Learn More About

Neuroinflammation Research

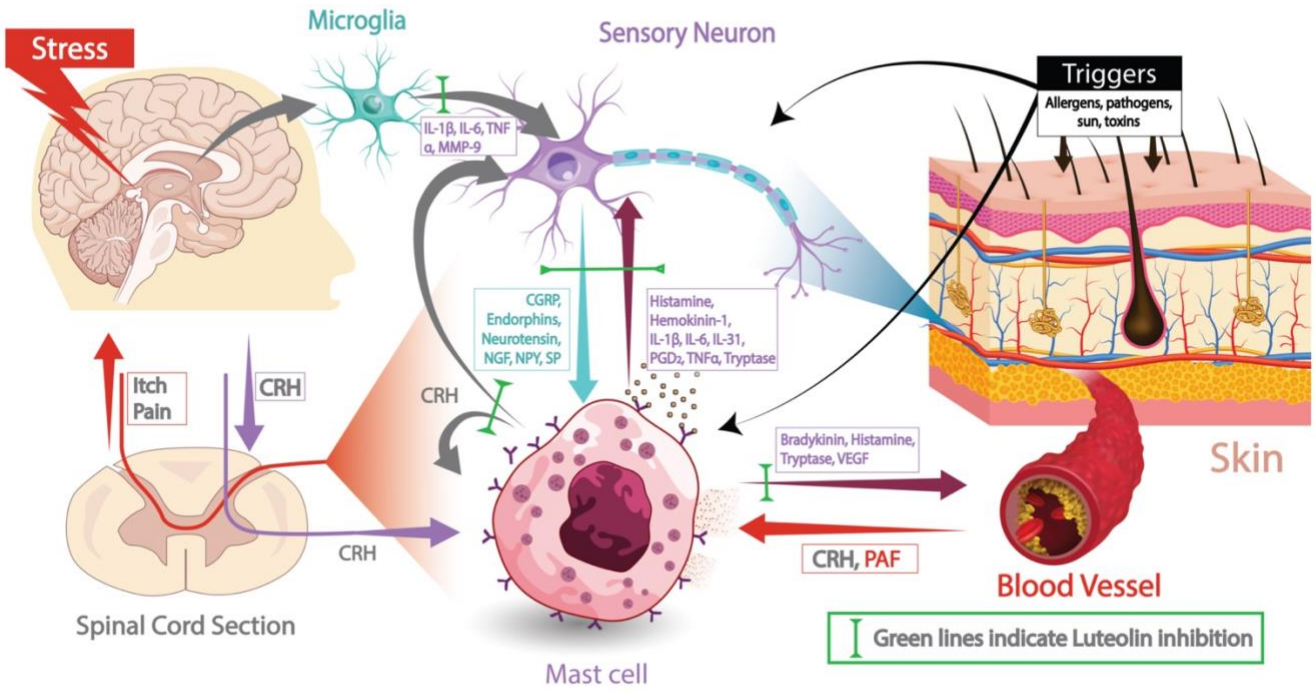

Figure 1. Diagrammatic representation of the proposed interactions involved in mast cell-microglia-neuron communications. Environmental, pathogenic and toxic triggers can damage central or peripheral neurons directly or via activation of mast cells and microglia that secrete neurotoxic molecules. These processes can be worsened by molecules such as corticotropin releasing hormone (CRH), released due to various stressful conditions, or the cytokine IL-33 released from other immune cells. The green lines indicate the pathways where natural flavonoids are expected to exert inhibitory/beneficial actions.

Research Strategy & Feasibility

Develop a human induced pluripotent stem cell (hiPSC) organoid brain-on-a-chip disease surrogate, using endothelial cells, mast cells microglia and neurons from patients and controls, to investigate pathogenetic mechanisms, especially the role of environmental toxins and stress, as well as screen potential inhibitors that can quickly translate into effective treatments.

We will assess neuronal health following stimulation of hiPSC-derived neurons co-cultured with endothelial cells, mast cells and microglial cells using the Axion Maestro Edge Microelectrode Array (MEA) System (Axion Biosystems, Atlanta, GA) (Fig. 2) coupled with the TriaLink microfluidic system (NETRI, Lyon, France) to create a human organoid brain-on-a-chip model. Using this human organoid system, we will investigate the effect of a single or combination of triggers to stimulate the release of neurotoxic molecules assayed by RNA-seq and enzyme-linked immunosorbent assay (ELISA) focusing on inflammation, neuronal connectivity and vascular integrity (Fig. 2). Human organoids composed of iPSC have been used to investigate some neurologic disorders and can also be used to screen for potential treatments.

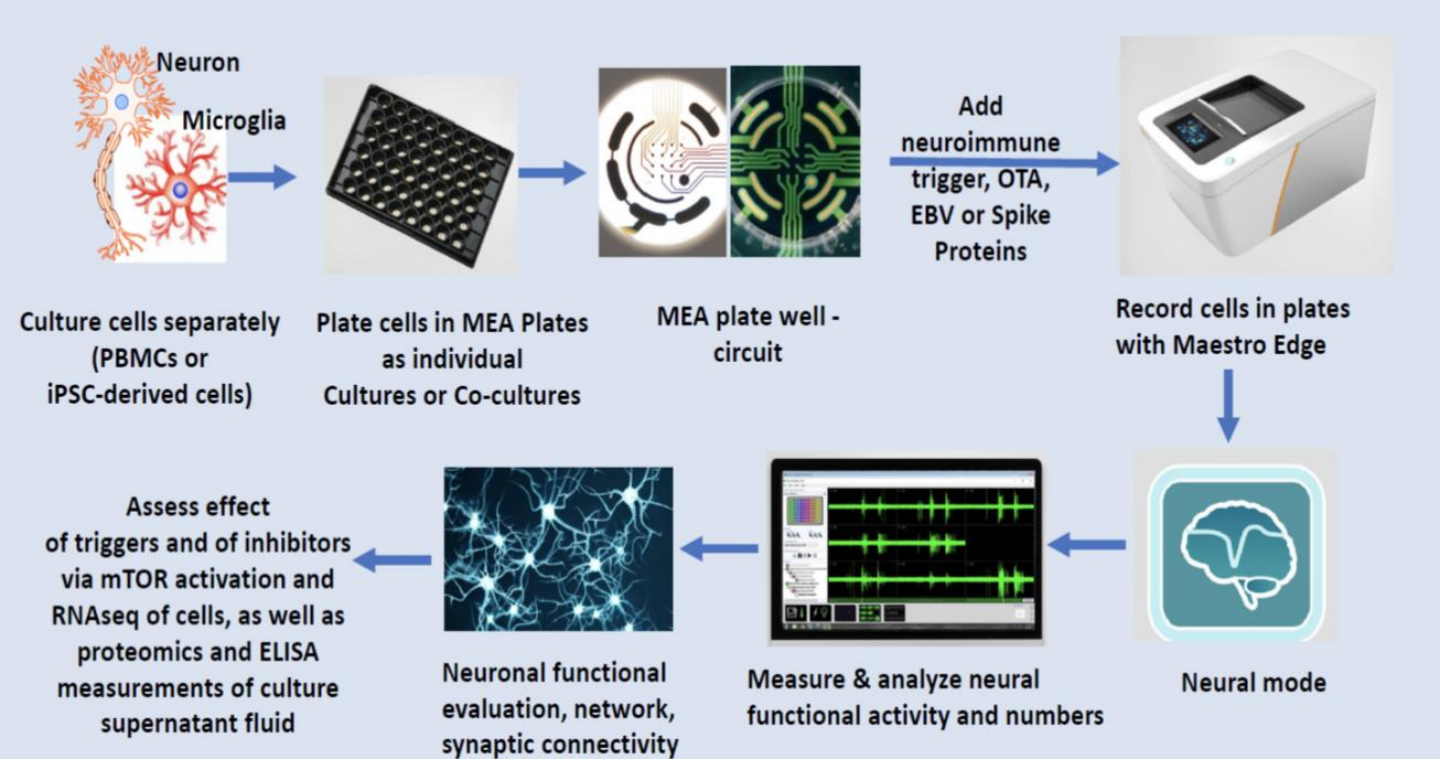

Figure 2. Diagrammatic representation of the *in vitro* organoid MEA system.

The Axion Maestro Edge system to analyze neuronal integrity and functional network in vitro is a benchtop self-contained 37^0C incubator with controlled temperature, humidity and O2 supply to maintain cell health and viability outside. The MEA Viability module allows for the simultaneous tracking of the viability of neuronal cultures while collecting electrical data to rule out whether the disruption in electrical activity is due to a disconnection/damage in the network alone or due to loss of cell viability in the culture. The Axion MaESTRO Edge is coupled with the NETRI NeoBento TriaLink MEA EDGE (Fig. 3), capable of high throughput microfluidic device permitting the study of the effects of different cell types on cultured neurons (Fig. 3). Using the microfluidic device allows the study of neural innervation and interaction with other cell types. Our preliminary results show the differentiation of hiPSCs into neurons in vitro (Fig. 4).

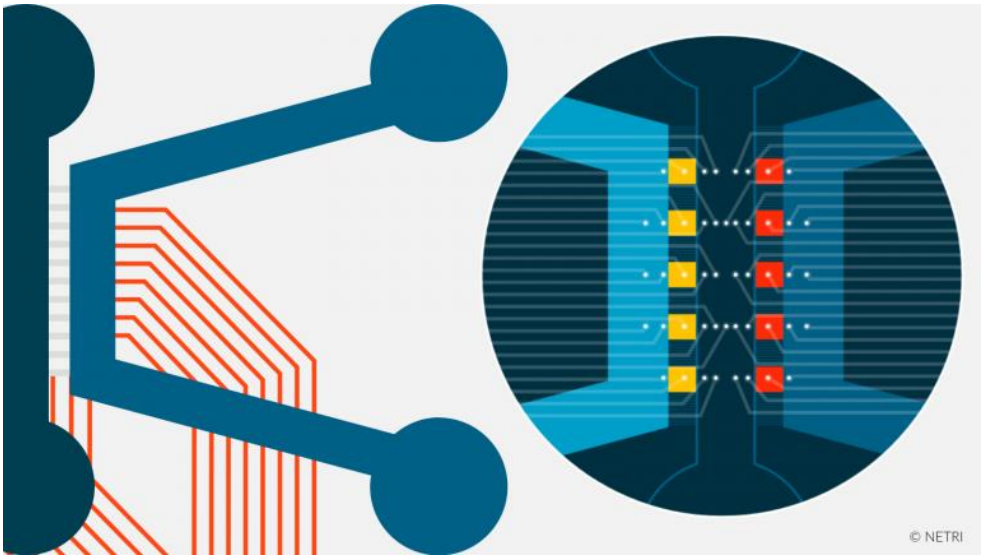

Figure 3. The TriaLinkTM Shift MEA microfluidic NeoBentoTM microchannels and electrophysiology technologies are combined in a single microfluidic chip to recreate physiological relevant cell populations integrated on a custom Axion Biosystems MEA and monitor their functional activity in addition to the central MEA edge with 336 electrodes to monitor neuron electrophysiological activity over time.

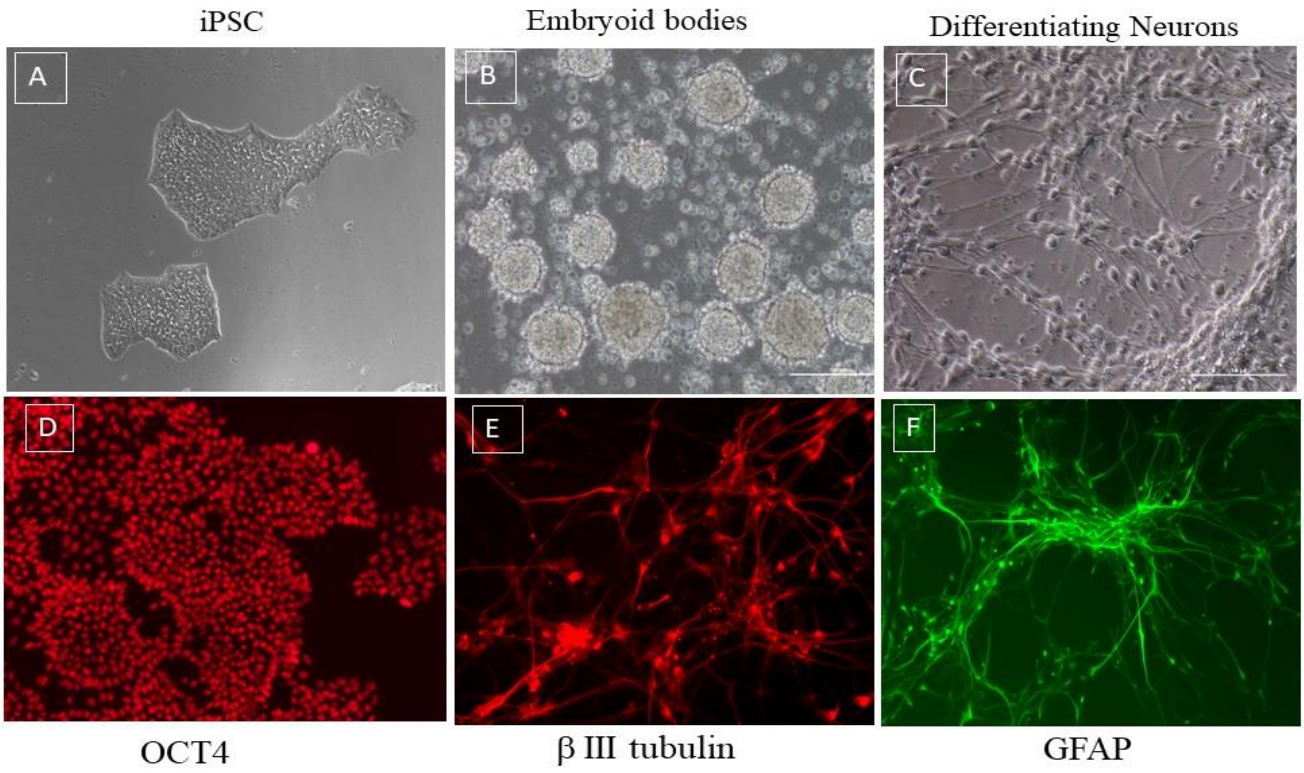

Figure 4. Photomicrographs of the various stages during differentiation of hiPSC into neurons. Upper Panel: (A) Undifferentiated iPSC, (B) embryoid bodies or cell aggregates in culture, and (C) differentiating neural progenitor cells (NPC). Lower panel: Immunostaining of (D) undifferentiated hiPSCs with pluripotent marker OCT4, (E) neurons with β III tubulin, and (F) glial cells with astrocyte marker glial fibrillary acidic protein (GFAP). Magnification 20X.

Firing of neurons in response to molecules released from the activated satellite cells is measured via recording of single or network activity of the neurons, while the health of neurons is measured via impedance (Fig. 5). The culture medium can be analyzed by proteomics and ELISA, while the actual cells can be analyzed by RNA-seq.

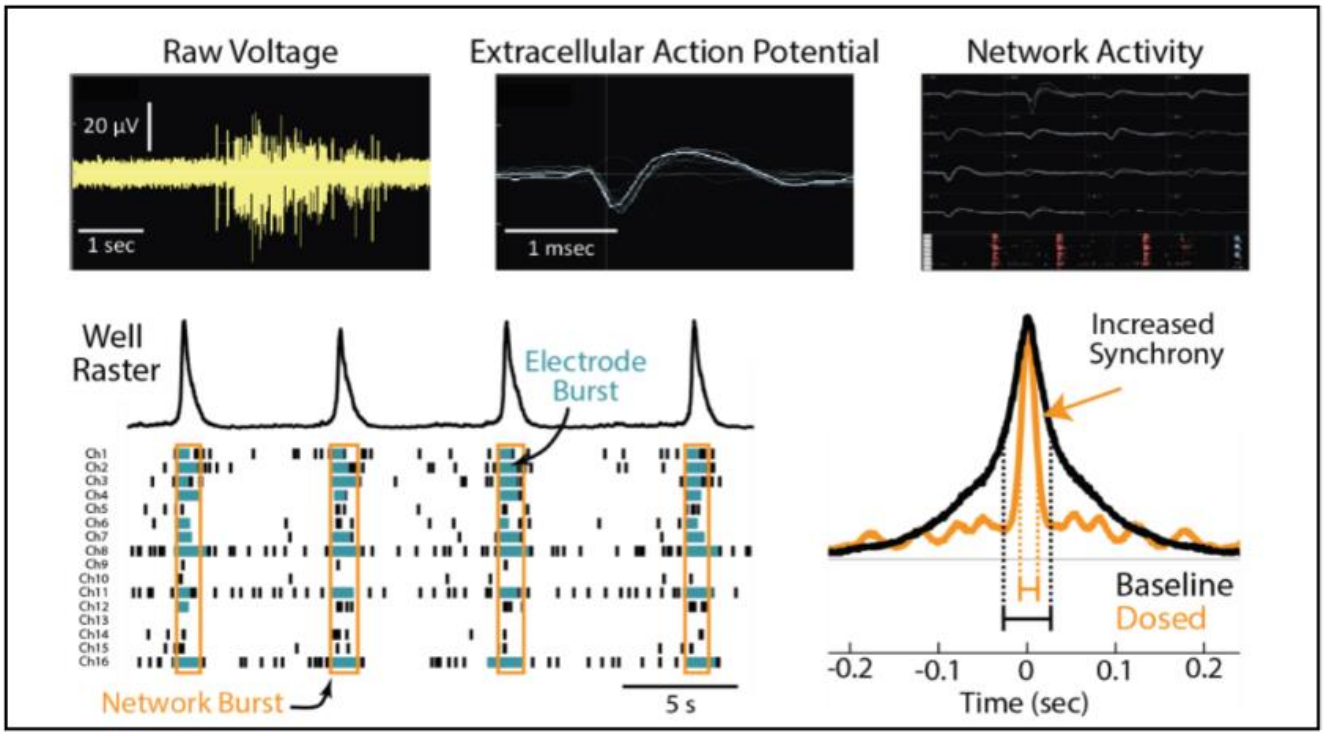

Figure 5. Representative recordings and graphs showing neuronal activity. It is critical to note that Axion can record individual neuronal firing, as well as neuronal bursts and synchronized firing which indicated healthy neuronal connectivity.