Supporting Online Material:

Supplemental Figure Legends

Figure S1. mGluR1 expression and biocytin labeling of OA-INs in hippocampal slice cultures.

(A) Immunocytochemical detection of mGluR1 expression (as described in Lapointe et al., 2004) in CA1 region of slice cultures. Note high level of expression in stratum oriens/alveus (or./alv.), absence in pyramidal cell layer (pyr.) and low expression in stratum radiatum (rad.). Scale bar, 50 µm.

(B) Representative OA-IN morphology. Biocytin-filled neurons were processed as described previously (Lapointe et al., 2004). For morphological identification, cells were examined with an Eclipse E600E light microscope (Nikon, Japan) equipped with a digital camera (Retiga 1300, QImaging, Burnaby, BC, Canada) and images were acquired with SimplePCI Imaging System (Hamamatsu Corp., Sewickley, PA). A total of 280 biocytin-labeled cells were identified after electrophysiological experiments. All cells were located in the CA1 oriens-alveus region. In 90% of labeled cells, the soma and dendrites were horizontally oriented and parallel to stratum pyramidale. The remaining 10% of cells had a vertical somato-dendritic orientation. In 168 cells, axonal projections were clearly labeled, allowing further classification. The large majority displayed characteristic morphology of Oriens-Lacunosum Moleculare (O-LM; n=163) cells with horizontally oriented cell body and dendrites and an axon projecting to stratum lacunosum-moleculare (l.m.) (Maccaferri, 2005). The other cells were bi-stratified subtypes (n=5; not shown) (Freund and Buzsaki, 1996). Scale bar, 50 µm.
Figure S2. Time course of cL-LTP_{mGluR1} following induction

(A) Representative EPSCs evoked by minimal stimulation at 2 h following sham-treatment (top) and repeated mGluR1 stimulation (bottom), showing larger responses after repeated mGluR1 activation. *Left:* Superimposed 20 successive events (EPSCs + failures; grey) with average EPSC (including failures; solid black line) of 100 events. *Middle:* Average of EPSC pairs (100 events) evoked by paired-pulse stimulation (50 ms inter-stimulus interval), showing reduction of paired-pulse facilitation after repeated mGluR1 stimulation. *Right:* Superimposed 1st and 2nd EPSCs of average pair. Black triangles indicate time of stimulation.

(B) Summary bar graphs for all cells showing increase in potency and reduction in paired-pulse ratio of EPSCs at 1-3 h after repeated mGluR1 stimulation, relative to sham-treated.

(C) Time course of effects on potency and paired-pulse ratio at 1, 2, 3, and 24 h after repeated mGluR stimulation, showing significant effects at every time point relative to sham-treated. There was no significant difference between effects at various time points. The data from Fig. 1C-D at 24 h following repeated mGluR1 stimulation was used for comparison at this time point in C.

Values expressed as mean ± s.e.m and * corresponds to p<0.01. Number above or below bar indicates number of cells.

Figure S3. cL-LTP_{mGluR1} occludes hebbian LTP at OA-IN synapses.

For occlusion experiments, we performed a hebbian pairing protocol (Perez et al. 2001). Briefly, EPSCs evoked by minimal stimulation were acquired for a 5-min baseline period,
and subsequently, LTP was induced by applying θ-burst stimulation (TBS; five bursts at 200-ms intervals (5 Hz), each consisting of four pulses at 100 Hz), paired with five depolarizing steps (each 60-ms duration) to −20 mV. The pairing protocol was repeated 3 times at 30-s intervals. After the induction protocol, EPSCs were recorded for at least 30 min. Hebbian LTP was examined only in one cell per slice from interleaved sham and repeated mGluR1 stimulation (3x DHPG/MPEP) groups. Experimenter was blind to treatment group.

(A) Representative example of cells showing LTP induced by a hebbian stimulation protocol in a slice that received sham-treatment 24 h previously (top), and absence of LTP in a slice that received previous 3 x mGluR1 stimulation (bottom). Left: Continuous scatter plot of EPSC amplitude before (Pre) and up to 30 min following the pairing protocol (TBS + depol.). Right: 20 consecutive traces (top and middle) and superimposed mean EPSCs (including failures; bottom) taken at indicated times. In slices with repeated mGluR1 stimulation, EPSCs in baseline conditions (Pre) were larger in amplitude than in sham-treated slices, but did not show LTP at 30 min post-pairing.

(B) Summary bar graphs for all cells. At 25-30 min post-pairing, EPSC amplitude (incl. failures) and potency (exc. failures) were increased, and failure rate was decreased in OA-INs from slices that received sham treatment 24 h previously (open bars). These effects were blocked in OA-INs from slices that received previous repeated mGluR1 stimulation (filled bars), indicating occlusion of hebbian LTP by prior induction of cL-LTP_{mGluR1}. Dotted lines represent mean baseline (100%). Values expressed as mean ± s.e.m and * corresponds to p<0.05. Number above bar indicates number of cells.
**Figure S4:** Lack of effects of treatment with inhibitors alone relative to sham treatment.  

(A) Summary of effects on evoked EPSCs. Application of indicated inhibitors alone did not affect EPSC potency and paired-pulse ratio compared to sham (p > 0.05 ANOVA, Tukey’s post-hoc comparison).

(B) Summary of effects on mEPSC amplitude and frequency. Application of indicated drugs alone did not affect mEPSC amplitude and frequency relative to shams (p > 0.05, Kolmogorov-Smirnov test, 6 neurons in each group, 200 events per neuron). Values are expressed as mean ± s.e.m.

**Figure S5:** Presence of mTOR and 4E-binding proteins 1 and 2 in OA-INs.  

(A) mTOR immunolabeling is observed in soma and dendrites of GAD67-GFP positive OA-INs, as well as in GFP-negative pyramidal cells (bottom panels).

(B, C) Both 4E-BP1 (B) and 4E-BP2 (C) were detected by immunofluorescence in GAD67-GFP positive OA-INs. Arrows point to GFP-positive interneurons expressing 4E-BP1 or 4E-BP2. Some other GFP-negative cell types were also found to express 4E-BP1 (arrow head).
Supplemental references


