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Supplemental Data
Long-Term Depression of mGluR1 Signaling
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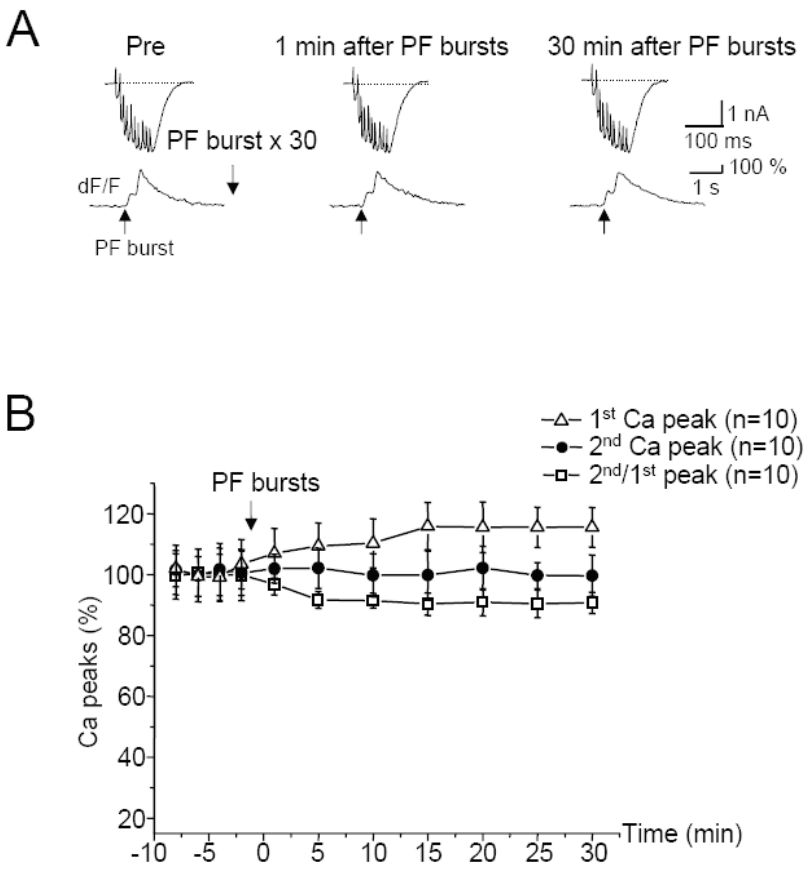

Figure S1. Strong, repeated parallel fiber burst activation delivered in current-clamp mode (I = 0) does not produce a persistent depression of parallel fiber-evoked mGluR1-mediated Ca transients.

A, Purkinje cells were loaded K-based internal saline supplemented with a Ca indicator (Oregon Green BAPTA-1, 200 μM) and dendritic measurements were made using the line-scan mode of a laser scanning confocal microscope. Representative single current and Ca indicator traces show responses to parallel fiber bursts (10 stimuli at 100 Hz) delivered in the presence of a maximal dose of picrotoxin (100 μM). Biphasic Ca responses with slow and fast peaks are evoked by this stimulation (expressed as dF/F). Responses are shown before (pre), and 1 min and 30 min after conditioning stimulation of the parallel fiber (each burst consisting of 5 - 10 pulses at 100 Hz, 30 – 50 bursts with an interval of 2 s, n=10).

B, The ratio of the slow to the fast peak amplitude of the Ca response was calculated as an index of the mGluR1-mediated Ca transient. The time course graph plots this ratio, averaged and normalized. The slight decrease in this ratio results from a small increase in the fast peak, not a decrease in the slow peak.

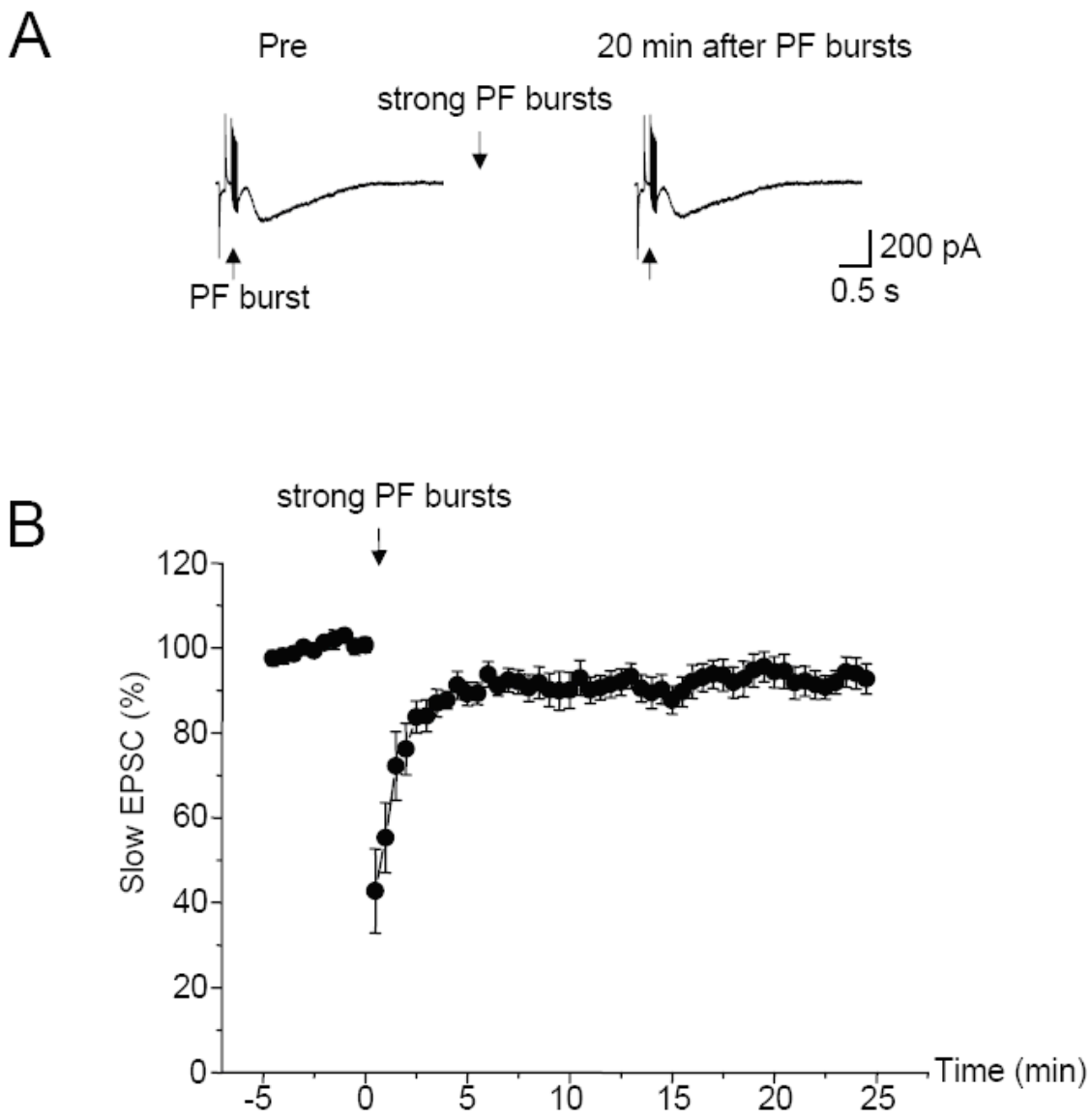

Figure S2. Strong, repeated parallel fiber burst activation delivered in voltage-clamp mode does not produce a persistent depression of parallel fiber-evoked mGluR1-mediated slow EPSCs.

A, Representative current traces show fast and slow components of the parallel fiber EPSC which were produced by bursts (10 stimuli at 100 Hz) delivered in the presence of a submaximal dose of NBQX (5 μM) and a maximal dose of picrotoxin (100 μM). The conditioning parallel fiber stimulation consisted of a 30 bursts (each burst consisting of 5 stimuli at 100 Hz) delivered with an interburst interval of 2 s

B, Population time courses of the percent changes in peak slow EPSC amplitudes induced by conditioning parallel fiber stimulation at the time indicated by the arrow (n = 11 cells).

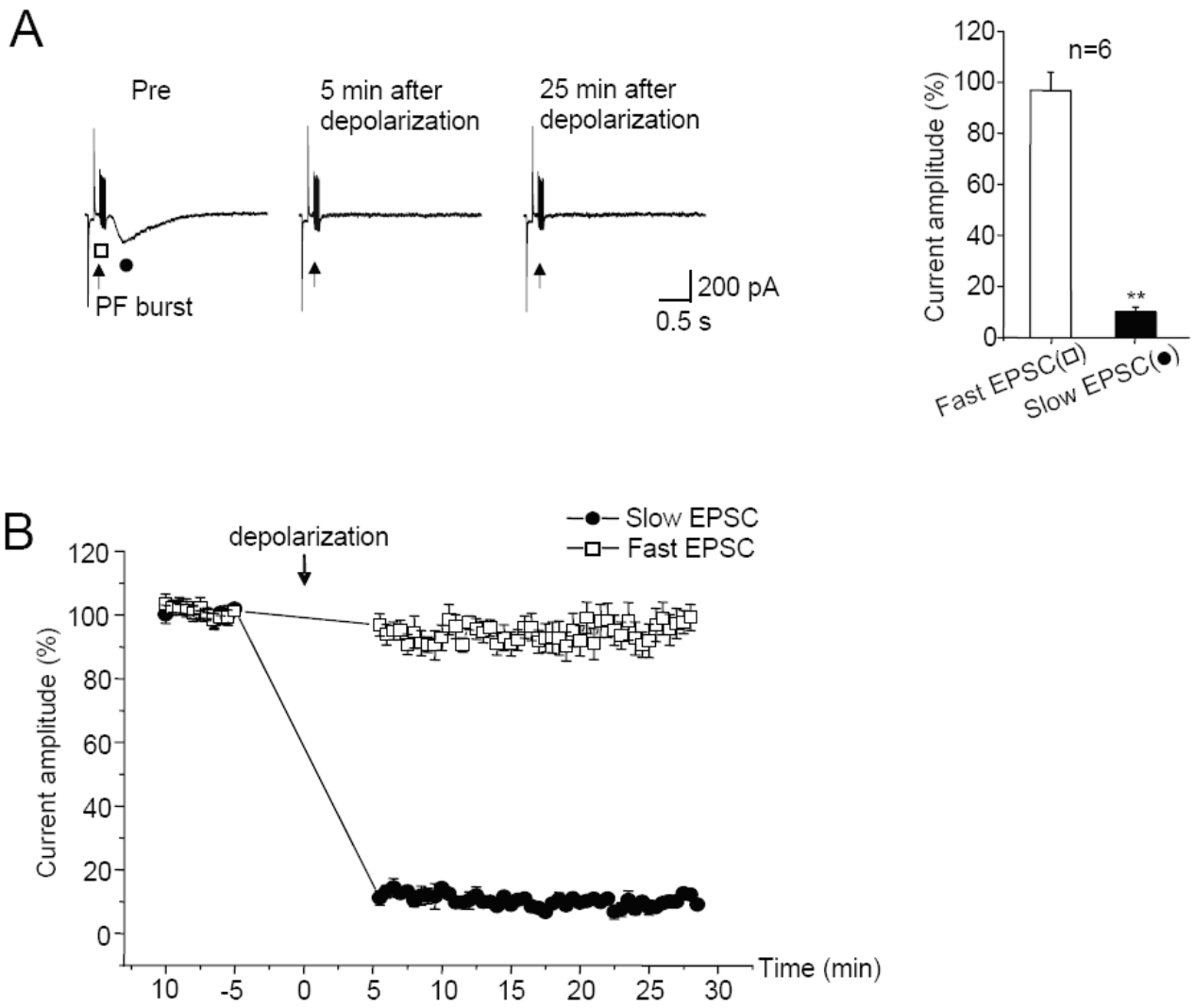

Figure S3. LTD(mGluR1) evoked by conditioning depolarization does not require associative activation of parallel fiber synapses.

A, Left: Representative current traces show fast and slow components of the parallel fiber EPSC which were produced by bursts (10 stimuli at 100 Hz) delivered in the presence of a submaximal dose of NBQX (5 μM) and a maximal dose of picrotoxin (100 μM). There was 5 minute interval between the last parallel fiber test stimulus and the conditioning depolarization of 5 s. Parallel fiber test stimuli were only resumed 5 min after the conditioning depolarization. Right: Population data for the fast and slow EPSC amplitudes are shown. $^{**}p < 0.01$ compared to pre by paired t-test.

B, Population time courses of the percent changes in peak fast and slow EPSC amplitudes induced by conditioning depolarization applied to Purkinje cells at the time indicated by the arrow.

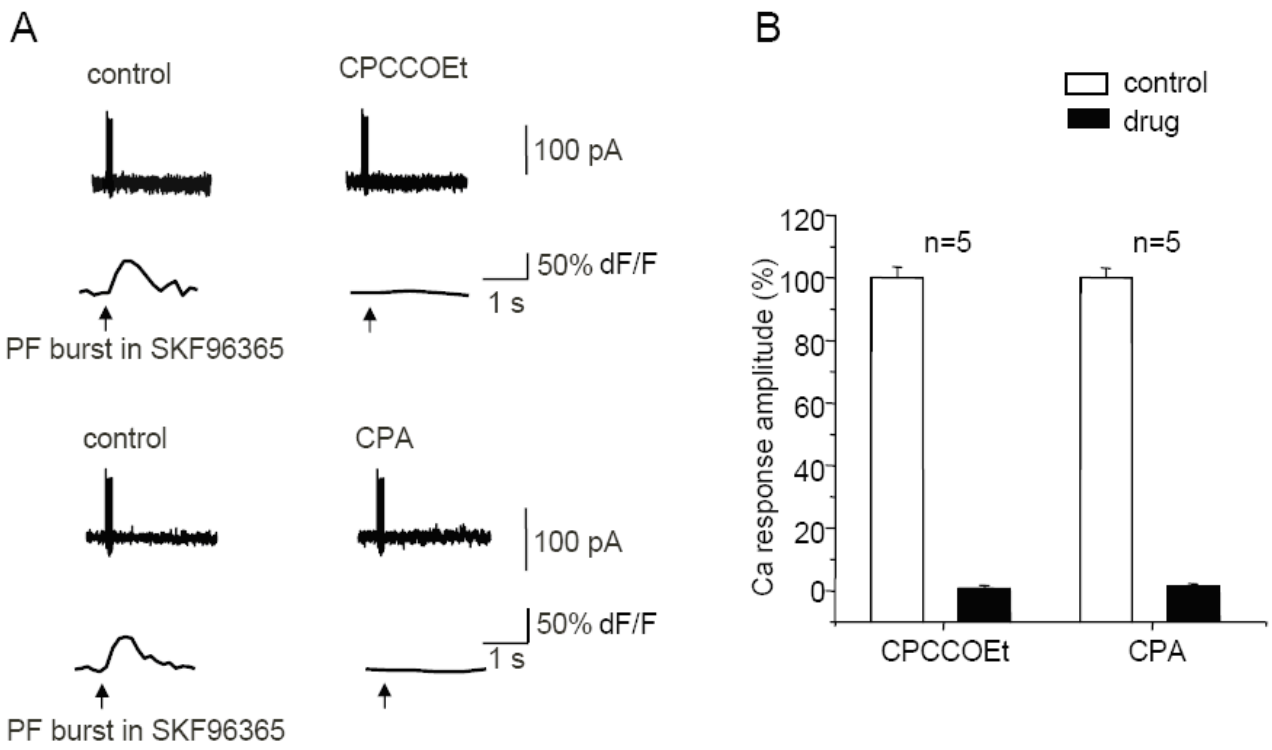

Figure S4. Ca responses to PF burst in the presence of SKF96365 are mediated by an mGluR1/IP3 cascade.

A, Parallel fiber burst stimulation in the presence of a nonselective antagonist of receptor-operated cation channels, SKF 96365 (10 μM), was used to trigger Ca mobilization without a discernable mGluR1-evoked slow EPSC. Before responses were measured, depolarizing pulses (100 ms) were applied to activate voltage-sensitive Ca channels and to load Ca stores. Representative current and Ca traces are shown before (control), and 10 min after drug application. Bath application of the mGluR1 antagonist CPCCOEt (100 μM) and the specific inhibitor of sarcoplasmic reticulum Ca-ATPases (CPA, 50 μM) abolished the evoked increase in dendritic Ca concentration.

B, Population data for the manipulations shown in panel A (n = 5 for both groups) .

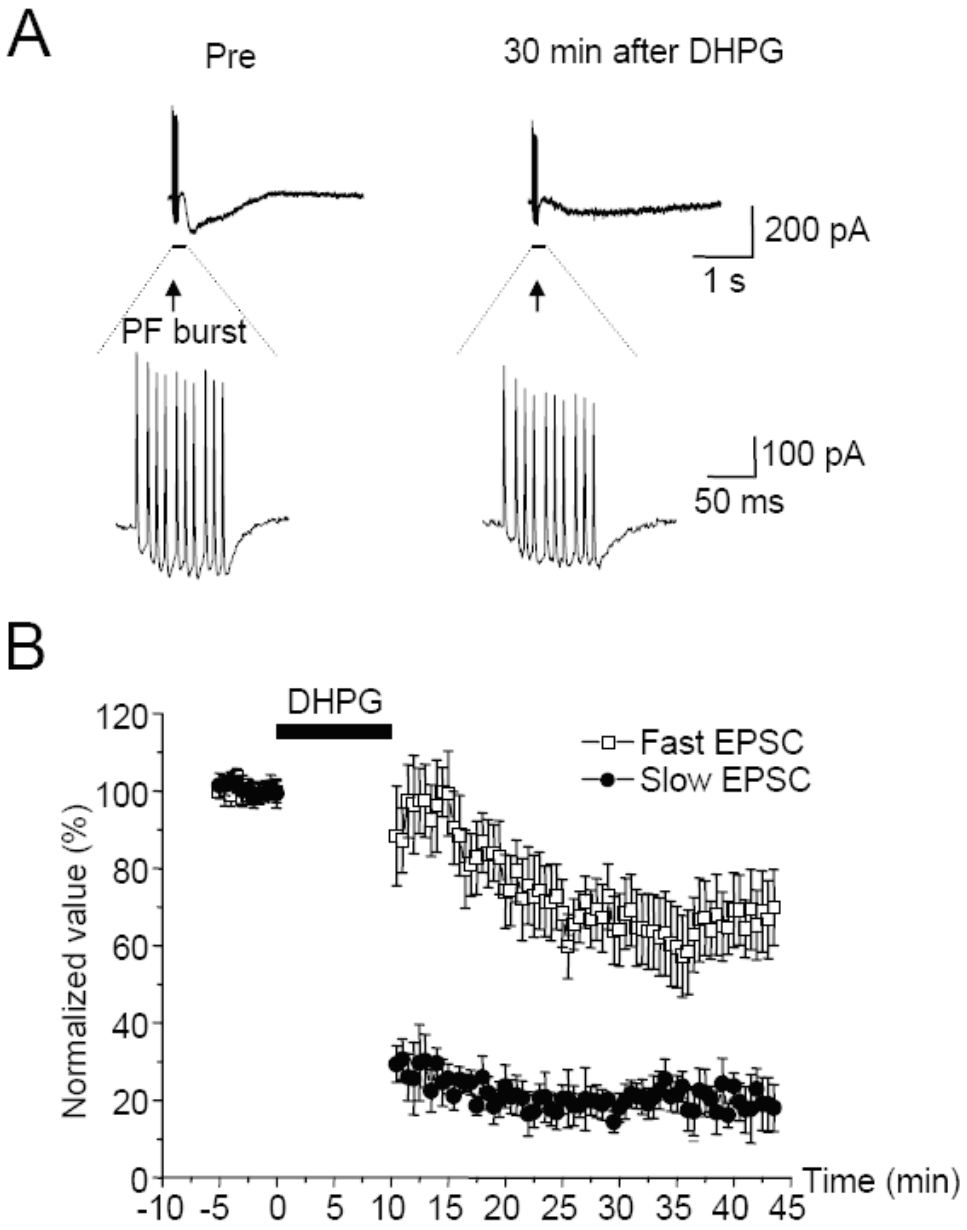

Figure S5. Prolonged DHPG application produced a sustained depression of both fast AMPA and slow mGluR1-mediated parallel fiber EPSCs.

A, Representative current traces show fast and slow components of the parallel fiber EPSC which were produced by bursts (10 stimuli at 100 Hz) delivered in the presence of NBQX (5 μM) and picrotoxin (100 μM). The expanded views reveal the fast current in more detail. Bath application of mGluR1 agonist DHPG (100 μM) for 10 minutes produced a sustained depression of both components. This depression was not associated with changes in the fast EPSC paired-pulse ratio (data not shown).

B, Averaged, normalized time courses are shown. N = 6 cells.

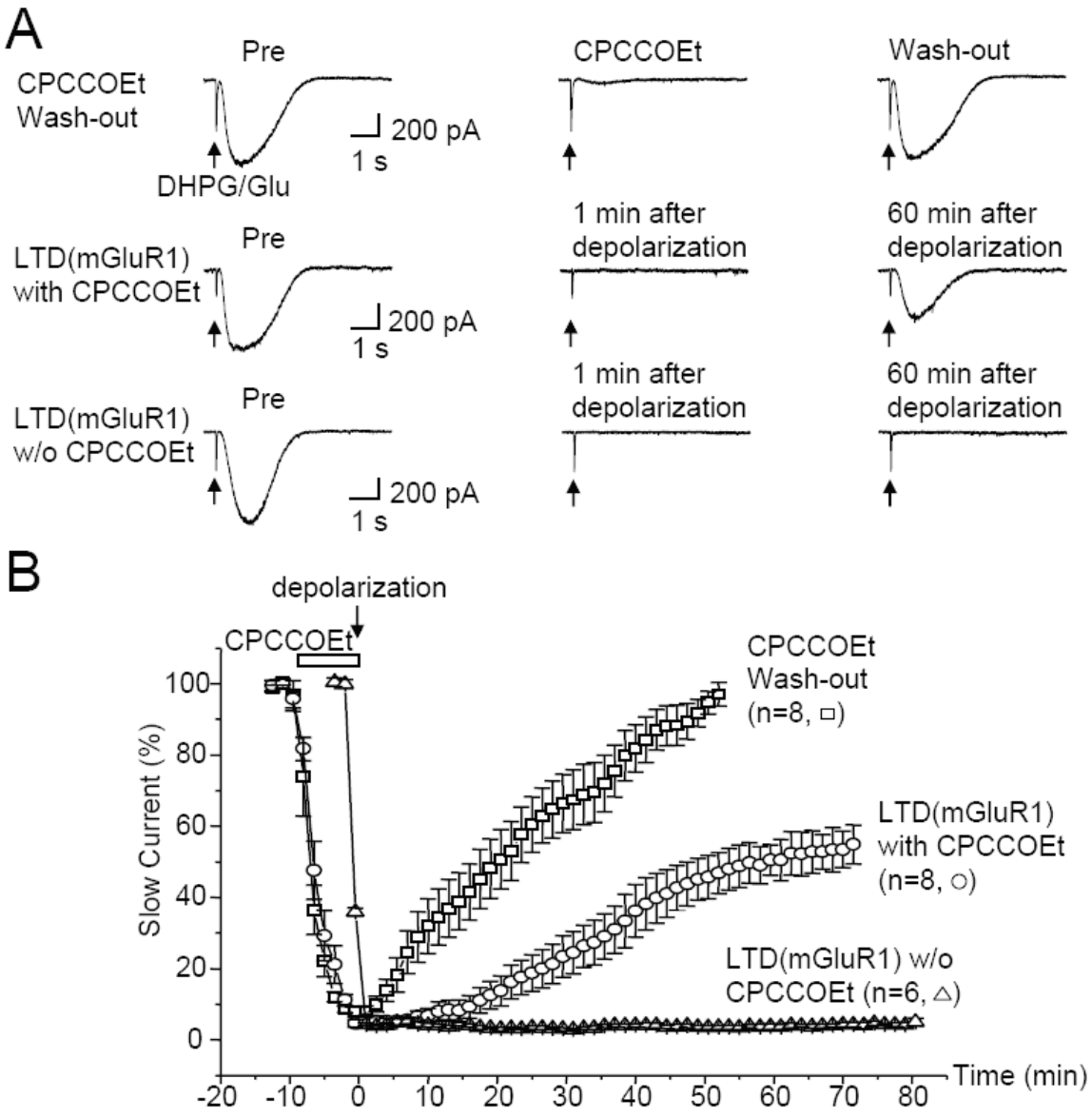

Figure S6. LTD(mGluR1) is partially attenuated by an mGluR1 antagonist.

A, Fast and slow currents were triggered by micropressure pulses (10 psi., 50 ms duration) of external solution containing DHPG (100 μM) and glutamate (40 -100 μM) delivered through a patch pipette. Following baseline recording, the mGluR1 antagonist CPCCOEt (100 μM) was added to the bath to produce a complete suppression of the slow EPSC. Immediately after a 5 sec long conditioning depolarization delivered in the presence of CPCCOEt, washout of CPCCOEt ensued. One control group shows CPCCOEt application and washout without conditioning depolarization, while another shows conditioning depolarization in the absence of CPCCOEt. Representative current traces are shown.

B, Averaged, normalized time courses of the changes in slow currents amplitudes for the manipulations shown in panel A (n=8, 8, and 6 respectively).