Supplemental Figures 1-4

Regulation of interneuron excitability by gap junction coupling with principal cells.

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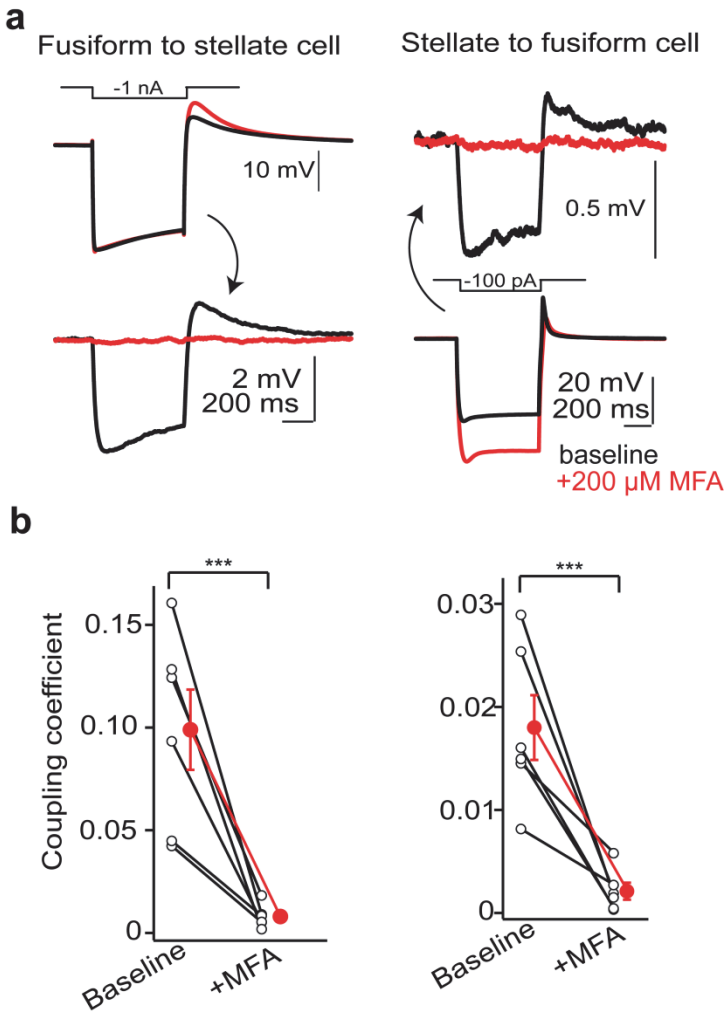
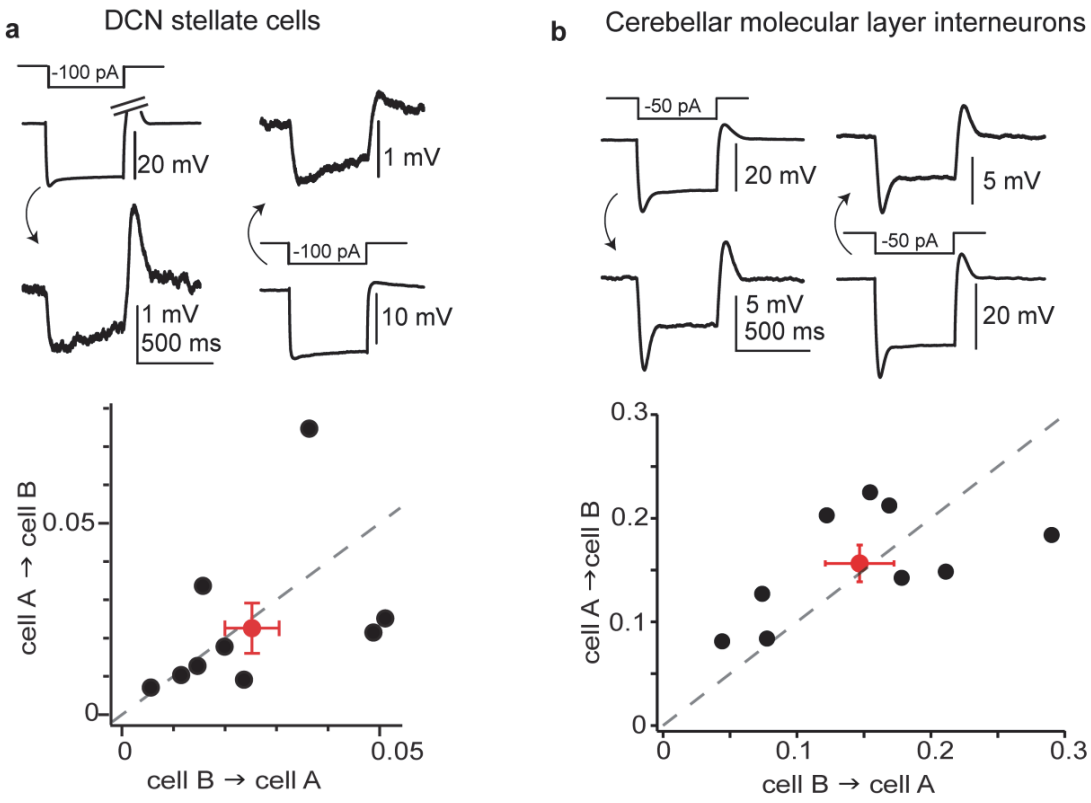


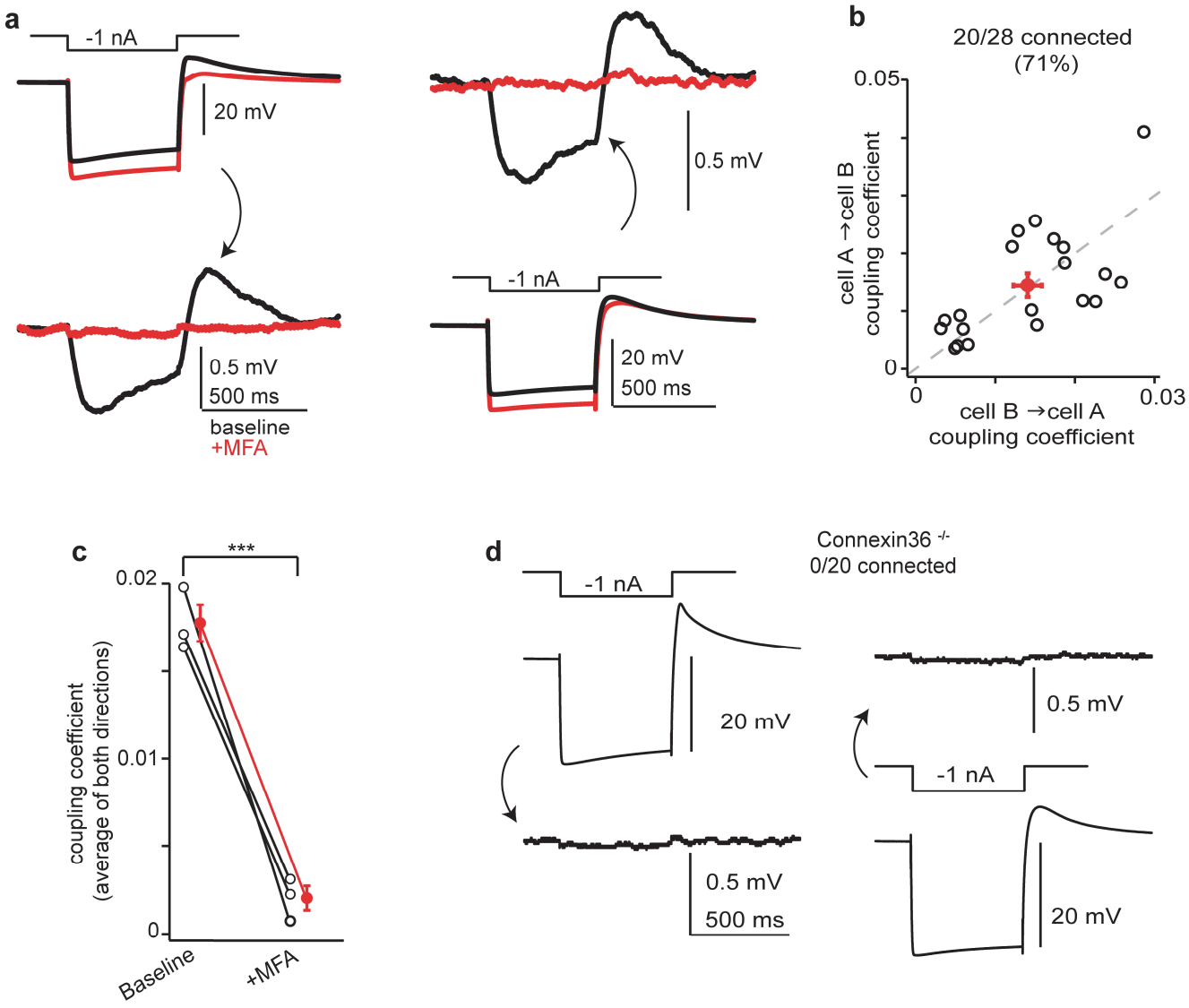
Supplemental Figure 1 The gap junction blocker MFA abolishes electrical coupling.
a) Average traces from a coupled fusiform-stellate cell pair (upper and lower traces, respectively). Black traces are the baseline period and red traces are after 15-30 min of incubation in the gap junction blocker MFA (100-200 µM). The increased amplitude in the stellate cell prejunctional waveform after MFA likely reflects a contribution of gap junction channels to the basal leak conductance[48].

b) Summary of the coupling coefficients from six pairs before and after application of MFA. Open circles represent individual experiments, red points are mean±SEM. Left and right panels represent fusiform-to-stellate and stellate-to-fusiform directions, respectively. MFA significantly reduced the coupling coefficient in the fusiform-to-stellate cell direction from 0.10±0.02 to 0.008± 0.002 ($t(5)=4.74$, $p=0.005$), whereas the stellate-to-fusiform coupling dropped from 0.018±0.003 to 0.002±0.001 ($t(5)=4.63$, 0.006, paired t-tests).



Supplemental Figure 2 DCN stellate cells are weakly electrically-coupled.
a) Top: Example average traces from an electrically-coupled pair of DCN stellate cells. Lower panel: Summary data plotting the coupling coefficients for 9 coupled pairs. The apparent directional asymmetry in some pairs is probably due to intrinsic variability in basal input resistance, as the group average (red point) falls on the unity line. The homologous coupling coefficient between DCN stellate cells (0.024±0.005, average of both directions) was significantly weaker than fusiform-to-stellate cell coupling ($F(2,123)=23.82$, $p=0.0006$, One way ANOVA + Dunnett's multiple comparisons tests) but not significantly different from stellate-to-fusiform coupling ($p=0.998$). The calculated junctional conductance for homologous coupling between DCN stellate cells was 0.14±0.04 nS.

b) Top: Example average traces from a pair of cerebellar molecular layer interneurons. 9/18 pairs showed coupling, and the coupling coefficient (0.15±0.02) was significantly greater than in DCN stellate cells ($t(17)=6.83$, $p<0.0001$, unpaired t-test). These values are in line with previous reports[13]. The junctional conductance for coupling between cerebellar stellate cells was 0.52± 0.07 nS.

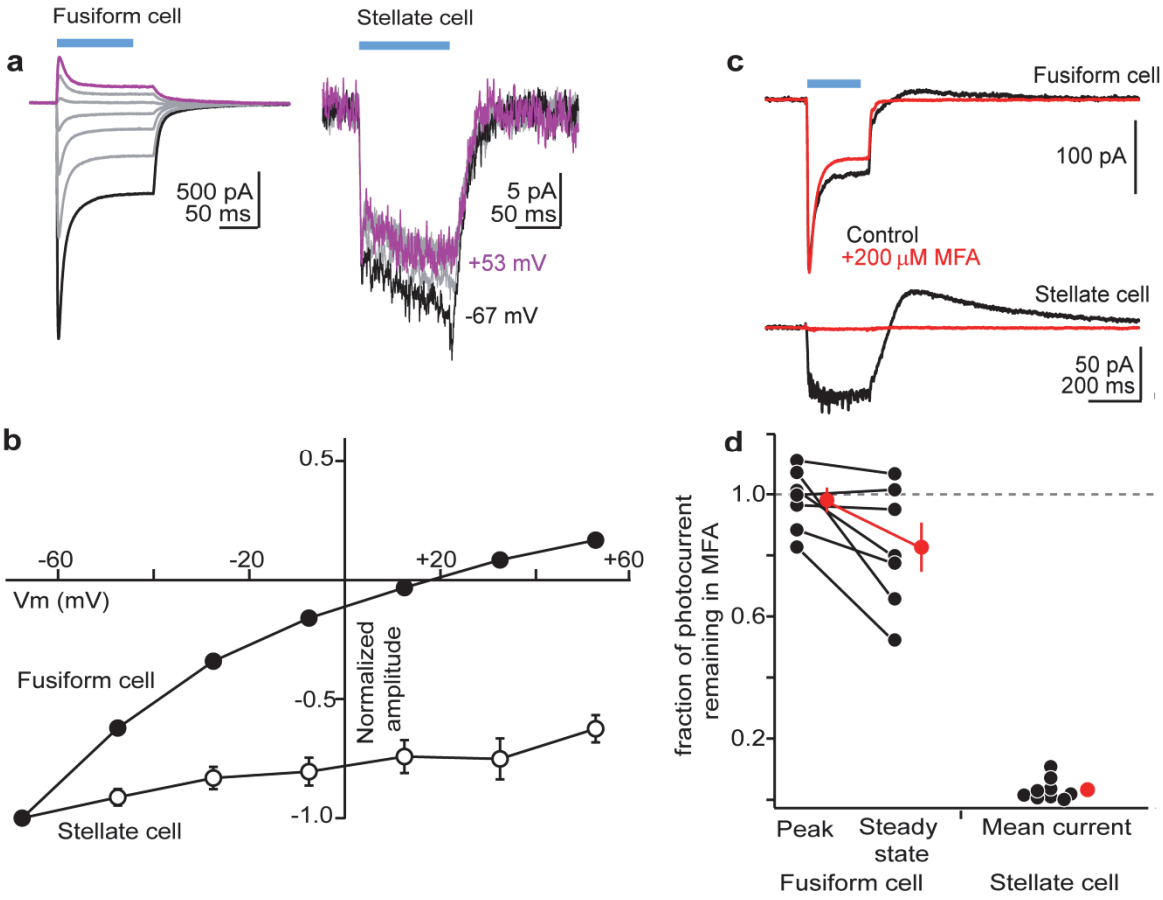


Supplemental Figure 3 Fusiform cells are weakly electrically coupled to one another.
a) Example average traces from a pair of fusiform cells. Current injection in one cell results in a small hyperpolarization of the other cell. Black and red traces are before and after addition of the gap junction blocker MFA, confirming that this effect is due to gap junction coupling.

b) Summary data showing the coupling coefficient of 20 similar pairs. Red symbol is mean±SEM. Dotted gray line is unity. Homologous coupling between fusiform cells (0.014±0.001, average of both directions) was significantly weaker than fusiform-to-stellate cell coupling ($F(3,143)=22.41$, $p<0.0001$, one way ANOVA and Dunnett's multiple comparison test), but not significantly different than stellate-to-fusiform or stellate-to-stellate coupling ($p=0.74$ and $p=0.95$, respectively). The junctional conductance for this data set was 0.50±0.07 nS.

c) Summary data showing that 100-200 μM MFA significantly reduced electrical coupling between fusiform cells ($n=3$ pairs; $t(2)=9.5$, $p=0.01$, paired t-test.) Open symbols are individual experiments, red is mean±SEM. Asterisks denote statistical significance.

d) Average traces from a fusiform cell pair in a Cx36-/- mouse. Electrical coupling was absent in fusiform cell pairs recorded in Cx36-/- mice (0/20 connected. $\chi^2(1)=22.64$, $p<0.0001$ compared to wild-type mice.)



Supplemental Figure 4 Stellate cells do not express ChR2

a) Voltage-clamped fusiform (left) or stellate cell (right) at different membrane potentials. The -67 and +53 mV traces are highlighted for comparison in black and mauve, respectively. Peak and steady-state photocurrents in fusiform cells are outward at +53 mV, while stellate cell responses remain inward at all potentials. Traces from a Thy1-ChR2 mouse; similar results were observed in VGluT2-ChR2 mice.

b) Average IV relationship for photocurrent responses in fusiform and stellate cells from ChR2 mice (n=10 cells each, solid and open symbols, respectively). Stellate cell responses were always inward, whereas fusiform cell photocurrents showed reversal. The positive reversal potential in fusiform cells may be due in part to electrically-coupled fusiform cells that are also depolarized during the light stimulus (Supplemental Figure 3). Moreover, the dendritic arbor of fusiform cells (see example cell in Figure 3, main text) may result in space-clamp limitations.

c) Differential block of fusiform and stellate cell light responses by the gap junction blocker MFA. Average photocurrent responses in a simultaneously recorded (uncoupled) fusiform/stellate cell pair from a Thy1-ChR2 mouse during baseline (black) and after 200 μM MFA (red). The peak photocurrent in the fusiform cell was unaffected, whereas the stellate cell response was entirely abolished. MFA caused a small reduction in the steady-state fusiform cell response, and blocked the small outward current upon light offset. This may result from electrical coupling of adjacent fusiform cells activated during the light stimulus.

d) Summary data showing the effects of 100-200 μM MFA on photocurrents in fusiform and stellate cells. The leftmost points show the fraction remaining in MFA (normalized to a pre-drug baseline) for the peak and steady-state amplitudes of the fusiform cell photocurrents. Black lines connect individual experiments, red points are mean±SEM. MFA had no effect on peak photocurrents (98± 4% remaining, n=7. $t(6)=0.34$, $p=0.7$, paired t-test). MFA slightly reduced the steady state photocurrent in 4/7 experiments, although this value was not statistically significant in grouped data (82± 8% remaining. $t(6)=2.09$, $p=0.08$, paired t-test). The rightmost data points represent the fraction remaining of the steady-state postjunctional photocurrent in stellate cells. MFA completely abolished photocurrents in stellate cells (3± 1% remaining, n=9. $t(8)=3.93$, $p=0.004$, paired t-test).