Compensatory changes in GABA and NMDA synaptic currents help recover synchronous activity during chronic AMPAergic blockade

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The emergence of synchronous bursts of action potentials is a hallmark of cultured neuronal networks during the first few weeks *in vitro* [1]. While it has often been assumed that pharmacological blockade of AMPAergic transmission using CNQX eliminates population bursts, we have shown that this effect is transient, and when CNQX is applied chronically, bursting returns within hours. Here, we sought to determine how population bursting homeostatically recovers in the absence of AMPAergic transmission and examined how other transmitter systems contributed to this recovery. Whole-cell voltage clamp recordings revealed an increase in the amplitude of inward synaptic currents within an hour of AMPAergic blockade. MEA recordings showed that NMDA mediates the recovery of population bursting during the first 48 hours of AMPAergic blockade, but that GABAergic transmission alone was sufficient to recover bursting during a more extended glutamatergic blockade. Together these results suggest that impaired AMPAergic transmission triggers compensatory changes in two other transmitter systems that help recover normal patterns of network activity. This form of plasticity offers a unique strategy for maintaining excitatory drive in cortical circuits when other avenues for excitation are chronically impaired.

Abbreviations: **MEA** microelectrode array • **DIV** days *in vitro* • **CNQX** 6-cyano-7-nitroquinoxaline-2,3-dione • **APV** D(-)-2-amino-5-phosphonopentanoic acid

Methods

Dissociated cortical cultures

- primary cultures of neurons and glia from E18 rat cortex
- polyethyleneimine- and laminin-coated coverslips or MEAs
- serum-containing growth medium changed every 3 days
- stored in incubator regulated at 35° C and 5% CO_2
- sterility and humidity maintained with Teflon or polydimethylsiloxane lids [2,3]

Drug treatments

- 40μM CNQX used to block AMPAergic transmission
- 50μM APV used to block NMDAergic transmission
- 20μM bicuculline used to block GABAergic transmission

MEA electrophysiology

- continuously recorded extracellular spikes from cultures grown on MCS 6-well MEAs for several days
- Neurorighter open-source, real-time electrophysiology platform used for multichannel data acquisition [4]
- all recordings performed in standard growth medium inside of incubator

Whole-cell recordings

- whole-cell voltage clamp used to record postsynaptic currents from pyramidal shaped cells in cultures grown on coverslips
- 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

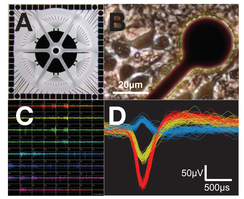

Figure 1: Microelecrode arrays. (A) Multi-well MEA with six pharmacologically-isolated recording chambers. **(B)** Phase-contrast micrograph of neurons near an MEA electrode. **(C)** Voltage traces recorded on each electrode using the Neurorighter acquisition system. **(D)** Sorted spike waveforms recorded on single MEA electrode.

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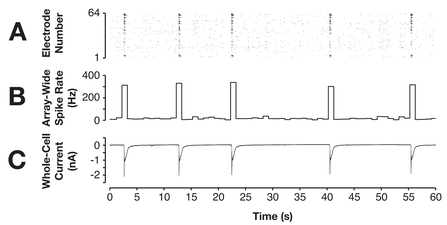

Figure 2: Population bursts can be assessed using MEA or whole-cell recordings. (A) Rastergram showing MEA-recorded spike times by electrode number. Five population bursts are shown. **(B)** Time histogram of spike times in A, collapsed across entire MEA. Bin size, 1s. **(C)** Whole-cell recording showing intracellular currents elicited during population bursts.

References:
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[4] Rolston JD, Gross R, Potter SM (2009). A low-cost multielectrode system for data acquisition enabling real-time closed-loop processing with rapid recover from stimulation artifacts. *Frontiers in Neuroengineering* 2.

Results

Population bursting recovers in absence of AMPAergic transmission

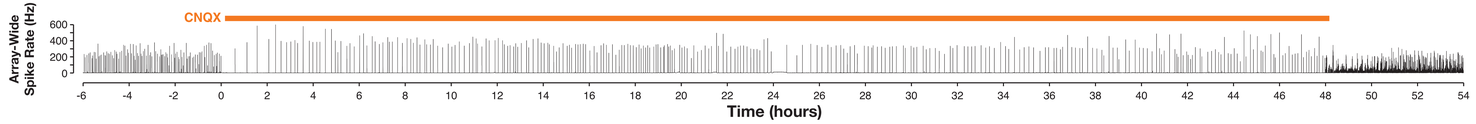

Figure 3: Chronic AMPAergic blockade. Array-wide firing rate histogram for culture treated with CNQX at time zero. The drug was replenished after 1 day and washed after 2 days. Bin size, 5s.

GABAergic inward synaptic currents increase in amplitude within an hour of AMPAergic blockade

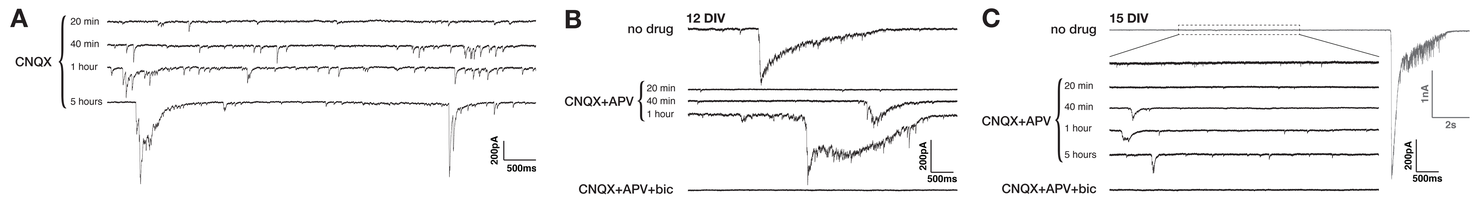

Figure 4: Amplitude of inward currents increases during glutamatergic blockade. (A) Postsynaptic currents recorded during CNQX treatment. The remaining currents increase with duration of treatment. **(B, C)** Postsynaptic currents recorded during a treatment with CNQX and APV at 12 or 15 DIV. The remaining currents increase with duration of treatment and are presumed to be GABAergic since they are abolished by bicuculline.

NMDAergic transmission is necessary for recovery of population bursting during first 48 hours of AMPAergic blockade

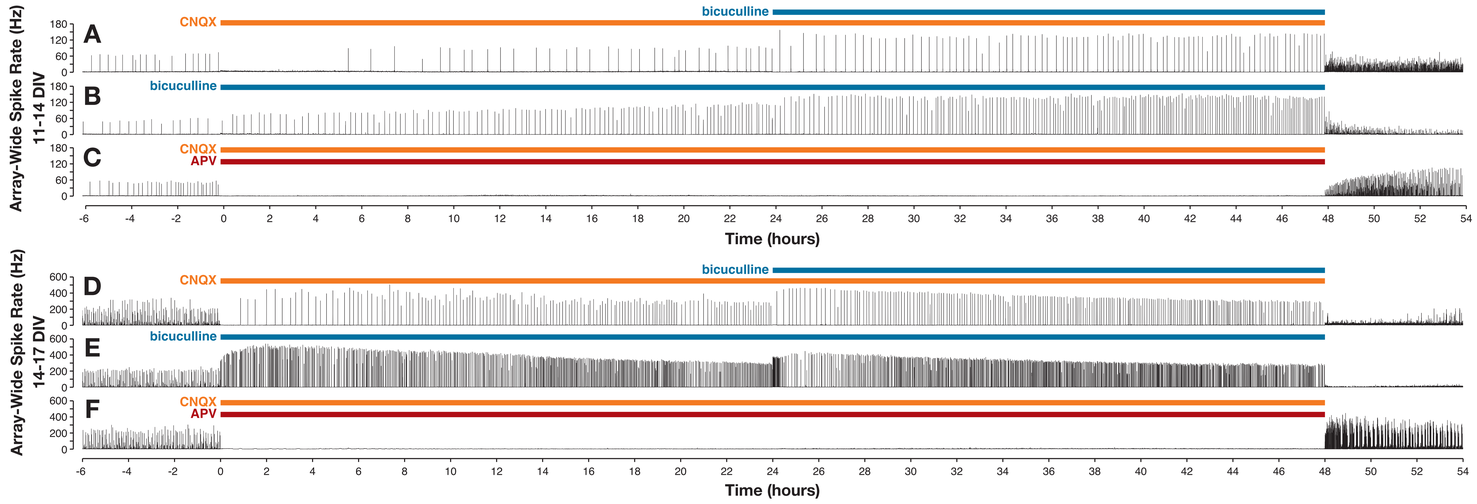

Figure 5: NMDAergic transmission can recover population bursting. (A) Array-wide spike histogram for culture treated with CNQX at 12 DIV, and bicuculline 1 day later. **(B)** Array-wide spike histogram for culture treated with bicuculline for 2 days. **(C)** Array-wide spike histogram for culture treated with CNQX and APV at 12 DIV. In all cases, drugs were washed at 14 DIV. For 2-day treatments, drugs were replenished after 24 hours. **(D-F)** Same as A-C, but with treatments beginning at 15 DIV and washed at 17 DIV.

GABAergic transmission can mediate recovery of population bursting over days

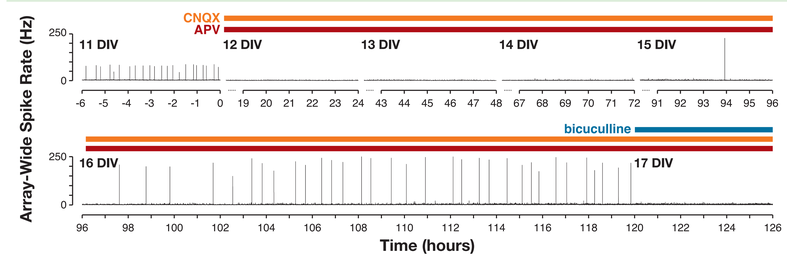

Figure 6: Glutamatergic transmission blockade elicits GABA-mediated bursting. Array-wide spike histogram showing firing rate during a 5-day treatment using CNQX and APV. Drugs were replenished every 24 hours. Bursting returns at the end of the 4 days, and is abolished by bicuculline. Bin, 5s.

Summary and Conclusions

- **Recovery of population bursts in the absence of AMPAergic transmission is mediated by NMDA.** Bursting returns within a few hours of CNQX application, but does not return for several days when CNQX and APV are co-applied.
- **Inward GABAergic currents increase after glutamatergic blockade.** This may provide a mechanism for restoring excitatory synaptic transmission in the absence of ionotropic glutamatergic transmission. It also may explain how GABA-mediated population bursts emerge after several days of CNQX and APV treatment.
- **Based on these results, we hypothesize that GABA and NMDA work in concert to recover network activity when AMPAergic transmission is chronically impaired.**

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