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

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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 multisite electrophysiology, closed-loop optogenetic stimulation, and pharmacology. First, we used 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 was still observed, even when normal firing rates were restored. Next, we blocked spiking activity while partially restoring transmission using an AMPAR modulator. We 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. These results raise questions about the functional role of synaptic scaling, and have implications for learning, memory, and neural injury.

Methods

Cell culture

- primary cultures of neurons and glia derived from E18 rat cortex plated onto polyethyleneimine- and laminin-coated MEAs
- transfected with AAV9-hSynapsin-ChR2(H134R)-eYFP at 1 DIV
- serum-containing growth medium changed every 3 days
- stored in incubator regulated at 35° C and 5% CO_2
- Teflon or polydimethylsiloxane lids using to maintain sterility and humidity [cite]

Treatment conditions

- 1 µM TTX used to block spiking
- 40 µM CNQX used to block AMPAergic transmission
- 40 µM CNQX used to block AMPAergic transmission while firing rate was restored using closed-loop optical stimulation
- 1 µM TTX + 20 µM cyclothiazide used to block spiking while enhancing AMPAergic transmission
- all treatment conditions lasted for 24 hours

Multisite electrophysiology

- MEA recordings performed before and during treatment conditions
- continuous recording of extracellular spikes from cultures grown on MEAs for ≥30 hours beginning during second week *in vitro*
- Neurorighter open-source, real-time electrophysiology platform used for multichannel data acquisition and closed-loop control of LED current driver [4]
- statistical significance assessed using Kruskal-Wallis tests followed by Wilconox rank-sum tests corrected for multiple comparisons

NeuroRighter | Real-time Electrophysiology
www.sites.google.com/site/neurorighter

Closed-loop optical stimulation

- blue LED (465 nm) driven by custom current source
- light uniformly distributed at 10.1 mW/mm^2
- 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 of stimulus delivered when integrated error between the target and measured firing rate became positive

Whole-cell recordings

- recorded mEPSCs from pyramidal shaped cells
- HEKA EPC8 amplifier and Patchmaster software used for acquisition
- all recordings performed in continuous perfusion of artificial cerebrospinal fluid bubbled with 5% CO2 and 95% O2
- statistical significance assessed using 1-way analysis of various t-tests corrected for multiple comparisons

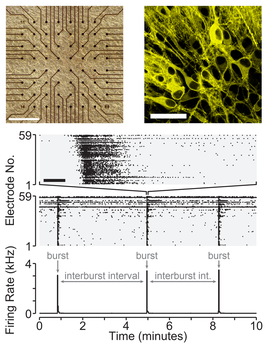

Figure 1: Micro-elecrode arrays. (A) Dissociated cortical culture on 59-channel MEA. (B) Confocal micrograph of cortical culture expressing ChR2-eYFP. Scale bar, 50 µm. (C) Top, rastergrams of spike times during a network-wide burst. Scale bar, 200 ms. Middle, rastergram during multiple bursts. Bottom, time histogram of spikes showing the MEA-wide firing rate (i.e. number of spikes occurring across entire MEA during each 1-second bin).

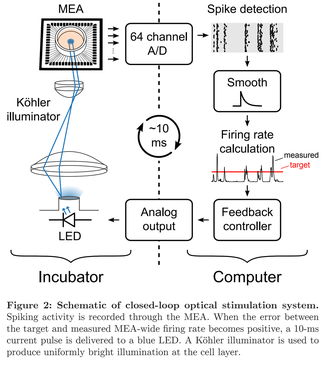

Figure 2: Schematic of closed-loop optical stimulation system. Spiking activity is recorded through the MEA. When the error between the target and measured MEA-wide firing rate becomes positive, a 10-ms current pulse is delivered to a blue LED. A Köhler illuminator is used to produce uniformly bright illumination at the cell layer.

Results

Closed-loop optical stimulation restores spiking and bursting activity

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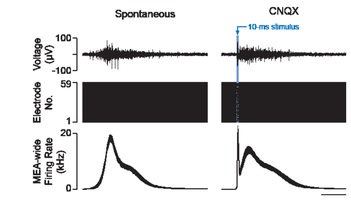

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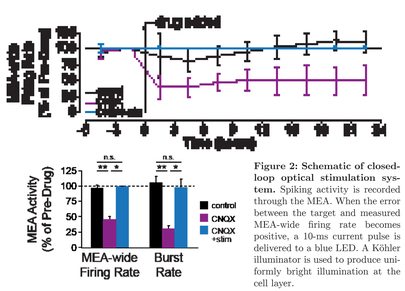

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Reductions in spiking are not required for CNQX-induced synaptic scaling

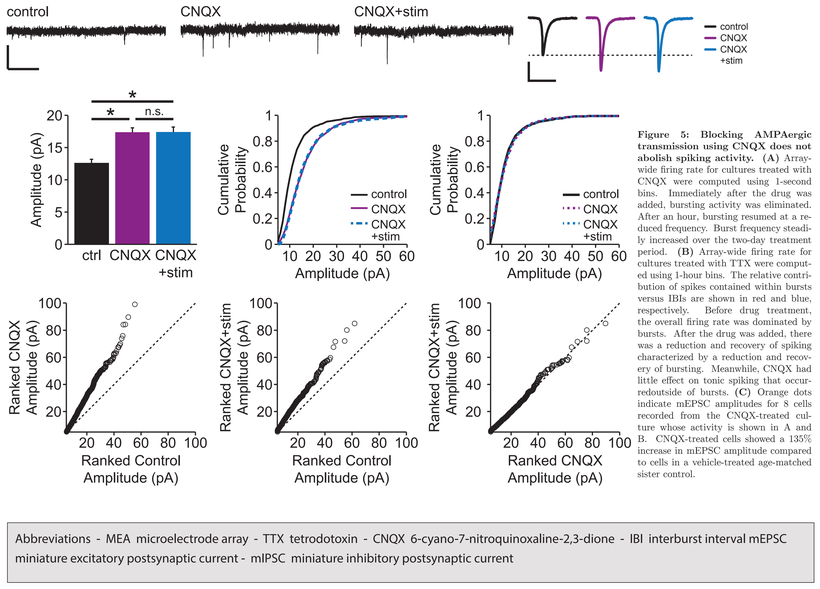
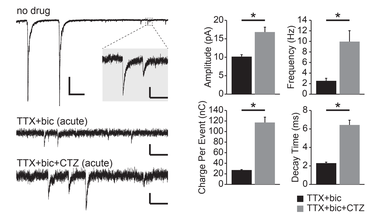


Figure 5: Blocking AMPAergic transmission using CNQX does not abolish spiking activity. (A) Array-wide firing rate for cultures treated with CNQX were computed using 1-second bins. Immediately after the drug was added, bursting activity was eliminated. After an hour, bursting resumed at a reduced frequency. Burst frequency steadily increased over the two-day treatment period. (B) Array-wide firing rate for cultures treated with TTX were computed using 1-hour bins. The relative contribution of spikes contained within bursts versus IBIs are shown in red and blue, respectively. Before drug treatment, the overall firing rate was dominated by bursts. After the drug was added, there was a reduction and recovery of spiking characterized by a reduction and recovery of bursting. Meanwhile, CNQX had little effect on tonic spiking that occur-redoutside of bursts. (C) Orange dots indicate mEPSC amplitudes for 8 cells recorded from the CNQX-treated culture whose activity is shown in A and B. CNQX-treated cells showed a 135% increase in mEPSC amplitude compared to cells in a vehicle-treated age-matched sister control.

Abbreviations - MEA microelectrode array - TTX tetrodotoxin - CNQX 6-cyano-7-nitroquinoxaline-2,3-dione - IBI interburst interval mEPSC miniature excitatory postsynaptic current - mIPSC miniature inhibitory postsynaptic current

Cyclothiazide increase quantal AMPAergic currents during TTX treatment



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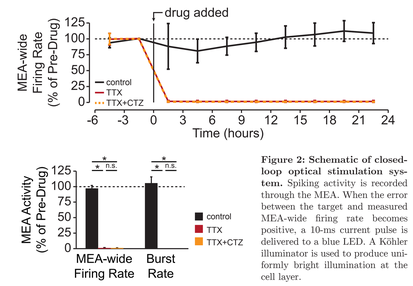

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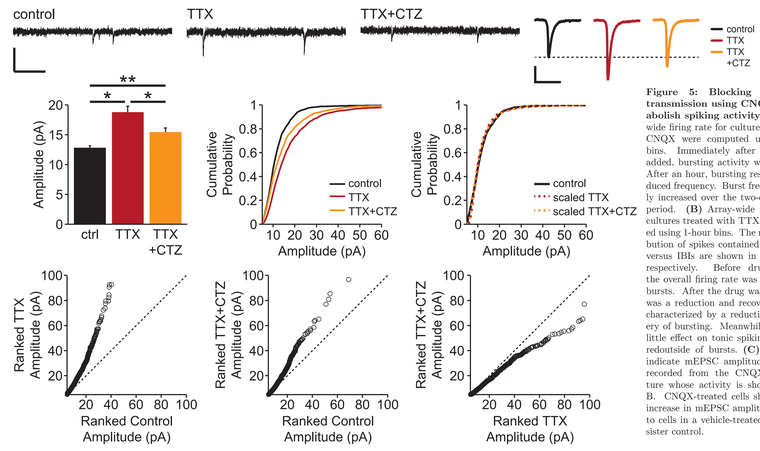
Reduced AMPA receptor activation is required for TTX-induced synaptic scaling



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Summary and Conclusions

- Reductions in AMPAergic transmission can trigger upward synaptic scaling even when firing rates are normal.
- AMPA receptor activation is necessary for both TTX- and CNQX-induced synaptic scaling.

References:

[1] Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 391:892-895.

[2] Wagenaar DA, Pine J, Potter SM (2006) An extremely rich repertoire of bursting patterns during the development of cortical cultures. BMC Neuroscience 7:11.

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