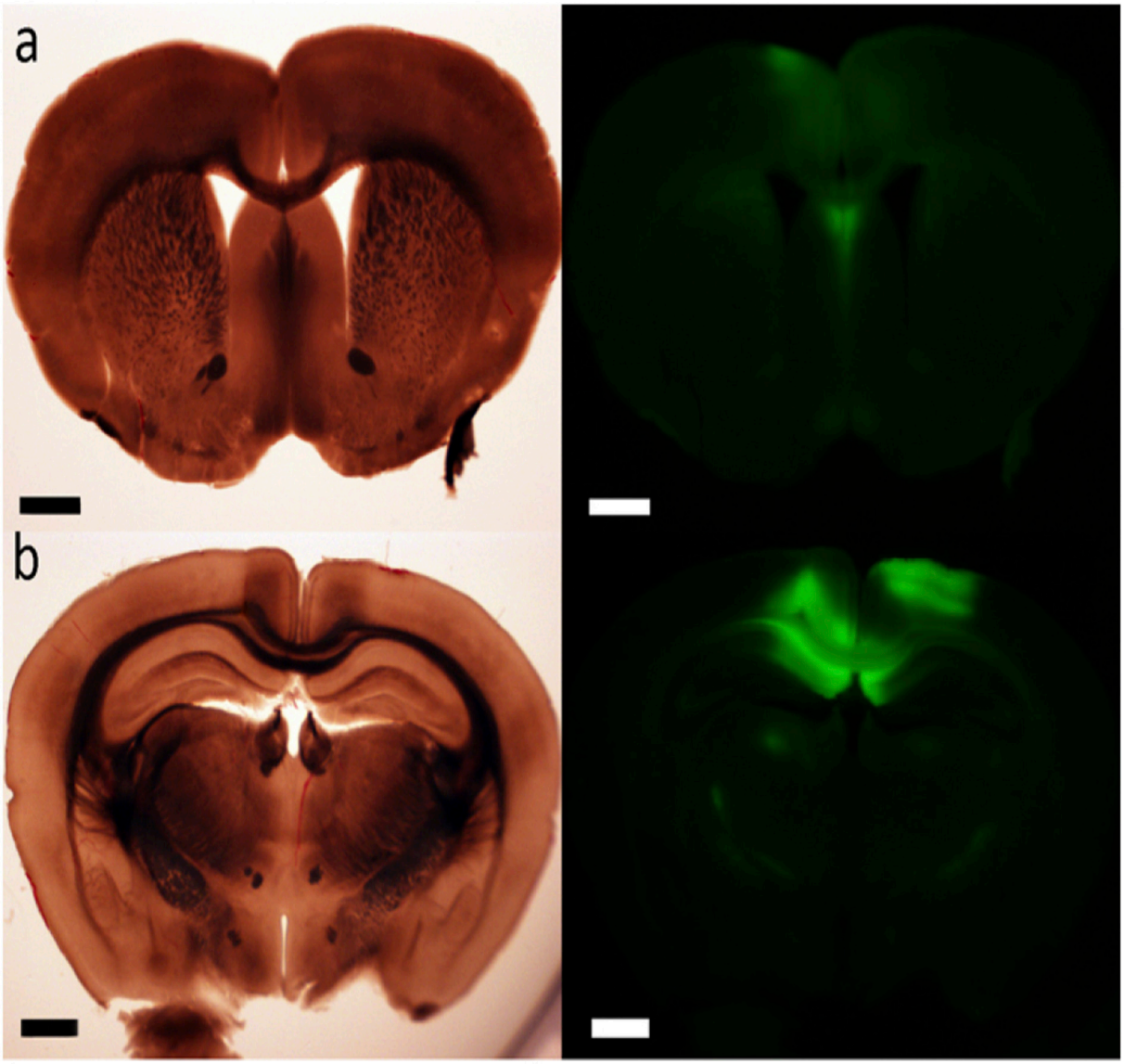

Supplementary Figure 6: Expression of ChR2-Venus in hippocampus does not result in striatal ChR2-Venus expression

(**a**) Lack of striatal expression of ChR2-Venus in striatum following an injection of AAV vector in hippocampus. Injection coordinates: A/P: -2.0, M/L: ± 0.35, D/V: -1.45. (**b**) AAV vector injection in hippocampus produces substantial ChR2-Venus expression at the injection site. Images are representative of 1 injected mouse. Scale bars: 1 mm.

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