Development of an *in vitro* Focal Neuronal Injury Platform With Simultaneous Neural Recording and Conducting Polymer/Graphene Oxide Nanocomposite-mediated Electrochemical Sensing

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Statement of Purpose: The challenge of treating traumatic brain injury (TBI) lies in the complex pathobiological cascade following injury that includes induction of oxidative stress, excitotoxicity, and energy failure, that ultimately leads to the activation of apoptotic pathways and widespread cell death. To better understand the mechanisms underlying TBI, simplified models that allow precise measurement of multiple components of the injury cascade are required. We are addressing this need by developing an *in vitro* focal neuronal injury platform with integrated neurochemical sensing and neurophysiology capabilities. The device, consisting of a planar multielectrode device (MED) with conducting polymer (CP) coated microelectrodes, will enable real-time, simultaneous monitoring of cultured neuronal network (CNN) electrical and chemical activity after focal injury. Graphene oxide (GO) is incorporated in the CP to provide functional groups for anchoring glutamate oxidase (GlutOx) and a custom-synthesized superoxide dismutase mimic (SODm) to the electrodes for electrochemical sensing of glutamate and reactive oxygen species (ROS).

Methods: Dissociated rat hippocampal neurons were cultured within a custom polydimethylsiloxane (PDMS) culture barrier on the surface of planar MED multielectrode devices (AlphaMED, Osaka, Japan) with two 32-electrode grids separated by 10 cm (Figure 1a). The CNNs were matured for 22 d then submitted to a focal crush injury with a 4 cm^2 PDMS stamp. Recordings of the network activity were collected every 2-3 days throughout the course of the experiment, and daily for 7 d after the injury. CP films composed of poly(3,4-ethylenedioxythiophene) doped with graphene oxide nanosheets (PEDOT/GO) were deposited on platinum iridium (PI) microelectrodes or the constituent indium tin oxide (ITO) electrodes of the MED64 probe using potentiostatic coulometry.

Results: Development of CNN firing activity was monitored with extracellular recordings from the electrodes of the MED64 probe. By 22 d, most electrode channels were active (> 70%) and were participating in robust synchronous bursting behavior. Application of the focal injury ablated all cells within the damaged area, creating two isolated networks within the culture region. The firing rate and burst rate on each side of the dish immediately increased following the injury, peaking at approximately 24 h before declining steadily over the next week. The bursting behavior within the two isolated networks diverged, creating two unique bursting patterns (Figure 1b) that did not resynchronize over the course of the experiment.

The PEDOT/GO coating decreased the impedance and increased the charge storage capacity of the PI and ITO electrodes significantly, demonstrating its potential as a recording electrode material. As proof of concept, multiple proteins were successfully immobilized on the surface of the PEDOT/GO film using carbodiimide chemistry, indicating that GlutOx and the amine-functionalized SODm may also be crosslinked to the film to impart biosensing functionality. In addition, the PEDOT/GO-coated electrodes outperformed bare electrodes in a dopamine sensing assay, likely due to the film's favorable electrical properties, suggesting that glutamate and ROS sensing may be similarly augmented.

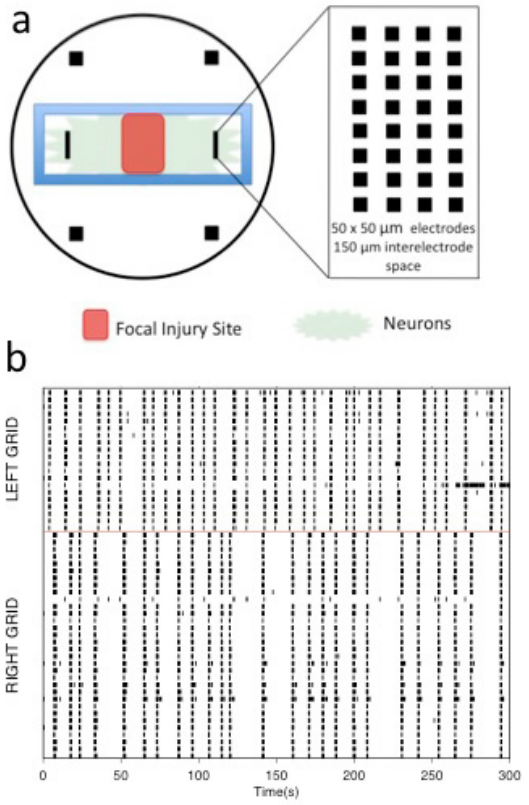

Figure 1. *In vitro* focal neuronal injury model. (a) Scheme summarizing the MED64 culture and damage platform. (b) Raster plot of neural activity on the left and right electrode grids after injury.

Conclusions: We have developed a novel *in vitro* model of focal neuronal injury that creates an exciting opportunity to study the dynamics of the injury cascade. After optimization of the CP-mediated electrochemical sensing capabilities of the platform, future studies will investigate the relationship between altered firing activity, excitotoxic glutamate activity, and oxidative stress after injury. Various parameters in our platform (injury severity, cell type, biosensor target) can be tuned, making the device a powerful research tool for elucidating the pathology of neuronal injury and developing future therapies.