Neocortical stimulation and classical eyeblink conditioning: A study of the Memory Index Theory

Introduction and Objectives

Memory is a fundamental cognitive process and is critical for learning and intelligence. Memory impairment associated with normal aging and brain disorders (e.g., dementia, Alzheimer's disease) has emerged as a public health problem with tremendous costs at the individual and societal levels. To resolve the problem, it is essential to understand how memory works in the brain and develop therapeutics for restoring or improving memory functions. According to one prominent view (also referred to as the Memory Index Theory), the brain encodes sensory inputs on the basis of current knowledge and stores the new data in raw form (i.e., episodic memory) across widely distributed neocortical (NC) modules (Figure 1). The view suggests that the hippocampal formation (HF) plays a crucial role in the process by generating a unique code ('index') that links together attributes of a given episode that are independently stored in weakly interacting NC modules (Teyler & DiScenna, 1986). The theory also suggests that spontaneous reactivation of the index patterns during sleep enables retrieval of recent memories and facilitates gradual extraction of 'semantic' knowledge from the memories by rewiring short and long-range connections among the NC modules (McClelland, McNaughton, & O'Reilly, 1995).

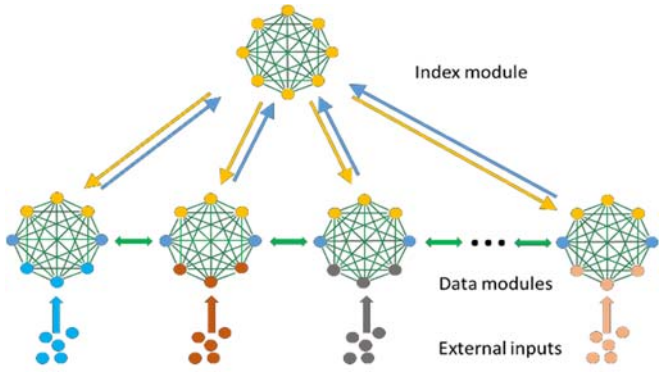

Figure 1. The essential components of the **Memory Index Theory.** The NC memory system consists of hierarchically arranged modules (for simplicity only two levels are shown). The index module (the highest located in the HF) generates a code unique to each distribution of patterns (attributes, context of our experience) in the data modules and adds that code to the stored patterns during acquisition. Data in lower modules can then be retrieved either bottom-up or top-down. Gradual rearrangement of inter- and intramodular connections (during slow-wave sleep) results in encoding of 'semantic' knowledge.

Although we have general understanding of the theory, it remains to be elucidated experimentally how the HF index contributes to neocortical dynamics during memory encoding and consolidation. The theoretically ideal strategy for an indexing system would be to allocate an arbitrary, random pattern to each episode such that partial or degraded input could retrieve the complete index. In fact, current evidence suggests that the HF index merely creates random patterns of neural activity (i.e., it works like a 'hash code')(Rich, Liaw, & Lee, 2014). Thus, it should be, in principle, possible to create artificial index codes during behavior, and re-stimulating these specific patterns during sleep might facilitate the persistence of memory (Barnes & Wilson, 2014). As a preliminary step to test this possibility, I will conduct a study to evaluate whether artificial cortical stimulation can be associated with learning experience in mice with intact brain. Continuous sequences of random patterns one for a short epoch of original learning experience will be generated and delivered as neocortical stimulation to animals expressing light-activated (blue light, 473nm) transmembrane ion channels (Channelrhodopsins) in cortical layer 5. Note, however, that the purpose of the study is not to test whether original neural patterns are re-expressed in the NC, merely to test the effect of artificial index code (merged with ongoing activity representing the event) on learning and memory.

To test the prediction that patterned cortical stimulation can be applied as artificial hippocampal index patterns, I will incorporate a type of classical conditioning paradigm. Classical eyeblink conditioning (EBC) is a model system widely used for studying behavioral and neural correlates of learning and memory (Thompson, 2005). Eyeblink conditioning usually consists of the pairing of a conditioned stimulus (CS, usually a light display or a tone) with an unconditioned stimulus (US, voltage shock in the eyelid or airpuff to the eye). The fundamental idea is that when the CS precedes the US over many times, the animal will learn to associate the two independent stimuli and exhibit a learned behavior (i.e., conditioned response) upon CS only presentation. The conditioned response (CR) is behaviorally similar to an unconditioned response (e.g., reflexive eyelid closure) but is mediated

by a different neuronal circuitry.

The goal of this project is to understand potential contribution of artificial hippocampal index replacement to learning and memory. As a direct way of testing this prediction, I will conduct a study by delivering optical stimulation to the mouse cortex (i.e., index replacement) and evaluate eyeblink conditioning performance (i.e., learning and memory) in mice.

Materials and methods

Animals

To assess the effect of the hippocampal information flow to the neocortex during and after learning, we will test transgenic mice (N = 8) that express light-activated channelrhodopsin in cortical layer 5. Animals will be handled and habituated to the stimulation setup.

Surgery

All animals will undergo headplate implantation and craniotomy following sham or lesion surgery. The headplate has a large cranial window (7 mm x 6 mm) covering the mouse cortex (bregma 2.5 to - 4.5 mm, lateral 0 – 6 mm), allowing wide-field stimulation across different cortical areas. A 2mm by 2mm cranial window will be created by removing the skull around the visual cortex. High-viscosity immersion oil will be added to the cranial window before coverslipping. Animals will be monitored post-operatively with Metcam (analgesic) and Baytril (antibiotic) injections.

Delay Eyeblink Conditioning Paradigm

We will take advantage of the classical eyeblink conditioning paradigm as a model system to test learning behavior induced by cortical stimulation (Figure 2). All animals will be handled and habituated to the head-fixation system. Training will continue for 7 – 10 days. Each day will consist of 100 trials, divided into 10 blocks. In each block, 9 trials will present paired CS and US and 1 trial will present only the CS. On each paired trial, an aversive stimulus (airpuff) will be delivered 250ms after the onset of the stimulation (i.e., 250ms delay with no stimulus). Eyeblink response will be captured by camera under infrared illumination. To test whether or not the animal learned the association, a test trial will be given without an airpuff.

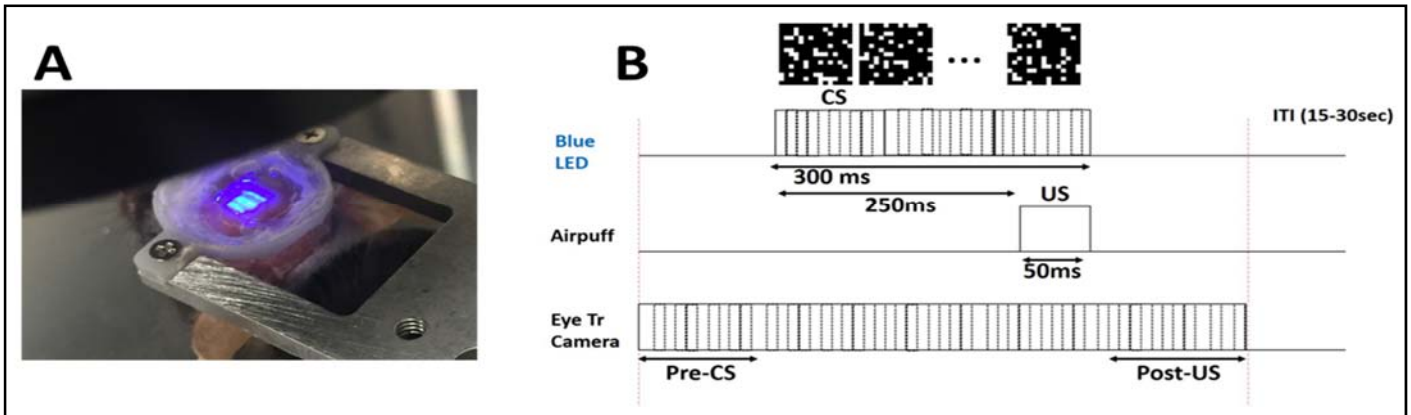

Figure 1. A. A photograph of optically stimulated (in stripe patterns) brain of an awake mouse expressing Channel Rhodopsin in Layer 5 of the cortex. B.A sequence of pseudorandom patterns of images will be illuminated in blue LED (473nm) for 300ms (30ms x 10 images). The patterns are created from bitmap images (600 by 600, size adjustable), which project onto the cranial window. A mild airpuff will be exerted via a solenoid valve open for 50ms, co-terminating with the light stimulation. A high-resolution eyetracking camera (~70 frames per second) will capture the eyelid movement. Learning is indicated as eyelid closure during the delay (i.e., occurring after the onset of the stimulation and before the onset of the airpuff).

Expected outcomes

We expect that mice will exhibit normal learning in eyeblink conditioning, by associating the cortical stimulation with airpuffs. The learning will occur when stimulation is paired with airpuff within short time window (i.e., 250ms delay), whereas no learning will occur when stimulation is delivered in a random manner.

Specific responsibilities of the student

I will be responsible for assisting the primary investigator of the project (Dr. Soyun Kim, Assistant project scientist in the laboratory) at various stages of the project. I will be taking a lead role in handling mice prior to surgery and running behavioral testing. I will be working on the optical stimulation system for training multiple animals across several days. I will be also responsible for preparing for surgery (craniotomy) and post-surgical care of animals. Besides experimental work, I will be responsible for reading relevant literature and presenting data in the lab meetings.

Timeline of the project

Fall Quarter	**Winter Quarter**	**Spring Quarter**
• Training in rodent surgery • Training in rodent handling • Training in rodent behavioral testing • Journal club	• Collect data from stimulation and eyeblink conditioning • Training in rodent behavioral testing • Journal club	• Data analysis • Training in programmable software (e.g., MATLAB) • Present the results at the UCI Undergraduate Research Symposium

Itemized budget and justification

Funds are requested for surgical supplies used for preparation of cranial window for stimulation ($250).
Funds are requested for animal maintenance ($0.65/cage/day x 10 cages x 100 days = $650).
Funds are requested for printing a poster for presentation at the annual UCI Undergraduate Research Symposium ($100).
Total requested: **$1,000**

References

Barnes, D. C., & Wilson, D. A. (2014). Slow-wave sleep-imposed replay modulates both strength and precision of memory. *J Neurosci, 34*(15), 5134-5142. doi:10.1523/JNEUROSCI.5274-13.2014

McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev, 102*(3), 419-457.

Rich, P. D., Liaw, H. P., & Lee, A. K. (2014). Place cells. Large environments reveal the statistical structure governing hippocampal representations. *Science, 345*(6198), 814-817. doi:10.1126/science.1255635

Teyler, T. J., & DiScenna, P. (1986). The hippocampal memory indexing theory. *Behav Neurosci, 100*(2), 147-154.

Thompson, R. F. (2005). In search of memory traces. *Annu Rev Psychol, 56*, 1-23. doi:10.1146/annurev.psych.56.091103.070239