**Multisensory Object Processing in a Mouse Model of Alzheimer's Disease**

## Background and Objective

Alzheimer's disease (AD) is the most common type of neurodegenerative disease characterized by an irreversible, progressive decline in multiple cognitive abilities. Despite growing evidence of several risk factors (aging, genetic, environmental) and pathological mechanisms of AD, few approaches to delay, prevent, or cure this disease exist. The Amyloid Cascade Hypothesis, a prominent view of AD pathogenesis, suggests that increased production and accumulation of amyloid beta (Aβ) plaques in the brain drives a cascade of neuropathologies (e.g., synaptic and neuritic disruption, oxidative injury, neuronal loss, circuitry dysfunction) that ultimately lead to dementia (1-3). Accordingly, Aβ plaque–driven synaptic disruption and dendritic spine reduction in the medial temporal lobe (MTL) are implicated in the memory deficits observed in early AD (4).

While the memory network in the MTL is thought to be particularly vulnerable to Aβ pathology, post-mortem brain examinations also indicate that the Aβ plaque burden gradually extends outside the MTL to the inferior temporal cortex, posterior parietal cortex, and prefrontal cortex, presumably accounting for marked disruption in cognitive functions other than the memory domain (5, 6). In fact, exhaustive neuropsychological assessment of mild cognitive impairment (MCI) patients showed multiple cognitive deficits in addition to memory impairment (7, 8). Current understanding is that certain perceptual capacities (e.g., visual, auditory, or tactile agnosia, prosopagnosia) as well as high-order sensory processing (e.g., audiovisual integration of speech) deteriorate over the course of AD (9-10). Yet, the precise neural mechanisms contributing to altered or disrupted sensory processing in AD is less understood.

Multisensory integration (MSI), the ability to integrate information from different sensory channels, is a fundamental part of our intellectual functioning (11). Most animals use MSI to identify and interpret objects in a complex environment. The integrative process has a clear adaptive advantage to recognizing objects more accurately and quickly (even without prior experience), thereby facilitating other cognitive processes (e.g., decision-making or memory) that guide future actions. Evidence from animal and human studies identified various brain regions mediating MSI, such as the perirhinal cortex and parietal cortex (12-15). While much research has identified brain networks supporting multisensory object processing in normal brain, few studies have investigated neural and behavioral correlates of multisensory object processing in the context of AD. Specifically, there is a lack of knowledge in the detailed patterns of neuronal circuitry underlying crossmodal object representation in AD. In the proposed project, neuronal properties representing crossmodal object representation in the perirhinal cortex of the 3xTgAD mice will be studied using a novel optical imaging instrument (the Miniscope, Figure 1).

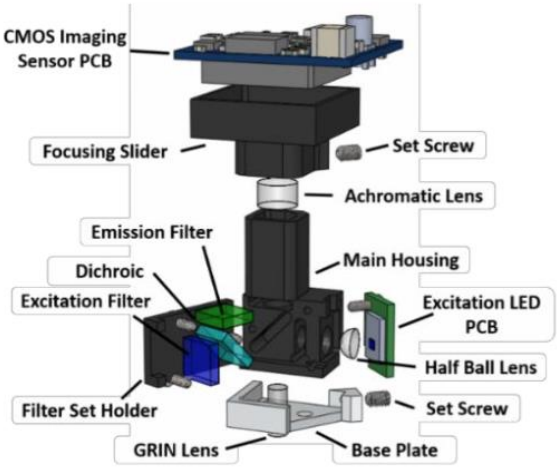


**Figure 1.** Parts configuration of the Miniscope (www.miniscope.org), a miniature fluorescence microscope used for wide-field calcium imaging to record neural activity in awake, freely behaving mice. Based on a design conceived by Mark Schnitzer's Lab, the Miniscope can be easily assembled with an excitation light source (473nm LED), lenses for focusing light, a fluorescence sensor to detect emitted light, and lightweight body materials. The chronically implantable gradient refractive index (GRIN) lens allows optical access to the deep brain structures that are inaccessible to conventional two-photon imaging technique.

**Approach**

Animals: A transgenic mouse model of AD (3xTg-AD) and non-transgenic control mice at the age of 6 -8 months will be used (N = 8 for each group). The 3xTg-AD mice start to exhibit Aβ plaques around 6 months of age and memory impairment is seen from 4 months of age (16).

Surgery: Animals will undergo three surgical procedures (Figure 2). In the first surgery, mice will be transfected by stereotaxic injection of AAV1. Syn. Flex. GCaMP6f.WPRE. SV40 (Penn Vector Core) into the perirhinal cortex. Second, a GRIN lens combined with a microprism will be lowered near the injection site. The microprism will enable vertical implantation of the lens for imaging the lateral cortex. Third, a baseplate will be affixed to the skull of each animal to support the Miniscope. During the third surgery, the Miniscope will be adjusted to reach a focal plane with the appropriate light intensity.

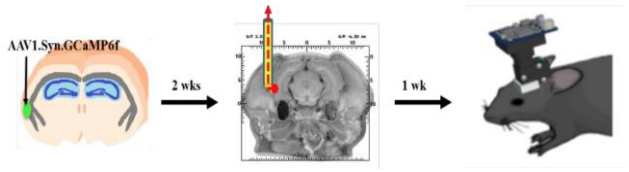


**Figure 2.** A schematic diagram of the experimental design. Mice will undergo stereotactic injection of GCaMP6f virus in the perirhinal cortex. Two weeks after surgery (to allow adequate GCaMP6f expression), GRIN lens-microprism will be implanted near the PRh. Following 1-week recovery, a baseplate will be cemented to the skull after adjusting the mounted Miniscope at the level when clear focal plane and appropriate light intensity are achieved.

Behavioral Paradigm: The proposed project will use a novel behavioral paradigm in which animals interact with objects based on different task demands (visual, tactile, or visuotactile exploration). We will use standard and modified versions of the spontaneous object recognition (SOR) task (17), exploiting the animal's natural tendency to preferentially explore a novel object compared to a familiar object (Figure 3). A Y-maze made with solid white Plexiglas will be used to minimize the influence of other spatial cues. Three-dimensional junk objects with different textures (e.g., plastic, glass) and shapes will be used as stimuli. Each trial will consist of a sample (visual, tactile, or visuotactile exploration of two objects) and a choice (visual, tactile, or visuotactile exploration of the novel object) phase, separated by a short retention delay (<1min). For the tactile task, the room will be illuminated with red light, preventing mice from acquiring visual information. Otherwise, the room will be illuminated with white light, allowing acute vision. The crossmodal task will involve both tactile-to-visual and visual-to-tactile conditions.

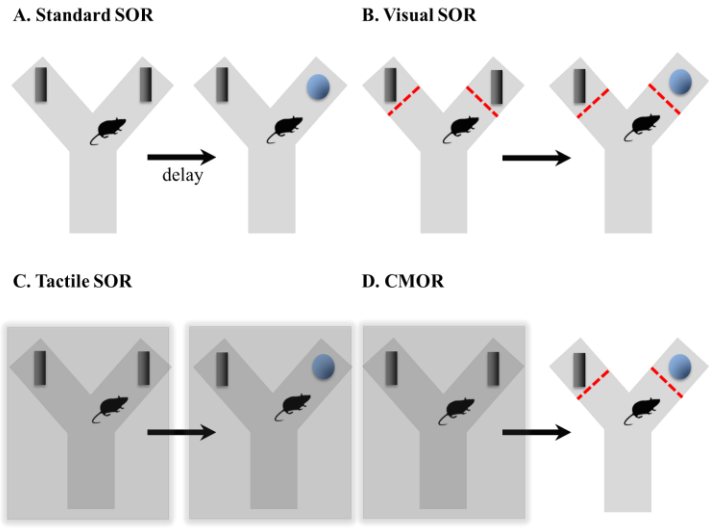


**Figure 3.** A schematic diagram of behavioral paradigms used in the proposed study. A Y-maze will be used with one arm assigned as the start location and the other two arms assigned for object placement. Animals are exposed to a new set of odorless objects on each trial. A brief retention delay will be used to minimize mnemonic demand. **A.** The standard spontaneous object recognition (SOR) task requires no external motivation or reward and can be completed with little training, given that animals naturally tend to explore novel objects more than familiar objects. **B.** The visual SOR prohibits tactile exploration by inserting a transparent barrier (red dotted line) between the animal and objects. **C.** The tactile SOR is performed under red-light illumination (darkness), prohibiting acute vision. **D.** The crossmodal object recognition (CMOR) task requires integration of tactile and visual cues for identifying the novel object during the 'choice' phase.

Imaging and Analysis: Data acquisition and analysis will be performed using open source software (Miniscope.org). Imaging will start 5 min prior to testing to ensure image quality and to record baseline activities. Each test session will not exceed 40 minutes to minimize the risk of photobleaching signals from the Miniscope. Images and behavioral data will be synchronized to generate $Ca^{2+}$ signals corresponding to different behavioral phases (sample, delay, and choice) of each trial. Following motion correction, regions of interest (ROIs) will be manually selected. The $Ca^{2+}$ activity traces measured by averaging pixels within each ROI will be plotted to create a fluorescent time series.

**Expected Outcomes**

We expect that 3xTgAD mice will exhibit intact visual and tactile SOR but impaired CMOR. Intact performance in the unimodal SOR tasks would be in agreement with findings from other mouse models or patients in early-AD stage, where basic perceptual function remain intact. Alternatively, the AD mice may not be impaired at CMOR, partly because the objects are very distinct from each other and hence, the perirhinal cortex is not required, or the task may be only sensitive to perirhinal cortical lesion (i.e., complete atrophy) and do not reveal impairments in progressively changing AD brain. In any of these circumstances, the findings will provide valuable information about the performance of AD mice on the CMOR task.

**Specific Responsibilities**

My responsibilities in the proposed project are to assist Dr. Soyun Kim (Assistant Project Scientist, who is the primary investigator of this project. I will be responsible for assembling the Miniscopes, assisting in the viral transfection and surgical implantation process, and carrying out the behavioral testing for animals used in the project. After collecting the behavioral data, I will also participate in data analysis.

**Timeline**

| Fall Quarter | Winter Quarter | Spring Quarter |
|---|---|---|
| • Assemble Miniscopes<br>• Test Miniscopes using open source software (Miniscope.org)<br>• Training in rodent surgery | • Train in rodent behavioral testing<br>• Collect behavioral data | • Data analysis<br>• Present the results at the UCI Undergraduate Research Symposium |

**Itemized Budget**

Funds are requested for ordering the AAV-GCaMP6f virus: $300

Funds are requested for ordering Miniscope parts: $600

Funds are requested for printing a poster for presentation at the annual symposium: $100

Total funds requested: $1,000

## References


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