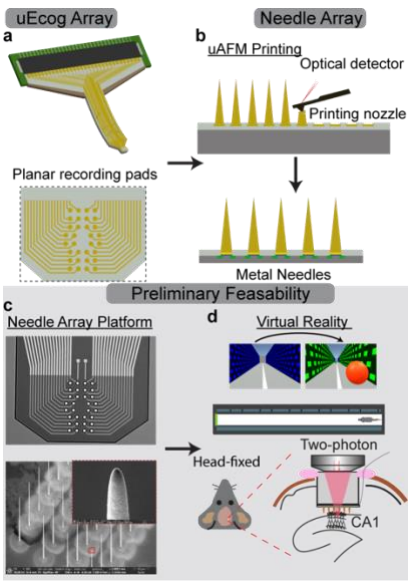
Graduate Research Plan Statement

Background: How do neurons in our brain integrate the myriad of distinct synaptic streams to form a memory? Although much work has investigated the biophysics of this question in acute brain slices *in vitro*, an outstanding challenge remains: mapping how behaviorally relevant features are bound and subsequently stored in *awake behaving animals*. A growing body of experimental and theoretical work has demonstrated that elaborate dendritic branching endows individual neurons their computational power[1]. Furthermore, the latest hypotheses for memory formation suggests that feature binding is strongly tied to somatic bodies and their dendritic branches[2]. A striking example of this computation is proposed to occur in the CA1 region of the hippocampus during active environmental exploration. During exploration, active regenerative dendritic events amplify somatic output and lead to synaptic potentiation, forming a cellular correlate of spatial memory[3]. Furthermore, during rest and immobility, the hippocampus undergoes short-bursts of high-frequency oscillations called sharp-wave ripples (SWRs)–a population correlate of *memory replay*, which is thought to reinforce previously potentiated synapses[4]. However, the underlying subcellular temporal and spatial activity profile during the SWR remains unclear. Disentangling the interplay between somato-dendritic coding and SWR can lead to transformative impacts in our understanding of single neuron computation - critical for neuromorphic AI based applications, and the genesis of spatial memory encoding in the CA1 - critical for health and disease. *To achieve this, we must overcome an incredible challenge: concomitant mapping of dendritic dynamics, somatic population coding, and sharp-wave ripples in awake behaving animals*. At present SWRs can only be measured electrically due to the nature of their temporal structure, while mapping dendritic dynamics requires high-resolution two-photon imaging. Moreover, combining two-photon imaging and high-resolution extracellular electrophysiology in the hippocampus is currently infeasible due to its sub-cortical location. *Thus, there is an urgent need for suitable tools and methods to perform simultaneous electrical and optical recordings in deep-subcortical structures.*

To address this challenge, *I propose a novel transparent nanoelectrode (Needle) array capable of mapping dendritic electrical activity and SWRs underlying memory formation.* Through this, I aim to simultaneously map large-scale somato-dendritic dynamics, both electrically and optically. Coupled with a virtual reality system, these nanoneedles will enable, for the first time, registry of multi-site single-unit activity, SWR events, and optical imaging of somato-dendritic coupling in awake behaving mice.

Previous Work: Preliminary electrophysiology and optical imaging studies suggest that hippocampal CA1 neurons can rapidly alter their somatic code based on environmental differences in virtual reality and that dendritic non-linearities play a critical role in the genesis of this memory formation. The Nano-Neurotechnology lab has developed a range of nanoelectrode methods compatible with two-photon imaging including nanopipette electrophysiology to interrogate extracellular and intracellular dynamics *in vivo* and *in vitro*[5]. Taken together, this allows me an opportunity to further investigate the finer processes of hippocampal neurons in the context of this proposal.



Aim 1: Development of Nanoneedle array for *in vivo* implantation. The needle array will be designed for both chronic and acute implantation. I will fabricate and characterize the nanoneedles using cutting-edge fabrication methods at Birck Nanotechnology Center at Purdue University. *The foundation of the 3D needle array will be based on a flexible uEcog platform that I have played a key part in developing in our lab **(Fig. 1a),** and nanoscale 3D electroplating using AFM technology **(Fig. 1b)***[6]. The platform will consist of a thin-film Parylene base, and gold-electrodes integrated onto a glass cannula, allowing for a soft and transparent interface to the CA1 region of the hippocampus **(Fig. 1c, top)**. After the array base is completed, the needles will be directly printed with submicron resolution using AFM based nanoscale 3D metal electroplating **(Fig. 1c, bottom)**. This direct-printing method of needles is a highly innovative approach for building scalable and tailorable arrays of nanoscale recording sites. My overall design boasts of 32 recording sites at present and

can be scaled up to 128. This platform by virtue of its 3D structure will facilitate mapping of somatic, dendritic, and SWR activity across the surface of the CA1. This device will be integrated with custom backend CMOS electronics to record neural data. I will characterize the impedance, flexibility, and insertion of the Needle array before hippocampal implantation.

Aim 2: Simultaneous recording of soma-dendritic activity during memory formation

After realizing the Needle array, I will implant the probe into the surface of the mouse hippocampus as they navigate a custom built-virtual reality (VR) environment that I have developed this past year. The nanoneedles will allow for recording hippocampal cellular and SWR electrical activity at an unprecedented resolution. The transparent nature of the array will allow simultaneous two-photon calcium imaging of population level coding. I will train animals in a VR setting to associate environmental cues with reward, thus forming memories in real-time that I will track electrically and optically (Fig. 1d). Optical imaging through the Needle array will reveal the role of somato-dendritic coupling across a large population. Concurrently, the high-density needle electrodes will reveal the relationship between single and multi-unit somato-dendritic computations and SWR activity during active memory formation.

Expectations and Pitfalls: Realization of the flexible Needle Array will enable 1) high signal to noise ratio (SNR) electrical and optical recordings, 2) mapping somato-dendritic electrophysiology at a fine-scale resolution, 3) soft 3D neural probes for tissue interfacing, and 4) the ability for chronic and acute implantation in awake behaving animals. If AFM printing does not yield desirable results (impedance is too high for example) 2-photon polymerization [cite] or high-aspect etching of highly doped silicon to define the needle array will be employed. With the latter, silicon pillars realized using a combination of isotropic and anisotropic etching will be thermally oxidized to refine the tips. Selective thin-metal deposition along with passivation will still allow for conductive recording of somato-dendritic electrical activity. Careful construction of the base electrode pads and the use of red-shifted IR imaging wavelengths will help render silicon transparent. Additionally, I will examine the use of PEDOT:PSS polymer coatings [cite] for impedance tuning and plasmonic materials to maximize transparency across electrode configurations. After implantation, I expect emergence of unique population dynamics during environmental traversal will be simultaneously registered on the needles and two-photon imaging plane. We will gain insight on how SWRs recruit and maintain dendritic populations and if these populations are altered in the presence of different virtual environments. *Using machine-learning source extraction algorithms, I have developed, I will analyze the link between SWRs and somato-dendritic computations across time.*

Intellectual Merit: *To date, simultaneous mapping of SWR consolidation and somato-dendritic coupling has remained out of reach*. This is in part due to a technological bottleneck for feasible optical and electrical interrogation in the hippocampus - a deep subcortical structure. With my experience in nanofabrication and device development, **I will fill this gap** by realizing a scalable platform that combines both modalities. The development of my multiscale pipeline will also present deeper insight in dendritic computations and memory consolidations. Creating a multifaceted tool for the neuroscience community will enable a more fundamental understanding of working memory.

Broader Impacts: I will work to increase the importance of structured multifaceted bioelectronics through 1) publications directed towards the neuroengineering and neuroscience community and 2) discussions with other disciplines researching biophysical mechanisms of dendritic computations. Additionally, I will share this technology with Dr. Laura Ewell, out collaborator working on understanding how dendrites and SWRs are affected under an epileptic disease model. In this collaborative effort we can work towards a more fundamental understanding of neural syntax in working memory and advance the field in understanding neurological disorders.

References.

[1]G. J. Stuart and N. Spruston, *Nature Neuroscience*, 2015, **18**, 1713-1721.[2]M. E. Sheffield and D. A. Dombeck, *Current Opinion in Neurobiology*, 2019, **54**, 1-11.[3]K. C. Bittner *et al.*, *Science*, 2017, **357**, 1033-1036.[4]G. Buzsáki, *Hippocampus*, 2015, **25**, 1073-1188.[5]K. Jayant *et al.*, *Cell Reports*, 2019, **26**, 266-278.e265.[6]N. Alsharif *et al.*, *Small*, 2018, **14**, 1800162.