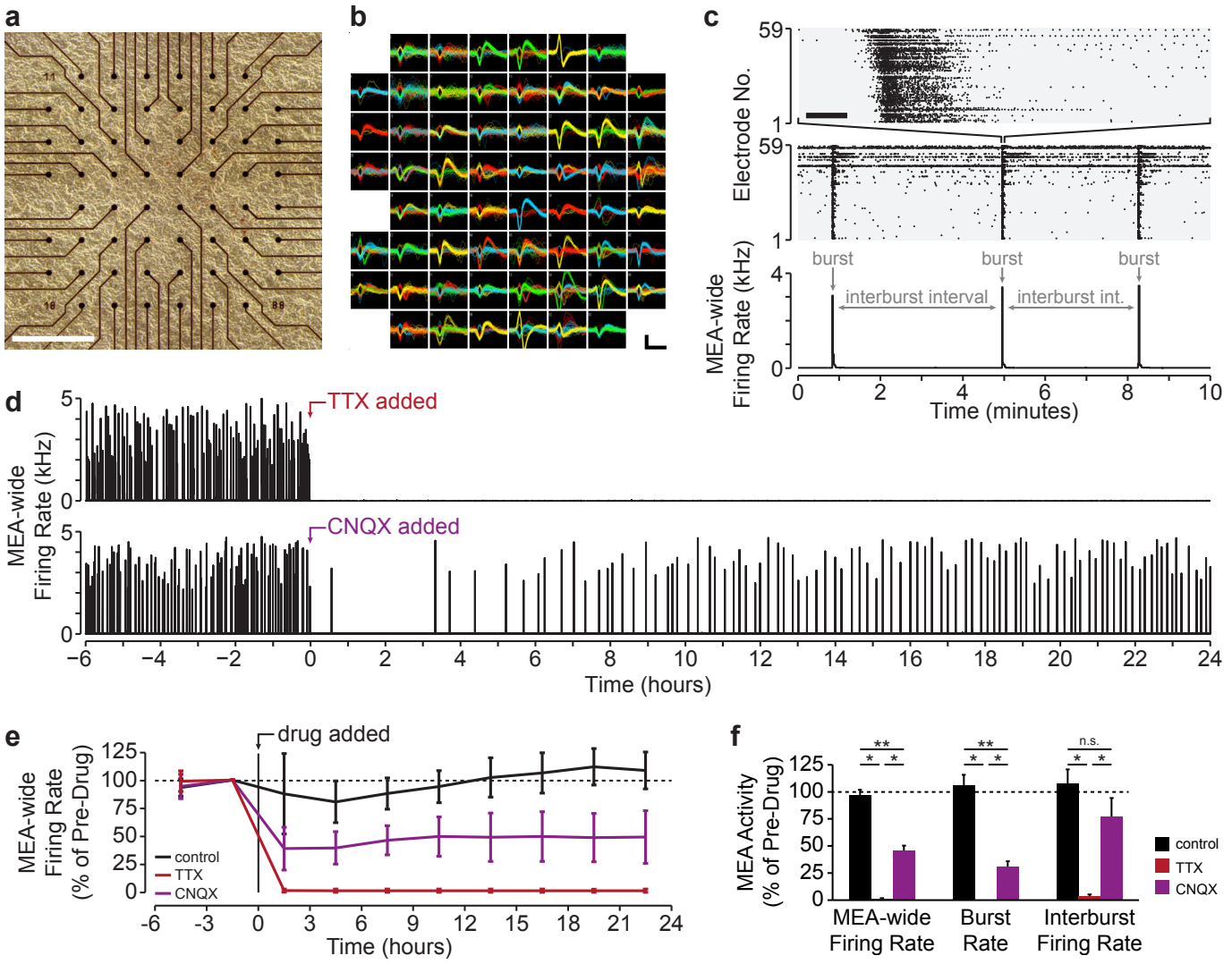

Figure 1: Spiking persists during blockade of AMPAergic transmission. (a) Phase-contrast micrograph of dissociated cortical culture grown on a planar MEA. Scale bar, 500 µm. **(b)** Extracellular spike waveforms recorded on each microelectrode shown in (a). For each electrode, colors denote different sorted units. Scale bars, 2 ms, 100 µV. **(c)** *Top*, rastergram of spike times occurring during a network-wide burst, typical of 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 represents the number of spikes occurring in each 1-second bin. **(d)** MEA-wide firing rates from example recordings before and during application of TTX or CNQX. Bin size, 1 s. **(e)** Mean MEA-wide firing rate over time in different conditions (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/vehicle application. Bin size, 3 h. Error bars, s.d. **(f)** Mean MEA-wide firing rate (control, 97.34±4.55%; TTX, 1.11±0.51%; CNQX, 46.20±4.12%; p<10-6), burst rate (control, 105.83±9.95%; TTX, 0±0%; CNQX, 31.18±4.76%; p<10^{-6}), and interburst firing rate (control, 108.12±12.72%; TTX, 3.60±1.50%; CNQX, 108.12±12.72%; p<10^{-4}) over the entire 24-hour treatment window, normalized to pre-drug values. Non-significant differences denoted by n.s. Significant differences denoted by *p<10^{-3}, **p<10^{-4}. Error bars, s.e.m.

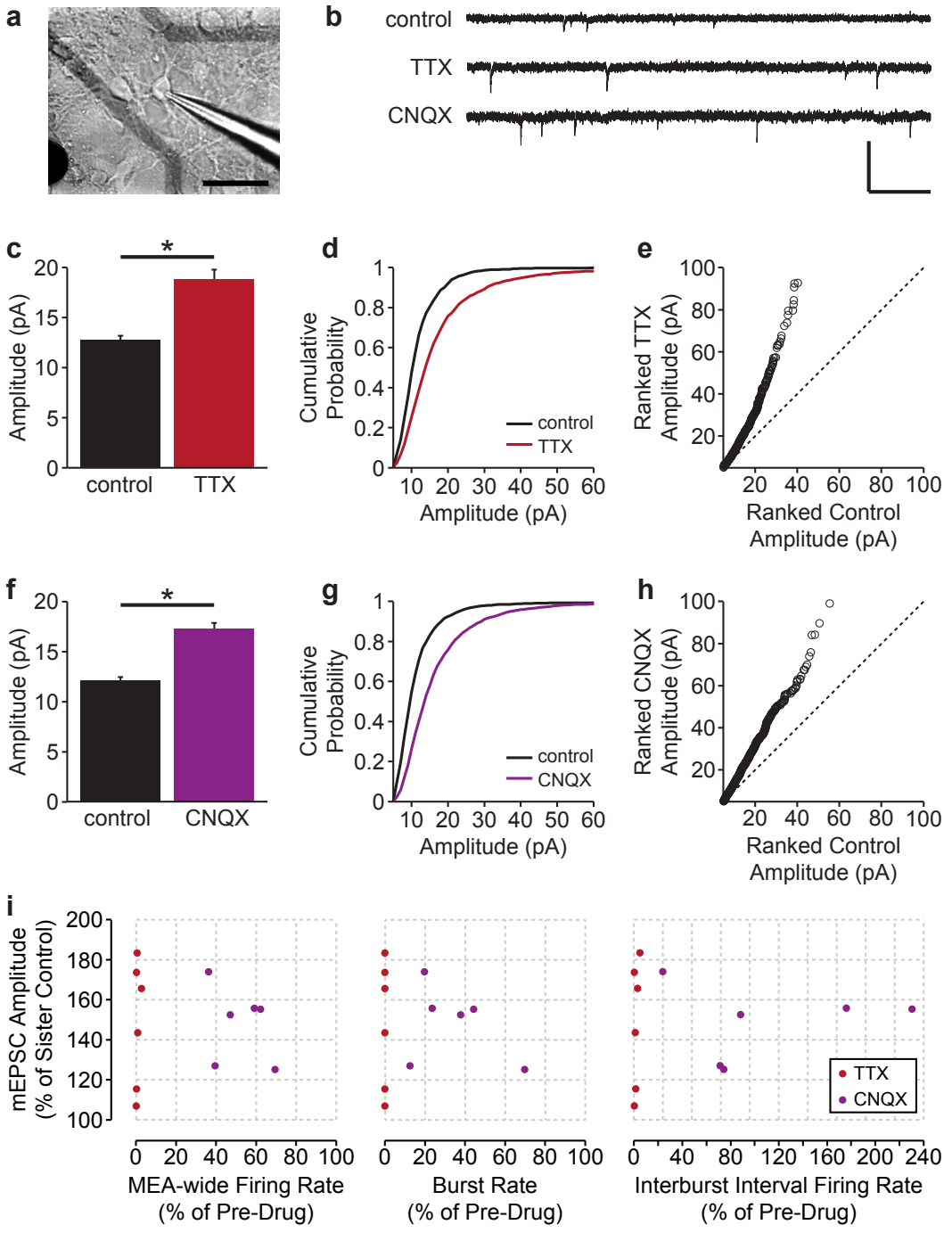

Figure 2: Reductions in spiking are not correlated to the the magnitude of synaptic scaling. (a) Pyramidal cell during whole-cell recording. Microelectrode (black, lower left) and electrode leads (dark grey) are visible. Scale bar, 50 µm. **(b)** Sample mEPSC recordings following 24-hour treatments. Scale bars, 50 pA, 200 ms. **(c)** Mean mEPSC amplitude from 6 sister culture pairs (control: 12.8±0.4 pA, n=47 cells; TTX: 18.8±1.0 pA, n=58 cells; $p<10^{-5}$). Error bars, s.e.m. **(d)** Cumulative distribution of mEPSC amplitudes following TTX or vehicle treatment. The 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 (control: 12.1±0.3pA, n=89 cells; CNQX, 17.3±0.5pA, n=94 cells; $p<10^{-12}$). **(g)** Cumulative distribution of mEPSC amplitudes following CNQX or vehicle treatment. The 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 (linear fit, r=-0.0466). *Center and right*, mean mEPSC amplitude plotted against burst rate and interburst firing rate, respectively (linear fits: burst rate, r=-0.1136, interburst firing rate, r=0.0435).

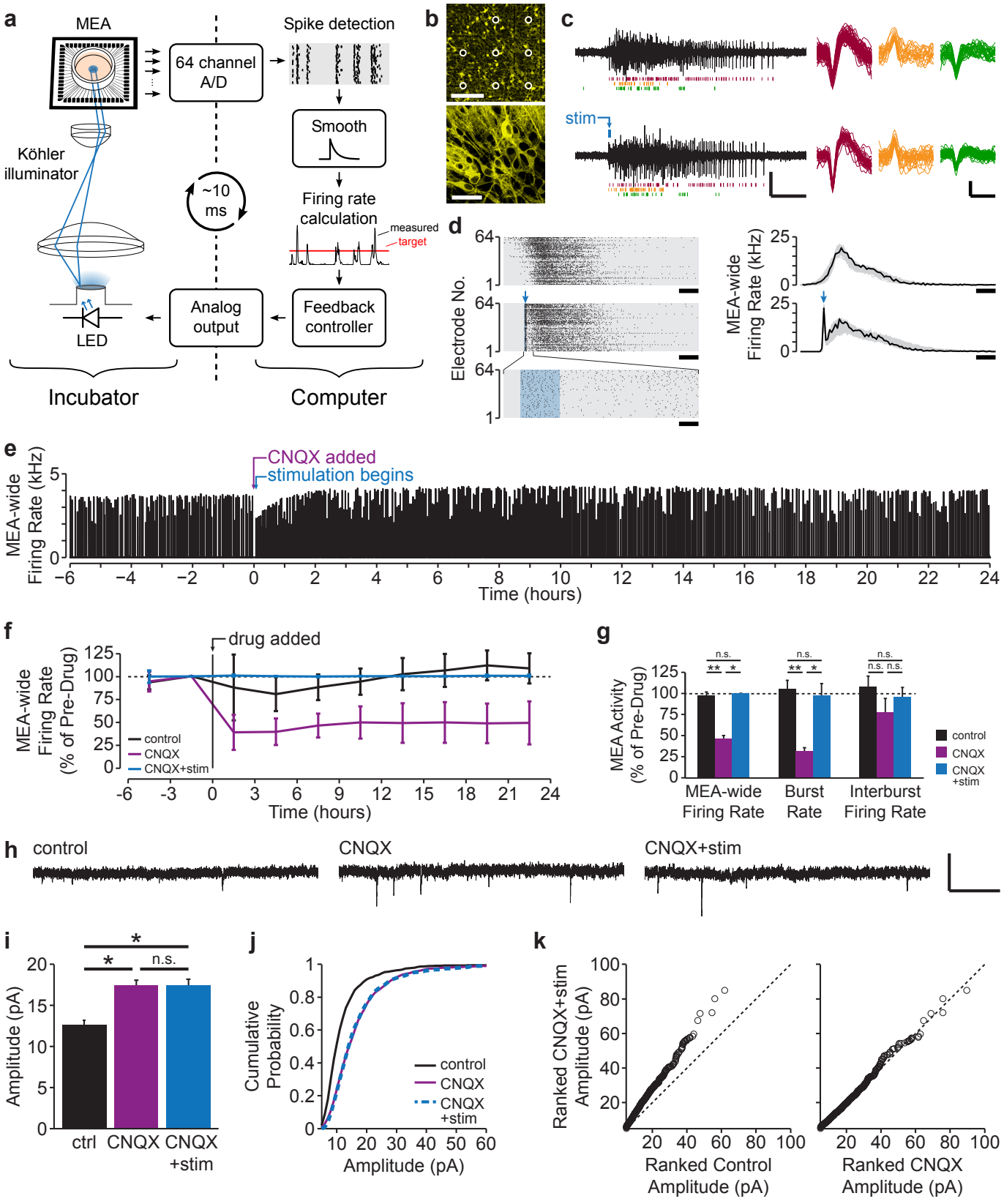

Figure 3: Reduced AMPAergic transmission directly triggers upward synaptic scaling. (a) Schematic of closed-loop optical stimulation system. Spiking activity is recorded through the MEA. When the error between the target and measured MEA-wide firing rate becomes positive, a 10-ms current pulse is delivered to a blue LED. A Köhler illuminator is used to produce uniformly bright illumination at the cell layer. **(b)** Confocal micrograph of cortical culture expressing ChR2-eYFP. Microelectrodes are circled in white. Scale bars, 200 µm (top), 50 µm (bottom).

[legend continued on next page]

Figure 3 (continued): (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 after addition of CNQX (bottom). The stimulation timing is noted, and the width of the blue rectangle indicates the duration of the light pulse, which is brief compared to the total burst duration. The colored vertical bars below each trace denote the timing of 3 different extracellular units captured on the electrode. *Right*, Expanded voltage traces showing all spikes detected during burst, separated by unit. Colors correspond to spike times at left. The similarity between the spike waveforms across the two conditions indicate that they are likely from the same neurons. Scale bars, 50 μV, 200 ms (left); 25 μV, 1 ms (right). **(d)** *Left*, rastergram showing spike times recorded across all electrodes during a spontaneous burst (top) and a photostimulation-evoked burst after addition of CNQX (middle). The recruitment of spikes across the entire MEA is similar between the two conditions. The blue arrow denotes the timing of the light pulse. An expanded rastergram shows spikes occurring at burst onset (bottom) and blue shading denotes when light is on. Scale bars, 100 ms (top and middle), 5 ms (bottom). *Right*, MEA-wide firing rate computed during bursts shown at left (black lines). All bursts occurring during the pre-drug condition (top) and 24-hour CNQX with photostimulation condition (bottom) are shown are plotted in grey. The blue arrow denotes timing of the light pulse. Bin size, 10 ms. Scale bar, 100 ms. **(e)** 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, 1 s. **(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, 3 h. Error bars, s.d. (g) Mean MEA-wide firing rate, burst rate, and interburst firing rate for CNQX+photostimulation cultures over the 24-hour treatment window, with control and CNQX values from Fig. 1f shown for comparison. CNQX-treated cultures with restored spiking showed no differences in MEA activity compared to vehicle-treated controls (MEA-wide firing rate, 100.23±0.41%, p<0.6; burst rate, 97.7±31.97%, p<0.9; interburst firing rate, 96.21±24.93%, p<0.9). Error bars, s.e.m. **(h)** Sample mEPSC recordings following 24-hour treatment with vehicle, CNQX, or CNQX+photostimulation. Scale bars, 30 pA, 200 ms. **(i)** Mean mEPSC amplitude for 5 sister culture pairs from the 3 treatment conditions (control: 12.6±0.6 pA, n=44 cells; CNQX: 17.4±0.7 pA, n=51 cells; CNQX+photostimulation: 17.4±0.8 pA, n=46 cells; $p<10^{-58}$; control vs. CNQX, $p<10^{-5}$; control vs. CNQX+photostimulation, $p<10^{-5}$, CNQX vs. CNQX+photostimulation, p>0.9). Error bars, s.e.m. **(j)** 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). **(k)** 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.

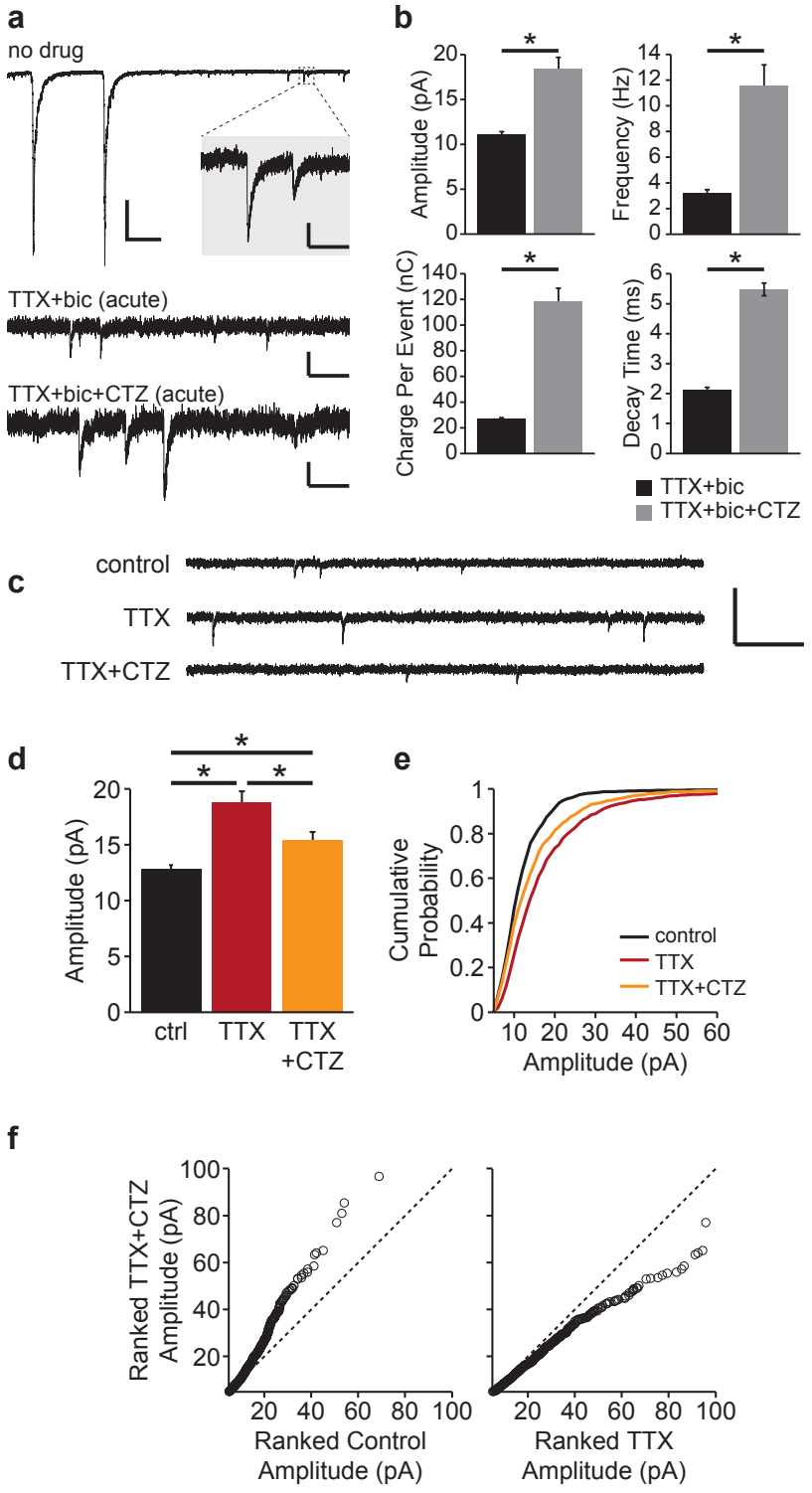

Figure 4: Upscaling that follows chronic spiking blockade is mediated by reduced AMPAR activation. (a) *Top*, sample post-synaptic currents recorded before any drugs are added. Scale bars, 200 pA, 1 s. The shaded inset showing lower-amplitude events. Scale bars, 10 pA, 100 ms. *Middle and bottom*, sample AMPAergic mEPSCs recorded before (middle) and after (bottom) addition of CTZ. Scale bars, 10 pA, 100 ms. **(b)** Mean mEPSC amplitude, frequency, charge per event, and decay time constant before and during CTZ. **(c)** Sample mEPSC recordings following 24-hour treatment with vehicle, TTX, or TTX+CTZ. **(d)** Mean mEPSC amplitude for 6 sister culture pairs treated from the 3 treatment conditions (control and TTX cultures same as Fig. 2c; TTX+CTZ: 15.5±0.7 pA, n=50 cells; $p<10^{-52}$; control vs. TTX+CTZ, $p<10^{-2}$, TTX vs. TTX+CTZ, $p<10^{-2}$). Error bars, s.e.m. **(e)** Cumulative distribution of mEPSC amplitudes following the 3 treatment conditions (control and TTX cultures same as Fig. 2d). The multiplicatively scaled TTX+CTZ distribution matches control ($p>0.5$), but is significantly different from the unscaled TTX distribution ($p<10^{-6}$). **(f)** 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.