Biological Unknown:

The concept of Neural Plasticity, the reorganization of a neural circuit to enhance adaptation to alterations of informational input and transmission, has recently absorbed an increasing amount of attention. It is known that ability for neurons to update their spatial location, to increase or decrease their communication via synaptic connection with other neurons, is essential to the concept of learning and storing knowledge. Details of potential that causes this dismantling and regeneration of neural structure is still unknown. Conceptualizing the stimulus that induces this behavior would result in increasing utility of this process to treat a wide variety of disorders caused by nervous system misalignment. These include Depression, Stroke victims, and even people with sensory malfunctions such as the blind and deaf [5].

Biological Question:

Does changing the potential for information in input stimulus promote neuroplasticity in mouse cortical neurons?

Model:

Primary Cortical Neurons will be extracted from wild type mice. In addition, a hippocampal brain slice will also be extracted. Cells and the brain slice will be organized into a custom microfluidic culture depicted []. Chambers 1-3 will contain cells stimulated by electrodes. Neurons in chambers 1-3 will be grown to make connections to neurons in chamber 4. Chamber 4 will be populated with a network of unmanipulated primary rat cortical neurons. Cell 5 will house a hippocampal brain slice that has grown to make connections to neurons in cell 4. (The purpose of the brain slice in chamber 5 is to give the signals transmitting through subject neurons a place to go. This is to ensure signal transmission through subject neurons is not affected by a lack of a receiver) [6]

Research Plan:

- Optogenetic techniques will be used to stimulate multiple rat neurons at once. Channelrhodopisn ChR2 will be introduced into neurons contained within chambers 1-3. Introduction ChR2 into the subject neurons be accomplished by the injection of AAV2-ChR2V (adeno-associated virus binded to ChR2) into the cells [1] . Neurons containing the ChR2 Opsin will react to light, with a wavelength of 472nm, by structurally reconfiguring to allow the flux of cations into the neuron [2]. This will effectively depolarize each neuron and induce an action potential. Neurons in cells 1-3 will be optically isolated, surrounded by blacked out walls. There will be 3 different laser sources for each chamber introducing pulses of 473nm blue light. These sources will be pulsing at different frequencies. In chamber one, the laser will supply pulses at a constant frequency. In chamber two, the associated laser will provide stimulus with random frequency pulses. In chamber three, the pulse frequency will be varied but in a systematic manner (slightly increasing and decreasing the rate of the applied pulses to mimic variation in sensory input that a mouse would experiences in sitsu). The variation in the

rate of stimulation will mimic a source of input that contains no information, infinite information, and an appropriate amount of information in chambers 1-3 respectively [4].

- Neurons within cell 4, will be loaded with single wavelength Rhod-2 Calcium indicator . The Rhod-2 in the cytosol of each neuron, will bind to the influx of Ca2+ that occurs to induce the exocytosis of synaptic vesicles containing neurotransmitter. A fourth laser will excite activated Rhod-2 (Rhod-2 bounded to Ca2+) by shining 560 nm light on the neurons in chamber 4. Upon excitation, the activated Rhod-2 will then emit a 580 nm wavelength. The intensities of the fluoresces emitted by Rhod-2 will be captured using microscopy cameras. Ca2+ influx is a product of an action potential and therefore an active synapse. An increase in active synapses should increase the fluoresce intensity, initiated by an activate Rhod-2, in the region of chamber four where more active synapses are present [3].
- Upon implementation of the Rhod-2 into the neurons of chamber 4, lasers stimulating chambers 1-3 will fire at the same constant rate. During this initial test, the fluoresce of Rhod-2 within neural synapse will be measured and in three sections of chamber 4 [7]. The three regions will be equally divided and will correspond to the regions of chamber 4 that chambers 1-3 interface with.
- The simulation frequencies of lasers 1-3 will then behave has described in bullet point 1. After the varied stimulation testing for an extended period, another measurement of intensity will be recorded and analyzed in those same three regions of chamber 4. Intensities from synapses derived for each third of chamber 4 will be normalized with intensities calculated from the initial test with all lasers firing at the same frequency (Florescence measurement in region 1 = [Intensity post varied stimulation in region 1] / [Intensity of constant rate of stimulation in region 1]) .
- If the fluorescence of Rhod-2 in synapses of neurons residing in the segregated regions of chamber 4 changes (indicating a change in influx of Ca2+ and therefore indicating a change in the activity associated with that synapse) , it could indicate a dependency of informational potential on Neural plasticity.
- Ex: If neurons in region 1 (constant stimulus; no information potential) show less fluoresce in synapses when compared to region three (calculated stimulus rate changes; appropriate amount of information potential) then it may be postulated that informational potential induces neural potentiation.

References

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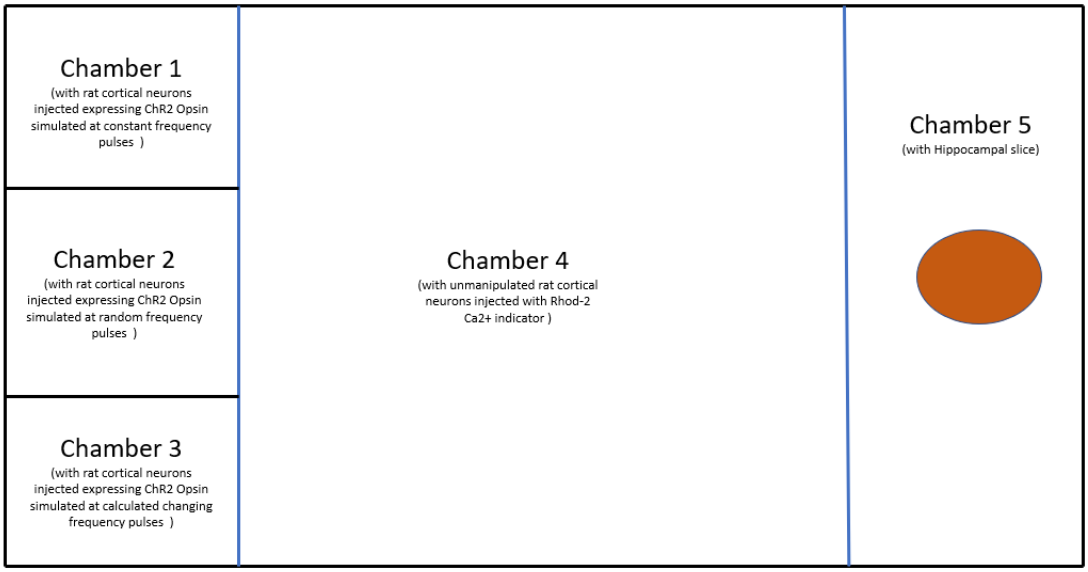
[5] DeMarse TB, Pan L, Alagapan S, Brewer GJ, Wheeler BC. Feed-Forward Propagation of Temporal and Rate Information between Cortical Populations during Coherent Activation in Engineered In Vitro Networks. Front Neural Circuits. 2016 Apr 22;10:32. doi: 10.3389/fncir.2016.00032. PMID: 27147977; PMCID: PMC4840215.

[6] Layout of Microfluidic Chamber



[7] Microfluidic chamber with parsed regions of chamber 4 indicated.