Long-Term Depression at Parallel Fiber to Golgi cell Synapses

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Supplementary material

Long-term depression of parallel fiber to Golgi cell synaptic responses, upon voltage-clamp based induction protocols in parasagittal slices

In a separate series of experiments we tested whether parallel fiber (PF)-Golgi cell (GoC) LTD could be induced in voltage clamp (V-clamp) on parasagittal slices. The GoC viability in the parasagittal orientation is better than in the coronal slice orientation, since GoCs are in general parasagittaly organized (Sillitoe et al. 2008; Barmack and Yakhnitsa 2008), but it was much more difficult to stimulate the cut PFs.

Methods were similar to the ones presented in the main text and only differences with the methods used in coronal slices are mentioned here. Slices from the vermis of the cerebellum were cut in the parasagittal plane at a thickness of 250-270 μm. A field-pipette filled with ACSF was employed to deliver monopolar extracellular electrical stimuli to the PFs. This electrode was placed in the ML and positioned so that EPSCs could be evoked.

Two different stimulation protocols were used: a 'burst' IP, similar to the one used in coronal slices (see main text, Methods) and a 'burst + depol' IP protocol. In the latter the stimulation train was combined with a depolarization to 0 mV: bursts of 20 pulses at 100 Hz paired with a postsynaptic depolarization step to 0 mV lasting 200 ms, repeated 30 times with an interburst interval of 2 seconds.

A schematic representation of the recording and stimulating pipette in the parasagittal slice are shown in Figure S1C. Cm was 131 ± 11 pF (range: 47-321 pF; *n*=30) and Rinput was 205 ± 18 MΩ (range: 72-511 MΩ; *n*=30) for GoCs recorded in parasagittal slices. These passive cell parameters are similar to the parameters measured in the coronal slice.

Control experiments, whereby no IP was applied but where EPSCs were continuously monitored at 0.1 Hz, showed a moderate decrease of the EPSC

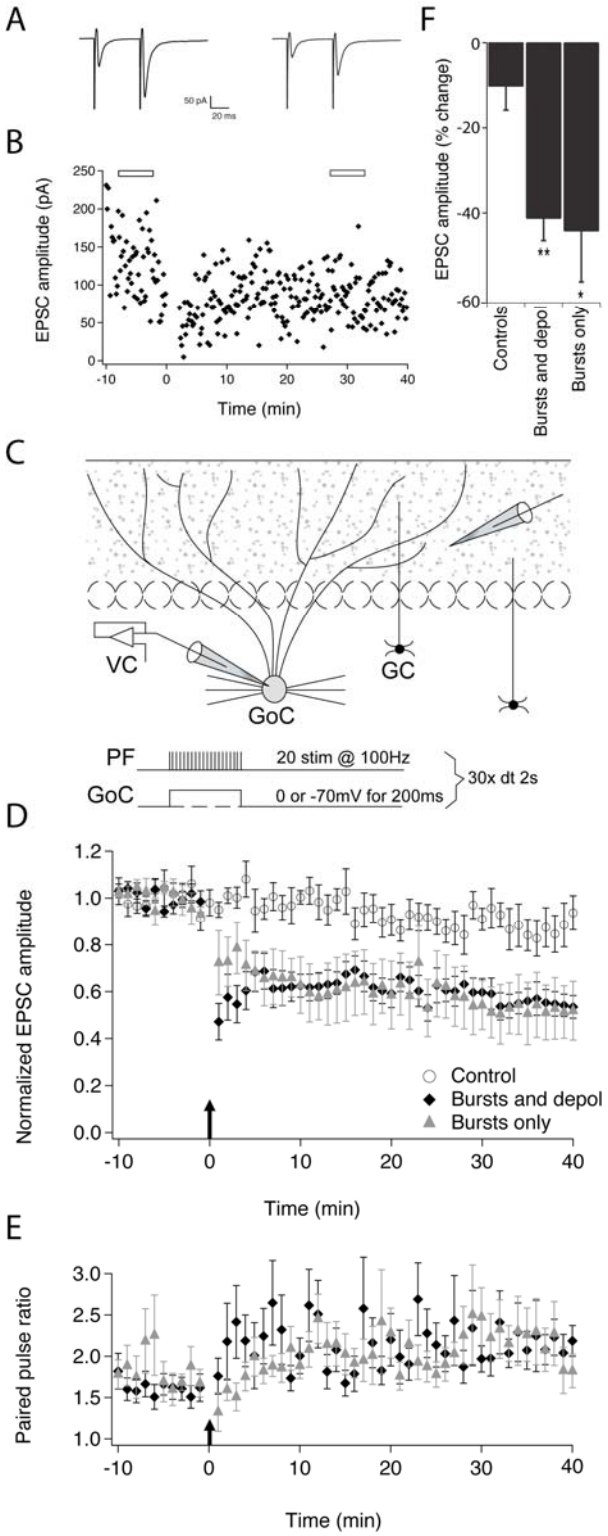
amplitude over the 50 min recording period (-10 ± 5% change at *t* = 25-35 min; *n* = 10) (Figure S1D). Robust LTD was evoked with the 'bursts + depol' IP (Figure S1B, D). Representative averaged traces of evoked EPSCs waveforms (Figure S1A) are reported in correspondence of 5 min pre-IP and of 30 min post-IP, from time points indicated by open bars in (Figure S1B). LTD of EPSCs amplitude is evoked by the 'bursts + depol' IP in a sample experiment (Figure S1B). This 'bursts + depol' IP generally resulted in an immediate and pronounced depression followed by a significant sustained decrease of the amplitude of the evoked EPSCs (-41 ± 5% change at *t* = 25-35 min post-IP; *n* = 14; $p<0.01$ compared to the control group) (Figure S1D, F). 8 out of 14 experiments showed both an immediate and sustained depression following the IP (Figure S1B), 5 out of 14 showed a slow progression towards depression, usually stabilizing 15-20 min post-IP, and one experiment did not show any change in activity.

Additional experiments were performed on parasagittal slices using the same IP but without the depolarization step. This 'bursts only' IP was also capable of inducing LTD, but showed a more progressive decrease following the IP. A sustained decrease in the amplitude of the EPSCs was also observed after about 20 min (-44 ± 12% change at *t* = 25-35 min post-IP; *n* = 7: $p<0.05$ compared to the control group; no significant difference between 'bursts + depol' IP and 'bursts only' IP) (Figure S1D, F).

Under the same experimental conditions, changes in the paired pulse ratios (PPRs) were investigated as a potential landmark of alterations in presynaptic release probability. We considered both induction protocols, including or excluding post-synaptic depolarization (Figure S1E). The LTD following 'bursts and depol' IP was associated with a sustained and significant increase in the PPR (1.65 ± 0.13 at 10 min pre-IP versus 1.94 ± 0.16 at *t* = 25-35 min post-IP; *n* = 14; $p<0.01$). The LTD following 'bursts only' IP was instead associated with a smaller and non significant increase of the PPR (1.82 ± 0.20 at 10 min pre-IP versus 2.01 ± 0.14 at *t* = 25-35 min post-IP; *n* = 7; $p=0.37$).

In (Figure S1F) for both IP protocols, the percentage change in EPSC amplitude averaged over 25 to 35 min post-IP is significantly different than in the control (*$p<0.05$ and **$p<0.01$).

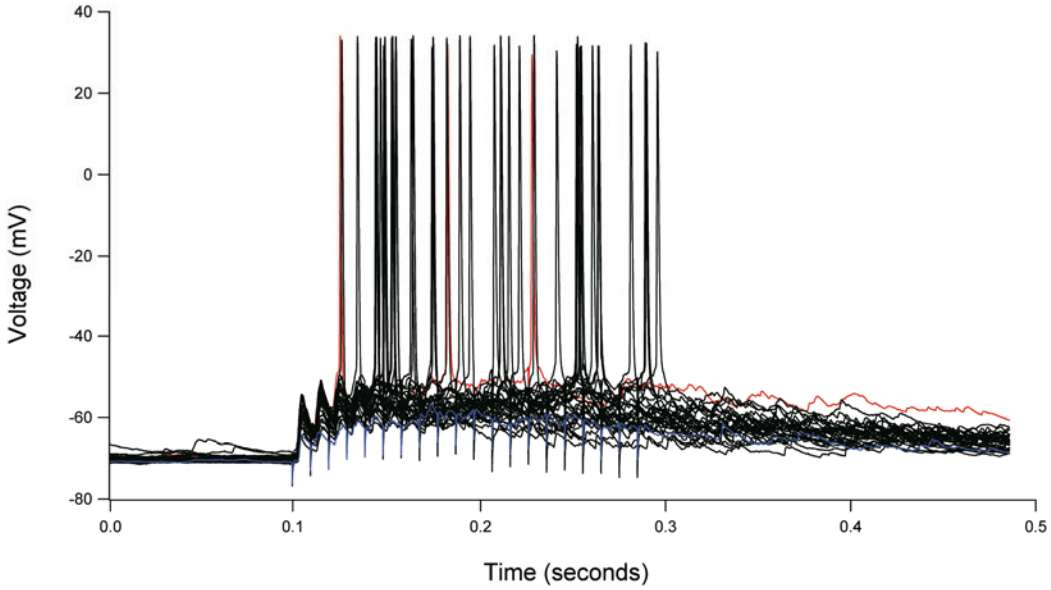
Supplemental Figure 1



Example response of Golgi cell during C-clamp IP

A superimposed image of the 30 traces during the C-clamp IP is shown where initially the cell spikes and progressively the EPSPs become smaller. The first trace is colored red and the last trace is colored blue.

Supplemental Figure 2



Relationship between EPSCs, PPR and extracellular Ca^{2+} concentration

Because GoC apical dendrites are quite thin and excitatory synapses from PFs are made on spine-like protrusions it cannot be excluded that small changes in PPR are difficult to detect at the level of the soma. Lowering the extracellular Ca^{2+} concentration is a purely presynaptic mechanism to induce depression of the EPSCs and the accompanying amount of change in the PPR recorded at the soma is indicative of the reliability of this parameter for detecting changes in presynaptic activity. To test this we performed a series of experiments whereby the responses of paired EPSCs and their respective PPRs were measured while over time the extracellular Ca^{2+} concentration was systematically lowered. On the basis of these measurements one can have an idea how reliable the PPR test is for discriminating between the pre- and postsynaptic locus of plasticity.

EPSCs were evoked by stimulating the ML at a certain stimulation intensity arbitrarily chosen at the beginning of each of the recordings. The stimulation intensity was afterwards unchanged all through the recording. Passive cell parameters were continuously tested during the whole recording and as soon as significant changes occurred in one or more of these parameters we stopped the recording. The EPSCs were evoked at a standard frequency of 0.1 Hz for 10 minutes in each new condition, leading up to a series of 60 consecutive paired EPSCs per extracellular Ca^{2+} concentration group. 5 minutes were allowed and were sufficient for change of concentration. EPSCs were monitored during this transition period at a frequency of 0.033 Hz. Perfusion speed of bubbled ACSF was 3-4 ml per minute. The EPSCs were recorded at 2.00, 1.75, 1.50, 1.25, 1.00, 0.50, 0.00 and back to 2.00 mM extracellular Ca^{2+} concentration. Changes in extracellular Ca^{2+} were compensated by a reciprocal increase of the Mg2+ concentration to keep the osmolality of the solution equal.

An example recording is shown in Figure S3A. What is shown is the mean EPSC of the 60 consecutive paired EPSCs per extracellular Ca^{2+} concentration group. At the end of the recording as a control the Ca^{2+} concentration was returned to initial value as a control of the quality of the recording and the cell. Figure S3B summarizes the normalized values for the EPSC amplitudes of 9 recordings. EPSCs were half their initial value when

moving from 1.50 to 1.25 mM extracellular Ca^{2+} concentration as shown in Figure S3B. The PPR is summarized for these 9 recordings in Figure S3C. The average and the variability of the PPRs increased while Ca^{2+} was decreasing. A quasi-linear increase was observed from 2.00 to 1.00 mM Ca^{2+}. From 1.50 mM there was a significant difference with the PPRs of the starting conditions.

The typical amount of depression observed during our PF-GoC LTD experiments was in the order of 25-30%. This corresponds to lowering the extracellular Ca^{2+} concentration to halfway between 1.75 and 1.50 mM. This corresponds in Figure S3C to an increase of the PPR of 10-15%, which is not significantly different from the PPR measured at 2.00 mM extracellular Ca^{2+} concentration. It is thus not excluded that small changes of the PPR at electrotonically distant sites, which are difficult to measure, could still contribute to the depression observed.

Measuring the PPR is still a valuable tool for determining the origin of the plasticity observed since an increase of the PPR is observed when a purely presynaptic depression of the EPSCs is induced, as was the case during the transient PTP that was sometimes observed.

Supplemental Figure 3