Hi All,

Nice paper. I believe you. Figures are gorgeous.

My strongest criticisms are as follows. I've annotated the text directly with smaller notes/criticisms.

1. The strongest elements of the paper are the statistical results and the methods used to achieve them. They are extremely convincing on their own. Basically, if a scientist in the field was presented with the figures, they would understand their importance and implications. However, the hard part is not relaying this message to them, but to a much wider audience. A good deal of the text is too specifically aimed at the homeostatic field and under motivated from a general neuroscience perspective. For example,
    a. The opening sentence of Discussion: "Current thinking in the field...". I don't think that should start the discussion since this is going to Nature, which is read in all fields of life science.
    b. A proper explanation of what synaptic scaling is not given until the last result section: "...a multiplicative relationship between the amplitude distributions..." Previous to that it was described as "…showed the scaling profile", which I did not understand.
    c. In my opinion, the true importance of your findings is not really explored in the discussion. In my mind, the most important part of your finding is that you have shown that one of the most elegant theories put forth in neurophysiology (cell-autonomous scaling) is wrong (at least in the upscaling direction), and this has profound implications for how memories are stored in the nervous system. I have always had a strong feeling that much of Turrigano's success in her early papers can be squarely attributed to Larry Abbott's knowledge of theoretical neuroscience. I would actually be as bold as to state that he first presented the idea of global scaling when looking at GGT's data. The reason I am confident saying this is that there are a ton of theoretical papers that came way before all of the synaptic scaling work and said (paraphrasing) *"There needs to be a global constraint on synaptic weights, which is autonomously regulated within a neuron, in order to allow synaptic weights to both store information and maintain cellular stability."* (e.g. Oja 1982, Miller and MacKay 1994). Aside from memory formation and stability, there are computational reasons why this type of constraint is good (Oja 1982 and many others). Basically, the synaptic scaling stuff seemed to be the answer to these theoretical studies - theory actually predicted something in neuroscience, a miracle! Only, according to you guys, it did not! So what are the implications?

       The implications are that we've lost the ability to maintain stability AND relative synaptic weights. I think that sucks, personally. I really liked GGT's way of doing things; it made more sense to me than the mechanism you guys revealed ☹. It seems to me that there MUST be a way for synapses to coordinate their strength in order for a cell to function. Many theories of memory require memories to be distributed across synapses

– how can this be the case if each synapse is regulated on its own? This paper sets up a bunch of experiments to try to figure out exactly what the global mechanism is. Or, it could mean that the idea of global constraint on synaptic strength is fundamentally wrong. And a whole bunch of theorists need to go back to their chalkboards.

A good place to get into this is when you say that

> *"Current thinking in the field suggests that reductions in somal action potentials (APs) lead to reduced VGCC opening and subsequent reductions in global calcium signaling, which then trigger the upscaling of AMPAergic quantal amplitude (Turrigiano, 2012). This would provide an elegant method for the homeostatic control of a cell's spiking activity."*

I would be much more satisfied if you stated why this hypothesis was elegant, and why the fact that it is wrong is such a big deal:

> *"Current thinking in the field suggests that reductions in somal action potentials (APs) lead to reduced VGCC opening and subsequent reductions in global calcium signaling, which then trigger the upscaling of AMPAergic quantal amplitude (Turrigiano, 2012). This would provide a elegant mechanism for maintaining neural stability since the relative weights of synapses are preserved during upregulation of synaptic strength. However, our results do not support this model of upscaling."*

Then at the very end of the paper mention that without a global constraint on synaptic weights, it is difficult to understand how the relative weights of synapses are coordinated across the cell and this opens huge questions for memory formation and maintenance.

2. There are portions of text that I would describe as 'combative'. The discussion is particularly aggressive. In my opinion, it is much more powerful to detail the correctness of your work than to portray others' work as flawed. Of course it is necessary to address conflicts, but they should not be the focus

    a. An example is the singling out of the Ibata paper as the paper which generated the model of cell-autonomous scaling. I would not go that far - it is a paper that provides experimental evidence for this model, which existed previous to the paper (back to Oja 1982).
    b. The discussion of VGCC's seems a bit tangential. If it is going to be brought up, the importance of local versus global Ca2+ signaling should be discussed as well. Right now it kind of seems be saying "GGT, you are wrong" and that's it. Perhaps the best way to

do this is to relate local Ca2+ signaling to the papers you cite that explore local synaptic modification at the end of the discussion.

- Jon

Mechanisms of homeostatic plasticity are thought to help foster stable activity in developing neural circuits. One well-studied form of homeostatic plasticity is synaptic scaling, a phenomenon in which the quantal amplitudes of all synaptic inputs onto a neuron are strengthened (upscaling) or weakened (downscaling) by a multiplicative factor to compensate for chronic changes in activity levels (Turrigiano, 2012). Synaptic upscaling is commonly elicited through prolonged pharmacological suppression of spiking or excitatory synaptic transmission. Because suppression of spiking reduces AMPA receptor (AMPAR) activation, and suppression of AMPAR activation reduces spiking, it has been difficult to distinguish between the effects of reduced spiking and reduced transmission in triggering upscaling (Rich and Wenner, 2007; Vitureira et al., 2011). Here we use a combination of multisite electrophysiology, optogenetics, and pharmacology to separate the roles of spiking and AMPAergic transmission on synaptic scaling. We show that chronic suppression of AMPAergic transmission triggers upscaling even when spiking activity is restored to normal levels. We also show that upscaling triggered by spiking activity blockade can be significantly attenuated by partial restoration of AMPAR activation. Together these results demonstrate that upscaling of excitatory synapses is triggered by reductions in AMPAR activation rather than reductions in spiking activity. Our findings suggest that the function of synaptic upscaling may be for compensatory regulation of synaptic strength rather than as a means of homeostatically controlling neuronal spiking activity.

Neurons are thought to tightly regulate their spiking activity through compensatory changes in synaptic strength and intrinsic cellular excitability. Although homeostatic synaptic

scaling has been observed in many different systems (Davis, 2006), it is unclear how perturbations to spiking activity trigger scaling. It is often assumed that blocking voltage-gated Na^+ channels with TTX (tetrodotoxin) or AMPARs with CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) both block spiking activity, which then triggers upscaling. To test this assumption, we used a planar microelectrode array (MEA; Fig. 1a-b) to continuously record extracellular spiking from hundreds of neurons embedded in dissociated cortical cultures during 24-hour TTX or CNQX application to examine the changes in spiking activity that are thought to lead to upscaling. We used the MEA recordings to detect network-wide bursts of action potentials occurring synchronously across many electrodes (hereafter referred to as bursts) and to assess a culture's overall firing rate by summing spiking levels throughout the array (Fig. 1c, Wagenaar et al., 2006).

Consistent with our expectations, blockade of TTX eliminated spiking activity for the entire 24-hour treatment (Fig. 1d-e). However, we found that CNQX only partially reduced spiking activity compared to pre-drug levels (Fig. 1d-e). The CNQX-induced reduction in firing rate was primarily due to a reduction in burst frequency (Fig. 1f). Meanwhile, spiking between bursts was not significantly affected (Fig. 1f). In most CNQX-treated cultures, bursting was significantly reduced during the first few hours, but began to recover by the end of 24 hours (Fig. 1d). While the reduction in activity following CNQX was variable across cultures, some degree of spiking and bursting always persisted, unlike those treated with TTX.

We hypothesized that cultures experiencing greater reductions in spiking activity might also experience more dramatic upscaling of miniature excitatory postsynaptic currents (mEPSCs). To test this hypothesis, we used whole-cell recordings to measure mEPSCs from

there are several instances where TTX and CNQX are used as verbs. This is an example of that. Sounds funny to me.

pyramidal cells following 24-hour application of TTX or CNQX (Fig. 2a-b). Consistent with previous literature (Turrigiano et al., 1998; Jakawich et al., 2010), chronic TTX or CNQX both produced similar increases in mEPSC amplitude over vehicle-treated sister control cultures (Fig. 2c, f; TTX, 146.81±7.98% of control; CNQX, 142.94±4.49% of control) and showed the scaling profile (Fig. 2d-e, g-h). This indicated that cells from cultures experiencing only moderate reductions in spiking scaled equally to cells that experienced complete elimination of spiking. To further explore 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 application to the increase in mean mEPSC amplitude recorded following the treatment (Fig. 2i). We observed no correlation between the TTX- or CNQX-induced change in firing rate and the resultant increase in mEPSC amplitude (r = -0.0466). Moreover, we could not find a relationship between the increase in mEPSC amplitude and any other features of spiking activity (Fig. 2i; burst rate, r = 0.1136; interburst firing rate, r = 0.0435). Overall, the lack of correlation between spiking and upscaling raise the possibility that reduced spiking activity does not trigger upscaling.

Instead of reduced spiking activity, it is possible 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. Since TTX and CNQX each reduce both spiking and AMPAR activation, either directly or indirectly, there are several possibilities for what activity signal could be triggering synaptic scaling: [I] reductions in spiking alone, [II] reductions in AMPAR activation alone, [III]

Need a reminder of CNQX's effect here.

huh?

studies

application of

ed

CNQX-treated, whereas

in TTX-treated cultures, whereas

quantify

reductions in

the degree of upscaling

informal. Maybe replace w/ "there was no correlation"

measurable

↳ there are many features, you only have access to a few.

reduced firing levels are not the feature of neural activity that triggers upscaling.

awkward.

I would either tabularize these possibilities or remove the numbering scheme. The reader is probably not going to check back to see what the # corresponds to and you use a text-based description in the subsequent text, anyway.

it seems you should search for "scaling" and replace w/ "upscaling" to maintain consistency.

concurrent reductions in both spiking and AMPAR activation, or [IV] reductions in either spiking or AMPAR activation.

In order to examine the effects of reducing AMPAR activation while leaving spiking intact, we developed a closed-loop optical stimulation system for restoring the normal levels of spiking activity during a 24-hour application of CNQX (Fig. 3a; Newman et al., in prep). We transfected neurons with channelrhodopsin-2 (ChR2) and observed expression throughout the culture within a week (Fig. 3b). Because the reduction in spiking following CNQX application is primarily due to a reduction in network-wide bursts (Fig. 1f), we selected a stimulation strategy that favored reinstatement of bursting. Brief pulses of blue light (10 ms, 465 nm, 5 mW·mm^{-2}) effectively reproduced spontaneous-like bursts in the presence of CNQX (Fig. 3c-d, Suppl. Fig. 3). Although each stimulus produced some short latency spikes that resulted directly from ChR2 activation, the vast majority of spikes were contained in longer latency barrages that occurred after the light pulse terminated. These longer latency barrages of spikes closely matched spontaneously-occurring bursts in terms of time course and profile of network recruitment of (Fig. 3c-d), and are presumably dependent on NMDAergic transmission (Suppl. Fig. 4). To precisely control firing rate at a particular setpoint, the timing of each stimulation pulse was determined in real-time based on spiking activity recorded through the MEA (Newman et al., in prep). 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. 3e). Closed-loop optical stimulation effectively restored control firing and burst rates (Fig. 3e-g).

to restore

you transfected w/ a virus, not ChR2. Also may want to say the promoter system and cell types since this is a big part of QG.

nevermind.

→ This is a lot of text to say that the bursts were similar.

to

pulse

occurred

These ChR2-evoked bursts

following

a real-time controlled

compared the measured firing rate to a target rate to determine when stimuli should be applied.

Also cite NR paper.

for the duration of the 24-hour drug application period.

Then

We recorded mEPSCs from triplicate sister cultures: [1] vehicle-treated control cultures experiencing normal AMPAR activation an (nevermind) normal spiking activity, [2] CNQX-treated cultures experiencing no AMPAR activation and reduced spiking, and [3] photostimulated CNQX-treated cultures experiencing no AMPAR activation but restored spiking activity (Fig. 3h). We found that stimulated and unstimulated CNQX-treated cultures had mEPSC amplitudes that were distributions.

scaled

~~elevated~~ over sister control cultures (Fig 3i) ~~and showed a scaled distribution~~ (Fig. 3j-k). :-K.

Further, we found that the mean and distribution of mEPSC amplitudes were ~~virtually identical~~ statistically indistinguishable in AMPAR-blocked sister cultures regardless of whether they experienced reduced or normal levels of spiking activity (Fig. 3j-k; P>0.9). The results ~~demonstrate~~ show that upscaling associated with AMPAR blockade is not triggered by reductions in spiking or spike-dependent processes (e.g. burst depolarizations, activation of voltage-gated calcium channels - VGCCs and consequent calcium signaling/presynaptic release). Therefore, reduced AMPAR activation by itself can trigger scaling, and reduced spiking is not necessary for upscaling (eliminates possibility I & III).

during the drug app. period.

se

ChR2 lets some weird ions into the cell. Do you really want to go down this road?

These sentences are so strong as to be defiant. I would attempt to use a similar style as the results text corresponding to figs 1 and 2.

Because reduced

~~Based on our observation that reduced~~ AMPAR activation can independently trigger scaling, we might expect that blocking activity with TTX triggers scaling because it ~~reduces~~ indirectly

upscaling?

eliminates

singular sounds weird.

spike-dependent release of neurotransmitter, and therefore reduces AMPAR activation. ~~We~~ Therefore, we

the

We

~~therefore~~ tested the importance of reduced AMPAR activation in TTX-treated cultures by partially restoring ~~some~~ AMPAR activation during TTX treatment. We used the AMPAR modulator, cyclothiazide (CTZ), to increase the amplitude and frequency of mEPSCs during TTX treatment (Fig. 4a), and these effects lasted for at least 12 hours (Suppl. Fig. 6). Like TTX, co-treatment of cultures with TTX and CTZ completely abolished spiking activity (Suppl. Fig. 7). The amplitude

To do this,

of mEPSCs recorded from cultures following 24-hour application of TTX and CTZ were significantly reduced compared to those treated with TTX alone (Fig. 4c). A multiplicative relationship between amplitude distributions existed for all 3 conditions (Figure 4d-e, Fig. 2e). These results demonstrate that partially restoring AMPAR activation during a spiking blockade reduces the upscaling observed following chronic TTX treatment. Together, the results suggest that upscaling after TTX or CNQX are both triggered by reductions in AMPAR activation (possibility II), rather than reduced spiking activity (possibility I or IV).

Current thinking in the field suggests that reductions in somal action potentials (APs) lead to reduced VGCC opening and subsequent reductions in global calcium signaling, which then trigger the upscaling of AMPAergic quantal amplitude (Turrigiano, 2012). This would provide an elegant method for the homeostatic control of a cell's spiking activity. However, our results do not support this model. First, upscaling still occurred when somal APs were restored during AMPAR blockade; second, upscaling was attenuated when AMPAR activation was partially restored while somal APs were eliminated. Rather, our results suggest upscaling is mediated by reduced AMPAR activation, and that any downstream calcium signaling that mediates it will be initiated locally in the dendrite as scaling was influenced by altering AP-independent quantal currents (CTZ and TTX). The spike-dependent model of upscaling is based on a study that blocked somal spiking and demonstrated an accumulation of AMPARs within hours (Ibata, Sun et al. 2008). Reasons for the differences between our study and that of Ibata et al. could be due to the method of scaling measurements (mEPSC amplitude vs. expression of AMPARs) or timing of the scaling process (24 hours vs. 3 hours). On the other hand, our observation that reductions in spiking alone are insufficient to trigger upscaling is consistent with

previous *in vitro* and *in vivo* studies showing that chronic reductions of spiking in single cells with unperturbed synaptic inputs did not increase mEPSC amplitude (Burrone et al., 2002; Pratt and Aizenman, 2007). Further, our finding that local synaptic signaling triggers compensatory changes in AMPAergic synaptic strength is consistent with a number of previous studies. These studies have shown that when receptor activation is chronically reduced at specific synapses, compensatory changes occur only at those synapses experiencing lowered transmission (Hou et al., 2008; Beique et al., 2011; Deeg and Aizenman, 2011; Sutton et al., 2006; Jakawich et al., 2010). Our finding that receptor activation triggers cell-wide synaptic scaling raises the possibility that upscaling of mEPSC amplitudes is merely the result of reducing AMPAergic transmission throughout the cell and therefore triggering local synaptic compensations at all synapses. This would suggest that upward scaling has little to do with homeostatic control of a cell's spiking activity, but instead is a transmission-dependent plasticity that facilitates local maintenance of synaptic strength.

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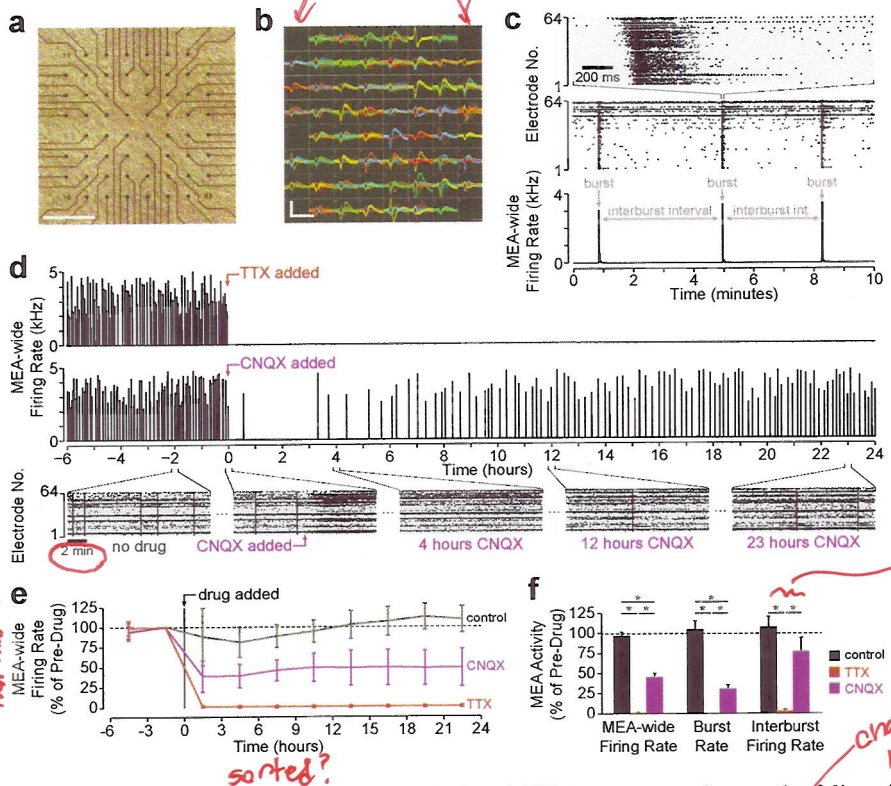
- usually the first sentence of a panel caption can be a fragment, but not the second (unless its something about scalebars or SEM etc) or bars.

so, e.g.

(b) Fragment. Full sentence.



get rid of black for absent chs, in the corners & Grounds.

Kind of hard to see two lines.

characteristic? Many other preps burst so might no be distinguishing.

detected

Normalized

sorted?

windows

instantaneous

redundant

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, 2 ms, 100 uV. **(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.

(e) Normalization explanation first. e.g.

Mean MEA-wide firing rate normalized by the firing rate 3-hrs prior to drug or vehicle treatment. (Normalize FR) Time bin 3 hrs. EB = SD.

(f). Normalized MEA FR, BR, IBI averaged over the 24 hr drug treatment period.

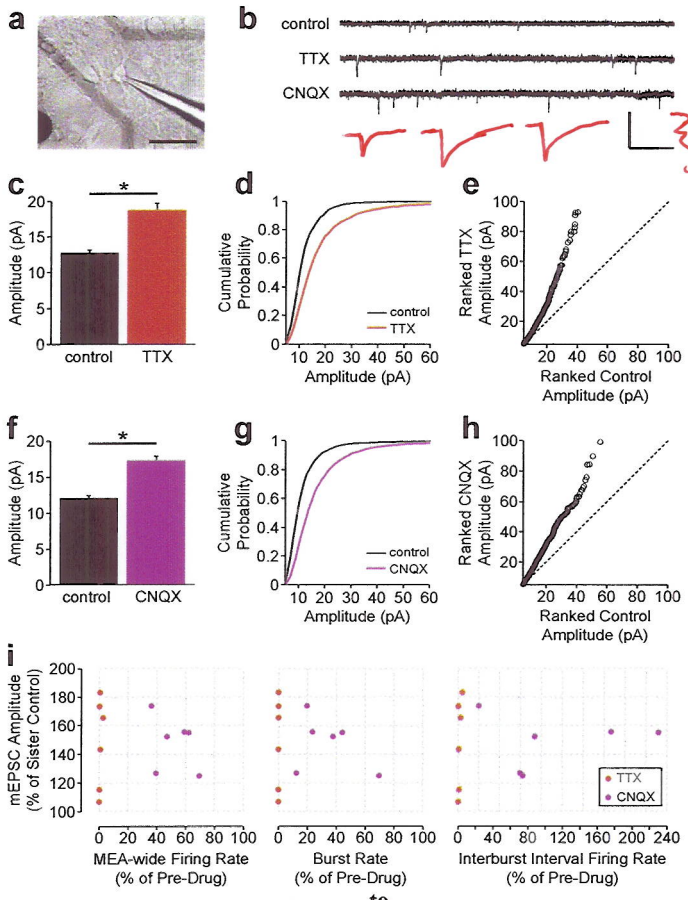


Figure 2: Spiking is not correlated ~~the~~ to the magnitude of synaptic scaling. (a) Pyramidal cell during whole-cell recording. Micro-electrode (black, lower left) and electrode leads (grey) are visible. Scale bar, 50 um. **(b)** Sample mEPSC recordings following 24-hour treatment with vehicle, TTX, or CNQX. **(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).

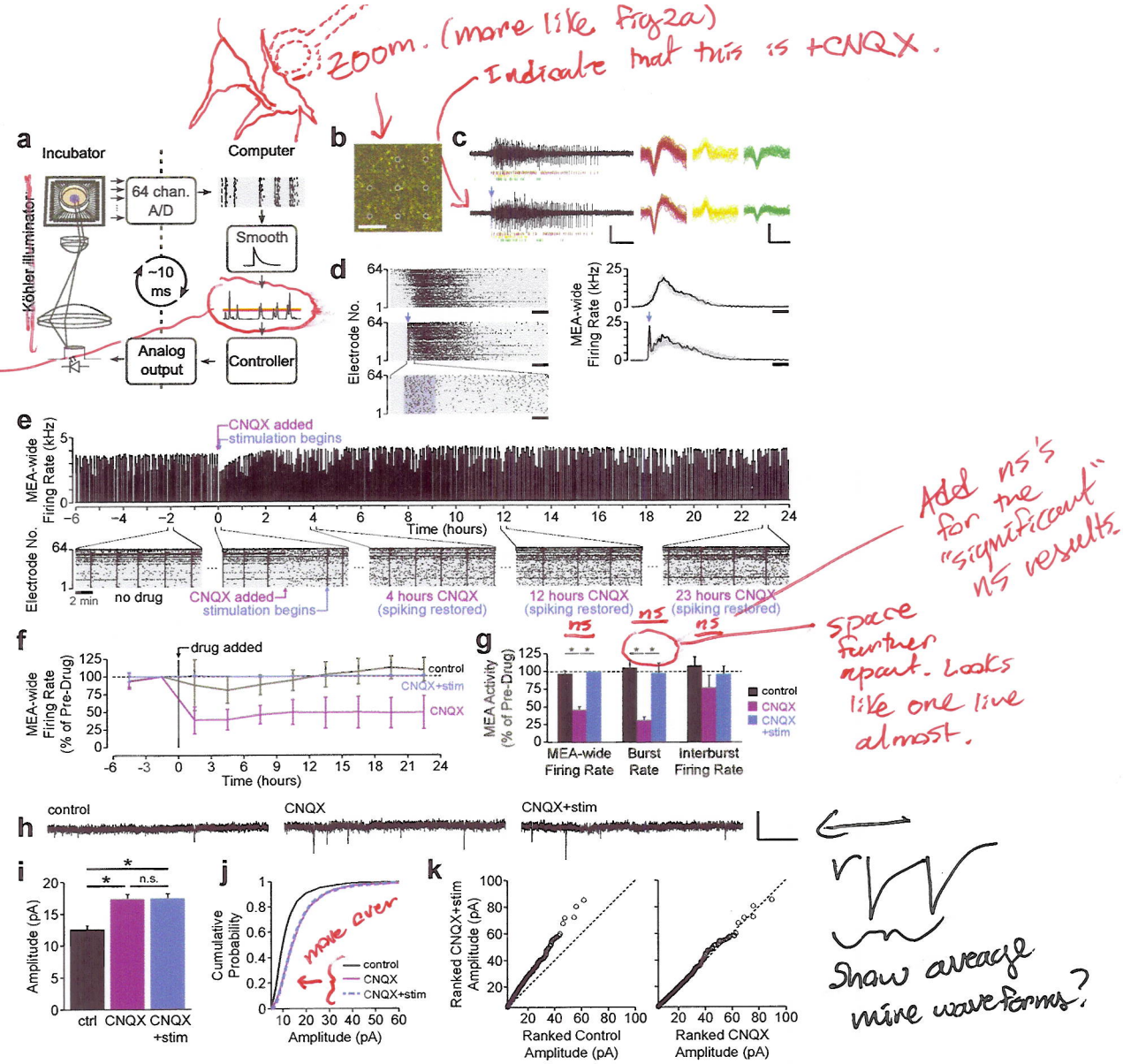


Figure 3: Reduced AMPAergic transmission directly triggers upward synaptic scaling. (a) Schematic of closed-loop optical stimulation system. **(b)** Neurons transfected with ChR2-eYFP. Microelectrodes are circled in white. Scale bar, 200 um. **(c)** *Left*, voltage traces recorded from a single microelectrode 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. **(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.

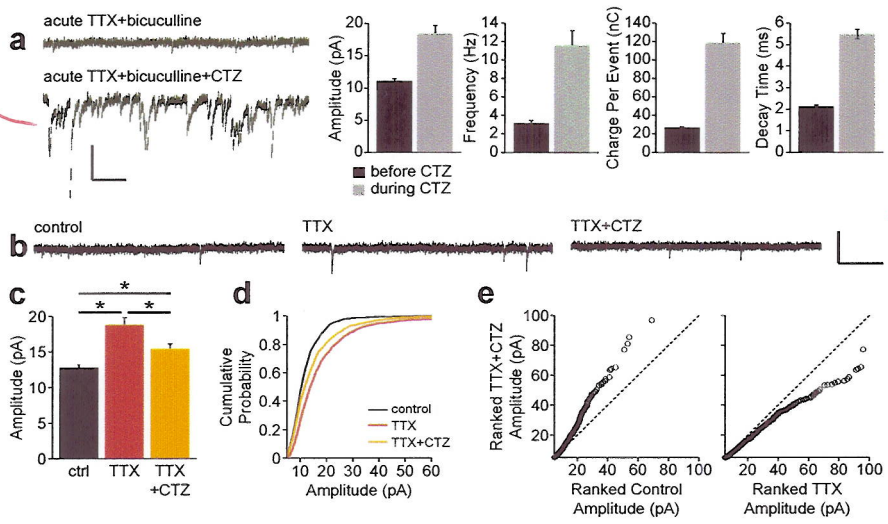


Figure 4: Reduced AMPA receptor activation mediates TTX-induced synaptic scaling. (a) *Left,* sample AMPAergic mEPSCs recorded before and after during CTZ. *Right,* Mean mEPSC amplitude, frequency, charge per event, and decay time constant before and during CTZ. **(b)** Sample mEPSC recordings following 24-hour treatment with vehicle, TTX, or CTZ. **(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.

Supplementary Methods (some of these will be ported to methods section in main text)

Cell culture

Primary cultures of neurons and glia were derived from E18 rat neocortex and grown on polyethylenimine- and laminin-coated microelectrode arrays (Multichannel Systems 60MEA200/30ir-Ti-pr) or glass bottom dishes (P35G-1.5-10-C) as described in Hales et al., 2010. Growth medium contained: 90% high-glucose DMEM, 10% horse serum, 0.5mM GlutaMAX, 1mM sodium pyruvate, 2.5ug/mL insulin (pH 7.2, 315 mOsm). Incubator was regulated at at 35°C and 5% CO2. Cultures were transfected with AAV9-hSynapsin-ChR2(H134R)-eYFP (from Dr. Karl Deisseroth via the University of Pennsylvania Vector Core) at 1 day in vitro. The following drug concentrations were used: TTX, 1uM; CNQX, 40uM; bicuculline, 20uM; cyclothiazide, 100uM. DMSO or water was used as vehicle, corresponding to the stable solvent used to dilute the drug that sister cultures were treated with. All experiments were performed during the second week *in vitro*.

Microelectrode array (MEA) recordings

MEA recordings were performed in ~~standard~~ growth medium in the cell culture incubator. Extracellular voltage waveforms were continuously sampled at 25kHz using the Neurorighter acquisition system (Newman et al., 2012, Rolston et al., 2009). Voltage recordings were filtered with a 3rd order Butterworth bandpass at ~~200-3000Hz~~, and action potentials were detected at threshold of ±5 times the root mean square error. Analysis of spike data was performed in MATLAB (The Mathworks). The pre-drug period was defined as a 3-hour segment preceding TTX, CNQX, or vehicle application. The treatment period was defined as the entire 24 hours during TTX, CNQX, or vehicle application. After the treatment period, all drugs were washed 4 times with standard growth medium.

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 $CaCl_2$, 1.5 $MgSO_4$, 1 NaH_2PO_4, 25 $NaHCO_3$, and 25 D-glucose, and saturated with 95% O2 and 5% CO2 (pH 7.4, 315 mOsm). To isolated AMPAergic mEPSCs, solution was supplemented with containing 1μM TTX and 20μM bicuculline. Temperature was regulated at 35°C using an inline heater (Warner ???). 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). mEPSCs were analyzed, blind to the treatment condition, using MiniAnalysis (Synaptosoft). Pipette resistances were 2-8Mohms. mEPSCs with amplitudes <5pA were excluded from analysis.

Optical stimulation

To deliver optical stimuli, a custom N-channel enhancement mode MOSFET current source (https://potterlab.gatech.edu/main/newman/wiki/index.php?title=Cyclops_Driver_R2) was used to drive a blue LED (46511 nm FWHM;

LZ4-00B200, LEDEngin, San Jose, CA). The LED was butt-coupled to a randomized fiber bundle (Schott AG, Mainz, Germany) which fed light to a Köhler illumination train mounted beneath the MEA amplifier. A full description and characterization of the closed-loop optical stimulation system used in this study is given in (Newman et al., 2013). Briefly, the average network firing rate was calculated every dt = 10 ms according to

$$f[t] = r[t] + (1 - \alpha) f[t - dt]$$

where = 5 sec/dt and and $r[t]$ = no. detected spikes/dt is the instantaneous firing at time t. The target rate, f^*, was defined as $f[t]$ averaged 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

$$e_f[t] = f^* - f[t].$$

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

$$if \sum_t e_f[t] > 0, \text{ apply } 10 \text{ ms pulse.}$$

(note: currently there's no way to notate summations properly in Pages; I will correct this when we port the document to a more supportive text editor)

Each stimulus pulse resulted in uniformly distributed 10.1 mW/mm^2 light the plane of the culture. The rise and fall times of each LED pulse were ~10 µs.

References for Supplementary Methods

Hales CM, Rolston JD, Potter SM (2010) How to culture, record and stimulate neuronal networks on micro-electrode arrays (MEAs). Journal of visualized experiments : 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. Frontiers in Neural Circuits 6:98.

Newman JP, Fong M-F, Potter SM (2013) Optogenetic feedback control of neuronal firing. Submitted

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. Frontiers in Neuroengineering 2:1-17.

List of Supplementary Figures

Supplementary Fig. 1: Computing firing rates, identifying bursts, etc
- show how bursts and interburst intervals were identified

Supplementary Fig. 2: mEPSC frequency and decay kinetics for TTX and CNQX
- bar charts comparing other mEPSC features

Supplementary Fig. 3: Optogenetic stimulation during CNQX treatment effectively mimics spontaneous bursts.
- Stimulation on all 59-channels for spontaneous vs. in CNQX
- Raster plot for snippets shown in (a)
- correlation plots for pre and post drug for CNQX vs. CNQX+stim

Supplementary Fig. 4: NMDAergic transmission is responsible for long-latency spiking during evoked stimulation.
(a) Chronic CNQX eliminates bursts, and when they recover they contain long-latency component, similar to spontaneous pre-drug bursts. (6-well)
(b) Chronic APV alone eliminates bursts, and when they recover contain are shorter than spontaneous pre-drug bursts. (6-well)
(c) APV+CNQX eliminates bursts for days.
(d) Bursts evoked during CNQX alone contain fast and slow components.
(e) Bursts evoked during APV alone contain only fast component. (get from jon)

Supplementary Fig. 5: mEPSC frequency and decay kinetics for CNQX and CNQX +stim
- bar charts comparing other mEPSC features

Supplementary Fig. 6: Cyclothiazide is effective at enhancing quantal AMPAR activation for at least 12 hours.
- bar chart showing amplitude, frequency, decay, charge for pre-drug, 0-3hr, 3-6hr, 6-9hr, and 9-12hr
- example mEPSC traces measured at each time point

Supplementary Fig. 7: Cyclothiazide does not change effects of TTX on spiking activity.
- equivalent of Fig. 1d-f and Fig. 3e-g for TTX+CTZ data (it looks the same as TTX only)

Supplementary Fig. 8: mEPSC frequency and decay kinetics for TTX and TTX +CTZ
- bar charts comparing other mEPSC features