Using multielectrode arrays to investigate the spontaneous firing patterns and functional connectivity in large neural assemblies

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INTRODUCTION
Spontaneous cortical activity induced without any sensory inputs has the potential to reveal crucial information about the neuronal network in its most native state. In fact, such patterns may provide certain functions to the network on their own. Previously, the difficulty in reliably eliciting and recording spontaneous cortical activity has made studying spontaneous firing patterns quite difficult. Here, we attempt to uncover the network structure and functional connectivity of the mouse barrel field through spontaneous activity recorded from acute brain slices of juvenile mice. With the high temporal resolution and spatial precision provided by multielectrode arrays (MEAs), we were able to resolve distinct waveforms from hundreds of neurons at a time. The temporal firing patterns of the neurons indicated clear correlations among cells.

METHODS
Coronal slice preparation. Juvenile (P15) C57BL/6 wild-type mice were anesthetized and decapitated. The brain was removed, and coronal slices were taken.

MEA recording. High density, perforated MEAs with 120 electrodes of 100 μm pitch from Multichannel Systems were used to obtain 10 minute recordings at a sampling rate of 50 kHz.

Data analysis. Data entered a wavelet-based spike sorting regime adapted from Quinlan 2004, and the processed data was additionally analyzed to determine functional connectivity as described in the Results section. All analysis was conducted in MATLAB 2015b (Mathworks).

RESULTS

Fig 1: Microelectrode array setup. A: Location on brain where coronal slices were taken. B: Brain slice placed on MEA. C: Perforated MEA field from Multichannel Systems.

Fig 2: Spike sorting. Spikes recorded from each individual electrode on the MEA were sorted into different clusters based on spike shape and temporal features. Electrodes often transduce 2-5 distinct waveform shapes, corresponding to different neurons.

Fig 3: Raster plot of spikes. Temporal firing patterns varied across neurons - while some cells appeared to be firing tonically, others were more dormant. Some cells also exhibited periodic firing, marked by small windows of inactivity occurring every few hundred milliseconds.

Fig 4: Covariance matrix of neuronal firing. The covariance between neuron pairs was calculated from the biograph. Neuron degree and firing frequency could be readily identified.

Fig 5: Biograph of neuronal connections. Based on the covariances and temporal patterns, correlational relationships between neuron pairs were identified and visualized as a biograph. Groups of heavily interconnected neurons can be clearly seen. The neuron degree was subsequently determined as the number of connections in the biograph.

Fig 6: Spatial map of neuron degree. Neuron degree was plotted for every neuron on the MEA grid to generate a spatial map. Clusters of highly connected neurons were observed in the upper portion of the MEA, corresponding to upper layers in the cortex.

Fig 7: Relationship between connectivity and firing frequency. Most neurons have a neuron degree between 3 and 6. These neurons demonstrated a wide range of mean interspike intervals (ISIs, reciprocal of mean firing rate). Although the anti-correlation between neuron degree and firing rate is not evident across all neurons, those with the most connections are exclusively high-firing neurons.

Fig 8: Network connectivity. Connectivity of a single neuron was compared against that of its first-order neighbors. A linear regression revealed that network functional connectivity was far from uniform, demonstrated by a deviation from the line of identity. Although well-connected neurons tended to interact amongst themselves, neurons with fewer connections were also recruited, suggesting that the network is moderately assortative.

CONCLUSIONS
1. Spontaneous cortical activity can be sustained and recorded with in vitro MEAs, which can detect hundreds of distinct neurons.
2. Hubs of highly connected neurons are identified within the network with high functional connectivity.
3. Future research could investigate the dynamics of neuronal interactions, and the physiological function and underlying mechanisms of spontaneous activity.

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