Title. A Systems Biology Approach for Understanding Memory

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Introduction. One of the most intriguing questions in neurobiology is how short term neural activity at synapses produces long-term memory. This process called synaptic plasticity is presently believed to result from the simple switch-like behavior of the following linear pathway: 1) presynaptically released glutamate binds post-synaptic AMPA receptors, 2) Ca^{2+} enters and increases the resting potential, 3) NMDA channel Mg^{2+} plug is expelled, 4) upon subsequent stimulation glutamate activates both AMPA and NMDA Ca^{2+} channels, 5) intracellular Ca^{2+} increases further, 6) a Ca^{2+}-dependent protein kinase cascade upregulates immediate early genes, 7) immediate early genes increase AMPA transcription resulting in increased basal calcium conductance, resting potential, and responsiveness to pre-synaptic signals [1]. However, a recent review of AMPA trafficking by Bredt and Nicoll indicates that AMPA expression is not switch-like, but rather is highly dynamic [2]. Furthermore, a recent theoretical study by Fusi showed that synaptic switching is an inefficient mechanism for memory storage and does not produce permanent memory [3].

Hypothesis. I propose to use a systems biology approach to test an alternative hypothesis that synaptic plasticity is not caused by a linear pathway, but instead arises from the dynamics of a complex biochemical network. A recent theoretical study by Fusi, Drew, and Abbot showed that unlike linear pathways, biochemical networks can plausibly account for memory storage and long-term retention [4]. I propose to use the newly emerging field of systems biology to investigate this theory. I propose to 1) verify my hypothesis by using electrophysiology to confirm two predictions of this study, and 2) use experimental and computational systems biology to build, test, and refine Bayesian and partial differential equation (PDE) models of the biochemistry of synaptic plasticity. The key feature of this approach is the use of systems biology to systematically refine models of synaptic plasticity. Specific features of this approach include: 1) use of high-throughput data and machine learning algorithms to objectively consider the space of all possible biochemical networks, 2) use of probabilistic Bayesian models to tolerate noise and encapsulate non-linear interactions, and 3) integration of an expected information gain metric to inform experimental design.

Research Plan and Anticipated Results. In the first months of the study, I plan to confirm two predictions of the Fusi-Drew-Abbott model: 1) repeated long-term potentiation decreases plasticity, and 2) depotentiation increases plasticity. To validate the former I plan to collaborate with Professor Mark Schnitzer to compare synaptic plasticity between sensory deprived and non-deprived mouse visual neurons. Specifically I will electrically stimulate pairs of pre- and post-synaptic cells in visual cortex slices to induce potentiation and measure resultant changes in post-synaptic resting potentials. Per my hypothesis, I expect to observe diminished potentiation in sensory non-deprived neurons. Similarly, I will evaluate the later prediction by comparing the post-synaptic resting potential in sensory non-deprived neurons stimulated with low frequency current with that of unstimulated sensory non-deprived neurons. According to my hypothesis, I expect to observe greater potentiation in the low-frequency stimulated cells.

I plan to spend the following six months using publicly available data to construct and refine an initial Bayesian model of the biochemical network responsible for synaptic plasticity. First I will build a consensus list of the genes and proteins involved in synaptic plasticity from those indicated in pathway and ontology databases. Second, I will construct my initial Bayesian model by connecting these biochemicals with interactions documented in biochemical pathway and protein-protein

interaction databases. Where applicable I will employ linear stability analysis to disambiguate the direction of each interaction [5]. Third, in collaboration with Dr. Karen Sachs, I will refine my model by applying a supervised Metropolis Bayesian structural inference algorithm to publicly available interventional microarray data. Specifically I will use my initial model to seed and constrain a search for the models with the highest posterior probability. This search will likely highlight edges for which only weak evidence existed in the literature as well as suggest several previously undocumented edges. During the following six months I will experimentally verify each of these predictions using yeast two-hybrid and co-immunoprecipitation assays. This will give me the opportunity to learn how to clone, transfect, and engineer fusion DNA, analyze protein complexes by gel electrophoresis and mass spectrometry, and culture mammalian and yeast cell lines. I expect these experiments to positively confirm approximately 50% of the previously unknown interactions and negatively confirm nearly all of the predicted extraneous edges.

I will spend the remainder of my second year developing a theoretical framework for modeling the complex spatiotemporal dynamics of synaptic plasticity with my research advisor Professor Markus Covert. Specifically I will develop a PDE model which relaxes the standard systems biology assumption of spatial isotropy. This is necessary because neurons have significant subcellular organization. I will employ the following assumptions: 1) separation of time scales – because electrical activity, biochemical signal propagation, and changes in gene expression occur on distinct time scales, each will be modeling assuming the other processes are time-independent, and 2) discrete anisotropy – neurons will be modeled as consisting of a small number of compartments. These simplifying assumptions will enable me to make quantitative, experimentally verifiable predictions of the dynamics of synaptic plasticity at minimal computational cost.

In parallel I will construct three experimental tools for observing the complex dynamics of synaptic plasticity: 1) fluorescent signaling protein fusion constructs, 2) FRET donor-acceptor constructs, and 3) siRNAs for each gene and protein-protein interaction in the network. The former two tools will permit me to use fluorescence microscopy during my third year to quantitate the expression, localization, and interactions of the multiple proteins involved synaptic plasticity with high spatiotemporal resolution. The later will enable me to iteratively improve my PDE model by 1) fitting the model's kinetic parameters to new interventional data, and 2) at each iteration using the refined model to select the next experiment as that with the maximum expected information gain.

Using the systematic approach outlined above, I expect to construct a quantitative, physiologically accurate model of synaptic plasticity within three years. In the future I plan to use my model to investigate how memory is perturbed in Alzheimer's disease by 1) testing amyloid β-protein theory, and 2) identifying drug targets for reversing its pathologic synaptic plasticity.

Personal Statement. With the support of my collaborators, I believe mapping the biochemical network of synaptic plasticity is feasible within the time frame outlined here. Furthermore, I believe this study will be very instructive for learning how to build, test, and refine models of cell physiology, and will prepare me for a career as an independent systems neurobiologist.

Statement of Originality. I hereby declare that this submission is my own work.

References

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