OVERCOMING LIMITATIONS OF ORGAN-ON-CHIP (OOC) TECHNOLOGIES TO ADVANCE THE CHARACTERIZATION AND MEDICAL MANAGEMENT OF CHEMICAL AND BIOLOGICAL (CB) THREATS

Recapitulating Complex Neuronal-glia Networks In An In Vitro System For Toxicant Characterization

Heather Enright Lawrence Livermore National Lab **Doris Lam** Lawrence Livermore National Lab **Aimy Sebastian** Lawrence Livermore National Lab **Jose Cadena** Lawrence Livermore National Lab **Chandra Bogguri** Lawrence Livermore National Lab **Nicholas Fischer** Lawrence Livermore National Lab

The neurological consequences and potential mitigation approaches for chemical and biological insults are often evaluated using simplified in vitro assays or in vivo in rodent models. While informative, translation of outcomes in these systems to the human condition is often poor. Therefore, recent efforts have focused on developing human-relevant in vitro systems that are designed to more closely recapitulate the function and microenvironment of the human brain. To more closely mirror the cellular complexity in the brain in vivo, we have developed a complex rat-based in vitro system (brain-on-chip, BOC) which incorporates glial cell types (e.g., astrocytes and oligodendrocyte precursor cells) in addition to cortical neurons on a multi-electrode array (MEA) device. Cultures were characterized using single cell RNA sequencing and immunostaining to profile the cell type and/or phenotype transcriptome, and to evaluate culture morphology and network maturity, respectively. Electrophysiology of neuronal networks was monitored using our embedded MEA. We are using both our rat and human-based (iPSC-derived neurons and astrocytes) BOC systems to evaluate chemicals of interest, such as fentanyl. Direct comparisons to rat in vivo exposures are also being conducted.

Using our approach, we have examined the neuroadaptive molecular and functional neural network changes following sub-chronic exposure to fentanyl (low, 0.01 mM; high, 10 mM). Sub-chronic fentanyl exposure resulted in a decline in neural (i.e., spiking) and network (i.e., bursting) activity, with diminished activity sustained for 5 days at the high dose condition. When treated with the mu-opioid receptor (MOR) antagonist CTOP and fentanyl, diminished activity was MOR-dependent in the low-dose condition but MOR-independent in the high-dose condition. These effects paralleled pre-and postsynaptic gene expression changes predominantly observed in the low-dose condition, while other biological processes (e.g., neurotransmitter synthesis, growth factor expression and cytokine signaling) were found altered in cultures exposed to the high dose. Ultimately, the creation and use of both rodent and human-based systems that mirror the complexity of the central nervous system in vivo will aid in understanding the neurological consequences of both chemical and biological insults, mixtures of insults and will aid in the development and optimization of countermeasures and therapeutics.

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