In vivo time-lapse imaging of Arc expression in freely behaving mice

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Introduction: Understanding neural network dynamics is key to identifying the biological basis of memory. This requires methods for simultaneously recording cellular activity of multiple neurons in freely behaving animals; current methods which observe neurons singly are insufficient. To meet the need for such experimental technologies, I propose to use my expertise in optics to develop a portable microendoscope (PME) for epifluorescence microscopy. I will then use the PME to study hippocampal expression of Arc (Activity Regulated Cytoskeleton-associated protein) [1], an immediate early gene (IEG) associated with learning and memory.

Arc is the most promising IEG to study in memory research due to its low basal expression, its regulation of synaptic plasticity, and its necessity for memory formation [2]. Most importantly, Arc expression has been shown to be upregulated in 40% of CA1 hippocampal neuronal dendrites immediately following patterned neuronal activity in response to a novel stimulus, peaking approximately 45 minutes post-stimulation, and slowly relaxing to basal levels over several hours [3]. Additionally, recent *in vitro* studies indicate that repeated exposures subsequently downregulate Arc expression in as much as 60% of those neurons [4]. The details of the complex regulation and function of Arc remain unknown. I believe that animal age and time of initial exploration contribute to the regulation of Arc expression. Consequently, I believe Arc expression must be studied in freely behaving individual animals. *In vitro* studies which explore Arc expression outside the context of behavior and head-fixed *in vivo* studies such as [5] which do not allow free exploratory behavior I believe are insufficient.

Traditionally neuroscience has attracted a wide array of researchers including physicists, psychologists, computer scientists, and electrical engineers, who have each advanced the field by contributing unique perspective. Physicists most notably advanced whole-brain functional imaging with the advent of nuclear magnetic resonance-based technologies such as fMRI. Here I propose to continue this tradition of collaboration between neuroscience and physics, by employing recent advances in optics to characterize the dynamics of Arc expression in individual animals, revealing new insights into the role of Arc in memory formation.

Hypothesis: I hypothesize: 1) the strength of Arc upregulation increases with initial exploration time; 2) younger mice have stronger and more persistent Arc expression compared to aged animals.

Research plan: I propose to study the temporal dynamics of Arc expression in two phases: 1) development (1 year) of the PME, and 2) experimental studies (2 years) which use the PME to quantitate Arc expression over time in individual freely behaving mice. With the guidance of my advisor, Prof. Mark Schnitzer, I believe the project is feasible within the presented timeline.

The PME will have two important improvements compared to Prof. Schnitzer's first generation of head-mounted microendoscopes [6] described in my previous research experience essay. First, the PME will have a light source and camera integrated into the microscope headmount to minimize motion artifacts by fixing the orientation of the camera with respect to the mouse brain. This improvement will eliminate troublesome de-rotation analysis of the data. Second, the PME will allow greater motion by, and chronic imaging of, the mouse. To realize the integrated camera, I will collaborate with Prof. Abbas El Gamal of the Electrical Engineering Department to incorporate a tiny CMOS camera in the PME design. Additionally I will collaborate with a mechanical engineering doctoral student in my lab to design and fabricate housing which is lightweight (less than 4g) and permits chronic implantation in freely behaving mice. Utilizing my expertise in optics theory, I will design the optics for the PME. The design will incorporate commercial and custom

micro-lenses, including gradient refractive index (GRIN) lenses, miniature achromatic lenses, and micro-ball lenses. Data acquisition from the imaging camera and power to the imaging camera and LED will be accomplished using a flexible, lightweight bundle of wires. Development of the PME is already in progress. The development phase has and will continue to allow me to work collaboratively in a group spanning multiple disciplines.

In the second phase of the study, I plan to investigate the temporal dynamics of Arc expression through behavioral experiments with heterozygous Arc-GFP knock-in mice. In these mice, the Arc promoter drives GFP as well as Arc expression, thus Arc expression is quantitated by the strength of GFP fluorescence. Broadly, I will allow a mouse to explore a novel environment several times over 4 days, using the chronic PME for time-lapse imaging over 4 hours following each exploration. I will image once every 20 minutes. Experiments will be structured by combining several variants of these 4-day/single environment trainings.

Specifically, to test my two-part hypothesis, I will begin by varying the time of initial exploration (TIE), setting subsequent exploration sessions to 10 minutes. On day 1, after mounting the PME on the mouse's head, I will visualize all of the neurons in the chosen CA1 hippocampal field of view by rapidly placing the mouse in several different novel environments. On day 2 I will begin several 4-day trials described above, varying the length of the TIE between 1 and 30 minutes. I will analyze the GFP fluorescence data for each 4-day trial to quantitate both the number of neurons expressing Arc and the strength of expression, using the initial neuronal map and a basal fluorescence signal for references. I will analyze the fluorescence data along with memory formation, as measured by behavioral analysis over the 4-day trials. I will compare my results to previously published *in vitro* and *in vivo* head-fixed studies to compare statistical trends. The entire procedure will be repeated for N=10 young mice. Secondly, I will test the effect of animal age on Arc expression. I will again use 4-day trials, repeating trials every two weeks for 6 months per mouse and allowing 10 minute exploration sessions. I will analyze the time-lapse fluorescence and behavior data following a similar protocol as noted above, repeating for N=10 young mice.

For experimental controls, I will repeat these experiments with homozygous knock-in mice, where the Arc promoter only drives GFP expression. I will repeat the experiments using wild-type mice to collect baseline behavioral data. Finally, I will do time-lapse imaging in heterozygous knock-in caged mice to obtain the basal Arc expression dynamics.

Anticipated results: I expect to see that initial Arc expression as well as memory formation will increase with TIE. I believe that Arc reactivation, shown by analysis of the percent of neurons that express Arc during later exposures, and memory formation will increase with TIE but decrease with age for a constant TIE. In the homozygous knock-in control mice, I expect that Arc expression will be regulated by session time and animal age, and memory formation will suppressed. I expect that basal Arc levels in caged animals will remain stable. Upon completion of the study, results will be reported in the neuroscience literature.

Personal motivation: With my training in physics and neuroscience I believe I am thoroughly prepared to conduct the proposed research. In particular, the optical design portion of the project will draw on my physics training. I am excited to propose this project. I believe it will provide excellent preparation for my career as a biophysics researcher and is worthy of support by the NSF.

Statement: I attest that this research proposal is my own work.

Literature citations: [1] GL Lyford *et al*. Neuron **14**:433, 1995. [2] N Plath *et al*. Neuron **52**:437, 2006. [3] JF Guzowski *et al*. Nat Neurosci **2**:1120, 1999. [4] JF Guzowski *et al*. PNAS **103**:1077, 2005. [5] KH Wang *et al*. Cell **126**:389, 2006. [6] BA Flusberg *et al*. Manuscript in preparation.