Supplemental Information

mGluR-LTD at Excitatory and Inhibitory Synapses in the Lateral Habenula Tunes Neuronal Output

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Figure S1. mGluR1-dependent LFS-eLTD and HFS-iLTD in the LHb. Related to Figure 1 and Figure 2.

(A). Low frequency stimulation (1 Hz, 15 min) eLTD is blocked by the mGluR1 antagonist LY367385. Bar graph and scatter plot show normalized averaged EPSCs ~40 min after the protocol (89.9±5%, t$_{8}$=1.941, p>0.05).

(B). Effect of the mGluR1 antagonist LY367385 on high frequency stimulation (100 Hz, 1 sec, at 0 mV)-driven iLTD (94.2±17.4%, t$_{5}$=0.394, p>0.05).

(C). Low frequency stimulation (1 Hz, 15 min) drives an iLTD (full circles) that remains not affected by the mGluR1 antagonist LY367385 (empty circles) (in ACSF, 77.4±8%, t$_{6}$=2.842, *p<0.05; in LY367385, 70.1.2±8.7%, t$_{8}$=3.415, **p<0.01). N in the figures indicates number of recorded neurons.
Figure S2. PPR analysis for EPSC and IPSC after mGluR activation. Related to Figure 1–4.

(A). Sample recordings indicating the parallel modulation of EPSCs and PPR after DHPG application.

(B). Same as A but for IPSCs.

(C). PPR of EPSCs after LFS (baseline 0.6±0.04 vs post-LFS 0.76±0.05, t_9=6.699, ***p<0.0001); PPR of EPSCs in the presence of intracellular GABA_α-β2 and γ2 peptide (GABA_α-β2, baseline 0.52±0.07 vs post-DHPG 0.73±0.05, t_6=4.127, **p<0.01; GABA_α-γ2, baseline 0.47±0.08 vs post-DHPG 0.68±0.09, t_7=4.303, **p<0.05). When not indicated time scale represents 5ms.

(D). PPR of IPSCs after HFS (baseline 0.79±0.1 vs post-HFS 0.79±0.1, t_5=0.1, p>0.05); PPR of IPSCs in presence of LY367385 (baseline 0.61±0.06 vs post-DHPG 0.65±0.06, t_8=1.166, p>0.05); of MTEP (baseline 0.63±0.12 vs post-DHPG 0.8±0.1, t_6=2.179, p>0.05); of PKC[19-36] in the recording pipette (baseline 0.83±0.08 vs post-DHPG 0.8±0.04, t_6=0.318, p>0.05); after PMA and DHPG (baseline 0.56±0.2 vs
PMA 0.56±0.16, t₄=0.107, p>0.05; baseline-PMA vs PMA-post-DHPG 0.5±0.2, t₄=0.794, p>0.05; PMA vs post-DHPG+PMA, t₄=1.812, p>0.05); in presence of NESS-0327 (baseline 0.82±0.09 vs post-DHPG 0.8±0.06, t₁₀=0.310, p>0.05); of dynamin inhibitor in the recording pipette (baseline 0.8±0.09 vs post-DHPG 0.83±0.2, t₈=0.280, p>0.05); of the GABA-β2 peptide in the recording pipette (baseline 0.79±0.12 vs post-DHPG 0.8±0.1, t₁₃=0.192, p>0.05); of the GABA-γ2 peptide in the recording pipette (baseline 0.57±0.08 vs post-DHPG 0.67±0.09, t₈=0.973, p>0.05). One way ANOVA among all baseline PPR conditions: F₈,₈₀=0.693, p>0.05. N in the figures indicates number of recorded neurons.
Figure S3. Bidirectional control of firing by mGluR-LTD, unaltered input resistance and action potential properties after mGluR activation. Related to Figure 5.

(A). Normalized effect of DHPG on synaptically-evoked APs number in the presence of GABA_A-β2 peptide in the internal solution (76.9±10.6, t_{13}=2.183, *p<0.05).

(B). Normalized effect of DHPG on synaptically-evoked APs number in the presence of NESS-0327 (158.2±21.8; t_{10}=2.667, *p<0.05).

(C). Averaged input resistance and scatter plot for recordings obtained in current clamp (baseline 495.5±63.6 vs post-DHPG 479.4±65.4, t_{13}=0.49, p>0.05).

(D). No correlation between mGluR-driven change in firing vs input resistance (r=0.35, p>0.05).

(E). Averaged action potential traces indicating the parameters analyzed before and after mGluR activation.

(F). Pooled data and scatter plots for AP amplitude (baseline 63.2±3.1 vs post-DHPG 59.8±3.1, t_{11}=1.31, p>0.05)

(G). Same as D but for AP width (baseline 1.06±0.1 vs post-DHPG 1.12±0.1, t_{11}=0.60, p>0.05)

(H). Same as D and E but for AP afterhyperpolarization amplitude (AHP, baseline 3.21±1.2 vs post-DHPG 3.22±1.2). No correlation of firing vs AP amplitude (r=0.36, p>0.05); vs width (r=-0.11, p>0.05); vs AHP (r=0.039, p>0.05). N in the figures indicates number of recorded neurons.
Figure S4. mGluR-dependent modulation of IPSCs, but not EPSCs, at EPN inputs onto the LHB. Related to Figure 1 and Figure 5.

(A). Experimental protocol and sample images representing stereotaxic injection of rAAV-ChR2 within the EPN, and EPN terminals within the LHB.

(B). DHPG effect on EPN\textsuperscript{LHB} EPSCs (109.4±6.5%, \( t_9 = 0.414 \), \( p>0.05 \)).

(C). DHPG effect on EPN\textsuperscript{LHB} IPSCs (65.4±6.5%, \( t_3 = 0.527 \), *\( p<0.05 \)). N in the figures indicates number of recorded neurons.
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**Supplemental Experimental Procedures**

**Stereotactic injections of viral constructs**

Bilateral injections (~300 nl per hemisphere) in anaesthetized mice were performed using a glass pipette on a stereotactic frame (Kopf, France). Viral constructs allowing the expression of Channelrhodopsin-2 or Cheta (rAAV2/1-CAG-hChR2(H134R)-mCherry; rAAV9-hSyn-Cheta-EYFP; titers: 1\texttimes{}10\textsuperscript{12}–1.3\texttimes{}10\textsuperscript{13} gc/ml; University of Pennsylvania, USA) were injected in the entopeduncular nucleus (EPN) (~1.25 mm AP, 1.80 mm ML, ~4.65 mm DV). Animals were allowed to recover for a minimum of 3 weeks before recordings were made. Only mice showing fluorescence within the lateral portion of the LHb were used for recordings.

**In vitro electrophysiology**

Sagittal slices (250 µm) containing the LHb were prepared as previously described (Maroteaux and Mameli, 2012). Slices were used for recordings in artificial cerebrospinal fluid (ACSF) equilibrated with 95\% O\textsubscript{2}/5 \% CO\textsubscript{2} and containing (in mM): NaCl 124; NaHCO\textsubscript{3} 26.2; glucose 11; KCl 2.5; CaCl\textsubscript{2} 2.5; MgCl\textsubscript{2} 1.3; NaH\textsubscript{2}PO\textsubscript{4} 1. The ACSF flow rate was 2 ml/min and the temperature was set at ~30°C. Currents were amplified, filtered at 2.5-5 kHz and digitized at 10 kHz. Access resistance was monitored by a 4 mV hyperpolarizing step, and experiments were discarded if access resistance increased >20\%. For voltage-clamp experiments the internal solution contained (in mM): CsCl 130; NaCl 4; MgCl\textsubscript{2} 2; EGTA 1.1; HEPES 5; Na\textsubscript{2}ATP 2; Na\textsubscript{3}GTP 0.6; Na\textsuperscript{+}- creatine-phosphate 5, QX-314 2, and spermine 0.1, pH 7.3, osmolarity ~300 mOsm. The liquid junction potential was ~3 mV and pipette resistance was 3-4 MΩ. Holding potential was ~50 mV. Synaptic currents were evoked through a glass pipette placed in the stria medullaris (60 µs at 0.1 Hz). Paired pulse ratio (PPR) was monitored (2 pulses, 20 Hz) and calculated as follows:
mGluRs were activated by R,S-3,5-dihydroxyphenylglycine (DHPG, 50 µM) in presence of NBQX (10 µM) and D-APV (50-100 µM) or Picrotoxin, 100 µM. mGluRs were synaptically activated using a low frequency stimulation protocol (LFS, 1 Hz, 15 min) or a high frequency stimulation protocol (HFS, 100 Hz at 0 mV for 1 sec; 5 times every 10 sec). Experiments assessing postsynaptic effects of DHPG (voltage-clamp) and output firing (current-clamp) were performed with internal solution containing (in mM): KGluconate 140; KCl 5; HEPES 10; EGTA 0.2; MgCl₂ 2; Na₂ATP 4; Na₃GTP 0.3; Creatine Phosphate 10, pH 7.3, osmolarity ~300mOsm. QX-314 2mM was included when blocking action potentials. Liquid junction potential (12 mV) was not corrected. AMPA-EPSCs current-voltage relationships were performed at -60, 0 and +40 mV. The rectification index was calculated as follows: \((I_{EPSC(-60)})/I_{EPSC(+40)})/1.5\). Miniature EPSCs (mEPSCs) or IPSCs (mIPSCs) were recorded at -60mV in presence of Tetrodotoxin (TTX, 1 µM) and frequency and amplitudes were computed during baseline and 20 minutes after DHPG. Current clamp recordings were performed in ACSF or in presence of the CB1-Rs neutral antagonist NESS-0327 (0.5 µM) and the GABAₐ-β2 peptide (50 µM) in the patch pipette. Cells were kept around their resting membrane potential (RMP –55 mV) throughout the recording. EPSPs and action potentials were evoked by 10 electrical pulses 50 ms apart through a glass pipette. Stimulation intensity was set to evoke ~50% of spikes out of the 10 delivered pulses. 10-20 min following DHPG the number of spikes were counted for a period of 3 minute (19 sweeps) and compared to the same period at baseline. The changes in firing following DHPG were normalized to the baseline number of action potentials. Only regular firing neurons were included in the analysis. The area under individual EPSPs (Synaptosoft Inc, USA) during baseline and following DHPG was averaged and the normalized total area was correlated to the normalized firing after DHPG. The input resistance was calculated via a 50ms hyperpolarizing current (I=20pA) step \((Ri=RMP/I)\). Action potential waveform analysis (Synaptosoft Inc, USA) of the amplitude, width and afterhyperpolarization amplitude for traces at baseline and after DHPG allowed to assess action potential properties changes.

**Non-stationary fluctuation analysis**

A peak-scaled non-stationary fluctuation analysis was made from mIPSCs (Synaptosoft Inc., USA). mIPSCs were selected by: fast rise time alignment; stable
baseline holding current; absence of spurious fluctuations during the mIPSC decay. Variance-amplitude relationship of mIPSC decay was plotted and fitted with the equation: \( \sigma^2 = iI - I^2/N + \sigma_b^2 \), \( i \): mean single-channel GABA current; \( I \): mean current; \( N \): the number of channels activated at the peak, \( N = \text{mean amplitude}/i;\ \sigma_b^2 \): baseline variance). \( i \) was estimated as the slope of the linear fit of the first portion of the parabola. The goodness of the fit was assessed with a least-square algorithm. Unitary current was converted in conductance based on holding potential (–60mV). Conductance and average IPSC amplitude, mean rise time, mean decay time, access resistance or background noise variance had no correlation \( (P > 0.4) \) (Maroteaux and Mameli, 2012).

Reverse Transcription-PCR
Total RNA from LHb (4 mice) was extracted using Trizol (Invitrogen) and transcribed into cDNA using SuperScript II Reverse Transcriptase (Invitrogen). PCR were conducted with Taq DNA polymerase (Invitrogen) using the following primers: mGluR1: forward primer 5’-CAAATCGCCTATTCTGCCAC-3’, reverse primer 5’-CCATTCCACTCTCGCCGTAATTCC-3’; mGluR5: forward primer 5’-GAGGAGGGGCTCGTCTGGGG-3’, reverse primer 5’-ACAGTCGCTGCCACAGGTGC -3’

Drugs and Peptides
Drugs were obtained from Abcam (Cambridge, UK), Tocris (Bristol, UK), Hello Bio (Bristol, UK) or Latoxan (France) and dissolved in water. TTX was dissolved in citric acid (1%), Picrotoxin, NESS-0327, WIN-55,212-2 and PMA in DMSO and LY367385 (in NaOH 10%). For PMA experiments, only cells responding to drug application were included in the analysis. When indicated peptides were included in the internal solution: PKC inhibitor PKC [19-36] (20 µM), dynamin inhibitor (QVPSRPNAP; 1.5 mM), GABA\(_A\)-R-\( \beta \)2 subunit peptide (KSRLRRASQLKITI; 50 µM), GABA\(_A\)-R-\( \gamma \)2 subunit peptide (SNRKPSKDKDKKKNPAPT; 50 µM). Peptide sequences for \( \beta \)2 and \( \gamma \)2 were custom-synthesized (GeneScript, USA). When recording in presence of peptides, baseline was taken ~10 min after entering in whole-cell to allow intracellular dialysis.
Analysis

Analysis was performed using IGOR-6 (Wavemetrics, USA) and MiniAnalysis (Synaptosoft Inc, USA). Cumulative plots for mPSCs were obtained by compiling all recorded neurons at baseline and after DHPG conditions. Data were binned at 2.5 pA and 150 ms to normalize the sample size across recordings and significance was assessed by the Kolmogorov-Smirnov test (KS). A linear fit was used for correlations and significance was tested with Pearson Coefficient (r). N in the figures indicates number of recorded neurons. All data are expressed as mean ± s.e.m. Significance was set at alpha=0.05 using paired t-test.