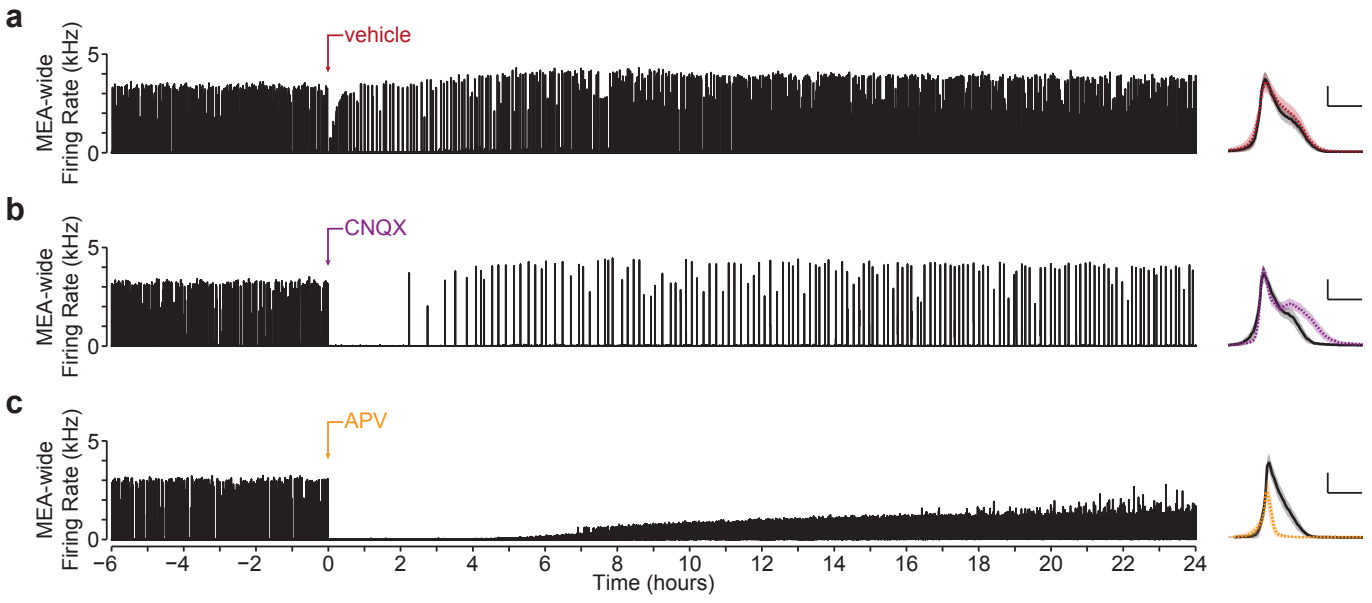

Figure 1: NMDAergic transmission is responsible for the late phase of bursts. (a) *Left*, MEA-wide firing rate for culture treated with vehicle. Bin size, 1 s. *Right*, average burst waveform before (black) and after (red) vehicle. Shading denotes s.d. Bin size, 10 ms. Scale bar, 1 kHz, 200 ms. **(b, c)** Same as (a) for CNQX- and APV-treated cultures, as labeled. 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 pronounced increase in burst duration. When NMDAergic transmission is blocked, burst duration is significantly reduced. Together these suggest that NMDAergic transmission is responsible for burst elongation.

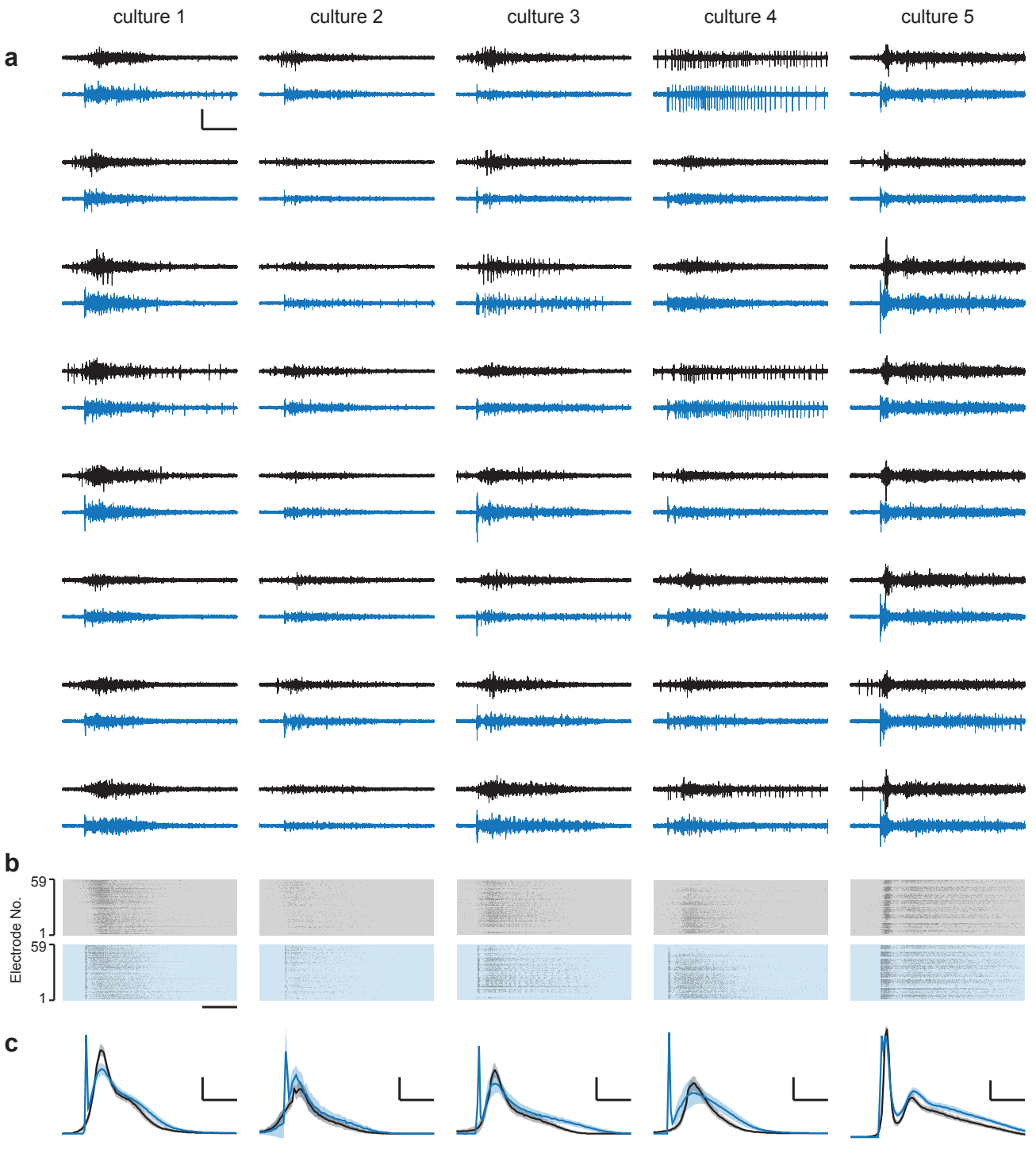

Figure 2: Optogenetic stimulation during CNQX treatment effectively mimics spontaneous bursts within individual cultures. (a) Raw voltage traces showing spiking activity on individual electrodes during a spontaneous burst in the absence of drugs (black), or a optically-evoked burst in the presence of CNQX (blue). Data is shown from all 5 chronically-photo-stimulated 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).

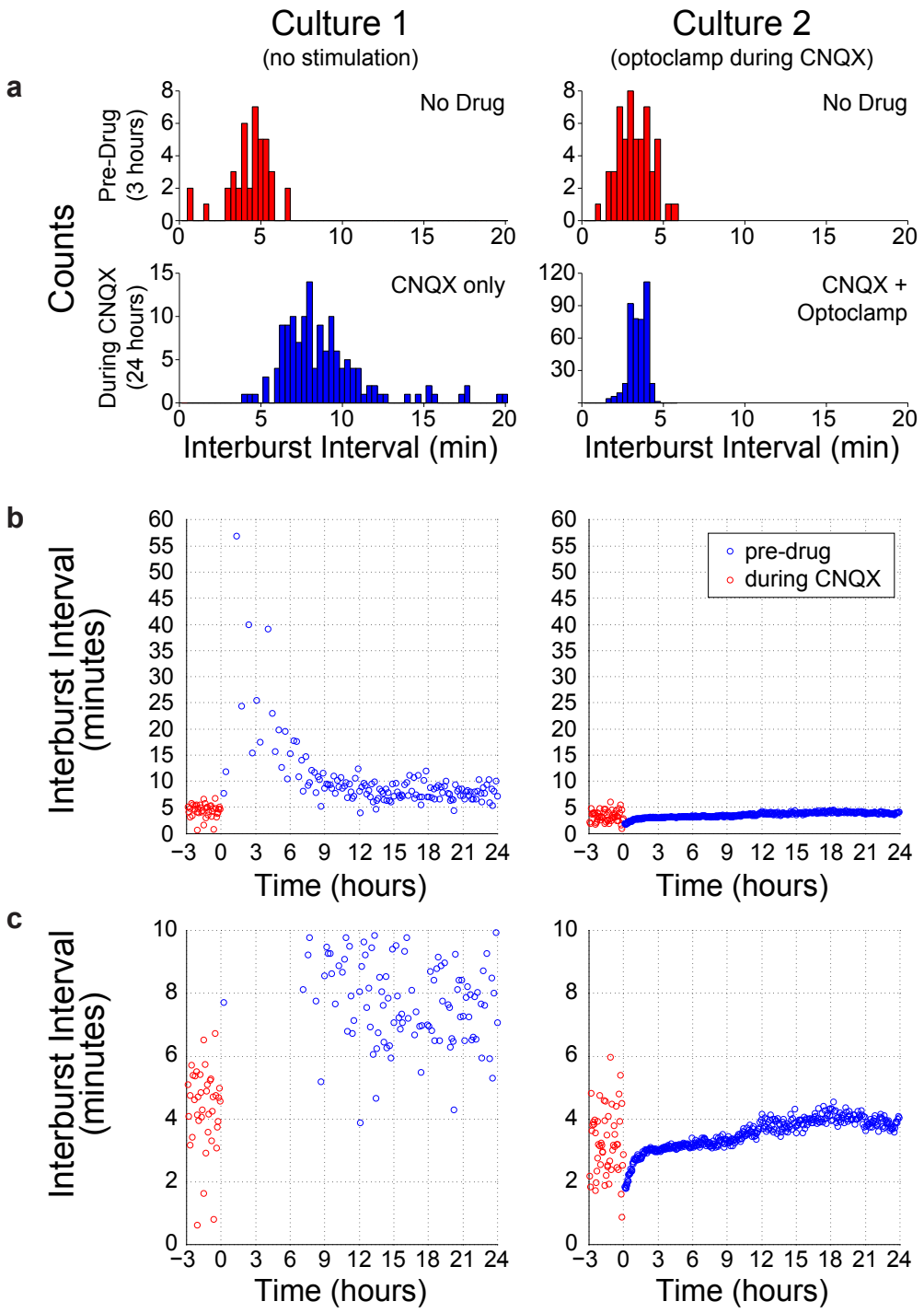

Figure 3: Interburst interval during CNQX or CNQX+optoclamp. For this figure, all data on left is for a culture treated only with CNQX, and all data on right is for a sister culture treated with CNQX but experiencing optically-restore firing rate across the MEA. **(a)** Histogram of interburst intervals during the 3-hour period before CNQX was added (top), or during the 24-hour CNQX treatment. CNQX shifts distribution to the right. Although CNQX+optoclamp prevents this shift, it also narrows the variance in interburst intervals. **(b,c)** Interburst intervals as a function of time, one zoomed out to show all data (b), and one zoomed in to show most of data (c). CNQX alone increased interburst intervals for the entire treatment, though the effect is attenuated after 6 hours. The addition of closed-loop optical stimulation decreased the variance in interburst interval over the entire 24-hour treatment period, though this effect is attenuated after 12 hours.

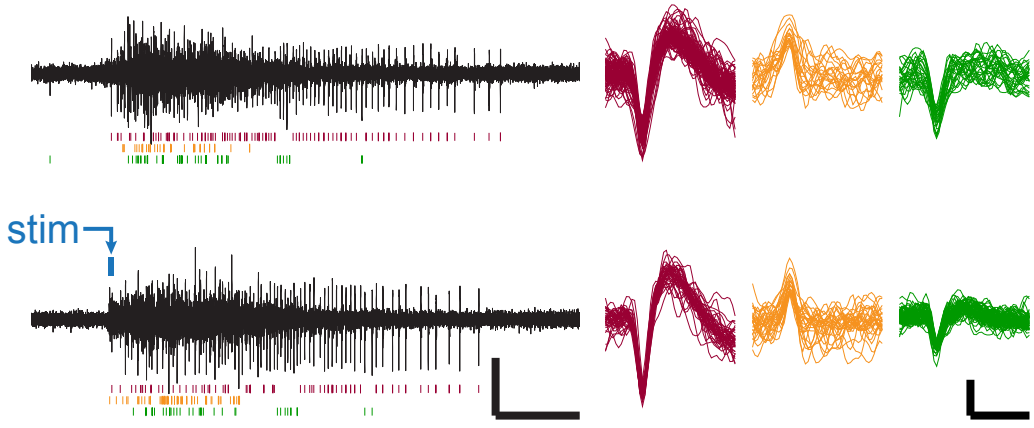

Figure 4: Individual units during spontaneous or optically-evoked burst. *Left*, voltage traces recorded from a single microelectrode during a spontaneous burst in the absence of any drug (top) and a photostimulation-evoked burst after addition of CNQX (bottom). The stimulation timing is noted, and the 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).

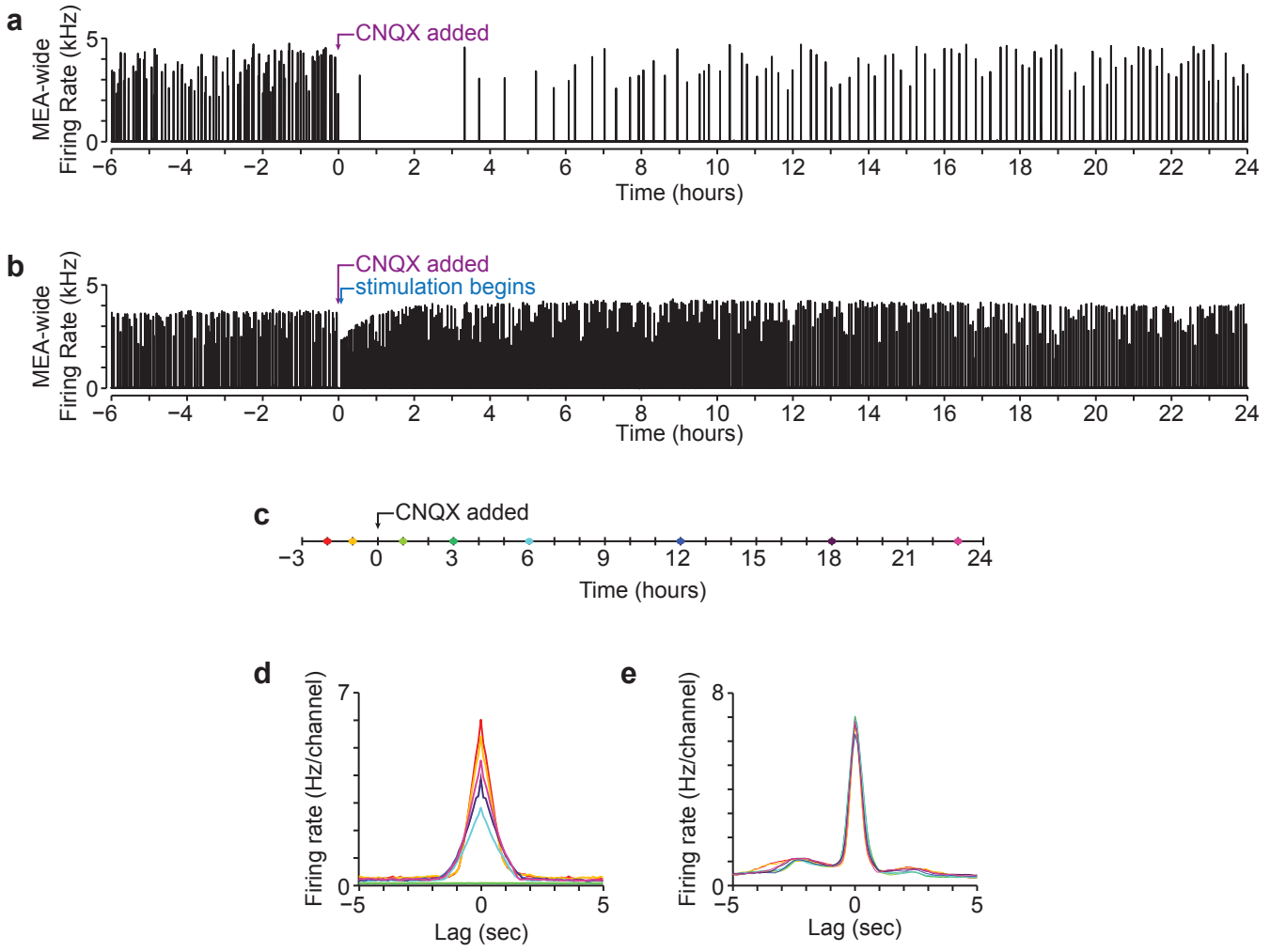

Figure 5: Optogenetic stimulation during CNQX treatment reproduces channel-to-channel correlations. **(a)** Array-wide firing rate for culture treated with CNQX. Bin size, 1 s. **(b)** Array-wide firing rate for culture treated with CNQX and with closed-loop optical stimulation used to restore overall firing rate recorded throughout the MEA. **(c)** Timeline showing time points (colored dots) when channel-to-channel correlations were calculated for recordings shown in (a) and (b). **(d)** Cross correlation function computed for each channel-channel pair at various timepoints, and averaged across pairs, for CNQX-treated culture shown in (a). Line colors correspond to dots in (c). Immediately after the drug is added, correlations are dramatically reduced , but start to recover after 6 hours. **(e)** Same as (d), but for CNQX-treated culture and experiencing optically-restored spiking levels shown in (b). Closed-loop stimulation restores pre-drug channel-channel correlations across the entire 24 hour treatment.

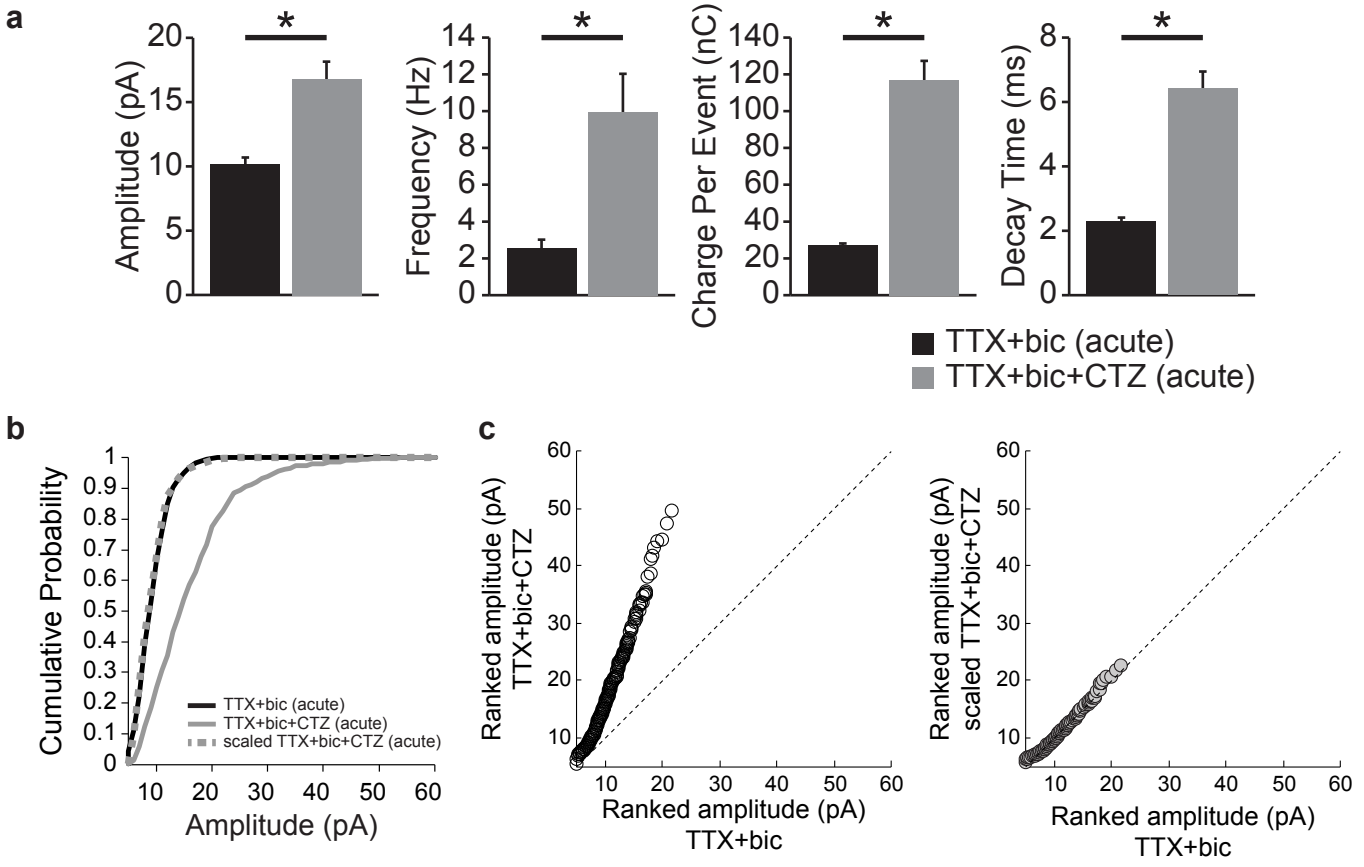

Figure 6: Acute effects of cyclothiazide on mEPSCs. (a) Mean amplitude ($p<10^{-3}$), frequency ($p<10^{-2}$), charge per event $p<10^{-7}$), and decay time constant $p<10^{-6}$) of AMPAergic mEPSCs before and during acute application of CTZ (before, n=10 cells; during, n=11 cells). TTX and bicuculline (bic) are used to isolated all synaptic currents to AMPAergic mEPSCs. **(b)** Cumulative distribution of AMPAergic mEPSC amplitudes before and during acute CTZ treatment conditions. The acute CTZ distribution, scaled by a multiplicative factor, is also shown. The multiplicatively scaled CTZ distribution matches pre-CTZ (i.e. TTX+bic only) condition (p>0.5). **(c)** *Left,* ranked TTX+bic+CTZ mEPSC amplitudes plotted against ranked pre-CTZ (i.e. TTX+bic only) amplitudes (linear fit, R^2=0.98684). *Right,* ranked TTX+bic+CTZ mEPSC amplitudes scaled using the best-fit line from plot on left, plotted against ranked pre-CTZ amplitudes. Dotted lines denotes the line of identity.

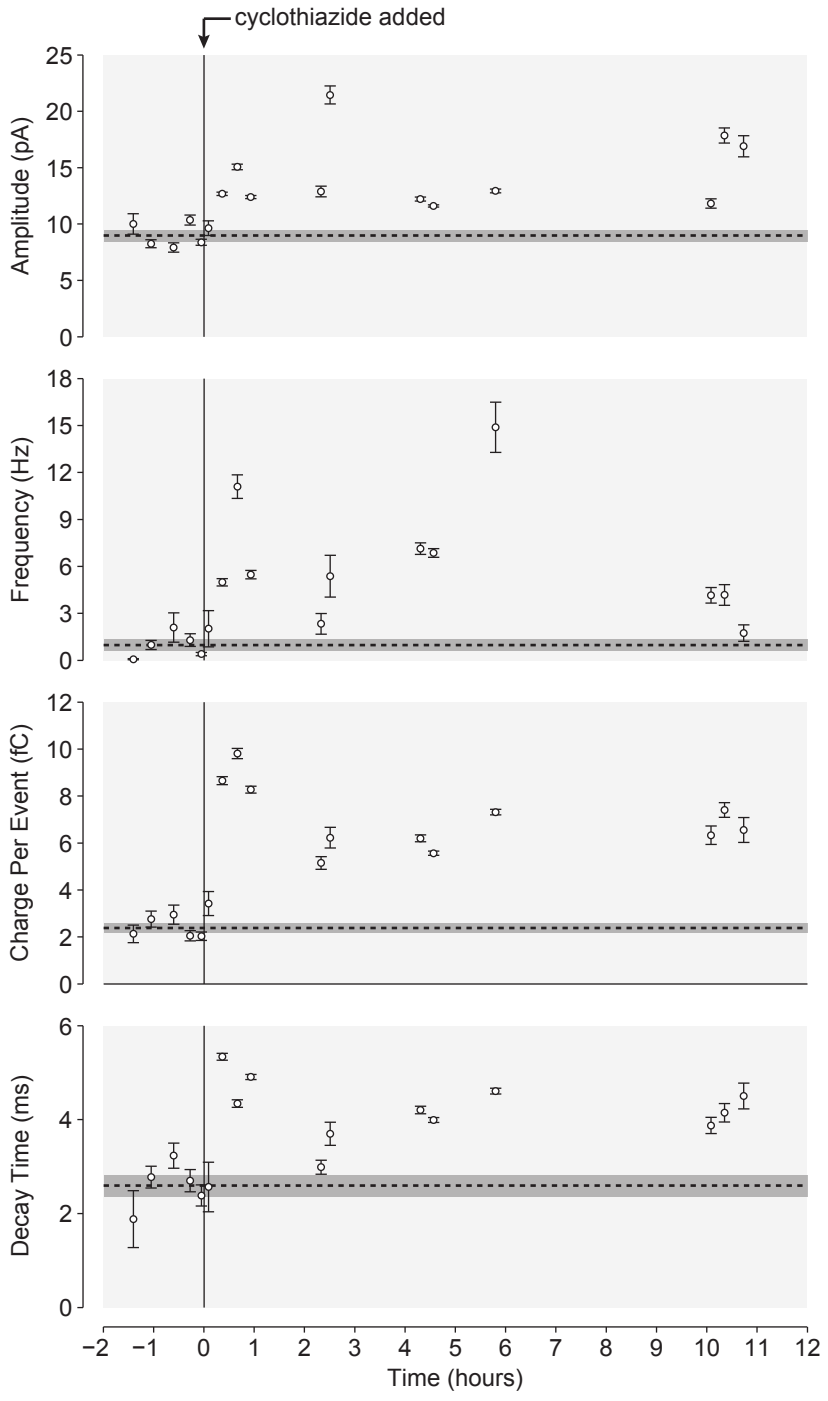

Figure 7: Cyclothiazide is effective at enhancing quantal AMPAR activation for at least 11 hours. mEPSCs were recorded from 4 different cells, using TTX and bicuculline to isolate AMPAergic events. After CTZ was added, mEPSCs were recorded from 11 additional cells at various time points over the course 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.

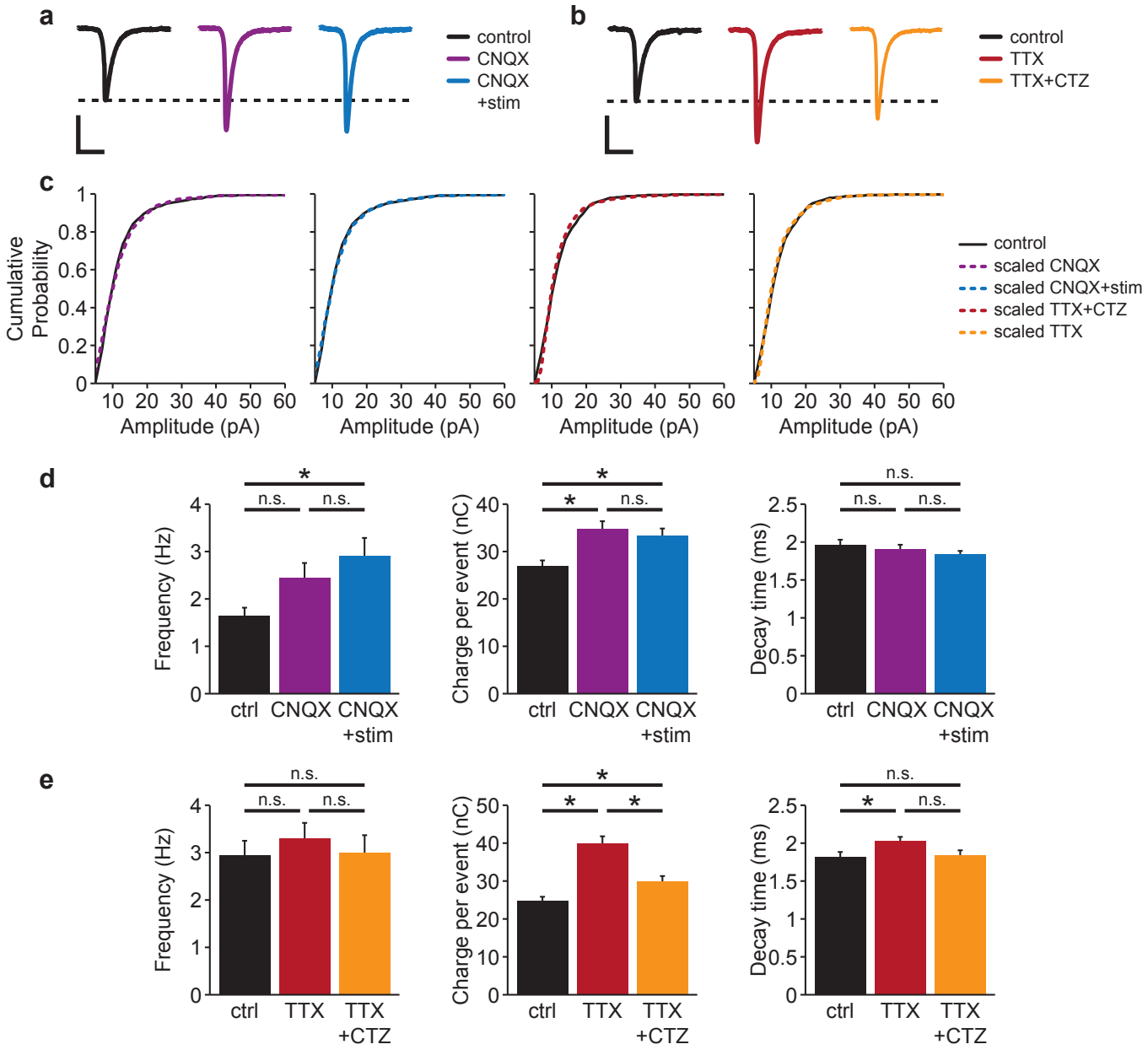

Figure 8: Scaled mEPSC amplitude distributions, and other mEPSC features. **(a,b)** Average mEPSC waveforms for cultures treated with vehicle, CNQX, CNQX+optical stimulation, TTX, or TTX+CTZ. (Different vehicle-treated controls are shown because they are taken from sister cultures corresponding to the different treatment groups). Scale bars, 5 pA, 10 ms. **(c)** 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, $p>0.5$). **(d)** 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, $p<10^{-3}$). **(e)** 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, $p<10^{-2}$).