>> Characterization of functional neuronal activity *in vitro* with local field potential signals

Key findings:

- Analyzing LFPs detected during network bursting events enables insights into LFP activity of interest.

- LFP power increases in relevant frequency bands throughout maturation.

- Pharmacological manipulation modifies the burst pattern and power distribution of LFPs.

Abstract

Advanced neuronal models, such as organoids, are promising platforms for studying brain development and establishing mature human phenotypes *in vitro*. Local field potentials (LFPs) provide an avenue to quantify the development of low frequency oscillations, which are reminiscent of *in vivo* brain rhythms. The objective of this work is to develop and validate an *in vitro* neuronal activity assay using low frequency LFP signals and traditional spike train analysis.

Introduction

Characterizing the collective activity of a cell population by assessing low frequency local field potentials (LFPs) formed in neuronal networks is a well-established method in neuroscience. Research has demonstrated that LFPs largely represent the summed input to a population of neurons by way of synchronized post-synaptic potentials.[1] Although spike train analysis indicative of the output of individual neurons has been the primary approach to microelectrode array (MEA) assays, LFPs carry additional information about the excitatory-inhibitory balance and functional activity of a neuronal network.

In electroencephalogram (EEG) literature, the appearance and prevalence of certain low frequency oscillations can be mapped to behavioral states such as deep sleep or heightened awareness.[2] As *in vitro* neuronal models grow more sophisticated through techniques employing 3D structures, co-cultures, and human induced pluripotent stem cells (hiPSCs), they may begin to exhibit meaningful low frequency electrical signals as well.[3] Development of easy-to-use tools for recording and analyzing LFP signals will complement existing assays of functional spiking activity to enhance characterization of neuronal models.

Here, we highlight the ability of the Maestro MEA platform to concurrently collect and analyze spike trains and LFPs from cultured neuronal networks. We also demonstrate that characteristics relating to the shape or spectral content of LFP signals can be reliably measured and used to describe and

differentiate neural activity patterns. For rat cortical neuron cultures, we demonstrate how the LFP shape changes over time and in response to modulating drugs.
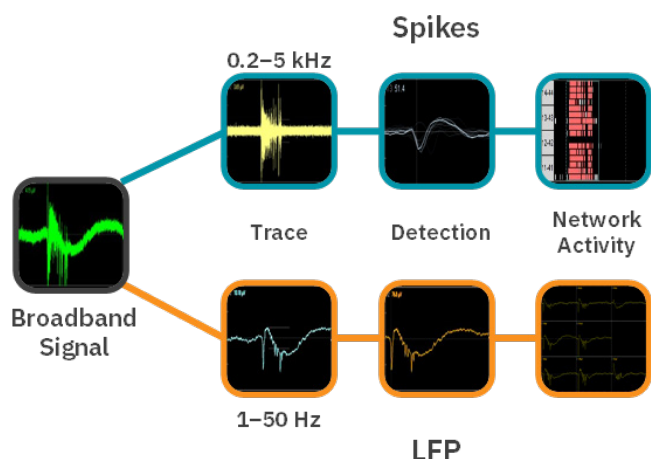
Figure 1: Broadband signal processing in AxIS Navigator. Broadband (1Hz – 5kHz) neural signals are recorded at a sampling rate of 12.5kHz. This broadband signal is then filtered into a Spike Trace (Butterworth bandpass filter 200 Hz – 5kHz) for spike and burst detection and an LFP Trace (median downsampling filter 1Hz – 50Hz) for LFP event detection.

Materials and Methods
Cells and reagents

Primary rat cortex neurons, RCN, (Cat. A1084002) were obtained from ThermoFisher (Waltham, MA). RCN media was composed of Neurobasal Plus Medium (Gibco, Cat. A3582901), Glutamax (Gibco, Cat. 35050061), B-27 Plus Supplement (Gibco, Cat. A3582801), and gentamicin (Gibco, Cat. 15710064). Amoxapine (Cat. 17347) was obtained from Cayman Chemicals (Ann Arbor, MI).

Cell plating

CytoView MEA 48- and 96-well plates were spotted with a 5 µL droplet of Poly-D-Lysine, PDL, (Gibco, Cat. A3890401) over the microelectrode array in

each well. After 1 hour incubation at 37°C and 5% CO_2, each well was washed with diH_2O four times to remove any excess PDL and allowed to dry overnight in the biosafety cabinet. Then, RCNs were thawed according to supplier recommendations. Next, the cell suspension was transferred to a 15 mL conical tube and centrifuged at 300 x g for 5 minutes. The supernatant was aspirated, being careful not to disturb the cell pellet. Cell density and viability were determined using a hemocytometer. Cells were resuspended and diluted in appropriate media to the desired working concentration of 16,000,000 neurons/mL. Cells were then spotted over the array in a 5 µL droplet for each well and the plate was incubated at 37°C and 5% CO_2 for 1 hour. Finally, additional media (200 µL for the 96-well plate and 300 µL for the 48-well plate) was added. Integrated humidity reservoirs on the CytoView MEA 96 were filled with sterile water to maintain humidity. Every 2-3 days during culture, 50% of the RCN media was replaced.

Drug dosing

At DIV 28, neurons were dosed with 3 µM amoxapine. Amoxapine was diluted from a stock solution in RCN media and was added at 10x the final concentration to treatment groups. Additional treatment groups included no treatment control (NTC) and 0.1% DMSO as a vehicle control. A 5-minute recording prior to dosing served as the baseline measurement. After drug dosing, the plate was incubated for 1 hour before another 5-minute recording was taken which served as the dosed condition.

### Neural activity recording with the Maestro MEA platform

The Neural Broadband Spontaneous configuration in AxIS Navigator was used to record spikes and LFPs from all electrodes in each well (**Fig. 1**) using a Maestro Pro. The temperature was set to 36°C,

and legacy heater mode was activated. Prior to recording, plates were pulled from the incubator, placed in the Maestro Pro, and allowed to equilibrate for 5 minutes. After recording, plates were returned to the incubator.

LFP and spike train analysis

Spike detection was performed using AxIS Navigator (Axion BioSystems). Spikes were detected with a threshold of 6 standard deviations above the noise. LFP and spike train analysis were performed with the Neural Metric Tool (Axion BioSystems).
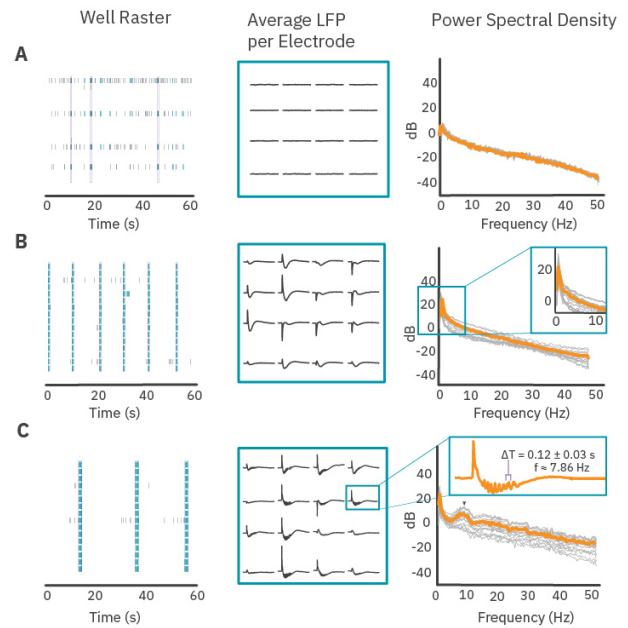
Figure 3: Peaks in the PSD correspond to patterns of spiking activity. Blue marks on raster plots indicate detected bursts on an electrode, while pink outlines indicate network bursting. Circles in the center show average LFPs on each electrode. (A) Neural activity with weak synchrony and poorly organized bursting results in very little deviation in LFP signal, corresponding to a smooth power spectrum which progressively decreases at higher frequencies. (B) Organized network activity displays large voltage deflections in LFP, producing a peak at low frequencies in the power spectrum. (C) More complex network activity with intra-event oscillations can produce additional peaks in the power spectrum at relevant rhythms. The average peak to peak time between ripples in an average LFP event is 0.127 seconds, which corresponds to the peak in the PSD at a frequency of 7.86 Hz.
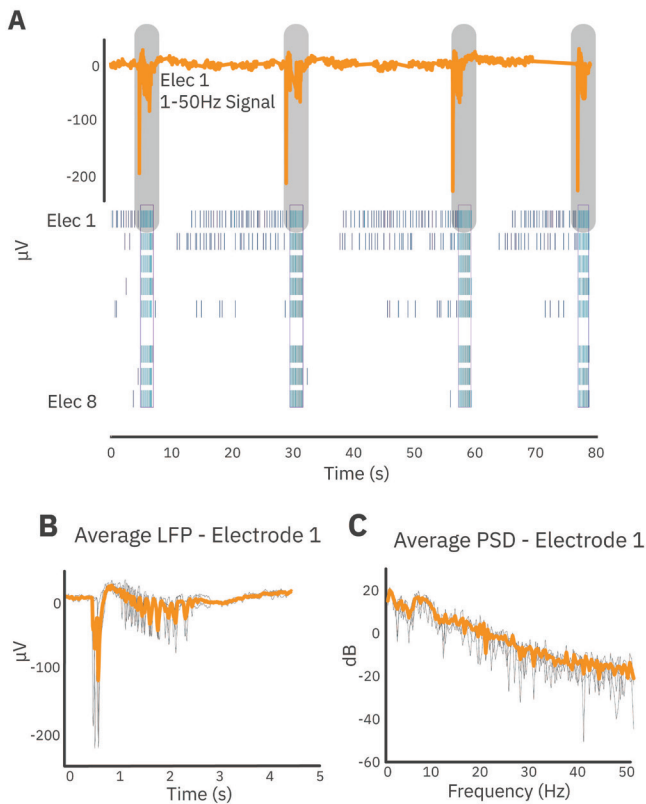
Figure 2: Network burst-triggered LFP analysis. After identifying network bursts in a well (A), network burst associated LFP traces for each electrode are sliced out to describe an average LFP event per electrode (B). Each event is transformed into the frequency domain (gray traces), and these are averaged to describe an average power spectral density curve (C) per electrode (black traces). From the average LFP and average PSD of each electrode, a well average LFP and PSD can be computed.

First, LFP events which occurred during network bursts were extracted (**Fig. 2**). For each electrode, LFPs were extracted starting 0.5 seconds before the start of the network burst and ending 4 seconds after the start of the network burst. Average LFPs and average power spectral density (PSD) curves were computed from these events using a periodogram approach. Prior to transformation to the frequency domain, LFP traces were downsampled using an anti-aliasing filter from the 12.5 kHz acquisition rate to 100 Hz, preserving frequency content up to 50 Hz. PSDs were computed in µV2/Hz and log-transformed for scaling. Endpoints from the PSD were calculated on a

per-well basis through integration of the well-average PSD according to the following defined frequency bands:

Band Name	Frequency Range
Low	0-1 Hz
Delta	1-4 Hz
Theta	4-8 Hz
Alpha	8-15 Hz
Beta	15-30 Hz
Gamma	30-50 Hz

Table 1: Definition of frequency bands for PSD analysis

Figure 4: LFP development during maturation of rat cortical neuronal cultures. (A) Average PSDs of network burst-triggered LFPs collected in a well are integrated over predefined frequency ranges to estimate power within the signal at different stages of maturation. (B) Power in the low band increases over time and begins to stabilize around DIV14, while alpha band power does not stabilize until the fourth week *in vitro*. The date of half-maximal power (dashed line) was determined for each frequency band. The development of alpha power lagged the low frequency power by ~6.5 days. Insets below (B) show examples of LFPs collected at DIV 7, 14, 21, and 28.
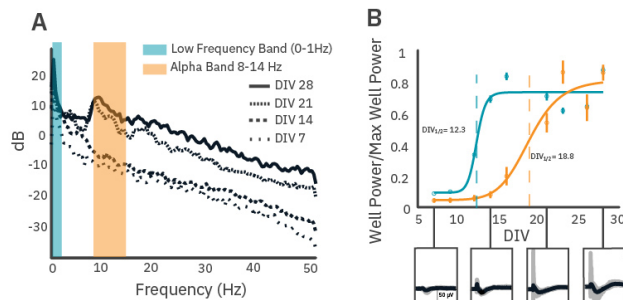
Results
Distinct LFPs emerge from spiking activity patterns

The PSD provides information about the oscillatory content of a signal. Here, we demonstrate the relationship between spiking activity, LFP shape, and oscillatory content of the PSD. Figure 3 presents three distinct examples of neuronal activity patterns in cultured cortical neurons at 28 days *in vitro*. Weakly synchronized network bursts in culture exhibited flat LFPs on all electrodes in the well (**Fig. 3A**), and the average PSD for LFP events decreases as a function of frequency without presenting a peak at any frequency.
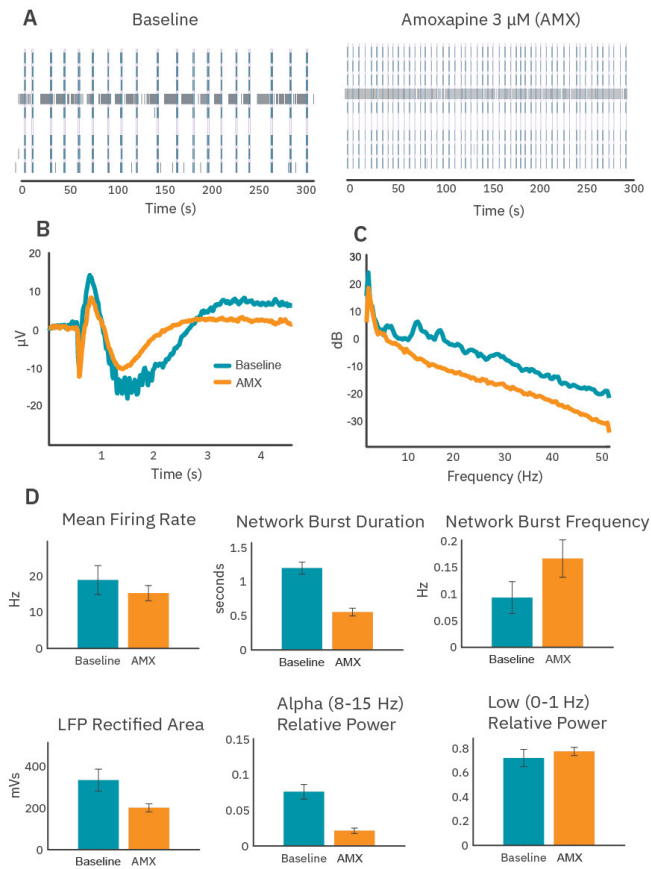
Coordinated, synchronized bursts exhibited slow deflections in voltage during network bursts. While the shape of an LFP was consistent on each electrode, electrodes in different parts of the well could have inverted shapes, different magnitudes, or little to no voltage deflection at all (**Fig. 3B**, middle). The average PSD reflects this increase in voltage deflections with a peak below 1 Hz (**Fig. 3B**). Cultures that contained oscillations within the network burst exhibited more complex LFPs with oscillations in voltage following an initial deflection. These oscillations in the low frequency signal (**Fig. 3C**) were very prominent and occurred with an average period of 0.127 seconds – a frequency of 7.86 Hz. The average PSD of LFP events in this well contains a peak near 7.86 Hz which is reflective of the presence of these oscillations in the LFP. This indicates that quantifying peaks in the PSD curves (both location and height of peak) may be an approach to characterize coordinated activity in neuronal cultures.

LFPs become more complex as neuronal cultures mature and develop

As cultures mature post-plating, significant changes can occur in the LFP shape and frequency content. A 96-well CytoView plate containing rat cortical neuron

Figure 5: Rat cortical neurons exhibit changes to bursting pattern, LFP shape, and LFP spectral content in response to amoxapine. (A) Network bursts (outlined in pink) become more frequent after addition of amoxapine. (B) Network burst-triggered LFPs are smaller and shorter, ripples are eliminated, and (C) spectral peaks in the alpha band and above are eliminated after amoxapine addition. (D) Although amoxapine produced a small decrease in mean firing rate, the impact on network burst frequency and duration, LFP shape, and power in low and alpha band frequencies are much more apparent.

cultures was monitored over 28 days using the Maestro Pro. At DIV 7, deflections in the LFP trace within network bursts are small in magnitude and brief. Total signal power increases over maturation and, by DIV 21, substantial peaks in alpha band power are identifiable (**Fig. 4A**). Large voltage deflections in LFPs, identifiable in the PSD by low frequency band power, appear relatively stable by as early as DIV14, whereas alpha band oscillations in the network burst-triggered LFPs first appear at DIV 16 and become more prevalent within wells after three weeks of maturation (**Fig. 4B**).

Amoxapine accelerates bursting and eliminates intraburst alpha band oscillations

After maturation, wells were dosed with 3µM amoxapine, a nonselective drug that primarily impacts serotonin and norepinephrine signaling. Amoxapine induced significant qualitative changes in the spiking activity (**Fig. 5A**), average LFP (**Fig. 5B**), and average PSD (**Fig. 5C**). Although amoxapine had only a small impact on the mean firing rate of the neurons in culture, the bursting organization changed substantially, with network bursts becoming shorter and more frequent (**Fig. 5D**).

Quantifiable changes in the LFP and PSD accompanied the changes in network burst dynamics. Average LFPs became shorter and smaller, and ripples were eliminated (**Fig. 5B**). Area under the curve of the rectified average LFPs and the power measured at low frequency both decreased (**Fig. 5D**), consistent with the decrease in network burst duration. In addition, power in the alpha band was effectively eliminated. In fact, all high frequency content in network burst-triggered LFPs was greatly diminished under amoxapine (**Fig. 5C**).

Conclusions

Neural signals can be filtered into high frequency spike traces and low frequency LFP traces and analyzed independently. During coordinated network activity, rat cortical cultures exhibit large voltage deflections in the LFP trace. Average power spectral density of these events can highlight features of the functional activity. For example, cultures with highly synchronous, regular network bursts display increased power in sub-1Hz frequencies, whereas cultures with strong oscillations display increased power at relevant frequencies. Also, LFP shape and spectral content change throughout maturation; as neural cultures mature, LFPs become larger in magnitude and may display higher frequency oscillations. Finally, LFP shape and spectral content can also be modified pharmacologically. Taken together, these results demonstrate that the quantification of LFP shape and spectral content provides a complementary technique for characterizing the functional activity of neuronal networks *in vitro*.

References

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