Microelectrode array recordings of cultured cortical networks help identify activity perturbations that trigger homeostatic synaptic plasticity

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Homeostatic plasticity provides a set of mechanisms for maintaining appropriate levels of spiking activity in developing neural circuits. When activity in cultured cortical networks was blocked for 2 days using TTX, there was a compensatory multiplicative increase in the amplitude of AMPAergic mEPSCs (synaptic scaling) [1]. Identical results have been observed when AMPAergic transmission was blocked for 2 days using CNQX. While changes in synaptic strength have been well-described in these experiments, network spiking activity is rarely tested. In this study, we continuously monitored network spiking activity in cortical cultures during a 2-day TTX or CNQX treatment using microelectrode arrays (MEAs). Drug treatments were followed by whole cell recordings of mEPSCs to assess synaptic strength. We found that TTX abolished all spiking activity. CNQX initially eliminated synchronous network-wide discharges or "bursts", but had little effect on tonic spiking activity outside of bursts. Surprisingly, bursting recovered in all CNQX-treated cultures. Both treatments were accompanied by upward synaptic scaling; however, the reduction in MEA-recorded activity and the degree to which synaptic strength increased was poorly correlated.

Methods

Dissociated cortical cultures

Primary cultures of neurons and glia were derived from E18 rat cortex and grown on polyethyleneimine- and laminin-coated MEAs. Serum-containing growth medium was changed every three days. Experiments were conducted during the second week *in vitro*.

MEA acquisition system

MEAs allow for continuous recording of extracellular action potentials from a grid of 59 microelectrodes embedded in a neuronal culture. All recordings were performed inside of an incubator in standard growth medium. Neurorighter (www.sites.google.com/site/neurorighter/) was used to acquire MEA data and detect spikes online. Spikes were sorted online and tracked throughout the recording. Spikes were re-sorted offline to validate waveforms and remove artifacts.

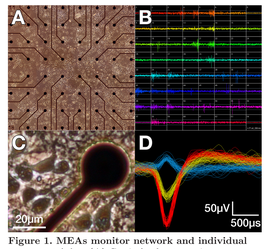


Figure 1. MEAs monitor network and individual neuron activity. **(A)** Cortical culture growing on an MEA. **(B)** Voltage traces recorded on each electrode using the Neurorighter acquisition system. **(C)** Phase-contrast micrograph of neurons near an MEA electrode. **(D)** Sorted pike waveforms recorded on single MEA electrode. This electrode shows 3 sorted units, presumably from three individual cells.

Firing rate and burst analysis

Network firing rate was computed by taking a time histogram of all spikes across the MEA using 1-second or 1-hour bins. A defining characteristic of dissociated cortical cultures is the development of synchronous spiking across the network, or "bursting" [2]. Spikes were classified as occuring during a burst or during an interburst interval (IBI) based on interspike interval taken across all electrodes.

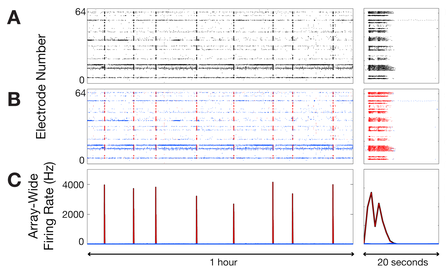


Figure 2. Firing rate analysis. **(A)** Spike rastergram from a bursting culture. **(B)** Same as A, but with colors distinguishing spikes occurring in bursts (red) versus the IBI (blue). **(C)** Array-wide firing rate histogram for 1-second bins, showing relative contributions of bursts versus IBIs to overall firing rate. Overall firing rate is dominated by spikes occuring in bursts.

Measuring AMPAergic synaptic strength

mEPSCs were recorded from pyramidal shaped cells using a HEKA EPC8 amplifier in the presence of 1μM TTX. In several experiments, 20μM bicuculline was added to eliminate GABAergic mIPSCs. We used this approach to pharmacologically isolate AMPAergic mEPSCs. We also used decay kinetics of AMPAergic mEPSCs (τ≤6ms) to eliminate GABAergic mIPSCs for recordings conducted in TTX alone.

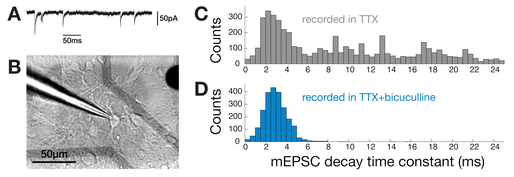
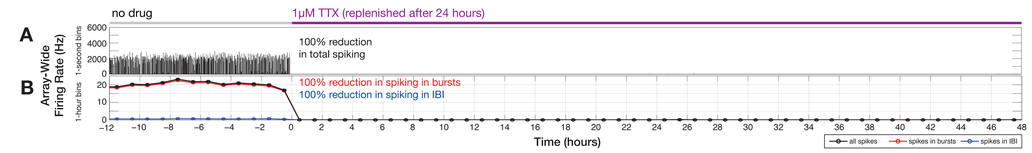


Figure 3: Whole-cell recordings for measuring AMPAergic synaptic strength. (A) A sample current trace showing mEPSCs recorded using a holding potential of -70mV. (B) Pyramidal shaped cell in cultured cortical network on MEA. (C) Histogram of decay time constants for glutamateric and GABAergic mEPSCs recorded in the presesnce of TTX. (D) Histogram of decay time constants for glutamateric mEPSCs in the presence of TTX and bicuculline.

Results

Chronic TTX treatment abolishes activity and induces homeostatic increases in synaptic strength



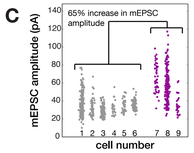
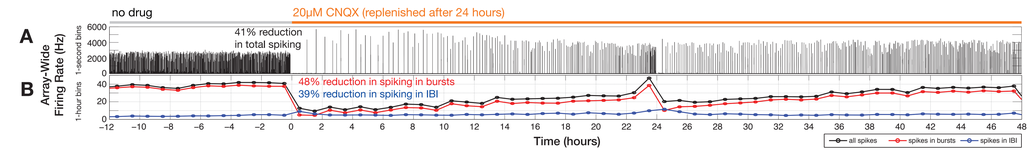


Figure 4: TTX effectively abolishes spiking activity. **(A)** Array-wide firing rate for cultures treated with TTX were computed using 1-second bins. After the drug was added, all spiking activity was eliminated. **(B)** Array-wide firing rate for cultures treated with TTX were computed using 1-hour 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, all activity (during both bursts and IBIs) was abolished. **(C)** Purple dots indicate mEPSC amplitudes for 3 cells recorded from the TTX-treated culture whose activity is shown in A and B. TTX-treated cells showed a 65% increase in mEPSC amplitude compared to cells in a vehicle-treated age-matched sister control.

Chronic CNQX treatment reduces, but does not abolish activity and induces homeostatic increases in synaptic strength



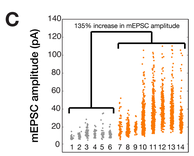


Figure 5: Blocking AMPAergic transmission using CNQX does not abolish spiking activity. **(A)** Array-wide firing rate for cultures treated with CNQX were computed using 1-second bins. Immediately after the drug was added, bursting activity was eliminated. After an hour, bursting resumed at a reduced 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 occurredoutside 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.

Chronic TTX and CNQX treatments lead to increases in network-wide and individual cell spiking activity after treatment is removed

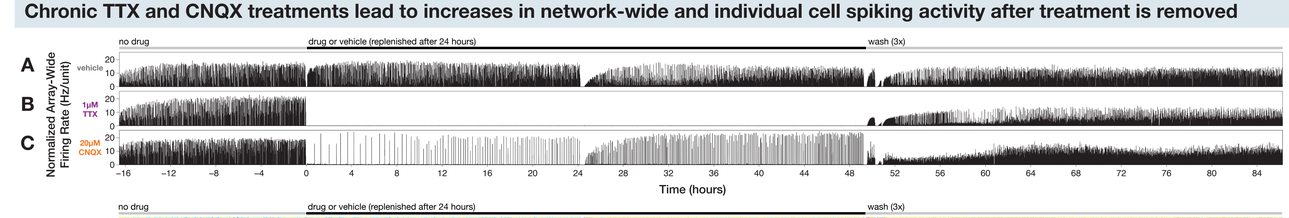


Figure 6: Chronic blockade of spiking or AMPAergic transmission leads homeostatic increases in network-wide activity after the drug is washed. **(A)** Unit-normalized array-wide firing rate for a vehicle-treated control culture. A moderate developmental increase in firing rate can be observed over the 100-hour recording. **(B)** Unit-normalized array-wide firing rate for an age-matched sister culture treated with TTX for 48 hours. Spiking activity is completely abolished. After the wash, burst frequency is elevated over pre-drug conditions. **(C)** Unit-normalized array-wide firing rate for an age-matched sister culture treated with CNQX for 48 hours. Spiking activity is initially reduced, but gradually recovers. After the wash, burst frequency is elevated over pre-drug conditions.

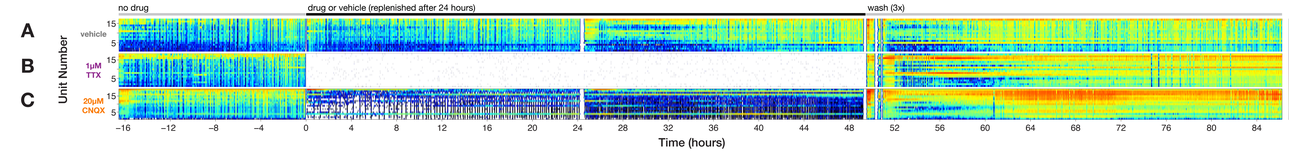


Figure 7: Chronic blockade of spiking or AMPAergic transmission leads homeostatic increases in individual cell activity after the drug is washed. **(A)** Firing rate for each unit in a vehicle-treated control culture (same recording as Fig. 6A). A moderate developmental increase in individual unit firing rate can be observed over the 100-hour recording. **(B)** Firing rate of individual units in an age-matched sister culture treated with TTX for 48 hours (same recording as Fig. 6B). After the wash, several units show a dramatic increase in firing rate compared to pre-drug conditions and the vehicle-treated culture. **(C)** Firing rate of individual units in an age-matched sister culture treated with CNQX for 48-hours (same recording as Fig. 6C). Initially, most units experience a dramatic decrease in firing rate, but each one recovers partial. After the wash, most units show a dramatic increase in firing rate compared to pre-drug conditions and the TTX- and vehicle-treated cultures.

Poor correlation between reduction in spiking activity and degree of synaptic scaling

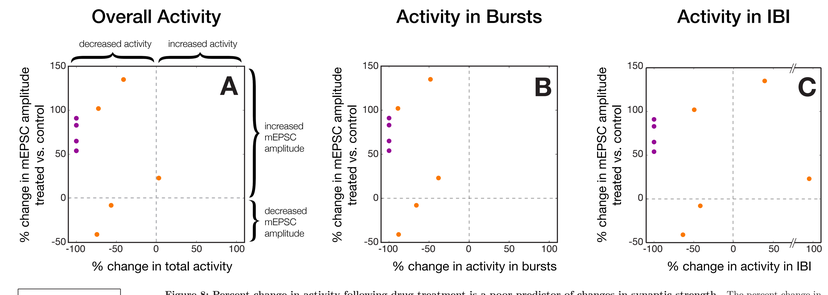


Figure 8: Percent change in activity following drug treatment is a poor predictor of changes in synaptic strength. The percent change in AMPAergic mEPSC amplitude was computed for each TTX- and CNQX-treated culture using vehicle-treated age-matched sister control cultures. These values are plotted as a function of **(A)** the percent change in total spiking activity, **(B)** the percent change in spiking activity occurring in bursts, or **(C)** the percent change in spiking activity occuring between bursts.

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

Summary and Conclusions

- **MEAs are useful tools for studying homeostatic plasticity *in vitro*.** MEAs provide a means to continuously monitor spiking during pharmacological perturbations and to follow the homeostatic recovery in cultured networks over days.
- **Reductions in spiking activity alone cannot predict the level of synaptic scaling.** TTX blocks spiking activity much more effectively than CNQX, and CNQX has variable efficacy in reducing spiking activity in different cultures. This suggests that neurons may be monitoring more features of network activity than spiking levels alone, or that reduction in spiking below a threshold is sufficient to trigger complete scaling.
- **Synaptic scaling is likely triggered by reducing synchronous network-wide spiking.** While TTX reduces spiking in both bursts and IBIs, CNQX primarily reduces spiking that occur in bursts. Both drugs elicit compensatory increases in mEPSC amplitude. Based on these findings, and the observation that approximately 90% of spikes occur in bursts, it is likely that a reduction in bursting activity triggers synaptic scaling.

References:

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