

Metaplasticity of Hypothalamic Synapses following In Vivo Challenge
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Supplemental Figures

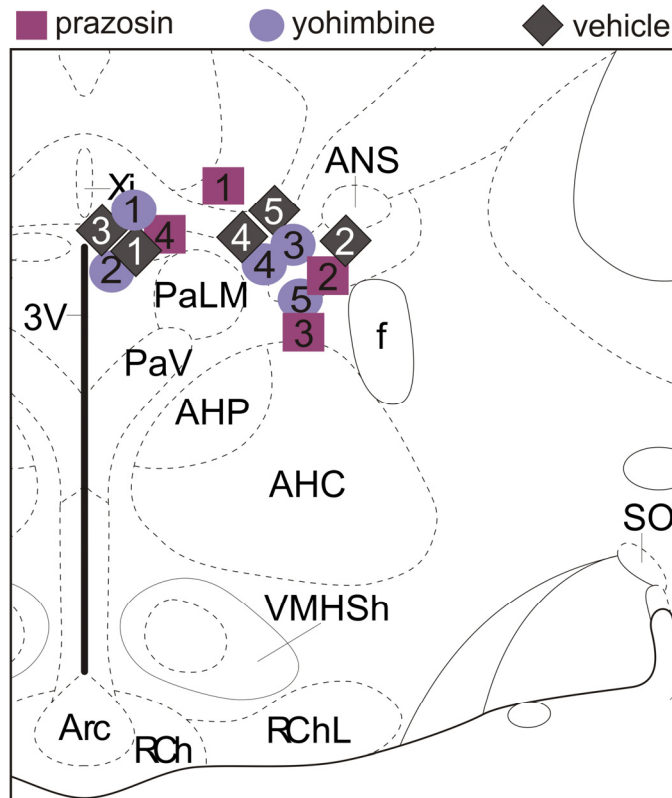


Figure S1. Microinjection of Adrenoceptor Antagonists into the PVN

Summary of *in vivo* microinjection sites in the PVN. Prazosin (squares), yohimbine (circles) and vehicle (diamonds) were mapped onto plates adapted from Paxinos and Watson (2005). The numbers refer to the individual injection sites. Injections from both the right and left hemisphere were mapped onto the same side in the diagram for simplicity. Refer to the Paxinos and Watson atlas for explanations of the abbreviations.

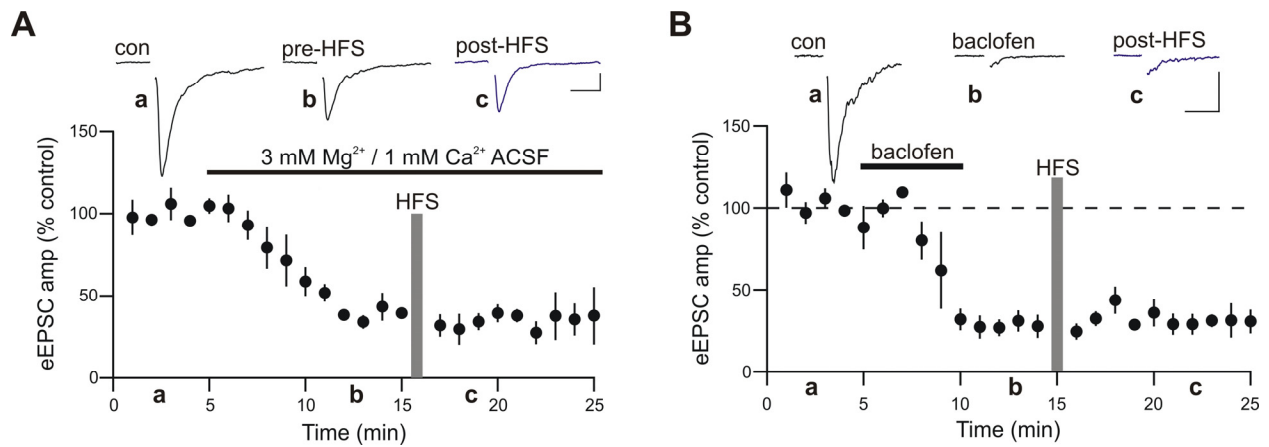


Figure S2. Failure of HFS to Induce LTP in Low Pr Conditions or Following Application of a GABA_B Receptor Agonist

(A) HFS (3×100 Hz - 1 s, 10 s interval) failed to induce plasticity in synapses where the probability of glutamate release was reduced with perfusion of ACSF containing 3 mM Mg²⁺ and 1 mM Ca²⁺ (10 min: pre-HFS 35.29 ± 4.32 % of control eEPSC amplitude; post-HFS 31.86 ± 6.33 %; $n = 5$; $p = 0.53$). Inset: averaged eEPSCs in control ACSF, after perfusion with ACSF containing 3 mM Mg²⁺ and 1 mM Ca²⁺ pre-HFS and post-HFS. Scale bars: 10 ms, 20 pA. **(B)** Application of the GABA_B receptor agonist baclofen (30 μ M) depressed evoked glutamate release. After a 5 minute wash of baclofen, subsequent application of HFS failed to recover synaptic currents to pre-baclofen levels ($n = 4$). Inset: averaged eEPSCs in control, after application of baclofen and following subsequent HFS. Scale bars: 20 ms, 40 pA.

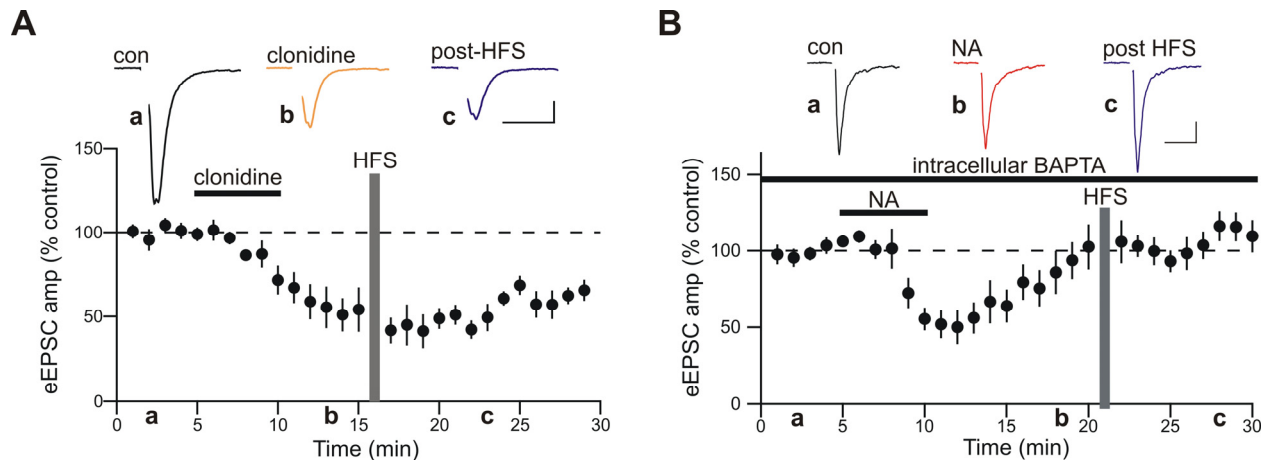


Figure S3. Failure to Induce Metaplasticity After Clonidine LTD or After Inhibition of LTD_{NA} with Intracellular BAPTA

(A) HFS failed to induce plasticity in synapses where LTD was induced with clonidine ($n = 6$).

Inset: averaged eEPSCs in control, after perfusion with clonidine pre-HFS and post-HFS. Scale

bars: 10 ms, 20 pA. **(B)** In neurons where 10 mM BAPTA was included in the patch pipette to

block induction of LTD_{NA} , subsequent HFS failed to potentiate synapses ($n = 6$). Inset shows

sample traces of eEPSCs in control (black), following NA pre-HFS (red) and after HFS (purple).

Scale bars: 20 ms, 50 pA.

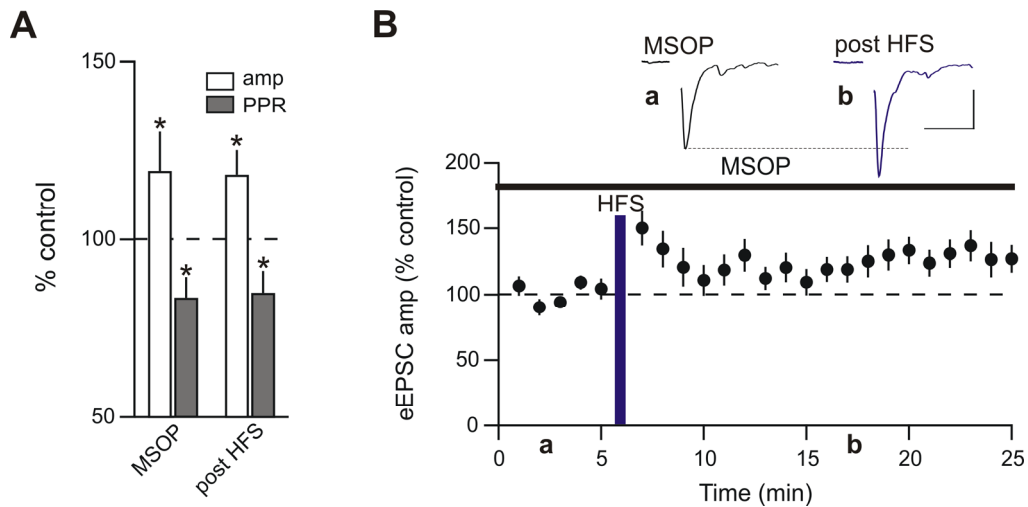


Figure S4. Group III mGluRs Are Tonicly Active and Prevent HFS Induced Potentiation

(A) Summary bar graph illustrating changes (expressed as a percentage of baseline control) in amplitude and PPR of evoked synaptic currents after application of the group III mGluR antagonist, MSOP (100 μ M; $n = 7$) and after HFS (post HFS compared to stable MSOP baseline) ($n = 10$; $*p < 0.05$). **(B)** Average time course showing the effect of HFS applied to slices continuously perfused with MSOP. Inset shows average eEPSCs before (black) and after HFS (purple). Scale bars: 20 ms, 40 pA.