Topic: Neuronal dynamics and plasticity oral/poster presentation

Identifying activity perturbations that trigger homeostatic synaptic plasticity

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Background/Aims

Homeostatic plasticity is a set of mechanisms for maintaining appropriate levels of spiking activity in developing neural circuits. When spiking in a cultured cortical network was blocked for 2 days using tetrodotoxin (TTX), there was a compensatory increase in excitatory synaptic strength (scaling). Upward scaling of synaptic strength also occurred when fast glutamatergic transmission was blocked using 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). While changes in synaptic strength following pharmacological perturbations have been well-described throughout the nervous system, network spiking activity is rarely monitored during these perturbations. In this study, we sought to examine the relationship between changes in network spiking activity and synaptic strength that follow chronic TTX or CNQX treatment.

Methods/Statistics

Dissociated cultures of neurons and glia were derived from embryonic (E18) rat cortex and grown on planar microelectrode arrays (MEAs). The growth environment was carefully regulated to promote long-term culture health and support MEA electronics. During the second week *in vitro*, we continuously recorded network and individual unit spiking activity via the MEA during a 2-day application of TTX, CNQX, or vehicle (replenished after 24 hours). Following each treatment, we washed the drug and either continued recording via the MEA to assess changes in spiking activity or performed whole-cell voltage clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) to assess differences in excitatory synaptic strength between the treatment conditions.

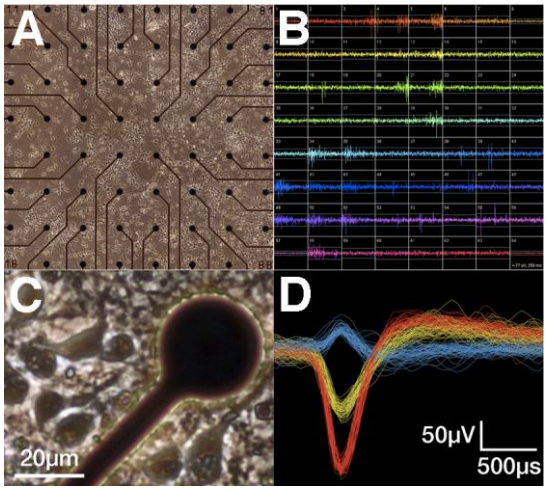

Figure 1: Monitoring network and individual neuron activity. (A) Phase-contrast micrograph of dissociated cortical culture on MEA. Electrode spacing, 200µm. **(B)** Voltage traces recorded on each electrode using the Neurorighter acquisition system. **(C)** Magnified view of (A) showing neurons near an MEA electrode. **(D)** Sorted spike waveforms recorded on a single MEA electrode.

Results

Under pre-drug or vehicle-treated conditions, the vast majority of spikes occurred within synchronous network-wide discharges or "bursts" (Fig. 2B). TTX effectively abolished spiking activity across the network compared to control conditions during the 2-day treatment window (Fig. 2). Meanwhile, CNQX reduced overall spiking and initially eliminated bursting; however, over the course of the 2-day CNQX treatment, bursting gradually recovered. After the drugs were washed, both TTX and CNQX-treated cultures showed increased unit and network spiking activity compared to the vehicle-treated sister controls. Both treatments were accompanied by compensatory increases in mEPSC amplitude. Interestingly, several CNQX-treated cultures exhibited a greater degree of synaptic scaling than TTX-treated cultures (Fig. 3C-D), even though CNQX was less effective at blocking spiking (Fig. 2). Overall, the reduction in MEA-recorded activity was poorly correlated to the degree of synaptic scaling.

Conclusion/Summary

MEAs provide an excellent tool for studying the activity dependence underlying mechanisms of homeostatic synaptic plasticity on the level of individual units and small neural circuits. The weak relationship between network spiking and the degree of synaptic scaling raises questions about what type of activity is monitored within neural circuits to trigger compensatory changes in synaptic strength.

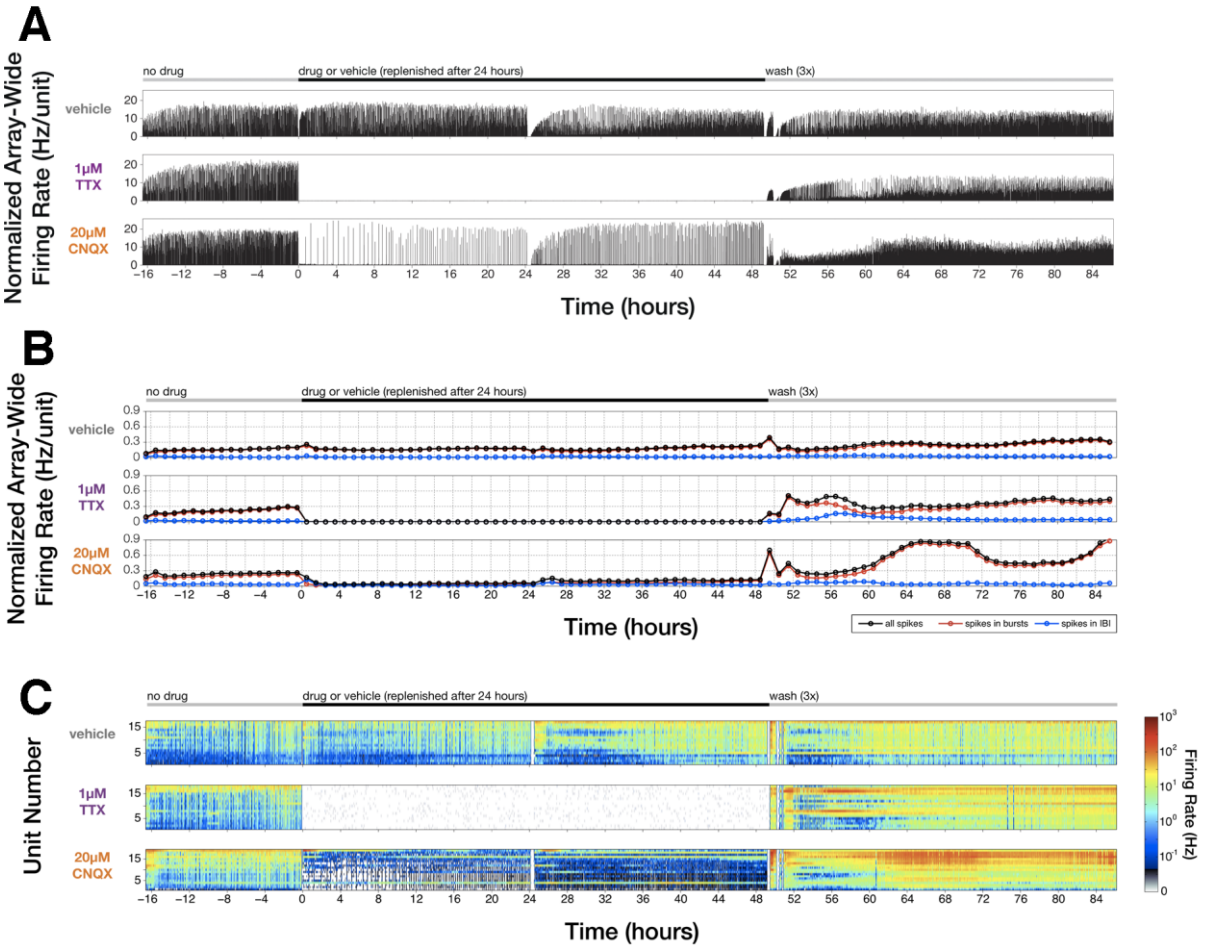

Figure 2: Chronic TTX and CNQX have distinct effects on spiking activity. **(A)** Unit-normalized array-wide firing rate for three sister cultures chronically treated with vehicle, TTX, or CNQX. TTX completely eliminates spiking while CNQX elicits a moderate reduction that slowly recovers. **(B)** Unit-normalized array-wide firing rate for spikes occurring within bursts (red) versus outside of bursts (blue). Most spikes are concentrated within bursts. **(C)** Firing rate for individual units before, during, and after treatment with vehicle, TTX, or CNQX. Recovery of spiking after CNQX is mediated both by increased firing rates in individual units, as well as a general elevation in firing rate across all units as seen in the vehicle-treated culture. After the wash, firing rate is especially heightened in the CNQX-treated culture.

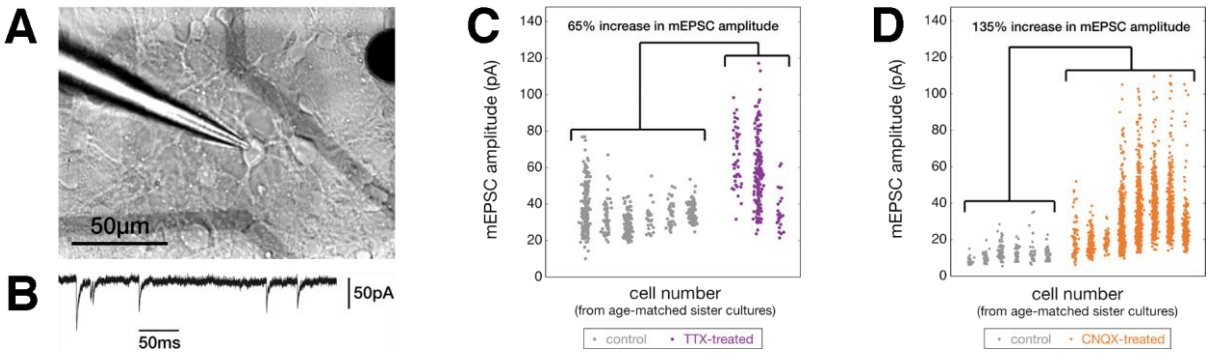

Figure 3: . **(A)** Pyramidal cell during patch recording. MEA leads and part of electrode are visible. **(B)** Miniature excitatory postsynaptic currents recorded using whole-cell patch clamp. **(C)** mEPSC amplitudes for 3 cells from a TTX-treated culture (purple) and 6 cells from a vehicle-treated sister control culture (grey). **(D)** mEPSC amplitudes for 8 cells from a CNQX-treated culture (orange) and 6 cells from a vehicle-treated sister control culture (grey).