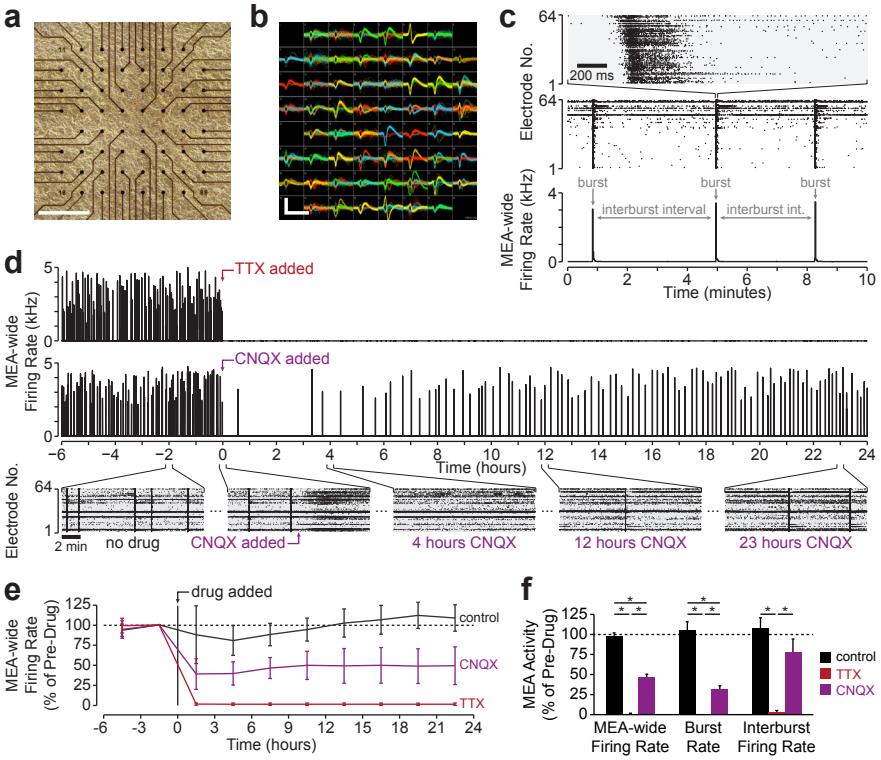

Figure 1: Spiking persists during blockade of AMPAergic transmission. (a) Phase-contrast micrograph of dissociated cortical culture grown on a planar MEA. Scale bar, 500 um. **(b)** Extracellular spike waveforms recorded on each microelectrode shown in (a). Scale bars, 100 uV , 2 ms. **(c)** *Top*, rastergram of spike times occurring during a network-wide burst, a distinguishing feature of spiking activity in dissociated cortical cultures. Scale bar, 200 ms. *Middle*, rastergram showing multiple bursts over several minutes. *Bottom,* time histogram of spikes occurring across the entire MEA over the same time course shown in middle panel. MEA-wide firing rate at each time point is computed by counting the number of spikes occurring during each bin, and dividing by the bin size. Bin size, 1 s. **(d)** *Top and middle*, MEA-wide firing rates from example recordings before and during application of TTX or CNQX. Bin size, 1s. *Bottom*, rastergrams for 15-minute snippets before or during CNQX treatment. Scale bar, 2 min. **(e)** Mean MEA-wide firing rate over time for all TTX- and CNQX-treated cultures (vehicle-treated controls, n=12 cultures; TTX, n=8 cultures; CNQX, n=13 cultures). Values are normalized to firing rate during 3 hour window before drug application. Bin size, 3h. Error bars denote s.d. **(f)** Mean MEA-wide firing rate, burst rate, and interburst firing rate for vehicle-, TTX-, or CNQX-treated cultures during the 24-hour treatment window. Values are normalized to firing rate during 3-hour window before drug application. MEA-wide firing rate was significantly different between all conditions (control, 97.34±4.55%; TTX, 1.11±0.51%; CNQX, 46.20±4.12%; Kruskal-Wallis, $p<10^{-6}$), with TTX being significantly less than both control and CNQX (both $p<10^{-3}$), and CNQX being less than control ($p<10^{-4}$) but greater than TTX. Burst rates followed a similar trend (control, 105.83±9.95%; TTX, 0±0%; CNQX, 31.18±4.76%; Kruskal-Wallis, $p<10^{-6}$; control vs. TTX, $p<10^{-3}$; control vs. CNQX, $p<10^{-4}$, TTX vs. CNQX, $p<10^{-3}$). Firing rate between bursts were also different between conditions (control, 108.12±12.72%; TTX, 3.60±1.50%; CNQX, 108.12±12.72%; Kruskal-Wallis, $p<10^{-4}$), again with TTX reduced compared to both control ($p<10^{-4}$) and CNQX ($p<10^{-4}$), and showing only a mild reduction compared to control ($p=0.02$, not significant at Bonferroni adjusted alpha=0.017). Error bars denote s.e.m.

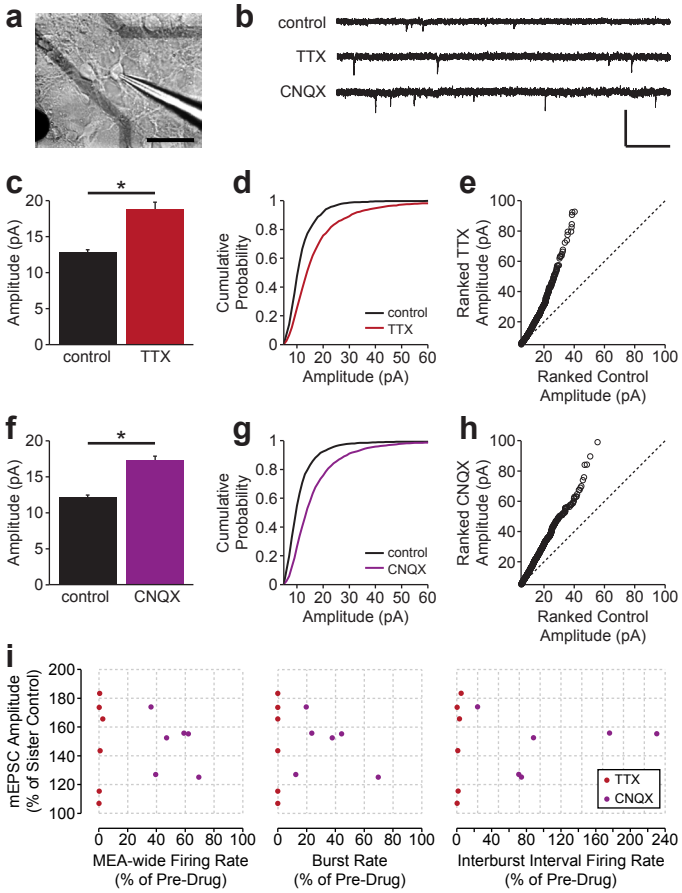

Figure 2: Spiking is not correlated to the magnitude of synaptic scaling. (a) Pyramidal cell during whole-cell recording. Micro-electrode (black, lower left) and electrode leads (grey) are visible. Scale bar, 50 um. **(b)** Sample mEPSC recordings following 24-hour treatment with vehicle, TTX, or CNQX. Scale bar, 50 pA, 200 ms. **(c)** Mean mEPSC amplitude for 6 sister culture pairs treated with vehicle or TTX (control: 12.82±0.36 pA, n=47 cells; TTX: 18.82±1.02 pA, n=58 cells; $p<10^{-5}$). Error bars denote s.e.m. **(d)** Cumulative distribution of mEPSC amplitudes following TTX or vehicle treatment. Multiplicatively scaled TTX distribution matches control ($p>0.6$). **(e)** Ranked TTX mEPSC amplitudes plotted against ranked control amplitudes (linear fit, $R^2 = 0.975$). Dotted line denotes the line of identity. **(f)** Mean mEPSC amplitude for 10 sister culture pairs treated with vehicle or CNQX (control: 12.1±0.34pA, n=89 cells; CNQX, 17.31±0.54pA, n=94 cells; $p<10^{-12}$). **(g)** Cumulative distribution of mEPSC amplitudes following CNQX or vehicle treatment. Multiplicatively scaled CNQX distribution matches control ($p>0.9$). **(h)** Ranked CNQX mEPSC amplitudes plotted against ranked control amplitudes (linear fit, R^2=0.996). **(i)** *Left*, Mean mEPSC amplitude for individual cultures plotted against the firing rate they exhibited during TTX or CNQX treatment. mEPSC amplitudes are normalized to corresponding sister control cultures, and MEA-recorded activity is normalized to pre-drug levels. *Center and right*, Mean mEPSC amplitude plotted against burst rate and interburst firing rate, respectively. (linear fits: MEA-wide firing rate, r=-0.0466; burst rate, r=-0.1136, interburst firing rate, r=0.0435).

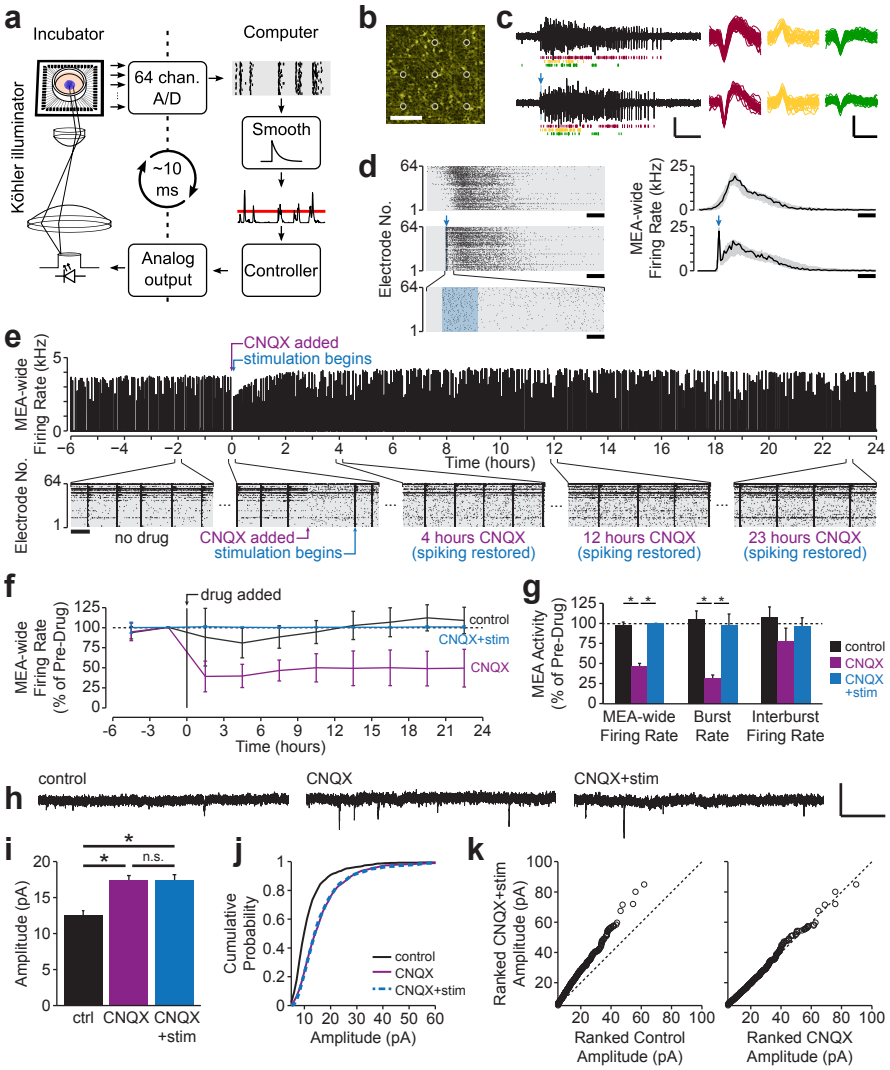

Figure 3: Reduced AMPAergic transmission directly triggers upward synaptic scaling. (a) Schematic of closed-loop optical stimulation system. **(b)** Neurons transfected with ChR2-eYFP. Microelectrodes are circled in white. Scale bar, 200 um. **(c)** *Left*, voltage traces recorded from a single microelectrode during a spontaneous burst in the absence of any drug (top) and a photostimulation-evoked burst in the presence of CNQX (bottom) from the same culture. Blue arrow denotes the timing of the light pulse. Shaded blue bar denotes 10-ms duration of the light pulse, which is brief compared to the total burst duration. Colored vertical bars below each trace denote the spike times for sorted extracelluar units on this microelectrode.Vertical colored bars denote spike times for individual extracellular units. The pattern of recruitment of units is similar between the two conditions. Scale bars, 50 uV, 200 ms. *Right,* extracellular units detected during the bursts shown on the left. Similarity between the spike waveforms across the two conditions indicate that they are likely from the same neurons. Scale bars, 50 uV, 1 ms. **(d)** *Left*, rastergram showing spike times recorded across all electrodes during a spontaneous burst (top) and a photostimulation-evoked burst in the presence of CNQX (middle). The recruitment of spikes across the entire MEA is similar between the two conditions. Blue arrow denotes the timing of the light pulse. Blue shading denotes when light is on, and grey shading indicates when light is off. Zoomed time scale of the CNQX+photostimulation condition (bottom) shows directly evoked spike times. Scale bars, 100 ms (top and middle), 5 ms (bottom). *Right*, MEA-wide firing rate computed during bursts shown at left (black lines). The 107 bursts that occurred during a 6-hour spontaneous recording (top) or the 416 bursts the 24-hour CNQX+photostimulation recording for this culture (bottom) are plotted in grey. Blue arrow denotes timing of the light pulse. Following the direct activation, the firing rates between the spontaneous and CNQX-+photostimulation conditions appear similar. Bin size, 10 ms. Scale bar, 100 ms. **(e)** *Top*, MEA-wide firing rates from example recordings before and during application of CNQX, with pre-CNQX firing rates restored using closed-loop photostimulation. The closed-loop controller begins 5 min after CNQX is added to verify that the drug has taken effect. Bin size, 1s. *Bottom*, rastergrams for 15-minute snippets before or during CNQX and photostimulation. Scale bar, 2 min. **(f)** Mean MEA-wide firing rate over time for CNQX-treated cultures with restored spiking (n=5 cultures). Control and CNQX values from Fig. 1e are shown for comparison. Closed-loop stimulation effectively locked firing rate to pre-CNQX levels. Bin size, 3h. Error bars denote s.d. **(g)** Mean MEA-wide firing rate, burst rate, and interburst firing rate for the 3 conditions during the 24-hour treatment window. CNQX-treated cultures with restored spiking showed no change in MEA activity (MEA-wide firing rate, 100.23±0.41%, p<xx; burst rate, 97.7±31.97%, p<xx; interburst firing rate, 96.21±24.93%, p<xx). Error bars denote s.e.m. **(h)** Sample mEPSC recordings following 24-hour treatment with vehicle, CNQX, or CNQX+photostimulation. Scale bar, 30 pA, 200 ms. **(i)** Mean mEPSC amplitude for 5 sister culture pairs from the 3 treatment conditions (control: 12.64±0.56 pA, n=44 cells; CNQX: 17.38±0.70 pA, n=51 cells; CNQX+photostimulation: 17.43±0.77 pA, n=46 cells; ANOVA, $p<10^{-58}$; control vs. CNQX, $p<10^{-5}$; control vs. CNQX+-photostimulation, $p<10^{-5}$, CNQX vs. CNQX+photostimulation, p>0.9). Error bars denote s.e.m. **(d)** Cumulative distribution of mEPSC amplitudes following the 3 treatment conditions. Multiplicatively scaled CNQX and CNQX+photostimulation distributions matched control (p>0.9 for both), and there was no difference between the unscaled CNQX and CNQX+photostimulation distributions (p>0.9). **(e)** Ranked CNQX+photostimulation mEPSC amplitudes plotted against ranked control or CNQX amplitudes (linear fits, $R^2 = 0.998$ and $R^2 = 0.995$, respectively). Dotted line denotes the line of identity.

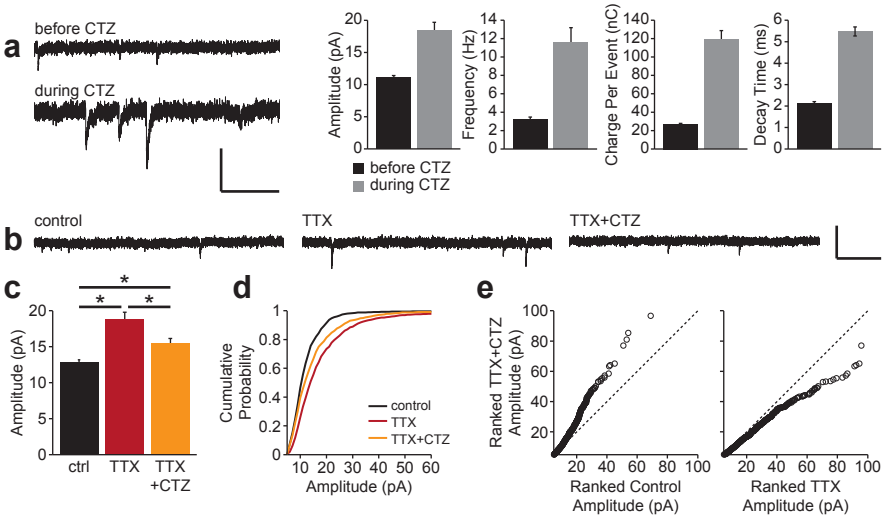

Figure 4: Reduced AMPA receptor activation mediates TTX-induced synaptic scaling. (a) *Left,* sample AMPAergic mEPSCs recorded before and after during CTZ. Scale bar, 25 pA, 200 ms. *Right,* Mean mEPSC amplitude, frequency, charge per event, and decay time constant before and during CTZ. **(b)** Sample mEPSC recordings following 24-hour treatment with vehicle, TTX, or CTZ. Scale bar, 30 pA, 200 ms. **(c)** Mean mEPSC amplitude for 6 sister culture pairs treated from the 3 treatment conditions (control: 12.82±0.36 pA, n=47 cells; TTX: 18.82±1.02 pA, n=58 cells; TTX+CTZ: 15.45±0.69 pA, n=50 cells; ANOVA, $p<10^{-52}$; control vs. TTX, $p<10^{-5}$; control vs. TTX+CTZ, $p<10^{-2}$, TTX vs. TTX+CTZ, $p>10^{-2}$). Error bars denote s.e.m. **(d)** Cumulative distribution of mEPSC amplitudes following the 3 treatment conditions. Multiplicatively scaled TTX and TTX+CTZ distribution match control ($p>0.7$ and $p>0.5$, respectively), and there is a significant difference between the unscaled TTX and TTX+CTZ distributions ($p< 10^{-6}$). **(e)** Ranked TTX+CTZ mEPSC amplitudes plotted against ranked control or TTX amplitudes (linear fits, $R^2 = 0.990$ and $R^2 = 0.989$, respectively). Dotted line denotes the line of identity.