Engineering memory augmentation through optogenetic stimulation following stroke

Introduction: Memory and cognition are cornerstones of the human experience, but a comprehensive understanding of the complex interactions regarding memory encoding, consolidation, and retrieval remains elusive[1]. One major difficulty in elucidating the functions of these networks has been the brain's immense interconnectivity; a perturbation in a single network can have far reaching effects[2]. With more sophisticated analytical techniques that assess connectivity and information flow between brain areas, and novel technologies such as optogenetics, we are now able to dissect the functional networks underlying memory formation more precisely than ever before.

Episodic memory is encoded through the cooperation of multiple brain regions, primarily hippocampal and parahippocampal areas. Ischemic stroke often impairs memory and cognition despite the absence of direct lesion to the hippocampus[3] and has been used as a tool to interrogate spatial learning[4]. The underlying neurophysiological mechanisms of the cognitive deficits following injury are poorly understood. Here, I propose to use optogenetics, a powerful tool to modulate neuronal networks, to assess the capabilities and flexibility of these functional networks.

Optogenetics allows high spatial and temporal control, cell-type specific manipulation, and simultaneous recording and stimulation by virally transducing neurons to be sensitive to light stimulation. Utilizing optogenetics will allow us to manipulate network activity during memory formation in real-time and observe the effects with uninterrupted electrophysiology.

Proposed Research: In order to gain a deeper understanding of the underlying electrophysiology of memory encoding following stroke, I will perturb memory formation in a rat model using focal injury remote to the hippocampus and use targeted real-time optogenetic stimulation to enhance and induce electrophysiological features related to memory encoding and measure behavioral outcomes. There are two candidate features that I will be manipulating in this proposal. Sharp wave associated ripples (SPW-Rs) are known to encode memories, and phase-amplitude coupling (PAC) is a general framework to view information flow and connectivity between brain regions.

Aim 1: Optogenetically lengthen SPW-Rs following stroke to augment memory encoding. SPW-Rs are high frequency oscillations in the hippocampus related to memory encoding[5]. My previous work has shown that the decrease in memory function following stroke is correlated with shortened SPW-Rs[6]. Recent work has shown that naturally occurring long-duration SPW-Rs are associated with better memory performance, and that lengthening short-SPW-Rs optogenetically increases performance and recruits more diverse neuronal assemblies[7]. I hypothesize that by prolonging SPW-Rs following stroke, the more diverse neuronal assemblies recruited may project to a wider set of potentially healthy cortical tissue, augmenting memory encoding. To test this, I will implement a closed-loop decoding algorithm to detect the occurrence of SPW-Rs in real-time during a behavioral task and use optogenetic stimulation to prolong SPW-Rs following stroke (see methods). I will quantify any change in memory encoding due to stimulation using both electrophysiology recordings during stimulation and behavioral metrics.

Aim 2: Optogenetically induce theta-gamma coupling following stroke to augment memory encoding. PAC is a method of measuring the interaction of local oscillations between brain regions[8]. The hippocampus has long been shown to coordinate function with theta (3-7 Hz)-gamma (30-60 Hz) coupling[9]. Recently I showed that theta-gamma coupling between the hippocampus and sensorimotor cortex in healthy rats breaks down following a cortical ischemic

stroke[6]. Furthermore, our lab has shown for the first time that PAC can be induced between two distant brain regions using optogenetic stimulation[10]. I hypothesize that optogenetically induced PAC will increase the relative potentiation between the two brain regions leading to an increase in communication and augmenting memory encoding. To test this, I will optogenetically stimulate the cortex and hippocampus simultaneously during memory demanding periods, inducing coupling between theta in the hippocampus and gamma in the cortex. I will quantify any change in memory encoding due to stimulation using both electrophysiology recordings during stimulation and behavioral metrics.

Methods for both aims: I will quantify the effects of manipulation using behavior and electrophysiology recordings during three memory demanding tasks: the M-maze test, cheese board test, and delayed non-match to sample test[11]. These tests utilize both egocentric and allocentric learning and vary memory demand by changing the inter-trial delays. Following training I will virally transfect rats with AAV5-CaMKIIa-hChR2(H134R)-EYFP —an optogenetic virus which encodes an excitatory opsin expressed in excitatory neurons— in the sensorimotor cortex and hippocampus to modulate excitation. I will separate control and stroke groups, performing distal middle cerebral artery occlusion in the left hemisphere of the stroke group. To allow for optical stimulation at the site of recording, I will implant four linear silicon μLED optrodes. I will place the optrodes such that recordings from cortex and hippocampus are captured simultaneously from both hemispheres. Rats will recover for one month post-operatively. Following recovery, I will split both stroke and control groups into three treatment groups with cohorts of ten rats each: no treatment, event-related stimulation, and random stimulation for a negative control. I will test for one month with optogenetic stimulation, collecting simultaneous recordings from the cortex and hippocampus and motion capture to quantify behavioral outcomes.

Expected challenges: The effect remote injury has on the electrophysiology of the hippocampus is not well understood, so predicting how memory encoding networks will react to optogenetic stimulation following stroke is difficult. If optogenetic stimulation fails to result in a change in behavior, electrical stimulation will be used. If neither stimulation modality elicits a behavioral response, the electrophysiological data will still be a valuable resource in looking at why the change in the features of interest did not illicit a response.

Intellectual Merit: SPW-Rs are a well-documented feature of memory encoding but modifying the characteristics of SPW-Rs with optogenetics has only recently begun to be explored. This work will be the first to attempt to modify SPW-Rs in a disease model to assess the potential for functional treatments.

Our group is the first to show that PAC can be induced through optogenetic stimulation[10].This work will be the first to use induced PAC to affect behavior, and to assess its ability to recover function in a disease model. Furthermore, it is translatable because PAC has been observed in multiple brain areas associated with disease phenotypes, such as Parkinson's in the basal ganglia[12].

Broader Impact: The precise neuromodulation achieved through optogenetic stimulation has the potential to fundamentally change the way we approach treatments to neurological disorders. The brain's vast interconnectivity necessitates a more thorough understanding of our ability to engineer communication between brain regions. This work will attempt to change how two brain regions communicate following injury, which is relevant to a multitude of neural disorders besides stroke such as traumatic brain injury and cerebral palsy. The insights from this project

will have a profound impact on the development of future therapies for a wide range of neurological disorders.

References:

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