Multiwell optogenetic stimulation for MEA-based disease modeling and drug discovery

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Introduction

Microelectrode Arrays (MEAs) reveal neuronal network interactions by monitoring and manipulating electrical activity at both the single neuron and network scale. Applications include:

- Screening of drugs, gene edits, or toxins for influence on single cell and network activity
- "Disease-in-a-dish" models capturing phenotypic components of specific clinical conditions
- Induced pluripotent stem cells (iPSCs) enable human cell-based models of neural diseases

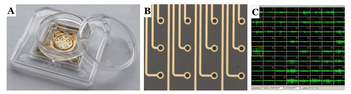


Fig. 1: (A) Single MEA culture well (B) A grid of 64 microelectrodes on the well bottom monitors overlying cell activity (C) Sample voltage recording traces from sixty-four electrode sites

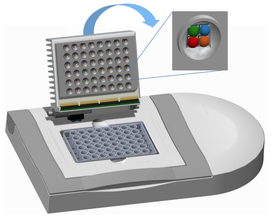
***Multiwell* MEAs enable high-throughput experimentation at reduced experimental time and cost.**

- Multiwell MEAs consist of multiple distinct culture wells on a single microplate, allowing for parallel experimentation with many experimental conditions, control cultures, and high 'N' counts.
- Axion's Maestro® system accommodates 12, 48, or 96 well plates, each with 768 microelectrodes distributed through the wells.

Fig. 2: (A, B) A 48-well MEA plate docks within (C) the Maestro system. (D) Spike-rate activity across 768 channels and 48 wells

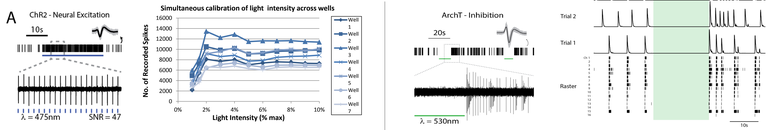
Optically stimulating multiwell plates allows for enhanced control and exploration of neuronal culture networks

- Existing electrical stimulation capabilities allow neural cultures to be perturbed during recordings for a more complete characterization of underlying network states.
- Optical stimulation of individual MEA wells provides enhanced capabilities for exploring / modulating cultured neural networks and characterizing disease states.
  - Cell-specific activation or inhibition of neuronal sub-types
  - Minimal stimulus artifacts during electrical recordings
  - Spatially uniform stimulus delivery across cultures
  - Tuning of activity levels and network states
  - Modulation of intracellular signaling / gene expression
  - Influence differentiating iPS cells



Multiwell light delivery device design

Initial device validation

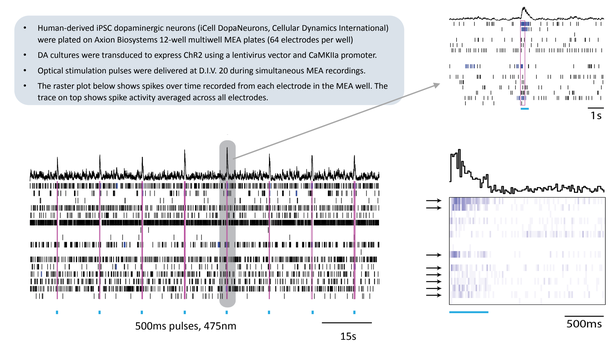


ChR2 mediated excitatory response: 10ms pulses of 475nm light stimulated ChR2⁺ cortical cultures

ArchT mediated inhibitory response: Spontaneous bursting in ArchT⁺ cultures was reliably silenced by continuous 530nm light.

Selective optical stimulation of human iPSC dopaminergic neurons

- Human-derived iPSC dopaminergic neurons (iCell DopaNeurons, Cellular Dynamics International) were plated on Axion Biosystems 12-well multiwell MEA plates (64 electrodes per well)
- DA cultures were transduced to express ChR2 using a lentivirus vector and CaMKIIa promoter.
- Optical stimulation pulses were delivered at D.I.V. 20 during simultaneous MEA recordings.
- The raster plot below shows spikes over time recorded from each electrode in the MEA well. The trace on top shows spike activity averaged across all electrodes.

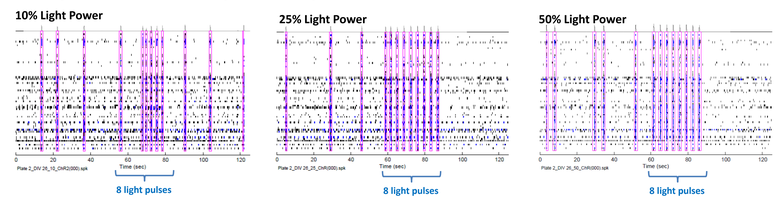


Zoom-in view of a DA culture response to a single optical stimulation pulse. Each optical pulse results in a network-wide burst within the MEA recording well.

Averaged response over eight stimulation pulses. Eight electrodes are shown to record light-evoked activity. This subset of light responsive cells from a larger recorded population is potentially the result of selective expression achieved using the CaMKIIa promoter, which targets excitatory cells.

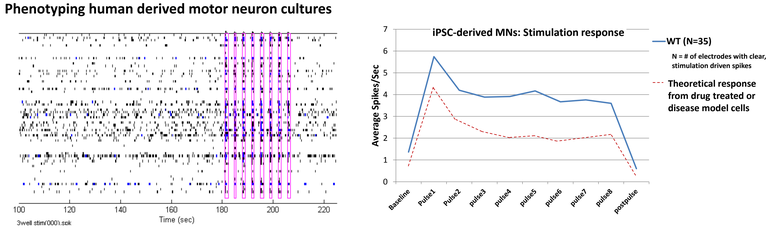
Phenotypic characterization of human iPSC-derived motor neurons

Pilot experiments to determine optical intensity thresholds



Stimulation test patterns (eight light pulses @ 500ms pulse width, 1.5s between pulses) were delivered to iPSC-derived motor neurons cultured within wells of an Axion 12-well MEA plate (64 electrodes per well). At 10% light power, not all optical pulses result in a network burst, and residual network firing existed post-stimulation. At higher light intensities, each optical pulse resulted in a network wide burst.

Phenotyping human derived motor neuron cultures



The same stimulation pattern was delivered at 50% intensity. A phenotypic profile was generated by quantifying the average spike rate in the spontaneous activity and after each optical pulse. Analysis of evoked activity provides an enhanced phenotypic characterization versus metrics of spontaneous activity alone. Enhanced phenotypic "fingerprinting" might be used in future testing to compare and differentiate wild type/control vs. disease model / drug treated cells.

Methods and device design

Optical design and testing

A multiwell optical stimulation device was developed to independently illuminate each well of a standard 48-well microplate. Toward this end, 192 independently addressable LEDs with center wavelengths encompassing the visible spectrum (475nm, 530nm, 612nm, 655nm) were laid out in forty-eight banks of four. An array of metallic reflectors concentrated and delivered light from each LED bank to the bottom surface of each MEA well. MEA plates were designed with an optically specialized lid, custom formulated white, opaque polymer walls for high specular reflection, and even diffusion of light within each MEA well. A heatsink with closed-loop, active cooling was designed to control LED board temperature. Electronics boards were designed to independently drive each of the 192 LEDs with 12-bit intensity resolution, updated every 100µs (10 kHz). Light delivery to the MEA surface was quantified using a silicon photodiode (FDS1010, ThorLabs) during 1ms test pulses at controlled currents. EMI noise and optoelectric artifact were investigated using Axion's AxIS software analysis system.

Human-derived motor neuron culture on MEA plates:

The differentiation method described in Maury et al, Nature Biotechnology 2014, was used to produce iPSC-derived motor neuron-like cultures. Dissociation and seeding of the embryoid bodies was done at day 14 of the differentiation protocol. Axion 12-well platinum MEA plates were prepared for cell plating. Using the lot specific dilution recommendation for Corning® Matrigel® hESC-qualified matrix, 40µL was slowly added to the center of each MEA well, taking care to maintain a 'micromass' within the central metal ring. Plates were then incubated for a minimum of 1h prior to use. The Matrigel® micromass was then aspirated gently to avoid touching the bottom of the plate or MEA electrode surface. Then, 40µL of the dissociated cell resuspension at 2.5 x10⁶ cells/mL was carefully added to the MEA well center in the same manner as the Matrigel®. These plates were immediately incubated for 1-2h before 500µL of motor neuron plating media was slowly added along the wall of each well. The plates were then returned to the incubator and left overnight. After 24h post-plating, an additional 500µL of fresh motor neuron growth media was added to each well in the same method. No media changes were done until 48-72h post-plating.

iCell DopaNeuron culture on MEA plates:

Cell were plated at 70k cells per well. Cells were transduced one week after plating to express ChR2 (lentivirus vector, CaMKII promoter.) MEA recordings were taken 20 days post-plating. Optical stimulation consisted of a 1m baseline recording followed by eight 500ms optical stimulation pulses (475nm, 25% intensity, 15s between pulse.)

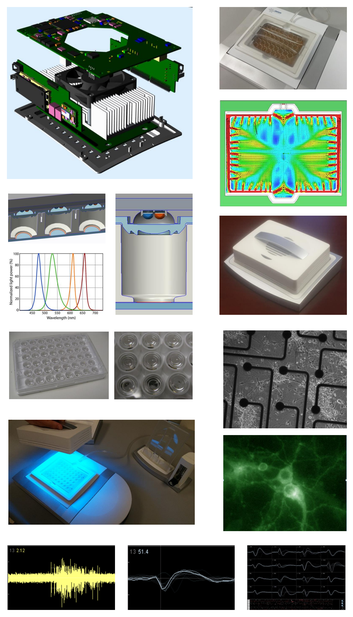
Rat cortical cell culture and viral transduction

Rat cortical neurons (QBM Cell Science) were cultured on 48-well MEAs. Neurons were transduced using one of two methods to express either Channelrhodopsin-2 (ChR2) or Archaerhodopsin (ArchT) along with a green fluorescent protein (UNC Vector Core) via AAV-9 viral vector and CAG promoter.

Transduction in the Tube:
ChR2 = rAAV9/C1-5-ChR2-GFP viral vector (3.9x10¹² viral molecules/mL) (Lot#AV4148C)
Rat cortical neurons (QBM Cell Science) were thawed, centrifuged, re-suspended in 37µL of Neurobasal media , and mixed with 3µL of ChR2 virus, yielding ~9.6*10⁹ viral particles in contact with 1.28x10⁶ neurons. Neurons were then plated at 160k cells per well (5µL drop size) into 24 wells of a white-walled 48-well MEA plate (Axion BioSystems).

Transduction in the Plate:
ArchT = rAAV9/CAG-ArchT-GFP viral vector (3x10¹² viral molecules/mL) (Lot#AV6221)
Rat cortical neurons were plated at 160,000 cells per well (5µL drop size) into 8 wells of a white-walled 48-well MEA plate. On day *in vitro* 14, the media was removed from all wells and replaced with 50µL each of serum free Neurobasal media containing 3µL of ArchT virus. The cells were incubated with the virus for 4h and then the media was replaced with complete Neurobasal media with B-27.

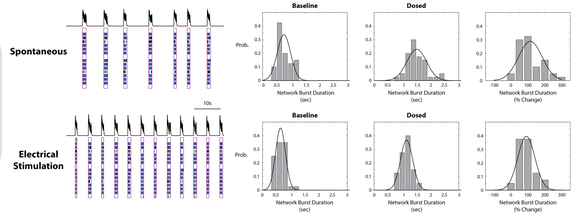
Cell Culture:
Neurons were cultured in Neurobasal media with B-27 for 30d with 50% media change every 3d. All experiments were conducted after 14d *in vitro* to allow networks to develop and spontaneous, synchronized bursting events to emerge. Optogenetic tests were conducted at least 14d post transduction to allow full expression of the opsin.



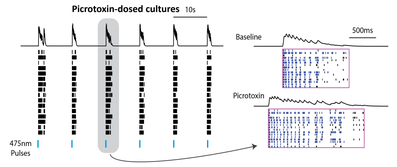
Enhancement of seizurogenic assays

To better assess anti-epileptic drugs and improve proconvulsant screening in drug safety, assays of seizurogenic neural activity must be improved. Quantification of network activity provides a multi-parameter phenotype for evaluating pro- or anti-convulsant compounds. In addition to the overall level of activity (mean firing rate), the organization of spikes into network-wide bursts of activity can be very sensitive to the addition of neuro-active compounds.

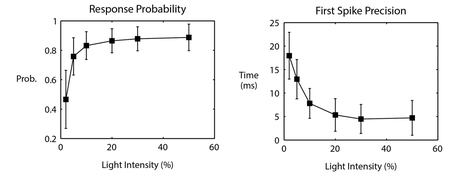
Electrical stimulation increases the reliability of the assay. Electrical stimulation was used to "pace" the network bursts across wells, leading to greater consistency across wells in the baseline and dosed (picrotoxin) condition, and increased sensitivity overall.



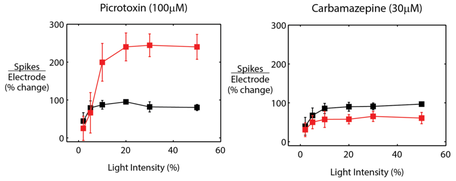
Advantages of optical stimulation. Optogenetic stimulation achieves the same benefits as electrical stimulation above, with a few additional advantages. Optical stimuli do not produce an artifact, so none of the neural response is obscured. This allows more accurate analysis of early portions of the response. Also, well-wide illumination ensures the stimulus is consistent from well-to-well, and not dependent on the proximity of cells to each electrode.



Optically evoked activity is reliable across wells (N=24 wells). The threshold light intensity, response probability (middle), and precision (right) were highly reliable. The lack of artifact with optogenetic stimulation enabled more accurate determination of spiking precision, as compared to electrical stimulation.



Optically evoked activity is sensitive to pro-convulsant and anti-epileptic compounds. When normalized to the highest stimulus intensity at baseline, the evoked response amplitude was highly sensitive to picrotoxin (middle) and carbamazepine (right), but not the vehicle control (left). Even illumination across wells produced more reliable results across wells, as compared to electrical stimulation.



Conclusion and future directions

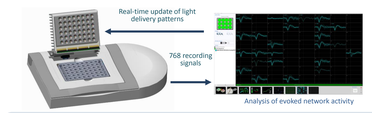
Conclusions

- Viral vectors, concentrations, and delivery techniques were efficiently optimized in parallel
- Human-derived iPSC networks were effectively modulated, with cell-type specific promoters
- Optogenetic stimulation provides enhanced metrics for evaluating drug response and mutation phenotypes in models of neural disease
- These findings demonstrate the potential of optically-integrated multiwell MEA systems to enable high-throughput drug screening and phenotypic modeling of neurological diseases.

Future directions and applications

- 96, 384 well architectures
- Chronic stimulation for control of network development
- Algorithms for automation and closed-loop control
- Application specific toolsets
- Pacing of cardiac cell cultures
- Modulation of intracellular signaling/gene expression
- Influence iPS cell differentiation

Closed-loop control of network states



Hardware and algorithms development to remove the operator from the "loop", enabling automated high-throughput experiments and closed-loop control of neural activity.