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Supporting Online Material for

Activity-Dependent Long-Term Depression of Electrical Synapses

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Methods

Slice preparation, recording, and drugs: Thalamocortical slices 400 µm thick were obtained from Sprague-Dawley rats aged P12 - P14. Slices were cut and incubated in sucrose solution (in mM: 72 Sucrose, 83 NaCl, 2.5 KCl, 1 $NaPO_4$, 3.3 $MgSO_4$, 26.2 $NaHCO_3$, 22 Dextrose, 0.5 $CaCl_2$, saturated with 95% O_2/5% CO_2). Slices were incubated at 33 ^oC for 20 min and returned to room temperature thereafter. The bath solution for recording contained (in mM): 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 10 dextrose and 2 $CaCl_2$, saturated with 95% O_2/5% CO_2. The submersion recording chamber was held at 32 - 33 ^oC (TC-324B, Warner). Micropipettes were filled with (in mM): 135 K-gluconate, 2 KCl, 4 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, and 10 phosphocreatine-Tris (pH 7.25 with 1 M CsOH or 1M KOH; 295 mOsm). The approximate bath flow rate was 2 ml/min. Voltages are reported as corrected for the liquid junction potential. When noted, the bath also included 1 µM TTX to block voltage-gated sodium channels.

Dye preparation. Dye was prepared fresh daily, starting with a 50 µg aliquot of Oregon Green 488 BAPTA-1 AM; 5 µL of 20% pluronic F-127 in DMSO was added to the dye vial, and vortexed for 5 min. Next 44 µL of ACSF was added and vortexed again for 5 min. The solution was sonnicated in an ice-water slurry for 30 min, then filtered with a centrifuge at 10 kRPM for 1 min.

Slice bulk-loading. ~20 µL of dye solution was loaded into the tip of a glass recording electrode. The electrode tip was broken against the bottom of the recording chamber, then moved to the area of interest, and inserted into the slice at a depth of 50-100 µm. Positive pressure was applied to the electrode with a Hamilton micro-syringe and left in place for 3 min.

Imaging. An X-Cite series 120 mercury lamp was manually shuttered for light exposures lasting several hundred ms; images and whole-cell data were collected before and during electrical stimulation of recorded cell(s). The filter set was 470 nm excitation, 525 nm emission with a 495 nm beam splitter in a Zeiss AxioSkop 2 FS Plus. Images were collected with AxioVision software driving an AxioCam MR3 for a 13-ms exposure into a 1388x1040 pixel image with 8-bit grey-level resolution. To raise the excitability of imaged neurons, the external ACSF was modified to contain 0.75 mM $CaCl_2$ and 7 mM KCl.

Numerical Analyses: The coupling coefficient is the attenuation of signal transfer between one cell and its electrically coupled neighbor, measured as a ratio of the postsynaptic voltage transmitted via the gap junction divided by the presynaptic voltage response.

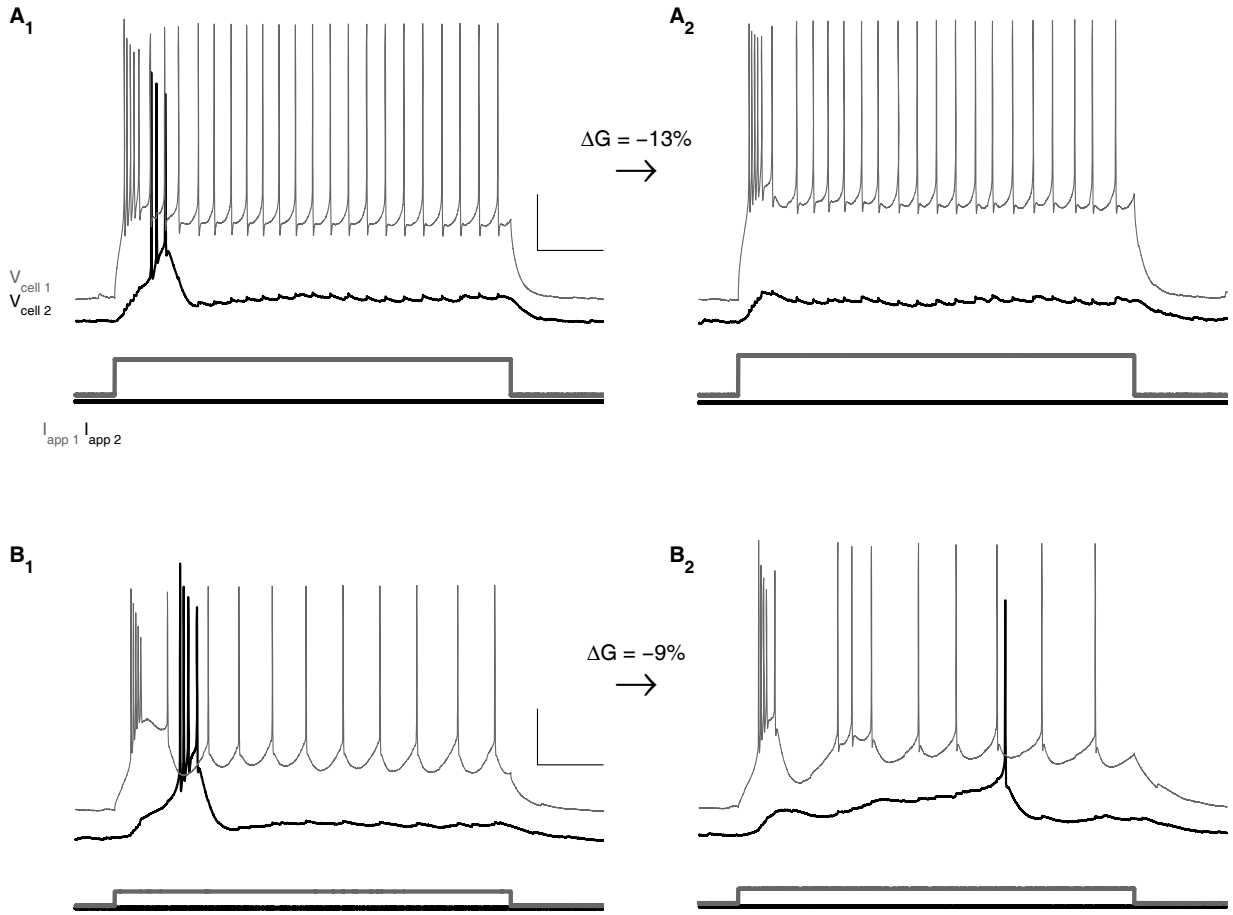
To measure ***cc***, we injected 100 – 200 pA hyperpolarizing current steps, each lasting 600 ms (1-sec ISI), and measured the steady-state voltage responses of the injected cell and its electrically coupled neighbor. The coupling coefficient $\mathbf{cc_{12}} = \Delta V_{cell\ 2} / \Delta V_{cell\ 1}$, where $\Delta V_{cell\ 1}$ is the change in membrane voltage in the injected cell and $\Delta V_{cell\ 2}$ is the change in membrane voltage in the coupled cell. Reported data of **cc** represent the average of 10 - 20 measurements.

Gap junctional conductance $\mathbf{G_C}$ was calculated by first calculating transfer resistance based on current injection into cell 2: $R_{21} = \Delta V_{cell\ 1} / I_{inj,\ cell\ 2}$), and similarly, R_{12} from current injection into cell 1. With $R_{in,1}$ representing the input resistance of cell 1, the resistance of the electrical synapse is taken as from Bennet (NY Academy of Sciences, 1966):

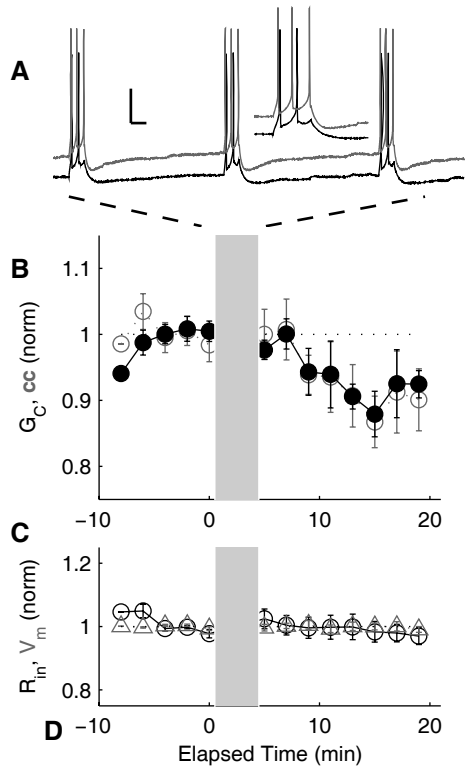
$$R_c = \frac{R_{in,1}R_{in,2} - R_{12}^2}{R_{12}}$$

Then $\mathbf{G_{12} = 1/R_C}$ when measured with current injection into cell 1, and $\mathbf{G_{21}}$ was measured by injection into cell 2. For $R_{12} << R_{in,1}$ and $R_{in,2}$, it is easily shown that the ratio $\mathbf{G_{12}/G_{21} \approx R_{12}/R_{21}}$ and is independent of input resistance.

Unless otherwise indicated, values in the text are mean ± SEM and significance was assessed by 2-tailed student's t-test, paired when appropriate (Fig. 4) and unpaired when direct within-pair comparisons could not be made (Fig. 2, 3). For all experiments, data were discarded from analysis for pairs in which R_{in} for either cell deviated by more than 20% from its baseline value, and for pairs in which the CV of the baseline values of **cc** exceeded 0.1. Reported average changes in **cc** and $\mathbf{G_C}$ are the average of the changes over all pairs comparing the entire pre- to the entire post-activity period; the plotted data **cc** and $\mathbf{G_C}$ were binned for each time point.



Supplementary Fig. 1. Gap junction-relayed activity before and after LTD. A_1). For one pair of neurons ($\mathbf{cc_{12}}$ = 0.2, $\mathbf{G_{12}}$ = 2.2 nS), spikes driven by current injection into cell 1 (gray traces; $V_{cell\ 1}$, $I_{app\ 1}$) initially elicited a burst in cell 2 (black traces). Scale bar 20 mV, 100 ms, 400 pA. A_2) After paired bursting resulting in LTD ($\Delta\mathbf{G_{12}}$ = - 13%; $\Delta\mathbf{cc_{12}}$ = -11%), the same stimulus and resulting spike train in cell 1 failed to elicit a burst in cell 2. B_1), B_2): Same paradigm as in (A) for a pair with initial $\mathbf{cc_{12}}$ = 0.18 and $\mathbf{G_{12}}$ = 1.3 nS, $\Delta\mathbf{G_{12}}$ = - 9%; $\Delta\mathbf{cc_{12}}$ = -8%. Scale bar 20 mV, 100 ms, 400 pA.



Supplemental Fig. 2. Paired sodium spikes without low-threshold calcium spikes. A) Coupled cells were driven with 50-ms current pulses from elevated rest voltages just below spike threshold (~V_m = -40 mV, set with steady current injection) to drive fast spikes without eliciting the low-threshold calcium spikes that underlie bursts. Pairings were repeated at 2 Hz for 5 min. Scale bar 20 mV, 50 ms. Inset, close-up of paired spiking. B) Paired fast spiking without bursting induced a delayed, smaller magnitude of LTD (mean $\Delta\mathbf{G_c}$ = -7.2 ± 2.0%, p = 0.03; mean $\Delta\mathbf{cc}$ = -7.0 ± 2.8% n = 8 pairs). C) Normalized input resistance (R_{in}) and resting membrane potential (V_m) for the cells shown in B.

For regular bursts (Fig. 2), each low-threshold calcium spike contained 2.9 ± 0.3 sodium spikes per burst, separated by 11.2 ± 0.1 ms (89.3 Hz). For sodium spikes stimulated without activating the low-threshold calcium spikes (above), stimuli yielded 2.9 ± 0.02 spikes per 50-ms stimulus but were separated on average by 22.2 ± 0.9 ms (45.0 Hz).