Reductions in AMPA receptor activation, rather than spiking, trigger upward synaptic scaling

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Chronic reductions in firing rate lead to upward synaptic scaling (upscaling), a form of homeostatic plasticity that is expressed as a cell-wide strengthening of excitatory synapses. Recent work, however, has suggested that reduced neurotransmission can also directly 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 multisite electrophysiology, optogenetic feedback control, and pharmacology. We performed micro-electrode array (MEA) recordings to continuously monitor spiking activity in cultured cortical networks. We then pharmacologically blocked AMPAergic transmission, while restoring normal spiking using closed-loop optogenetic stimulation delivered based on MEA-recorded activity. We found that upscaling still occured, even when normal firing rates were restored. Next, we blocked spiking activity while partially restoring transmission using an AMPA receptor modulator and found that changes in AMPA receptor 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. These results raise questions about the functional role of synaptic scaling, and have implications for learning, memory, and neural injury

Abbreviations: MEA microelectrode array | **TTX** tetrodotoxin | **CNQX** 6-cyano-7-nitroquinoxaline-2,3-dione | **bic** bicuculline | **CTZ** cyclothiazide | **ChR2** channelrhodopsin-2 | **mEPSC** miniature excitatory postsynaptic current

Background

Upward synaptic scaling

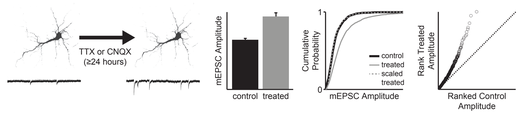
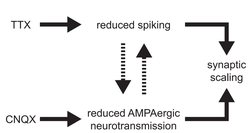


Figure 1: Typical experimental assessment of synaptic scaling. Cultured neurons chronically treated with TTX or CNQX show increased mEPSC amplitude, and this increase is multiplicative across the entire mEPSC distribution [1].

Triggers for scaling



Methods

Cell culture

- Primary cultures from E18 rat neocortex grown on planar MEAs [2,3]
- Transfected with AAV9-hSynapsin-ChR2(H134R)-eYFP at 1 DIV

Treatment conditions

- 40 μM CNQX used to block AMPAergic transmission
- 40 μM CNQX + closed-loop optical stimulation to restore firing rate
- 1 μM TTX used to block spiking
- 1 μM TTX + 20 μM CTZ used to block spiking while enhancing AMPAergic currents

Multisite electrophysiology

- Continuous recording of extracellular spikes from cultures during second week *in vitro*
- NeuroRighter real-time electrophysiology platform used for multichannel data acquisition and closed-loop control of LED current driver [5,6]

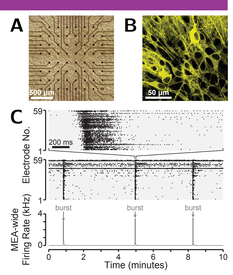
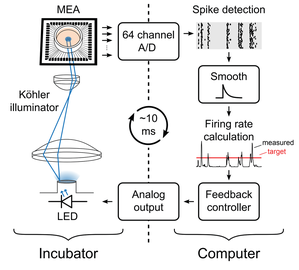


Figure 2: Microelectrode arrays. (A) Dissociated cortical culture on 59-channel MEA. **(B)** Confocal micrograph of culture expressing ChR2-eYFP. **(C)** *Top*, rastergram of spike times during network-wide burst. *Middle*, rastergram during 3 bursts. *Bottom*, time histogram of spikes (i.e. MEA-wide firing rate) [4].



Closed-loop optical stimulation

- Blue LED (465 nm) driven by custom driver
- Average firing rate calculated every 10 ms
- Target firing rate set to average firing rate during 3-hour epoch prior to CNQX treatment
- 10-ms pulse delivered at 10.1 mW/mm^2 when integrated error between target and measured firing rate became positive

Whole-cell recordings

- Recorded mEPSCs from pyramidal shaped cells
- 1 μM TTX + 20 μM bicuculline to isolate AMPAergic events
- Analysis performed blind to treatment condition

Figure 3: Schematic of closed-loop stimulation system. Spiking activity is recorded through the MEA. When the integrated error between the target and measured 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.

References: **[1]** Turrigiano et al., 1998. *Nature* 391:892-895. **[2]** Potter & DeMarse, 2001. *J Neurosci Methods* 110:17-24. **[3]** Hales et al., 2010 *JoVE* 1–7. **[4]** Wagenaar et al., 2006. *BMC Neurosci* 7:11. **[5]** Newman, et al., 2013. *Front Neural Circuits* 6:98. **[6]** Rolston et al., 2009. *Front Neuroeng* 2:1-17. **[7]** Sutton et al., 2006. *Cell* 125:785-799. **[8]** Hou et al., 2008. *PNAS* 105:775-780. **[9]** Beique et al., 2011. *PNAS* 108:816-821. **[10]** Deeg & Aizenman, 2011. *Nat Neurosci* 14:548-550.

Results

Closed-loop optical stimulation restores spiking during AMPAergic blockade

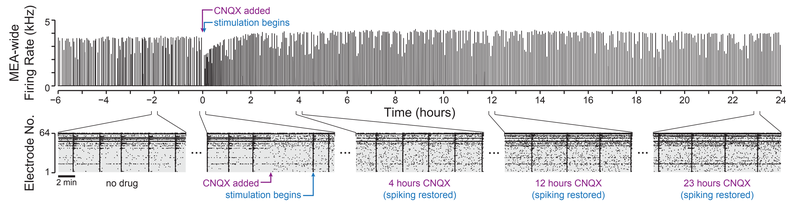


Figure 4: Spiking restored during CNQX treatment. Spontaneous MEA-wide firing rate is monitored for several hours. CNQX is added (time=0 hours), and closed-loop optical stimulation begins 5 minutes later. The controller's target firing rate is set to the average spontaneous rate during the 3-hour period before CNQX treatment. *Top*, MEA-wide firing rate over time before and during the treatment period. Bin size, 1 s. *Bottom*, rastergrams showing 15-min segments of spiking data on all electrodes at various time points during recording.

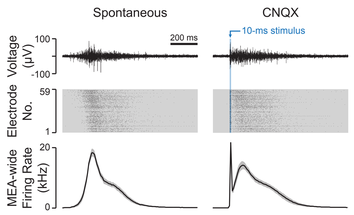


Figure 5: Bursts evoked during CNQX treatment resemble spontaneous bursts. *Top*, voltage recording on a single microelectrode during a spontaneous burst (left) or an optically-evoked burst during CNQX treatment (right). *Middle*, rastergram of spikes detected on all electrodes during bursts shown above. *Bottom*, average burst firing rate profile during 6-hour period before CNQX (left), or during 24-hour CNQX treatment with closed-loop optical stimulation (right). Shading, s.d.

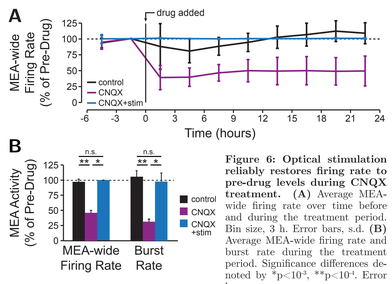


Figure 6: Optical stimulation reliably restores firing rate to pre-drug levels during CNQX treatment. (A) Average MEA-wide firing rate over time before and during the treatment period. Bin size, 3 h. Error bars, s.d. **(B)** Average MEA-wide firing rate and burst rate during the treatment period. Significance differences denoted by *p<10^{-3}, **p<10^{-4}. Error bars, s.e.m.

Reductions in spiking are not required for CNQX-induced synaptic scaling

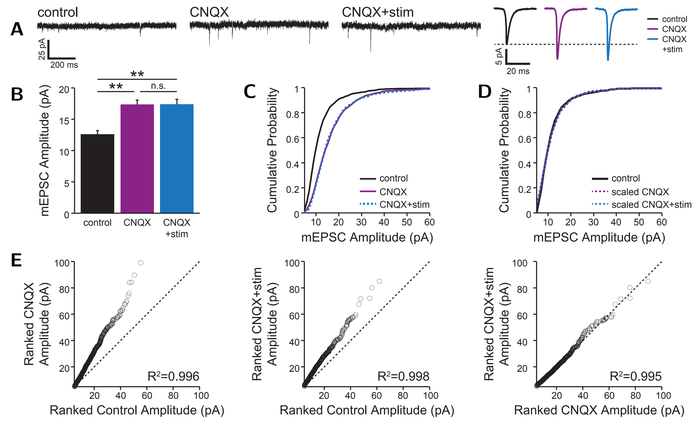


Figure 7: CNQX-induced scaling persists with spiking is restored. (A) *Left*, sample mEPSCs recorded from cells treated with vehicle, CNQX, or CNQX and closed-loop photostimulation. *Right*, average waveform of all mEPSCs recorded for each treatment condition. **(B)** Mean mEPSC amplitude for the 3 treatment conditions. 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. The two CNQX-treated distributions (with and without photostimulation) are statistically indistinguishable (p>0.9). **(D)** Cumulative distribution of multiplicatively scaled mEPSC distributions following CNQX treatment (with and without photostimulation). Scaled distributions match the control distribution (p>0.9 for both). **(E)** Ranked mEPSC amplitudes for the three treatment conditions plotted against one another. Dotted line denotes line of identity.

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Cyclothiazide increases quantal AMPAergic currents during spiking blockade

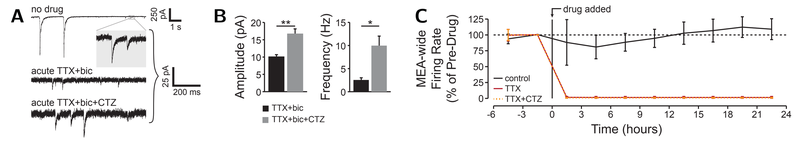


Figure 7: Quantal AMPAergic currents augmented during TTX treatment. (A) *Top*, whole-cell recording of typical synaptic currents, with shaded box zooming in on smaller events. *Middle and bottom*, sample AMPAergic mEPSCs before (middle) and during (bottom) acute CTZ treatment. **(B)** Average mEPSC amplitude and frequency of AMPAergic mEPSCs during acute CTZ treatment. Significance differences denoted by *p<10^{-2}, **p<10^{-3}. Error bars, s.e.m. **(C)** Average MEA-wide firing rate over time before and during the treatment period. Bin size, 3 h. Error bars, s.d.

Reduced AMPA receptor activation is required for TTX-induced synaptic scaling

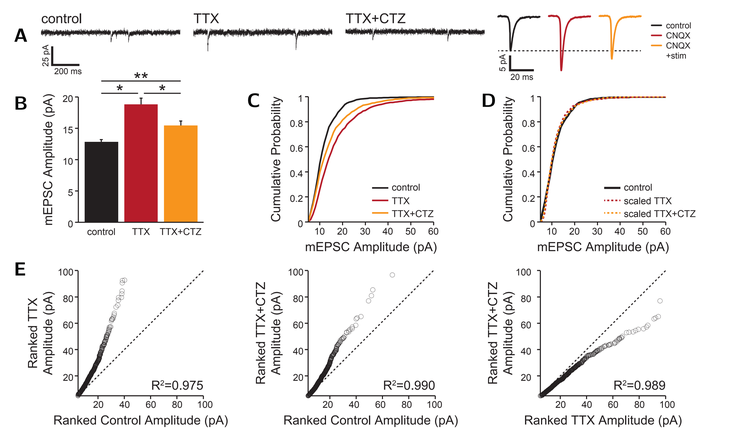
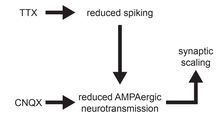


Figure 9: Cyclothiazide attenuates TTX-induced scaling. (A) *Left*, sample mEPSCs recorded from cells treated with vehicle, TTX, or TTX+CTZ. *Right*, average waveform of all mEPSCs recorded for each treatment condition. **(B)** Mean mEPSC amplitude for the 3 treatment conditions. Non-significant differences denoted by n.s. Significant differences denoted by *p<10^{-2} and **p<10^{-5}. Error bars, s.e.m. **(C)** Cumulative distribution of mEPSC amplitudes following the 3 treatment conditions. The distribution of mEPSC amplitudes is significantly different between the TTX and TTX+CTZ conditions (p<10^{-6}). **(D)** Cumulative distribution of multiplicatively scaled mEPSC distributions following TTX or TTX+CTZ treatment. Scaled distributions match the control distribution (TTX, p>0.7; TTX+CTZ, p>0.5). **(E)** Ranked mEPSC amplitudes for the 3 treatment conditions plotted against one another. Dotted line denotes line of identity.

Summary & Conclusions

- Optogenetic feedback control of neuronal firing is a powerful tool for separating spiking from causally-related variables (e.g. neurotransmission)
- Reductions in AMPAergic transmission are sufficient to trigger upward synaptic scaling even when spiking and bursting activity are normal.
- Reduced AMPA receptor activation is necessary for both TTX- and CNQX-induced synaptic scaling.
- Upward synaptic scaling acts to regulate AMPAergic transmission rather than neuronal firing rates.
- Because synapse-specific compensations have been described following local reductions in transmission [7-10], it is possible that cell-wide synaptic scaling represents many local synaptic compensations occurring throughout a neuron.



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