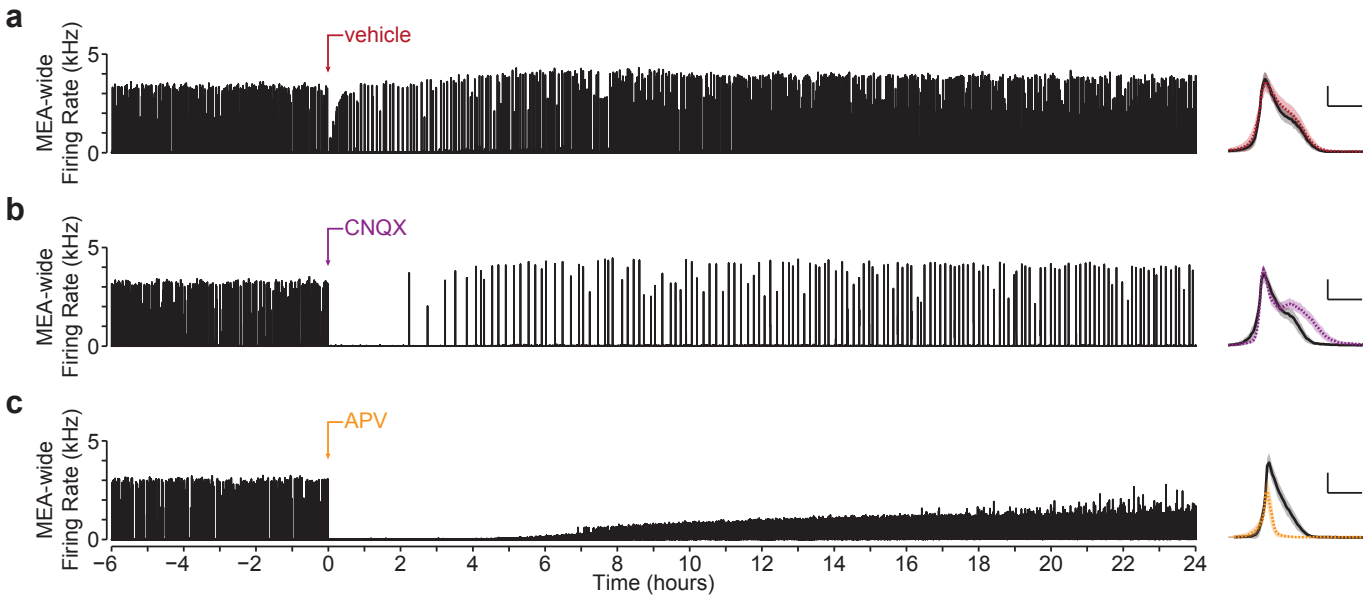
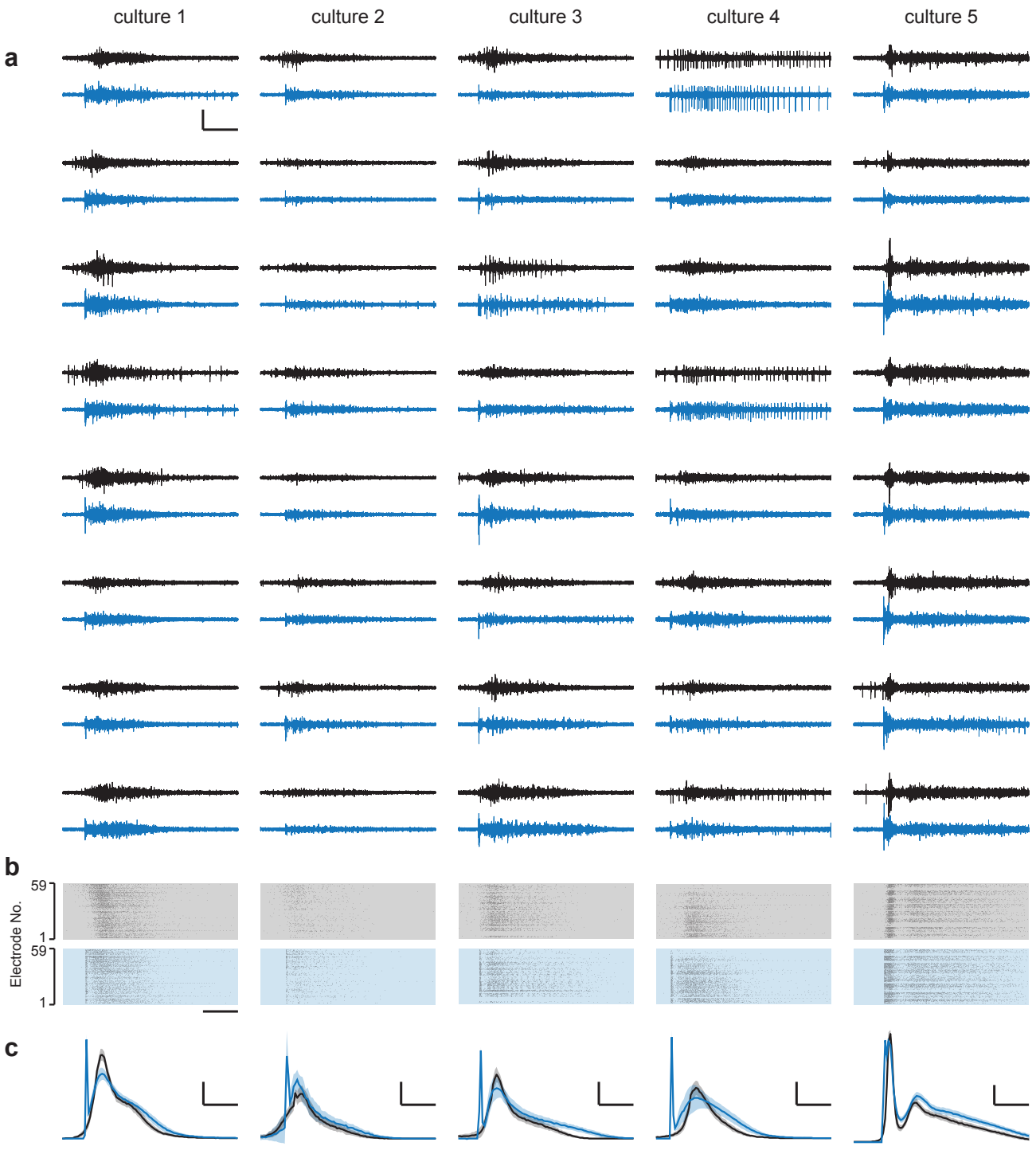
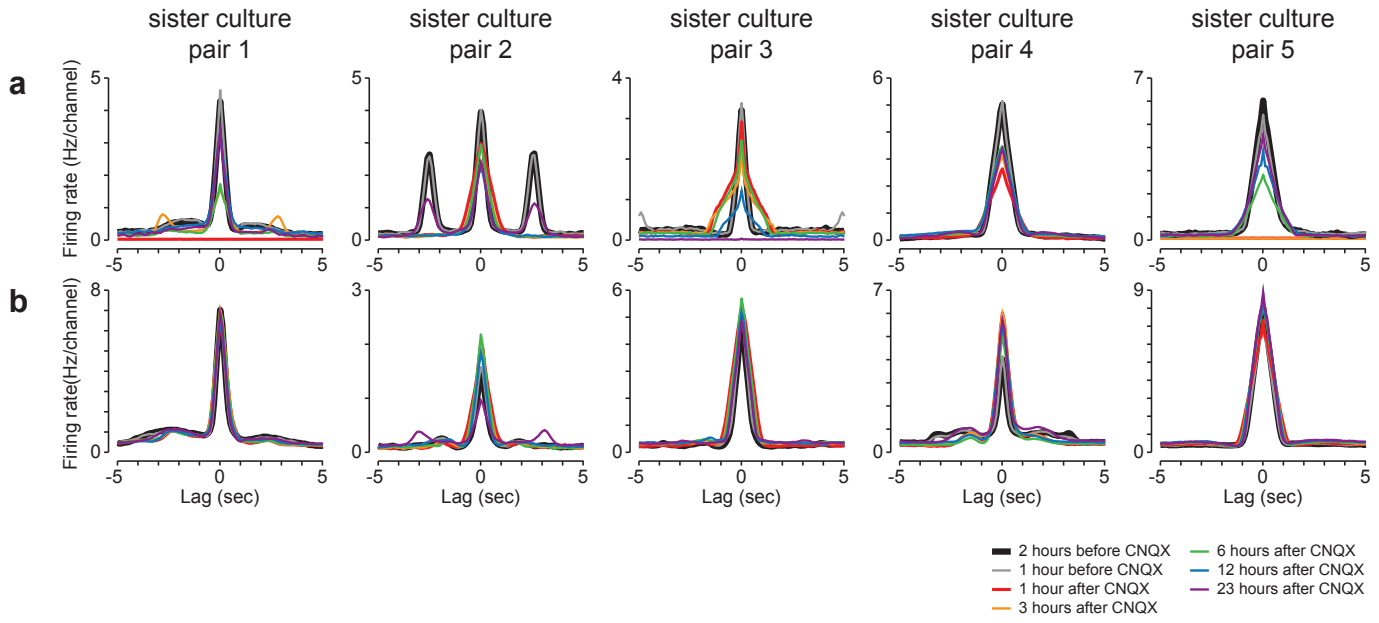


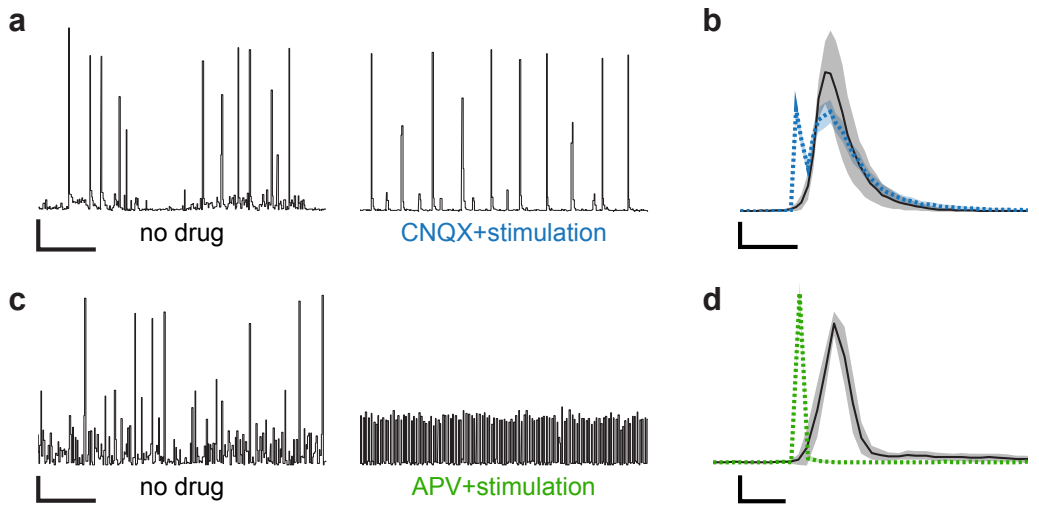
Supplementary Figure 1: NMDAergic transmission is responsible for the late phase of bursts. (a) *Left*, MEA-wide firing rate for culture treated with vehicle. Bin size, 1 s. *Right*, average burst waveform before (black) and after (red) vehicle. Shading denotes s.d. Bin size, 10 ms. Scale bar, 1 kHz, 200 ms. **(b, c)** Same as (a) for CNQX- and APV-treated cultures. All recordings shown are from sister cultures were plated on a multi-well MEA, with each well containing 9 microelectrodes. When AMPAergic transmission is blocked, there is a slight delay in burst onset, but a pronounced increase in burst duration. When NMDAergic transmission is blocked, burst duration is significantly reduced. Together these suggest that AMPAergic transmission facilitates burst initiation and that NMDAergic transmission is responsible for burst elongation.



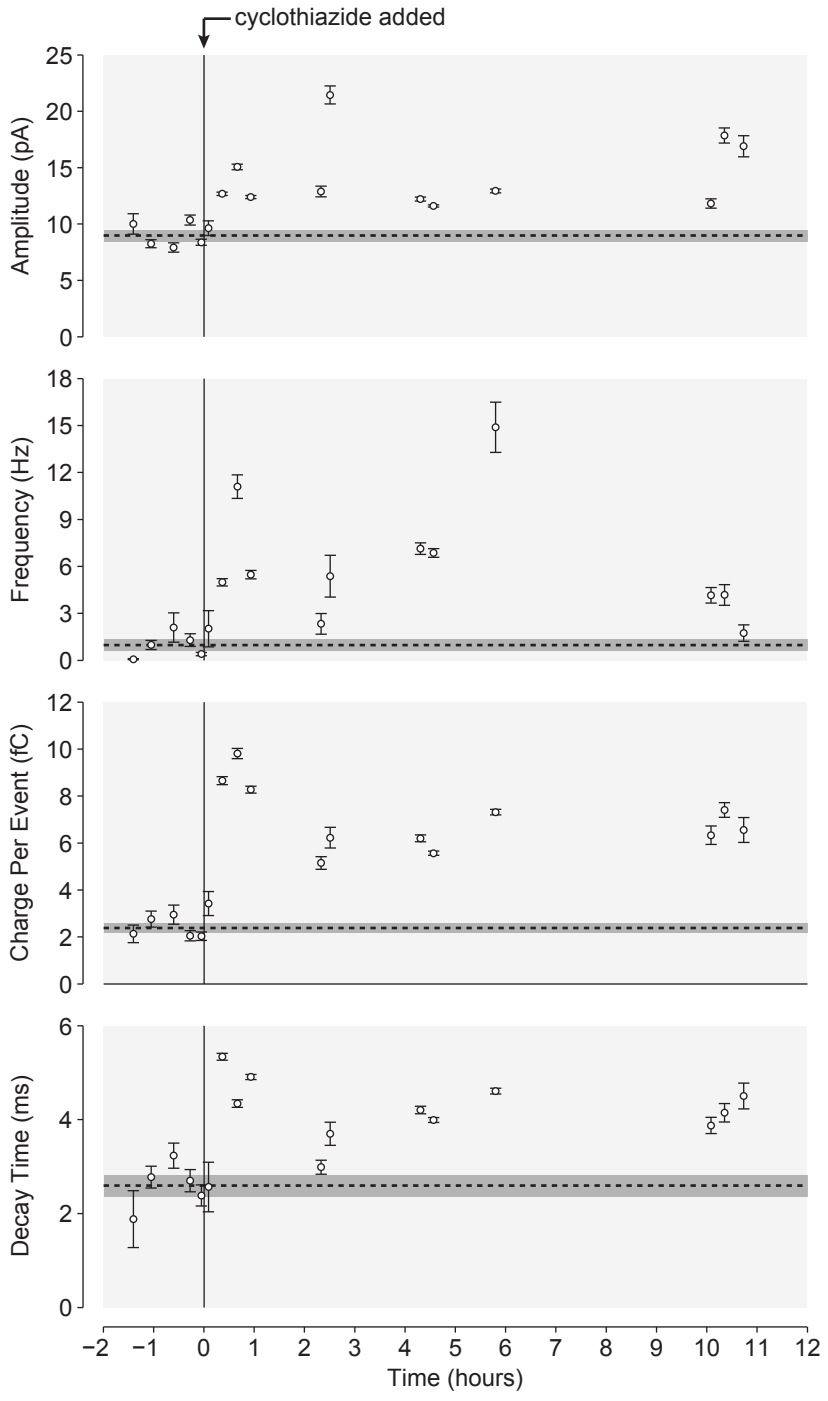
Supplementary Figure 2: Optogenetic stimulation during CNQX treatment effectively mimics spontaneous bursts within individual cultures. (a) Raw voltage traces showing spiking activity on individual electrodes during a spontaneous burst in the absence of drugs (black), or a optically-evoked burst in the presence of CNQX (blue). Data is shown from all 5 chronically-photostimulated cultures, and the 8 electrodes that were most active during the pre-drug period were selected for display. Scale bars, 100 μV, 200 ms. **(b)** Rastergrams showing spike times for all MEA electrodes corresponding to burst shown in (a). Grey background denotes spontaneous data, and blue background denotes condition with CNQX and optically-restored spiking. Scale bar, 200 ms. **(c)** Average MEA-wide firing rate during a burst (spontaneously-occurring, black, 6 hours of burst data; optically-evoked during CNQX, blue, 24 hours of burst data). Shaded regions denote s.d. Bin size, 10 ms. Scale bars, 5 kHz (cultures 1, 3, 4, 5), 2 kHz (culture 2), 200 ms (all).



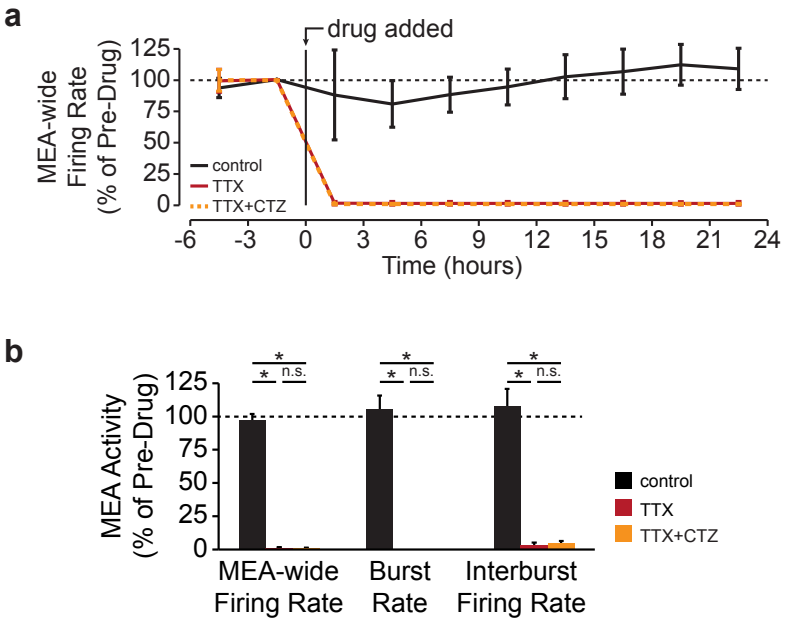
Supplementary Figure 3: Optogenetic stimulation during CNQX treatment reproduces channel-to-channel correlations. **(a)** Cross correlation function computed for each channel-channel pair at various timepoints, and averaged across pairs, for five CNQX-treated cultures. Spiking across channels becomes less correlated during CNQX treatment, though generally improves over 24 hours. **(b)** Same as (a), but for CNQX-treated cultures and experiencing optically-restored spiking levels. Cultures are displayed below the corresponding unstimulated CNQX-treated sister culture. Closed-loop stimulation maintains pre-drug channel-to-channel correlations immediately after CNQX treatment, and this effect is sustained over 24 hours.



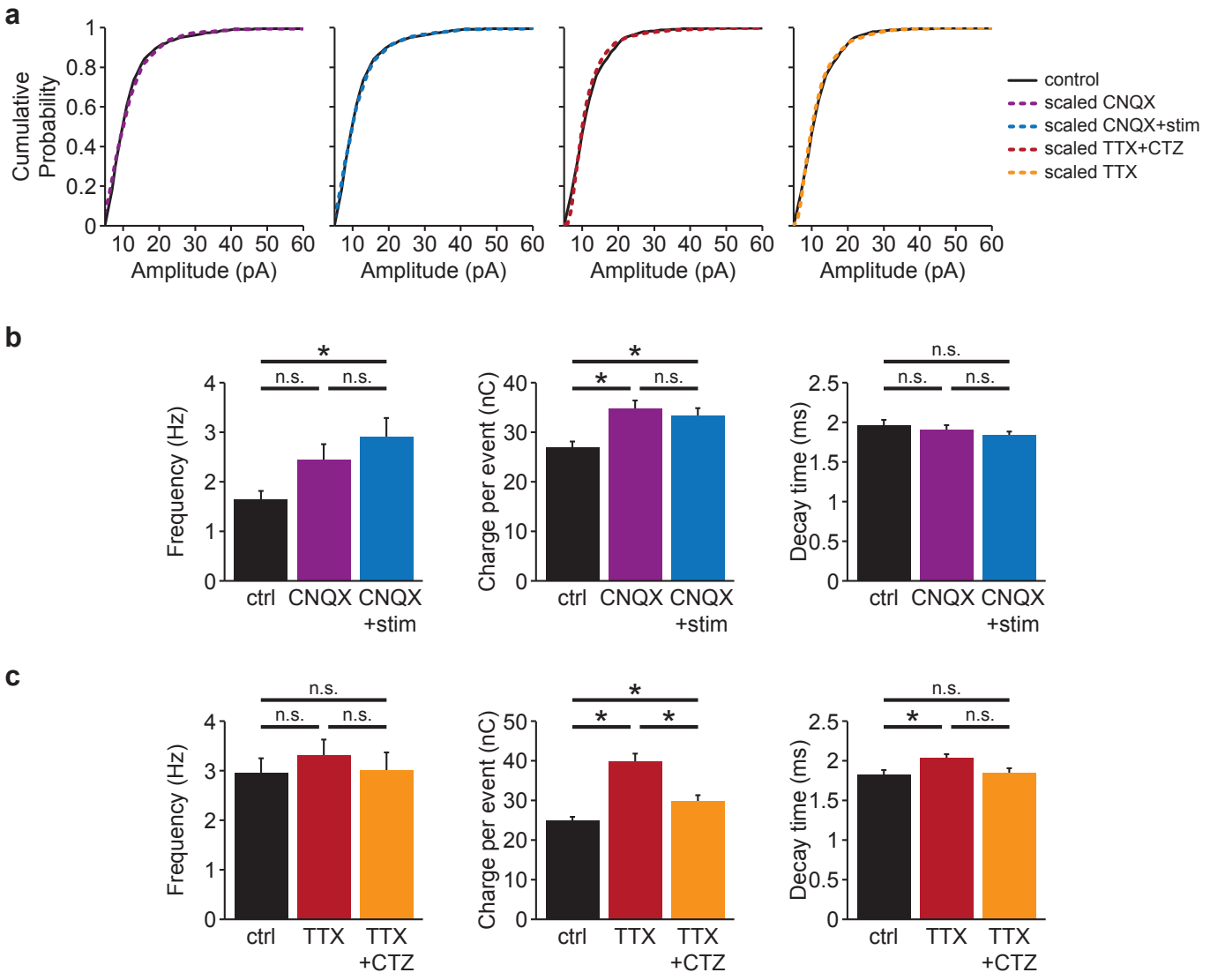
Supplementary Figure 4: NMDAergic transmission facilitates normal bursting during optical stimulation. (a) MEA-wide firing rate for a culture before drug treatment (left), and during CNQX treatment with optogenetically-restored firing rate (right). Bin size, 1 s. Scale bars, 200 Hz, 1 min. **(b)** Average burst waveforms for the two conditions pre-drug (black) and CNQX+photostimulation (blue) conditions. Data used to generate averages was taken for an hour before and after traces shown in (a). Bin size, 10 ms. Scale bar, 2 kHz, 100 ms. **(c)** MEA-wide firing rate for a culture before drug treatment (left), and during APV treatment with optically-restored firing rate (right). Bin size, 1 s. Scale bars, 200 Hz, 1 min. **(d)** Average burst waveforms for the pre-drug (black) and APV+stimulation (green) conditions. Data used to generate averages was taken for an hour before and after trace shown in (c). Bin size, 10 ms. Scale bars, 800 Hz, 50 ms. Data shown in this figure was generated from cultures infected with AAV2-CaMKIIa-ChR2(H134R)-mCherry, and recordings were performed at 26 DIV (a,b) and 33 DIV(c,d). All other plating, recording, and stimulation parameters were the same as other experiments previously described.



Supplementary Figure 5: Cyclothiazide is effective at enhancing quantal AMPAR activation for at least 11 hours. mEPSCs were recorded from 4 different cells, using TTX and bicuculline to isolate AMPAergic events. After CTZ was added, mEPSCs were recorded from 11 additional cells at various time points over the course of 11 hours. Mean mEPSC amplitude, frequency, charge per event, and decay time is shown for all 15 cells. Points just before and after CTZ is added represent the same cell. Dotted line denotes the pre-CTZ average of the 4 cell means. Shading and error bars denote s.e.m.



Supplementary Figure 6: Cyclothiazide does not change effects of TTX on spiking activity. (a) Mean MEA-wide firing rate over time for cultures co-treated with TTX and CTZ (n=5 cultures). Control and TTX values from Fig. 1e are shown for comparison. Both TTX-treated cultures eliminate spiking, regardless of whether CTZ is present.. Bin size, 3 h. Error bars, s.d. **(b)** Mean MEA-wide firing rate, burst rate, and interburst firing rate for the TTX+CTZ condition during the 24-hour treatment window, with control and TTX values from Fig. 1f shown for comparison. TTX+CTZ completely abolished spiking and bursting, and the effect on MEA activity is not different than TTX alone (MEA-wide firing rate, 1.056±0.002%, p>0.3; burst rate, 0±0%, p=1; interburst firing rate, 4.26±1.91%, p<0.4). Error bars, s.e.m.



Supplementary Figure 7: Scaled mEPSC amplitude distributions, and other mEPSC features. **(a)** Scaled mEPSC amplitude distributions for CNQX, CNQX+photostimulation, TTX, and TTX+CTZ (same data as Figs. 3 and 4). Scaled distributions are no different that those from sister controls (CNQX, $p>0.9$; CNQX+stim, $p>0.9$; TTX, $p>0.7$; TTX+CTZ, $p>0.5$). **(b)** Mean frequency (control, 1.645±0.168 pA, $n=44$ cells; CNQX, 2.282±0.270 pA, $n=51$ cells; CNQX+photostimulation, 2.640±0.253 pA, $n=46$ cells; $p<0.02$), charge per event (control, 26.957±1.209 nC; CNQX, 34.804±1.643 nC; CNQX+photostimulation, 33.527±1.373 nC; $p<10^{-3}$), and decay time (control, 1.973±0.062 ms; CNQX, 1.908±0.062 ms; CNQX+photostimulation, 1.841±0.044; $p>0.2$) for CNQX+photostimulation experiments. There are signficance difference in frequency of control vs. CNQX+photostimulation conditions ($p<10^{-2}$), and in charge per event of control vs. both CNQX cases (control vs. CNQX, $p<10^{-3}$; control vs. CNQX+photostimulation, $p<10^{-3}$). **(c)** Mean frequency (control, 2.962±0.297 pA, $n=47$ cells; TTX, 3.321±0.368 pA, $n=58$ cells; TTX+CTZ, 3.008±0.368 pA, $n=50$ cells; $p>0.6$), charge per event (control, 24.909±0.928 nC; TTX, 39.965±1.931 nC; TTX+CTZ, 29.867±1.439 nC; $p<10^{-10}$), and decay time (control, 1.832±0.056 ms; TTX, 2.037±0.053 ms; TTX+CTZ, 1.850±0.061; $p<0.02$) for TTX+CTZ experiments. There are signficance difference in charge between all conditions (control vs. TTX, $p<10^{-8}$; control vs. TTX+CTZ, $p<10^{-2}$; TTX vs. TTXCTZ, $p<10^{-4}$), and in decay time for cultures treated with TTX (control vs. TTX, $p<10^{-2}$).