**Upward synaptic scaling is dependent on neurotransmission rather than spiking**

**Abstract**

Chronic reductions in firing rate are thought to directly trigger upward synaptic scaling (upscaling), where a cell’s excitatory synaptic strength is increased in order to homeostatically maintain spike rate. Recent work, however, has suggested that reduced neurotransmission can trigger compensatory synaptic strengthening. In order to separate the importance of spiking and AMPAergic transmission in triggering upscaling, we independently manipulated these two variables through a combination of multi-electrode recording, closed-loop optogenetic stimulation, and pharmacology. Upscaling was triggered when AMPAergic transmission was blocked, even if spiking was optogenetically restored. Further, upscaling was attenuated when AMPAergic currents were enhanced while spiking was blocked. We demonstrate that upscaling is triggered by reduced AMPA receptor activation, not reduced spiking. Our results challenge the prevailing belief that the function of upscaling is to homeostatically maintain firing rate.

**Introduction**

In order for neural networks to function properly they must express specific levels and patterns of spiking activity. Because aberrant levels of activity often develop following neural injury and disease, it is important to identify the triggers and mechanisms of plasticity that influence network excitability 1. Homeostatic plasticity represents a set of mechanisms that ensures cells or networks maintain appropriate levels of spiking activity 2,3. The best studied form of homeostatic plasticity is AMPAergic synaptic upscaling, where AMPAergic quantal amplitudes throughout the cell are multiplicatively increased following chronic reductions in spiking activity 4,5. Such compensations are bidirectional, as chronic elevation of spiking activity triggers downward AMPAergic scaling 5-7.

Despite the importance of these synaptic compensations, our understanding of the triggers of synaptic upscaling is incomplete. The prevailing hypothesis is that reductions in somatic spiking trigger AMPAergic upscaling by reducing calcium entry and signaling, which in turn cause accumulation of synaptic AMPA receptors (AMPARs) 4,8,9. Therefore, neurons are thought to tightly control their spiking levels by monitoring spike-dependent calcium entry at the soma. Consistent with this idea, synaptic upscaling is observed following chronic blockade of either voltage-gated sodium channels or AMPARs, both of which dramatically reduce spiking activity 10. However, blocking action potentials or AMPARs both reduce glutamatergic transmission as well, and recent studies have suggested that reduced transmission by itself can trigger compensatory synaptic strengthening 9,11,12. When neurotransmission is reduced chronically by altering spiking in a subset of presynaptic fibers, local compensatory synaptic strengthening was observed only at those synapses experiencing reduced transmission. It is not clear how or if transmission-dependent synaptic compensations are related to cell-wide synaptic scaling.

In the current study, we independently manipulated spiking and AMPAergic transmission to identify the effect of each on upscaling. First, we continuously monitored spiking activity across cultured networks with 30-hour micro-electrode array (MEA) recordings. We showed that reductions in spiking did not correlate with the magnitude of upscaling. Next we pharmacologically blocked AMPAergic transmission, while restoring normal spiking through optogenetic stimulation controlled by feedback from our MEA recordings. We found that spiking had no influence on upscaling. Finally, we blocked spiking activity while partially restoring transmission using an AMPAR modulator, and found that changes in AMPAR activation were critical to the upscaling process. We conclude that cell-wide multiplicative upscaling is directly triggered by reduced AMPAergic transmission, and not reduced spiking. Because previous work has suggested that reductions in transmission trigger synapse-specific compensations 13-16, it is possible that upscaling is a cell-wide version of local transmission-dependent compensations. If true then this would suggest that upscaling functions to maintain local synaptic strength, rather than as a mechanism to homeostatically maintain firing rate. The findings have significant implications for both homeostatic and Hebbian forms of plasticity. Our findings demonstrate the power of optogenetic feedback control for separating the roles of spiking and transmission during plasticity.

**Results**

***Spiking persists during chronic AMPAergic transmission blockade***

We sought to examine the nature of spiking activity in a developing cultured neural network, and to assess how this activity is altered during perturbations that trigger synaptic scaling. To this end, we used planar MEAs (Fig. 1a-b) to record extracellular spiking activity from hundreds of neurons embedded in dissociated cortical cultures during their second week *in vitro*. For each culture, the overall firing rate was assessed by summing spikes across all MEA electrodes, and dividing by the elapsed time interval 17. Consistent with previous work 17-19, MEA recordings showed that most spiking was associated with network-wide population bursts (hereafter referred to as bursts), but low levels of asynchronous spiking were also observed during the interburst interval (Fig. 1c).

We next examined how 24-hour blockade of either voltage-gated sodium channels or AMPA/kainate receptors modulated spiking and bursting activity. Both perturbations are thought to trigger upscaling by reducing spike activity. Consistent with our expectations, the voltage-gated Na+ channel blocker TTX, eliminated spiking activity for the entire 24-hour treatment (Fig. 2a-b). In contrast, the AMPA/Kainate receptor antagonist CNQX, only partially reduced spiking activity compared to pre-drug levels (Fig. 2a-b; 20-22). The CNQX-induced reduction in firing rate was primarily due to a reduction in burst frequency, while asynchronous spiking during the interburst interval was not significantly affected (Fig. 2c). Bursting was significantly reduced during the first few hours of CNQX treatment, but typically began to recover by the end of 24 hours (Fig. 2a, Suppl. Fig 1b), likely due to NMDAergic transmission (Suppl. Fig 1, 21,23). While the CNQX-induced reduction in spiking was variable across cultures, some degree of spiking and bursting always persisted, unlike cultures treated with TTX. The distinct effects of TTX and CNQX on spiking activity might suggest that they also have different effects on activity-dependent processes such as synaptic scaling.

***Homeostatic reductions in spiking activity are not correlated with changes in synaptic strength***

We hypothesized that cultures experiencing greater reductions in spiking activity would also experience more dramatic upscaling of quantal amplitude. To test this hypothesis, we used whole-cell voltage clamp recordings to measure miniature excitatory postsynaptic currents (mEPSCs) from pyramidal cells following 24-hour application of TTX or CNQX (Fig. 3a-b). All analyses of mEPSCs throughout the study were carried out blind to the treatment condition. Consistent with published results 5,20, chronic TTX or CNQX treatment produced similar increases in mEPSC amplitude over vehicle-treated sister control cultures (Fig. 3b-c, f; TTX, 146.8±8.0% of control; CNQX, 142.9±4.5% of control) and the distributions of mEPSC amplitudes scaled multiplicatively (Fig. 3d-e, g-h; Suppl. Fig. 5a). Thus, surprisingly, cells from CNQX-treated cultures experiencing only moderate reductions in spiking scaled equally to cells in TTX-treated cultures that experienced complete elimination of spiking.

To quantify the relationship between spiking and scaling, we compared changes in spiking and mEPSC amplitude for individual sister culture pairs. For each culture, we compared the reduction in MEA-recorded firing rate during the 24-hour TTX or CNQX treatment to the increase in mean mEPSC amplitude recorded following the treatment (Fig. 3i). We observed no correlation between either the TTX- or CNQX-induced change in firing rate and the resultant increase in mEPSC amplitude. Moreover, there was no correlation between the increase in mEPSC amplitude and other measurable features of spiking activity (Fig. 3i). Overall, the lack of correlation between spiking and scaling demonstrated that the degree of upscaling could not be fully explained by changes in spiking activity.

There are several reasons why changes in synaptic strength might be poorly correlated with changes in spiking activity. It is possible that any reduction in spiking beyond a certain threshold triggers the same scaling response. The rate of insertion of AMPARs that mediates the expression of scaling might saturate, such that further increases in quantal amplitude are not possible during the 24 hour treatment period. Alternatively, it is possible that postsynaptic spiking is not the activity signal being monitored to trigger scaling, and that reductions in AMPAR activation might directly trigger scaling.

It has been difficult to distinguish between the independent effects of spiking and AMPAR activation on synaptic scaling because they are highly-coupled processes. Blockade of AMPAR activation using CNQX eliminates depolarization of cells, and thus decreases postsynaptic spiking. Conversely, blockade of spiking using TTX eliminates evoked neurotransmitter release, and thus reduces the amount of glutamate that can activate AMPARs. Since TTX and CNQX each reduce spiking and AMPAergic transmission, either directly or indirectly, we developed two strategies for examining how chronic reductions in spiking and transmission independently affect synaptic scaling; we block AMPARs while maintaining normal spiking activity, or block spiking but partially restore AMPAR activation.

***Closed-loop optical stimulation restores normal spiking during AMPAergic transmission blockade***

In order to examine the effects of reducing AMPAergic transmission independent of spiking, we developed a closed-loop optical stimulation system for restoring normal levels of spiking activity during chronic CNQX treatment (Newman et al., in review). We used an adenoassociated virus to drive expression of channelrhodopsin-2 (ChR2) H134R mutant 24 in cultured neurons. We observed expression throughout the culture within a week (Fig. 4a). To deliver optical stimuli, we used a custom current source to drive a blue LED (465 nm, see methods). Blue light was passed through a randomized fiber bundle and fed to a custom optical train, providing uniformly-distributed illumination in the plane of the culture (Fig. 4c; 10.1 mW/mm2).

Because the reductions in firing rate that accompany CNQX application are primarily due to reductions in network-wide bursts (Fig. 2c), we used a stimulation strategy that reinstated bursts. Each brief (10-ms) pulse of blue light reliably evoked short-latency spikes that resulted directly from ChR2 activation, followed by a longer latency barrage of action potentials. These longer latency barrages of spikes, which occurred tens to hundreds of milliseconds after the blue light pulse terminated, closely resembled spontaneously-occurring bursts in terms of time course and profile of network recruitment (Fig. 4b, d, e; Suppl. Fig. 2-3). We suspect these bursts are dependent on NMDAergic transmission, since spontaneous-like bursts could not be reproduced during NMDA receptor blockade (Suppl. Fig. 4).

To achieve precise control of MEA-wide firing rate, we controlled optical stimulation in real-time, based on spiking activity recorded through the MEA (Newman et al., in review; 25). The MEA-wide firing rate was calculated every 10 ms, and the 10-ms pulse of blue light was delivered if the integrated error between the target and measured firing rate became positive (Fig. 4a). In order to restore normal levels of spiking during an AMPAR blockade, we treated cultures with CNQX and began closed-loop optical stimulation with a target spiking level set to the pre-drug firing rate (Fig. 5a). Closed-loop optical stimulation effectively restored the pre-drug firing rate throughout CNQX application (Fig. 5b) while preserving spiking correlations between electrodes (Suppl. Fig 3). These similarities in spiking statistics suggest that spike-dependent processes, such as vesicular release, were also maintained. Further, our controller effectively restored burst rate (Fig. 5c) and burst shape (Suppl. Fig. 2) suggesting that normal levels of somatic calcium influx, which is primarily associated with network-wide bursts in neuronal cultures 19,26,27 was also well-preserved.

***Reductions in spiking are not required to trigger upward synaptic scaling***

Having restored normal spiking during AMPAergic transmission blockade (Fig. 5), we next examined whether this restored spiking activity would prevent upward synaptic scaling. To this end, we recorded mEPSCs from triplicate sister cultures: [1] vehicle-treated control cultures experiencing normal AMPAergic transmission and normal spiking activity, [2] CNQX-treated cultures experiencing no AMPAergic transmission and reduced spiking, and [3] photostimulated CNQX-treated cultures experiencing no AMPAergic transmission but restored spiking activity (Fig. 6a). Consistent with our previous results, mEPSC amplitudes from CNQX-treated cultures experiencing reduced spiking were scaled up, compared to sister control cultures (Fig. 6, Suppl. Fig. 5a). Interestingly, mEPSC amplitudes from CNQX-treated cultures experiencing restored spiking also scaled up, and were statistically indistinguishable from CNQX-treated cultures experiencing reduced activity (Fig. 6a-d; P>0.9). These results demonstrate that reductions in spiking are not required to trigger upward synaptic scaling. Instead, reduced AMPAergic transmission can directly and independently trigger upscaling even when spiking is normal.

***Reductions in AMPA receptor activation trigger upward synaptic scaling***

Based on our results that reduced AMPAergic transmission triggers upscaling that is independent of changes in spiking, we hypothesized that chronic TTX application leads to upscaling because it prevents spike-dependent release of neurotransmitter and therefore reduces AMPAergic transmission. Alternatively, it is possible that upscaling can be triggered in multiple ways, and that reductions in spiking or in AMPAergic transmission can each trigger scaling independently.

To test the importance of reduced AMPAergic transmission on TTX-induced synaptic scaling, we sought to enhance the AMPAergic quantal currents that remained during a spiking blockade. AMPARs mediate fast glutamatergic transmission and desensitize quickly after binding glutamate. In order to enhance AMPAergic mEPSCs we used cyclothiazide, known to disrupt AMPAR desensitization and thus increase receptor open time 28, as well as increase presynaptic release 29. In our cultured cells, co-treatment with TTX and CTZ significantly increased the amplitude and frequency of AMPAergic mEPSCs compared to TTX alone (Fig. 7a-b), and this effect persisted for at least 11 hours (Suppl. Fig. 6).

In order to test the effect of partially restoring AMPAergic transmission during a spiking blockade, we treated sister cultures with either TTX, TTX and CTZ, or vehicle. As with previous experiments, we recorded spiking activity through the MEA during the 24-hour treatment period (Suppl. Fig. 7) and recorded mEPSCs after washing out the drugs (Fig. 8a). Like TTX treatment, co-treatment with TTX and CTZ completely abolished spiking and bursting activity (Suppl. Fig. 7). However, mEPSC amplitudes from cultures co-treated with TTX and CTZ were significantly reduced compared to sister cultures treated with TTX alone (Fig. 8a-b). Co-treatment of TTX and CTZ were significantly increased compared to vehicle-treated controls. This intermediate increase in synaptic strength is likely because CTZ only increased quantal currents and did not fully restore normal postsynaptic currents observed in control cultures with intact spiking (Fig. 7a, top trace). A multiplicative relationship between mEPSC amplitude distributions existed for all three conditions (Figure 8c-d, Suppl Fig. 5a), indicating that the increases in synaptic strength were consistent with synaptic scaling. These results show that partially restoring AMPAR activation during a spike blockade can significantly attenuate TTX-induced synaptic scaling. This demonstrates that the reduction in AMPAergic transmission is a critical component in triggering TTX-induced upscaling, and suggests that this form of scaling is the same as that observed following chronic CNQX treatment. Together, our results provide strong evidence that AMPAR activation is the activity feature monitored in cortical networks to trigger compensatory increases in synaptic strength.

**Discussion**

Cell-wide homeostatic synaptic scaling is thought to be triggered by changes in spiking activity. Our present study challenges this basic tenet and suggests an alternative trigger for synaptic scaling. First, we found that reductions in spiking activity were not correlated to changes in synaptic strength. We then showed that reductions in spiking activity were not required to elicit upward synaptic scaling. Lastly, we found that reductions in AMPAR activation in the absence of spiking, directly and independently triggered upscaling. These findings challenge computational and experimental models of synaptic scaling, and have important implications for compensatory plasticity in the context of learning, memory, development, and disease.

**A challenge to the spike-dependent model of upscaling**

Synaptic scaling rules were proposed as a mathematically tractable way to curb unbounded synaptic strengthening or weakening predicted by models of Hebbian learning 30-33. Since then, there has been a wealth of evidence in support of both upward and downward scaling across a range of experimental contexts 2,10. While these early models hypothesized that scaling could be triggered by changes in firing rate, the dependence of scaling on spiking appears to be more nuanced.

In the case of upscaling, the prevailing model suggests that chronic reductions in the frequency of somatic action potentials alter global calcium signaling, and trigger upscaling of AMPAergic currents 4,8,9. However, this model is incompatible with our results for several reasons. First, optogenetic restoration of spiking and bursting in the presence of CNQX, which presumably restores spike-dependent calcium signaling, did not attenuate AMPAergic upscaling (Fig. 6). The absence of any correlation between reductions in spike rate and the average increase in synaptic strength also suggested that reduced spiking did not trigger upscaling (Fig. 3i). Finally, TTX-induced scaling was lessened when quantal AMPAR activation was pharmacologically enhanced despite a complete blockade of spiking activity (Fig. 8). Our results demonstrate that upscaling is triggered by reduced AMPAR activation, not reduced spiking.

Previous studies that reduced spiking in postsynaptic cells without changes in the activity of presynaptic inputs have had mixed results concerning AMPAergic upscaling. In one set of studies, spiking was chronically reduced in individual postsynaptic cells by overexpression of an inwardly-rectifying potassium channel (Kir2.1), *in vitro* and *in vivo* 34,35. The Kir2.1-expressing cells homeostatically recovered spiking activity over a few days without showing changes in quantal amplitude, arguing against a role for reduced spiking in AMPAergic upscaling. However, in a more recent study, accumulation of AMPARs was observed when spiking was blocked by puffing TTX locally on the soma for a few hours, while leaving dendritic neurotransmission largely intact 8. This observation supported the idea that reduced spiking triggers upscaling. These conflicting results are likely explained by differences in the duration of treatment (days vs. hours), and the way synaptic strength was assessed (mEPSC amplitude vs. GluA2 fluorescence). Our finding that upscaling was triggered by reducing AMPAergic transmission, even when spiking is restored, is consistent with the first set of studies and provides strong evidence that changes in spiking are not required to trigger upscaling.

As discussed for the Kir2.1 transfection studies, dramatic reductions in spike rate and altered spike timing in individual cells did not trigger upscaling 34,35. Similarly, significant alterations of spiking occur in 24-hour NMDA receptor blockade (Suppl Fig. 1, 21,23), but this does not appear to trigger upscaling 5,15. These findings argue against the possibility that some subtle change in cellular spiking (e.g. timing) is the trigger for upscaling.

**Reduced neurotransmission triggers AMPAergic upscaling**

We have shown that upscaling is triggered when spiking is normal but AMPARs are blocked, and that the magnitude of scaling is lessened if spike blockade is accompanied by partial restoration of AMPAR activation. The findings demonstrate that upscaling is triggered by reduced AMPAR activation. A similar transmission-based compensatory plasticity has been described, both *in vivo* and *in vitro*, although studies of these compensations have occurred in a synapse-specific manner (for reviews see -9,11,12). Such studies have shown that reductions in spiking activity in a subset of presynaptic inputs triggered compensatory synaptic strengthening at only those synapses with reduced transmission; this was achieved by local application of a receptor antagonist 15, by presynaptic overexpression of Kir2.1 13,14, or by altering sensory input *in vivo* 16. Because we have determined that reduced receptor activation triggers cell-wide upscaling, we propose that upscaling produced by chronic TTX or CNQX treatment may be the result of blocking or reducing AMPAergic transmission throughout the cell, thus triggering local synaptic compensations at all synapses. Consistent with upscaling being a synapse-specific plasticity, we found that we could influence upscaling by simply enhancing quantal activation of AMPARs while blocking spiking activity; therefore signaling pathways associated with upscaling could be initiated locally in the dendrite, independent of spiking activity. This would suggest that upscaling is a transmission-dependent plasticity that facilitates local maintenance of synaptic strength.

The possibility that upscaling is the result of a synapse-specific plasticity raises questions about its relationship to learning and memory. Hebbian plasticity adjusts the strength of individual synapses, thereby contributing to the varied distribution of synaptic strengths within a cell. This form of synaptic plasticity is widely believed to underlie memory encoding and storage. A cell-autonomous model of synaptic scaling represents an elegant way to stabilize a neuron’s activity levels without disrupting the relative synaptic strengths established through Hebbian plasticity. Conversely, a synapse-specific model (where scaling emerges when activity at all synapses is similarly disrupted) suggests that relative synaptic strengths are vulnerable to homeostatic modification that act in opposition to Hebbian strengthening or weakening. It will be important for future studies to examine the consequences of these seemingly antagonist forms of synaptic plasticity.

**Functional Implications**

Cells can recover their firing rates following perturbations that reduce spiking 35,36. Therefore, reduced spike rate must trigger compensatory mechanisms that increase a cell’s excitability. AMPAergic upscaling has been the prime candidate for such a compensatory mechanism as it is thought to be triggered by reductions in postsynaptic firing rate and would be expected to increase spiking. However, we found that upscaling is not triggered by reduced spiking, but rather is dependent on AMPAR activation. This suggests that the purpose of upward scaling is not to homeostatically control a cell’s spiking activity, but instead functions to regulate excitatory synaptic transmission. This represents a fundamental shift in the perceived function of synaptic upscaling.

If not AMPAergic upscaling, then what contributes to the homeostatic control of spiking activity? Several possibilities exist, including intrinsic cellular excitability 34,37-39, changes in probability of release 35, and GABAergic scaling 40. In addition, downward AMPAergic scaling can contribute to homeostatic control of spiking following perturbations that elevate postsynaptic spiking levels 41,42, though notably, transmission-dependent compensations have also been described following activity elevation of individual presynaptic inputs 43.

Understanding the compensatory plasticity that is triggered following perturbations to either spiking or neurotransmission has important implications across multiple fields. In certain disease states and following neural injury, aberrant spiking levels can develop, and result in seizure activity or spasticity 44-48. In these states homeostatic plasticity fails to compensate adequately and prevent these dysfunctional outcomes. On the other hand, rather than suppressing inappropriate activity, homeostatic plasticity could actively participate in producing the aberrant activity; perturbations in spiking or transmission associated with neural damage might trigger maladaptive compensations that lead to hyperactive states 1,49. Therefore, identifying the triggers and functional goals of these compensatory mechanisms will be crucial for understanding a network’s response to neural injury.

**Experimental Procedures**

***Cell culture***

Sterilized microelectrode arrays (MEAs; Multichannel Systems, 60MEA200/30ir-Ti-pr) and glass bottom dishes (GBDs; Mattek, P35G-1.5-10-C) were coated with polyethyleneimine (Sigma, P-3143) and laminin (Sigma, L-2020)**.** Neocortical hemispheres were isolated from embryonic day 18 (E18) rats or equivalent tissue was purchased from BrainBits, LLC (part number: cx). Tissue was enzymatically dissociated using 20 U·mL-1 activated papain (Roche, 10108014001) at 36.5 °C, mechanically dissociated by trituration, and stained to assess viability using Trypan Blue (Invitrogen, 15250). The resulting cell suspension was diluted to 2,500 live cells·μL-1, and 35,000 cells were plated as a 2 mm diameter drop over the center of the grid of MEA electrodes or the GBD culturing surface. Growth medium was modified from Jimbo et al., 1999, and contained: 90% high-glucose DMEM, 10% horse serum, 0.5 mM GlutaMAX, 1 mM sodium pyruvate, 2.5 μg/mL insulin (pH 7.2, 315 mOsm); no antibiotics or antimycotics were used. Medium was fully exchanged at 1 day *in vitro* (DIV), and half the medium was exchanged every 3 days thereafter. MEA and GBDs were sealed with fluorinated ethylene-propylene (Potter and DeMarse, 2001) or polydimethylsiloxane (Blau et al., 2009) membranes. Cultures were maintained in an incubator regulated at 35 °C, 5% CO2, and 65% relative humidity. Further details of our culturing procedures are described in Hales et al., 2010. All protocols were in compliance with the National Research Council's Guide for the care and use of laboratory animals using a protocol approved by the Georgia Tech IACUC.

***Pharmacology***

Drugs were used in the following concentrations (in μM): TTX, 1; CNQX, 40; bicuculline, 20; cyclothiazide, 100; APV, 50. Cyclothiazide was obtained from ENZO. All other drugs were obtained from Sigma. The vehicle used to treat control cultures was DMSO or water, depending on the solvent used to dilute drug in experimental group of comparison.

***Transfections***

AAV9-hSynapsin-ChR2(H134R)-eYFP was produced by the University of Pennsylvania Vector Core using DNA from Dr. Karl Deisseroth. All cultures used in ChR2 experiments, including controls, were transfected at 1 DIV.  The genomic titer was 1x1013 c.f.u.·mL-1, and 0.5 μL was added to 1 mL growth medium at 1 DIV during the first medium exchange. Expression of the eYFP reporter protein was verified during the first week *in vitro* using a confocal microscope (Zeiss LSM 700).

***MEA recordings***

Experiments began after 8-12 days *in vitro* (DIV). MEA recordings were performed in standard growth medium in a cell culture incubator (35 °C, 5% CO2, 65% relative humidity). Voltages recorded through micro-electrodes were amplified and bandpass filtered from 1 Hz and 5 kHz using a 60-channel analog amplifier (Multichannel Systems, MEA60-Up) and digitized at 25 kHz using the Neurorighter acquisition system (Newman et al., 2012, Rolston et al., 2009). Voltage recordings were digitally filtered with a 3rd order Butterworth bandpass at 200-3000Hz, and action potentials were detected at threshold of ±5 times the root mean square noise. Offline analysis of the recorded spike data was performed in MATLAB (The Mathworks). The pre-drug period was defined as a 3-hour segment preceding drug or vehicle application. The treatment period was defined as the entire 24 hours during drug or vehicle application. After the treatment period, cultures were washed 4 times with standard growth medium. Statistical significance for firing and burst rate data was determined using a Kruskal-Wallis test followed by Wilconox rank-sum tests with Bonferroni correction for multiple comparisons.

***Whole-cell recordings***

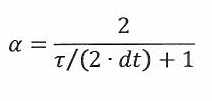
Miniature excitatory postsynaptic currents (mEPSCs) were recorded from pyramidal-shaped cells in a continuous perfusion of artificial cerebrospinal fluid containing (in mM): 126 NaCl, 3 KCl, 2 CaCl2, 1.5 MgSO4, 1 NaH2PO4, 25 NaHCO3, and 25 D-glucose, and saturated with 95% O2 and 5% CO2 (pH 7.4, 315 mOsm). To isolate AMPAergic mEPSCs, the external solution was supplemented with 1 µM TTX and 20 µM bicuculline. The solution temperature was regulated at 35°C using an inline heater (Warner 64-0102). Internal solution contained (in mM): 100 K-gluconate, 30 KCl, 10 HEPES, 2 MgSO4, 0.5 EGTA, 3 ATP (pH 7.4, 290 mOsm). mEPSCs were recorded using an EPC8 amplifier (HEKA). Pipette resistances ranged from 2-8 MΩ. mEPSCs were analyzed, blind to the treatment condition, using MiniAnalysis (Synaptosoft), and mEPSCs with amplitudes less than 5 pA were excluded from analysis. Statistical significance for mEPSC data was determined using a 1-way analysis of variance followed by t-tests with Bonferroni correction for multiple comparisons. mEPSC amplitude distributions were compared using the Kolmogorov-Smirnov test. mEPSC characteristics are summarized in Suppl. Fig. 5.

***Optical stimulation***

To deliver optical stimuli, a custom N-channel enhancement mode MOSFET current source (<https://potterlab.gatech.edu/main/newman/wiki>) was used to drive a blue LED (465±11 nm FWHM; LEDEngin, LZ4-00B200). The LED was butt-coupled to a randomized fiber bundle (Schott AG, A21045), which fed light to a custom Köhler illumination train mounted beneath the MEA amplifier. The average network firing rate was calculated every *dt* = 10 ms according to



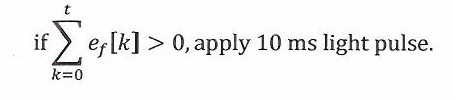
where



defines a first-order averaging filter with a τ = 2.5 second time constant and *r*[*t*] = no. detected spikes/*dt* is the instantaneous firing at time *t*. The target rate, *f*\*, was defined as *f*[*t*] over a 3 hour period prior to CNQX application. Five minutes following the application of CNQX to the culturing medium, an error signal was generated between the target and measured firing rate according to



Finally, an on-off controller was used to determine stimulus application according to



Each stimulus pulse resulted in uniformly distributed 10.1 mW/mm2 light the plane of the culture. The rise and fall times of each LED pulse were ~10 μs. A full description and characterization of the closed-loop optical stimulation system used in this study is given in Newman et al., in review.

**Methods References**

Jimbo, Y, Tateno, T, Robinson, HP (1999) Simultaneous induction of pathway-specific potentiation and depression in networks of cortical neurons. *Biophys J* 76:670–678.

Potter SM, DeMarse TB (2001) A new approach to neural cell culture for long-term studies. *J Neurosci Methods* 110:17-24.

Blau A, Neumann T, Ziegler C, Benfenati F (2009) Replica-moulded polydimethylsiloxane culture vessel lids attenuate osmotic drift in long-term cell cultures. *J Biosci* 34:59-69.

Hales CM, Rolston JD, Potter SM (2010) How to culture, record and stimulate neuronal networks on micro-electrode arrays (MEAs). *JoVE*:1–7.

Newman JP, Zeller-Townson R, Fong M-F, Arcot Desai S, Gross RE, Potter SM (2012) Closed-loop, multichannel experimentation using the open-source NeuroRighter electrophysiology platform. *Front Neural Circuits* 6:98.

Newman JP, Fong M-f, Millard DC, Stanley GB, Potter SM (in review) Optogenetic feedback control of neuronal firing. (Manuscript included in supporting materials)

Rolston JD, Gross RE, Potter SM (2009) A low-cost multielectrode system for data acquisition enabling real-time closed-loop processing with rapid recovery from stimulation artifacts. *Front Neuroeng* 2:1-17.

# Bibliography

1. Rich, M.M. & Wenner, P. Sensing and expressing homeostatic synaptic plasticity. *Trends Neurosci* **30**, 119-125 (2007).

2. Marder, E. & Goaillard, J.M. Variability, compensation and homeostasis in neuron and network function. *Nat Rev Neurosci* **7**, 563-574 (2006).

3. Davis, G.W. Homeostatic control of neural activity: from phenomenology to molecular design. *Annu Rev Neurosci* **29**, 307-323 (2006).

4. Turrigiano, G. Homeostatic synaptic plasticity: local and global mechanisms for stabilizing neuronal function. *Cold Spring Harb Perspect Biol* **4**(2012).

5. Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C. & Nelson, S.B. Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391**, 892-896. (1998).

6. O'Brien, R.J.*, et al.* Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* **21**, 1067-1078. (1998).

7. Lissin, D.V.*, et al.* Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proc Natl Acad Sci U S A* **95**, 7097-7102. (1998).

8. Ibata, K., Sun, Q. & Turrigiano, G.G. Rapid synaptic scaling induced by changes in postsynaptic firing. *Neuron* **57**, 819-826 (2008).

9. Wang, G., Gilbert, J. & Man, H.Y. AMPA receptor trafficking in homeostatic synaptic plasticity: functional molecules and signaling cascades. *Neural Plast* **2012**, 825364 (2012).

10. Turrigiano, G.G. The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* **135**, 422-435 (2008).

11. Vitureira, N., Letellier, M. & Goda, Y. Homeostatic synaptic plasticity: from single synapses to neural circuits. *Curr Opin Neurobiol* (2011).

12. Bassani, S., Folci, A., Zapata, J. & Passafaro, M. AMPAR trafficking in synapse maturation and plasticity. *Cell Mol Life Sci* (2013).

13. Hou, Q., Zhang, D., Jarzylo, L., Huganir, R.L. & Man, H.Y. Homeostatic regulation of AMPA receptor expression at single hippocampal synapses. *Proc Natl Acad Sci U S A* **105**, 775-780 (2008).

14. Beique, J.C., Na, Y., Kuhl, D., Worley, P.F. & Huganir, R.L. Arc-dependent synapse-specific homeostatic plasticity. *Proc Natl Acad Sci U S A* **108**, 816-821 (2011).

15. Sutton, M.A.*, et al.* Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* **125**, 785-799 (2006).

16. Deeg, K.E. & Aizenman, C.D. Sensory modality-specific homeostatic plasticity in the developing optic tectum. *Nat Neurosci* **14**, 548-550 (2011).

17. Wagenaar, D.A., Pine, J. & Potter, S.M. An extremely rich repertoire of bursting patterns during the development of cortical cultures. *BMC Neurosci* **7**, 11 (2006).

18. van Pelt, J., Wolters, P.S., Corner, M.A., Rutten, W.L. & Ramakers, G.J. Long-term characterization of firing dynamics of spontaneous bursts in cultured neural networks. *IEEE Trans Biomed Eng* **51**, 2051-2062 (2004).

19. Opitz, T., De Lima, A.D. & Voigt, T. Spontaneous development of synchronous oscillatory activity during maturation of cortical networks in vitro. *J Neurophysiol* **88**, 2196-2206 (2002).

20. Jakawich, S.K.*, et al.* Local presynaptic activity gates homeostatic changes in presynaptic function driven by dendritic BDNF synthesis. *Neuron* **68**, 1143-1158 (2010).

21. Corner, M.A., van Pelt, J., Wolters, P.S., Baker, R.E. & Nuytinck, R.H. Physiological effects of sustained blockade of excitatory synaptic transmission on spontaneously active developing neuronal networks--an inquiry into the reciprocal linkage between intrinsic biorhythms and neuroplasticity in early ontogeny. *Neurosci Biobehav Rev* **26**, 127-185 (2002).

22. Chiappalone, M.*, et al.* Networks of neurons coupled to microelectrode arrays: a neuronal sensory system for pharmacological applications. *Biosens Bioelectron* **18**, 627-634 (2003).

23. Legrand, J.C., Darbon, P. & Streit, J. Contributions of NMDA receptors to network recruitment and rhythm generation in spinal cord cultures. *Eur J Neurosci* **19**, 521-532 (2004).

24. Nagel, G.*, et al.* Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. *Curr Biol* **15**, 2279-2284 (2005).

25. Newman, J.P.*, et al.* Closed-Loop, Multichannel Experimentation Using the Open-Source NeuroRighter Electrophysiology Platform. *Front Neural Circuits* **6**, 98 (2013).

26. Minerbi, A.*, et al.* Long-term relationships between synaptic tenacity, synaptic remodeling, and network activity. *PLoS Biol* **7**, e1000136 (2009).

27. Murphy, T.H., Blatter, L.A., Wier, W.G. & Baraban, J.M. Spontaneous synchronous synaptic calcium transients in cultured cortical neurons. *J Neurosci* **12**, 4834-4845 (1992).

28. Partin, K.M., Patneau, D.K., Winters, C.A., Mayer, M.L. & Buonanno, A. Selective modulation of desensitization at AMPA versus kainate receptors by cyclothiazide and concanavalin A. *Neuron* **11**, 1069-1082 (1993).

29. Diamond, J.S. & Jahr, C.E. Asynchronous release of synaptic vesicles determines the time course of the AMPA receptor-mediated EPSC. *Neuron* **15**, 1097-1107 (1995).

30. Oja, E. A simplified neuron model as a principal component analyzer. *J Math Biol* **15**, 267-273 (1982).

31. Miller, K. & MacKay, D. The role of constrains in Hebbian learning. Neural Computation. *Neural Computation* **6**, 100-126 (1994).

32. Abbott, L.F. & Nelson, S.B. Synaptic plasticity: taming the beast. *Nat Neurosci* **3 Suppl**, 1178-1183 (2000).

33. Turrigiano, G.G. & Nelson, S.B. Hebb and homeostasis in neuronal plasticity. *Curr Opin Neurobiol* **10**, 358-364. (2000).

34. Pratt, K.G. & Aizenman, C.D. Homeostatic regulation of intrinsic excitability and synaptic transmission in a developing visual circuit. *J Neurosci* **27**, 8268-8277 (2007).

35. Burrone, J., O'Byrne, M. & Murthy, V.N. Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature* **420**, 414-418 (2002).

36. Turrigiano, G., Abbott, L.F. & Marder, E. Activity-dependent changes in the intrinsic properties of cultured neurons. *Science* **264**, 974-977 (1994).

37. Desai, N.S., Rutherford, L.C. & Turrigiano, G.G. Plasticity in the intrinsic excitability of cortical pyramidal neurons. *Nat Neurosci* **2**, 515-520 (1999).

38. Wilhelm, J.C., Rich, M.M. & Wenner, P. Compensatory changes in cellular excitability, not synaptic scaling, contribute to homeostatic recovery of embryonic network activity. *Proc Natl Acad Sci U S A* **106**, 6760-6765 (2009).

39. Prinz, A.A., Bucher, D. & Marder, E. Similar network activity from disparate circuit parameters. *Nat Neurosci* **7**, 1345-1352 (2004).

40. Kilman, V., van Rossum, M.C. & Turrigiano, G.G. Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA(A) receptors clustered at neocortical synapses. *J Neurosci* **22**, 1328-1337. (2002).

41. Leslie, K.R., Nelson, S.B. & Turrigiano, G.G. Postsynaptic depolarization scales quantal amplitude in cortical pyramidal neurons. *J Neurosci* **21**, RC170 (2001).

42. Goold, C.P. & Nicoll, R.A. Single-cell optogenetic excitation drives homeostatic synaptic depression. *Neuron* **68**, 512-528 (2010).

43. Hou, Q., Gilbert, J. & Man, H.Y. Homeostatic regulation of AMPA receptor trafficking and degradation by light-controlled single-synaptic activation. *Neuron* **72**, 806-818 (2011).

44. Wissel, J., Manack, A. & Brainin, M. Toward an epidemiology of poststroke spasticity. *Neurology* **80**, S13-19 (2013).

45. Herman, S.T. Epilepsy after brain insult: targeting epileptogenesis. *Neurology* **59**, S21-26 (2002).

46. Nielsen, J.B., Crone, C. & Hultborn, H. The spinal pathophysiology of spasticity--from a basic science point of view. *Acta Physiol (Oxf)* **189**, 171-180 (2007).

47. Coull, J.A.*, et al.* Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* **424**, 938-942 (2003).

48. Boulenguez, P.*, et al.* Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. *Nat Med* **16**, 302-307 (2010).

49. Frohlich, F., Bazhenov, M. & Sejnowski, T.J. Pathological effect of homeostatic synaptic scaling on network dynamics in diseases of the cortex. *J Neurosci* **28**, 1709-1720 (2008).

**Figure legends**

**Figure 1: Overview of micro-electrode array recordings for monitoring spiking activity. (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.

**Figure 2: Spiking and bursting persist during AMPAergic transmission blockade. (a)** MEA-wide firing rate histograms from example recordings before and during application of TTX or CNQX.  Bin size, 1 s. **(b)** 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.  **(c)** Mean MEA-wide firing rate (control, 97.3±4.6%; TTX, 1.1±0.5%; CNQX, 46.2±4.1%; *p*<10-6), burst rate (control, 105.8±10.0%; TTX, 0%; CNQX, 31.2±4.8%; *p*<10-6), and interburst firing rate (control, 108.1±12.7%; TTX, 3.6±1.5%; CNQX, 77.4±16.8%; *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 3: Reductions in spiking are not correlated to the the magnitude of synaptic scaling. (a)** Pyramidal cell during whole-cell recording.   Scale bar, 50 μm. **(b)** *Left*, sample mEPSC recordings following 24-hour treatment with vehicle, TTX, or CNQX.  Scale bars, 25 pA, 200 ms. *Right*, average mEPSC waveforms. Scale bars, 5 pA, 20 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*=-0.047).  *Center and right*, mean mEPSC amplitude plotted against burst rate and interburst firing rate, respectively (linear fits: burst rate, *r*=-0.114, interburst firing rate, *r*=0.044).

**Figure 4: Optogenetic stimulation re-creates spontaneous-like bursting during AMPAergic transmission blockade. (a)** Confocal micrograph of neurons in a cortical culture expressing ChR2-eYFP.  Microelectrodes are circled in white.  Scale bars, 200 μm (left), 50 μm (right).  **(b)** *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 blue arrow denotes the timing of the stimulus, and the width of the blue rectangle indicates the duration of the light pulse (10ms), which is brief compared to the total burst duration.  The rastergrams (colored vertical bars) below each voltage trace denote the spike times of 3 different extracellular units captured on the electrode. *Right*, expanded voltage traces showing all spikes detected during burst, separate units are displayed in different colors. 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). **(c)** Schematic of closed-loop optical stimulation system.  Spiking activity is recorded through the MEA. When the integrated 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. **(d)** Rastergram showing spike times recorded across all electrodes during a spontaneous burst (top) and an optically-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). **(e)** MEA-wide firing rate computed during bursts shown in (d), denoted by black lines. All bursts occurring during the 3-hour pre-drug condition (top) and 24-hour CNQX with photostimulation condition (middle) are shown are plotted in grey. Zooming out in time (bottom) shows that each stimulus reliable evokes a burst. Blue arrows denote timing of the light pulse. Bin size, 10 ms. Scale bars, 100 ms (top and middle), 2 min (bottom).

**Figure 5: Closed-loop optical stimulation restores normal spiking activity throughout chronic AMPAergic transmission blockade. (a)** *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, 1 s. *Bottom*, rastergrams show 15-minute segments of spiking activity at different time points throughout the recording. Neurons throughout the culture contribute to restored spiking activity during the entire 24-hour CNQX treatment. Scale bar, 2 min.  **(b)** Mean MEA-wide firing rate over time for CNQX-treated cultures with restored spiking (*n*=5 cultures).  Control and CNQX values from Fig. 2b are shown for comparison.  Closed-loop stimulation effectively locked firing rate to pre-CNQX levels.  Bin size, 3 h.  Error bars, s.d.  **(c)** 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. 2c shown for comparison.  CNQX-treated cultures with restored spiking showed no differences in MEA activity compared to vehicle-treated controls (MEA-wide firing rate, 100.2±0.4%, *p*<0.6; burst rate, 97.7±32.0%, *p*<0.9; interburst firing rate, 96.2±24.9%, *p*<0.9).  Error bars, s.e.m.

**Figure 6: Reduced AMPAergic transmission directly triggers upward synaptic scaling.**  **(a)** *Left*, sample mEPSC recordings following 24-hour treatment with vehicle, CNQX, or CNQX+photostimulation.  Scale bars, 25 pA, 200 ms. *Right*, average mEPSC waveforms. Scale bars, 5 pA, 20 ms.  **(b)** 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*<10-6).  Non-significant differences denoted by n.s.  Significant differences denoted by \**p*<10-5. Error bars, s.e.m.  **(c)** 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).  **(d)** 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 7: 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 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). **(c)** *Left*, sample mEPSC recordings following 24-hour treatment with vehicle, TTX, or TTX+CTZ. Scale bars, 25 pA, 200 ms. *Right*, average mEPSC waveforms. Scale bars, 5 pA, 20 ms. **(d)** Mean mEPSC amplitude for 6 sister culture pairs treated from the 3 treatment conditions (control and TTX cultures same as Fig. 3c; TTX+CTZ: 15.5±0.7 pA, *n*=50 cells; *p*<10-5).  Significant differences denoted by\**p*<10-2 and \*\**p*<10-5. Error bars, s.e.m.  **(e)** Cumulative distribution of mEPSC amplitudes following the 3 treatment conditions (control and TTX cultures same as Fig. 3d).  The distribution of mEPSC amplitudes is significantly different between the TTX and TTX+CTZ conditions (*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.

**Supplementary Figure 1: NMDAergic transmission is responsible for the late phase of the burst. (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. When AMPAergic transmission is blocked, there is a pronounced increase in burst duration. When NMDAergic transmission is blocked, burst duration is significantly reduced. These results suggest that NMDAergic transmission is responsible for burst elongation. The recordings shown are from sister cultures grown on a multi-well MEA (Multichannel Systems, 60-6wellMEA), with each well containing 9 microelectrodes. All other plating and recording procedures are the same as described in Online Methods.

**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 before adding CNQX (black), or an optically-evoked burst after the addition 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 during an optically-evoked burst. 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: Closed-loop optogenetic stimulation during CNQX treatment reproduces channel-to-channel firing correlations.***Top*, spike detection rate (bin size, 10 ms) cross-correlation was computed for each pair of channels, and averaged across all pairs, for five CNQX-treated cultures (no stimulation).  This analysis was performed at several time points before and after CNQX was added, denoted by different colored lines. Spiking across channels became less correlated during first few hours after CNQX application, though spiking correlations generally increased over the 24-hour treatment.  *Bottom*, same as top, but for CNQX-treated cultures experiencing optically-restored spiking levels. Closed-loop stimulation maintained pre-drug channel-to-channel firing correlations immediately after CNQX treatment, and this effect was 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, 100 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 described in Online Methods.

**Supplementary Figure 5: mEPSC features following different chronic treatments. (a)** Scaled mEPSC amplitude distributions for CNQX, CNQX+photostimulation, TTX, and TTX+CTZ (same data as Figs. 3 and 4). Scaled mEPSC distributions for drug-treated cultures were no different than distributions from control sister cultures (CNQX, *p*>0.9; CNQX+stim, *p*>0.9; TTX, *p*>0.7; TTX+CTZ, *p*>0.5). **(b)** Mean frequency (control, 1.6±0.2 pA, *n*=44 cells; CNQX, 2.3±0.3 pA, *n*=51 cells; CNQX+photostimulation, 2.6±0.3 pA, *n*=46 cells; *p*<0.02), charge per event (control, 27.0±1.2 fC; CNQX, 34.8±1.6 fC; CNQX+photostimulation, 33.5±1.4 fC; *p*<10-3), and decay time (control, 2.0±0.06 ms; CNQX, 1.9±0.06 ms; CNQX+photostimulation, 1.8±0.04; *p*>0.2) for CNQX+photostimulation experiments. There are significant differences 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, 3.0±0.3 pA, *n*=47 cells; TTX, 3.3±0.4 pA, *n*=58 cells; TTX+CTZ, 3.0±0.4 pA, *n*=50 cells; *p*>0.6), charge per event (control, 24.9±0.9 nC; TTX, 40.0±1.9 nC; TTX+CTZ, 29.9±1.4 nC; *p*<10-10), and decay time (control, 1.8±0.06 ms; TTX, 2.0±0.05 ms; TTX+CTZ, 1.9±0.06; *p*<0.02) for TTX+CTZ experiments. There are significant differences in charge between all conditions (control vs. TTX, *p*<10-8; control vs. TTX+CTZ, *p*<10-2; TTX vs. TTX+CTZ, *p*<10-4), and in decay time for cultures treated with TTX (control vs. TTX, *p*<10-2).

**Supplementary Figure 6: 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 was 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 7: 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. 2b 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. 2c 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, 1.1±0.002%, *p*>0.3; burst rate, 0%, *p*=1; interburst firing rate, 4.26±1.91%, *p*<0.4). Error bars, s.e.m.