IOS Preliminary Proposal: COLLABORATIVE: Physiological and Molecular Analysis of Single Hippocampal Principal Neurons during Social and Spatial Learning

PROJECT DESCRIPTION

I: Personnel

Dr. Johann “Hans” A. Hofmann, PI
Department of Integrative Biology, Center for Computational Biology and Bioinformatics, Institute for Cellular & Molecular Biology, Institute for Neuroscience
The University of Texas at Austin
1 University Station – C0990, Austin, TX 78712

STATUS: PI

TITLE: Professor; Director, Center for Computational Biology and Bioinformatics; Co-Director, Neural Systems and Behavior Course

ROLE: Dr. Hofmann will be involved in all aspects of the proposed research, including project design, supervision of trainees, overseeing behavioral and molecular experiments and analyses, teaching, presenting findings at scientific conferences, writing manuscripts.

Dr. André A. Fenton
Center for Neural Science
New York University
4 Washington Place (Room 980)
New York, NY 10003-6621

STATUS: PI

TITLE: Professor; Co-Director, Neural Systems and Behavior Course

ROLE: Dr. Fenton will be involved in all aspects of the proposed research, including project design, supervision of trainees, overseeing behavioral and electrophysiological experiments and analyses, teaching presenting findings at scientific conferences, writing manuscripts.

II. Project

1. CONCEPTUAL FRAMEWORK

Identifying the neuromolecular basis of individual variation is a major challenge for genotype to phenotype mapping. Diversity in neuronal structure and function is one of the most striking sources of variability in organisms with nervous systems and gives rise to complex behavior and cognition. Yet our understanding of the molecular processes underlying neuronal individuality and plasticity is still very limited and made all the more difficult because we now know that experience changes gene expression, which itself changes neural function and thus the subsequent neural functions in future experience (Ginsberg et al. 2004, Eberwine et al. 2012, Park et al. 2014). Our innovative approach to understand neuronal variability aims to compare neuron-to-neuron variation in synaptic function (physiology and plasticity) and molecular expression in identifiable hippocampal neurons due to regional specialization, and in response to experience using robust spatial and social learning paradigms in mice [active place avoidance or social memory tasks (Cimadevilla et al. 2000, Hitti & Siegelbaum 2014)]. The use of transgenic "Arc-GFP" mice allows us to identify morphologically and/or functionally distinct populations of neurons.

The proposed research will investigate the influence of cell type and activity state on single cell variability. Unlike previous research in this area, our approach will evaluate endogenous transcriptional variability in single hippocampal neurons in relation to synaptic function and behavior, and determine the extent to which learning alters transcriptional and physiological functional variability of neurons (Fig 1).

2. RATIONALE AND SIGNIFICANCE

Progress in systems neuroscience depends on integrating data across levels of organization in order to gain a more comprehensive understanding of the neural basis of behavior. Consequently, modern research and training requires cross-disciplinary programs that bring together concepts and methods from diverse disciplines. While imaging and genetic approaches have long complemented single cell electro-physiological analyses, it has now also become possible to conduct molecular level analyses on a single neuron level.

This innovative and integrative research proposal will transform our understanding of molecular and physiological processes underlying neuronal and cognitive variability that give rise to individual differences in neural circuits. Specifically, we will provide fundamental new insights into how transcriptional and electrophysiological variation arises both within and between major principal cell classes of the hippocampus that are crucial for learning, memory, and social cognition.

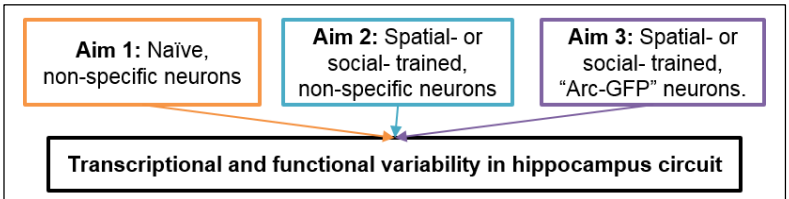

Fig. 1. Experimental Overview of Proposed Research. Each aim provides increased insight into individual cellular variability in transcriptional and physiological function. For details see text.

3. RESEARCH QUESTION

Does forming different types of memory produce distinct changes in synaptic function and molecular activity in defined regions within the hippocampal circuit? The consensus "synaptic plasticity and memory" hypothesis poses that memory is based on long-term changes in the function of a sparsely distributed set of synapses within neuronal circuits via specific modulation of molecular mechanisms that regulate synaptic function, and that acquisition and maintenance of different types of memory are associated with distinct region-specific changes at the level of single cells (Mayford et al. 2012, Takeuchi et al. 2014).

4. RESEARCH APPROACH

Overall Rationale: Using complementary approaches, PIs Hofmann and Fenton have made significant progress in understanding neuron-to-neuron variation in synaptic physiology and plasticity in response to spatial learning (Fenton) and molecular variability in response to changes in social behavior (Hofmann). Collaboratively, their combined expertise will shed light on individual, neural, and cognitive variability in response to two forms of hippocampal dependent learning experiences. The two PIs and their graduate students have been working together conducting proof-of-principle experiments to validate the approaches used in this study. This research increases our ability to map genotypes to phenotypes by shedding light on the molecular and physiological mechanisms that give rise to single neuron variability.

Aim 1: Investigate key features of the synaptic plasticity and memory hypothesis by examining differences between classes of principal cells in the CA1 and CA2 hippocampal fields.

Rationale & Significance: We will investigate key features of the "synaptic plasticity and memory" hypothesis first by examining differences between distinct but related classes of principal cells in the CA1 and CA2 hippocampal fields. Our research will identify the extent to which these neurons vary in baseline physiology and gene expression of naïve mice.

Experimental Design: The hippocampus of naïve c57b6 mice will be dissected and processed for *ex-vivo* slice physiology. We will identify and record from CA1 principal cells in the CA1 and CA2 hippocampal fields. We will record evoked field responses to estimate input-output functions of CA1 and CA2 as an assay of network synaptic function, as well as record single cell currents under voltage clamp to determine whether the different cell types express different baseline distributions of excitatory and inhibitory and AMPA- and NMDA-mediated currents as well as their ratios to estimate excitation/inhibition balance and LTP/LTD status, which we cannot determine from the field recordings (Mercer et al. 2007, Zhao et al. 2007). These studies will establish the baseline against which further studies are compared. Furthermore, they will extend prior work because we will then micro-aspirate the cell body of these neurons and process the material for gene expression analysis using a validated pipeline for automated RNA isolation using (Harris, Otopalik, et al. 2014) and gene expression analysis of using Nanostring® technology from single neurons (Harris, Chung, et al. 2014) (Fig 3D). These functional and molecular datasets will be investigated separately as well as in an integrated functional network analysis to characterize the interactions amongst these variables at multiple levels of biological organization (Fig. 2).

Potential Pitfalls and Alternative Approaches: We do not anticipate any difficulties as we have validated that sufficient high quality RNA can be isolated from micro-aspirated single neurons using RNA amplification techniques (Whitaker et al. 2011). If the RNA isolation proves difficult we will perform the isolation analysis on small groups of ~5 neurons, which we have demonstrated is ample for the Nanostring® analysis (Fig 3D).

Aim 2: Estimate the impact of hippocampus-dependent learning on the distribution of the physiological and transcriptional network measures in the CA1 and CA2 hippocampal subfields of wild-type mice.

Rationale and significance: CA1 as the main output of the hippocampus circuit is most well-known for its role in spatial memory (O'Keefe & Nadel 1978, Cimadevilla et al. 2001), while CA2 has recently been selectively implicated in social discriminative memory (Bielsky et al. 2004). We will investigate how the experiences of hippocampus-dependent learning in social and spatial paradigms changes the distributions of physiological and molecular measures of single neurons. These assays will be performed on single cell samples from the trained and untrained populations of the CA1 and CA2 pyramidal cells, independent of whether or not a cell was specifically recruited for the learning experience. The prediction according to the sparseness assertion of the consensus hypothesis is that differences will not be detectable between the trained and untrained animals due to sparseness of the learning recruited cells, which may partly contribute to high between-

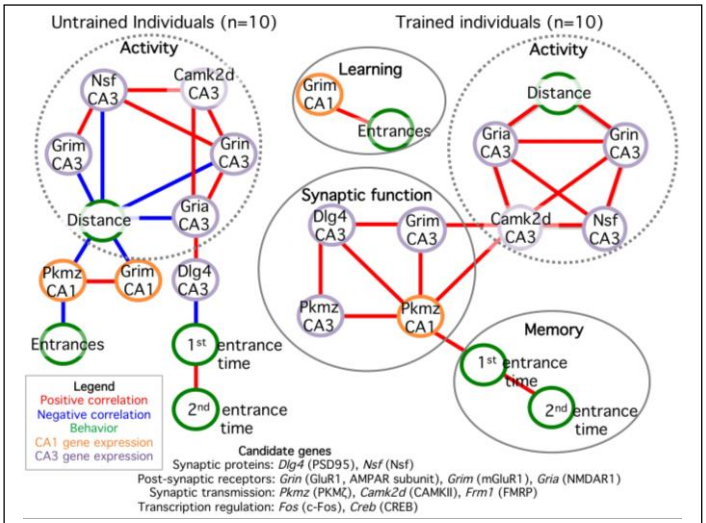

Fig 2. Distinct networks of behavior and gene expression associations in trained and untrained mice. After testing in place avoidance, trained (3 10-min trials in 1 day) or untrained (no shock) mice, RNA was isolated from CA3 and CA1 tissue punches for qPCR analysis of 10 candidate genes. Pair-wise correlations between gene expression values and behavioral variables were computed and statistically significant correlations are visualized as network of associations using the R package igraph (Csardi & Nepusz 2006). The association networks for the trained and untrained mice are distinct, and largely consistent with hypothesized roles for these genes. Given the central role of PKMZ in memory maintenance, it is remarkable that CA1 PKMZ gene expression is a hub that links the memory and activity variables to genes that regulate synaptic function. [Figure best viewed in color.]

and within-animal variability in the single cell features.

Experimental Design: Mice will be trained in either hippocampus-dependent spatial (active place avoidance (Cimadevilla et al. 2001) or social memory tasks (Cimadevilla et al. 2000, Hitti & Siegelbaum 2014) to examine how the electrophysiological and molecular profiles of in the CA1 and CA2 circuit nodes differ due to the learning experiences. The same physiological and molecular analysis will be performed as described in Aim 1 experimental design.

Preliminary data: Remarkably, our preliminary data suggest that learning-induced changes at multiple levels of biological organization can be detected between regions CA1 and CA3 after spatial learning (Fig. 2). Here, a particular innovation will be to include distinct behavioral estimates of activity, learning, and memory, with the physiological estimates of function and the estimates of molecular expression to characterize the functional networks of interactions amongst these variables, integrated across multiple levels of biological organization (Fig. 2). Furthermore, these results suggest potential challenge to the sparseness assertion of the consensus hypothesis, because the networks of interaction amongst the gene expression and physiology measures differ between the trained and untrained groups of mice.

Potential Pitfalls and Alternative Approaches: The active avoidance and social discrimination memory paradigms are robust and in use in the Fenton laboratory so we anticipate no difficulty in using these tests. The two tests are unlikely to provide the same intensity of gene expression changes, which is why we will focus on the correlations between the intensity measures, rather than their absolute values (Fig. 2). Furthermore, we will be able to use the respective untrained "tethered" control animals to compute relative change in the individual trained animals because the tethered controls are exposed to the same physical conditions as the trained animals but without experiencing the explicit learning component.

Aim 3: Compare learning-recruited and not recruited cells to estimate the impact of learning on single neurons.

Rationale & significance: Aim 3 will investigate the consensus hypothesis further by examining how learning changes synaptic function and molecular expression in single cells and how these changes relate to behavioral variables. We will investigate whether or not the acquisition and persistence of different types of memory alter the cellular profile of hippocampal principal cells that have been identified to participate in the encoding (Arc-GFP+) compared to cells that are identified not to participate in the encoding of memory (Arc-GFP-). We will examine only animals that received training in the social or spatial learning tasks and compare in each animal the GFP+ learning-recruited principal cells and the adjacent GFP- cells that are less likely to have been vigorously active during the training experience. Spatial learning is expected to activate CA1 and less so CA2 whereas social learning is expected to strongly activate CA2. According to the consensus hypothesis, particular changes in synaptic function and gene expression will be detected in the GFP^+ cells compared to the GFP^- cells, and these changes will correlate with measures of learning and memory performance.

Experimental Design: We will use activity dependent expression of GFP in an "Arc-GFP" mutant mouse to identify "learning-recruited" (GFP+) neurons (Fig. 3 A1, red arrow). This mouse is generated by crossing ArcCreERT2 mice and R26R-STOP-floxed-GFP mice. 4OH-tamoxifen injection five hours before initial training in a rapidly learned active place avoidance task as well as a social learning task activates Cre leading to GFP expression in the subpopulation of neurons that transcribed Arc during training. Because Cre expression is transient, only in the presence of the drug, and once activated GFP expression is

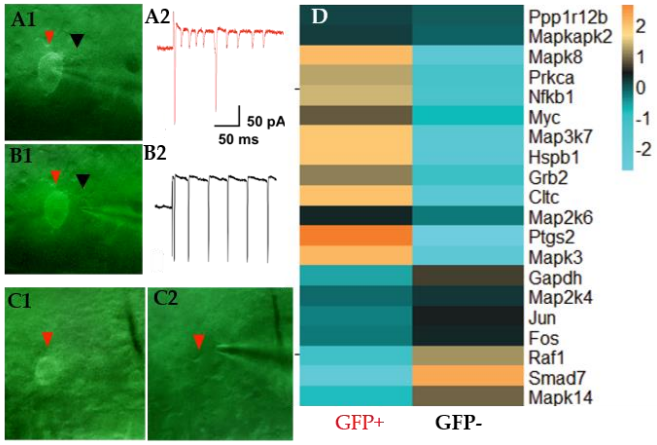

Fig 3. Comparing learning-recruited GFP^+ and non-recruited GFP^- neurons. Following training, we will identify and record from learning-recruited GFP^+ neurons (red arrows, A1, A2) and from non-recruited GFP^- neurons (black arrow, B1, B2). Then, we will micro-aspirate these neurons for molecular profiling, usual visual cues to confirm successful micro-aspiration (red arrows, C1, C2). Nanostring® gene expression assays will be used to quantify differential gene expression between GFP^+ and GFP^- cells (D). [Figure best viewed in color.]

persistent, GFP is an indelible marker for the principal cells that were activated during the memory training. GFP^+ cells are enriched with the active subset, and GFP^- neurons are enriched with the inactive subset. We will then compare the differences in electrophysiological and molecular profiles of single learning-recruited neurons (GFP+) (Fig. 3 A2, D) with the features of GFP- neurons (Fig. 3 B2, D) that were unlikely to have been recruited by different learning episodes (Denny et al. 2014).

Potential Pitfalls and Alternative Approaches: We anticipate no difficulties as the mice are actively in use in the Fenton lab. If the spatial and social tasks do not differentially activate CA1 and CA2, respectively, we will nonetheless proceed because the two tasks are very distinct and the central question is whether the two different experiences produce different patterns of transcriptional and physiological changes and whether these changes are sub-region specific.

5. BROADER IMPACTS

Participation in existing institutional infrastructure for education and outreach. The Neural Systems & Behavior (NS&B) course, jointly directed by PIs Fenton and Hofmann, has provided intensive training in the concepts and methodology of behavioral neurobiology and systems neuroscience to outstanding pre- and postdoctoral students since 1978. NS&B offers multiple training opportunities in modern approaches to the study of neural systems and behavior to the next generation of behavioral neuroscientists during early stages of their research careers: intensive lectures and discussion, one-on-one interaction with internationally renowned scientists, and extensive hands-on laboratory training with a variety of invertebrate and vertebrate preparations using state-of-the-art techniques and equipment. The integrative nature of this project provides unique and outstanding educational opportunities for trainees at all stages of their career. This research will be partially conducted alongside students in the Neural Systems and Behavior course at the Marine Biological Laboratories where scientific careers are transformed through learning and discovery-driven research (www.mbl.edu/nsb).

Development of innovative educational activities and broadening dissemination. In 2013, PIs and NS&B Co-Directors Hofmann and Fenton introduced single-cell molecular and genomic techniques to the NS&B curriculum to complement and extend the electrophysiological characterization of specific neuronal subclasses including identified neurons in invertebrate preparations. Working in pairs, students learned the value of integrating molecular and physiological experiments and were incredibly excited to successfully conduct these single-cell techniques in student-developed projects (Fig. 4A). Furthermore, PIs Hofmann and Fenton have dramatically broadened the reach of NS&B resources and expertise by webcasting all the lectures (Fig. 4B) and creating animated video tutorials of complex concepts and techniques taught by expert NS&B instructors (Fig. 4C). The webcast lectures and the animated video tutorials are hosted on our website at http://www.mbl.edu/nsb/about/course-videos/.

Figure 4. Broadening the scope and impact of the Neural Systems & Behavior (NS&B) course with technological and educational innovation. The single-neuron molecular and physiological approaches described herein have been incorporated into course laboratory exercises in 2013 (A1) and 2014 (A2) with great success. For the first time ever in 2014, all ~80 lectures associated with the course were webcast live and achieved on our website (B). We also created video tutorials for teaching complex concepts and techniques so that the expertise of NS&B instructors can be disseminated world-wide (C).

Benefiting Society. PIs Hofmann and Fenton and their trainees have published experiments from the course in a technical report (Harris, Otopalik, et al. 2014) and presented these findings at five scientific conferences (Fenton et al. 2014, Harris, Chung, et al. 2014). All data will be open access, which will be explained in detail in a full proposal, if invited.