Development of amygdala organoids (AOs) for studying bipolar disorder

Introduction: Bipolar disorder (BD) is defined as a "chronic and progressive psychiatric illness characterized by mood oscillations with episodes of mania and depression" [1]. BD afflicts about **1 out of 25 people**, and BD patients have a **15-fold higher chance of committing suicide** and a **reduced lifespan by 15 years** [2, 3]. Treatment typically entails a **trial-and-error methodology** with polypharmacy of lithium salts and antiepileptic drugs during stable mood phases and antipsychotic drugs during unstable/extreme mood phases [3]. These drugs have a **small therapeutic window** and can have **detrimental side effects** if administered in the wrong phase [3]. BD affects emotional centers of the brain and involves possible molecular mechanisms such metabolic, oxidative stress response, and calcium signaling dysfunction/dysregulation [1, 3, 4]. Even with this knowledge, the aetiology and mechanism of BD is **"frustratingly limited"** [3].

Background: **Organoids** are *in vitro* derived multicellular structures that **emulate complex structures and functions of an organ**. Organoids are developed by culturing stem cells such as human pluripotent stem cells (hPSCs) in 3D culture environments with tailored chemical and mechanical stimuli. Brain organoids have expanded our ability to study the brain by yielding anatomic structures, cell types, and functions that more closely mimic the embryonic brain than existing traditional 2D culture methods. Studies have shown that controlled exogenous factors can help create region-specific brain organoids such as hippocampal organoids [5]. 3D brain organoids allow testing hypotheses on human models to study diseases and possible treatments. Brain tumor organoids have been used to investigate tumorigenesis and for drug discovery and screening [5]. Researchers have created **many different region-specific brain organoids but not amygdala organoids** [5]. The **amygdala is integral to emotional response**, emotional memory consolidation, stress response, and social behavior [4]. Studies have shown that the **amygdala plays a critical role in neurodevelopment** because damage to the amygdala in early development causes downstream effects on other brain regions [4]. The amygdala's functions suggest implications in emotional and social disorders such as BD and attention-deficit/hyperactivity disorder (ADHD) [4]. Generally, organoid development faces challenges with long-term culture due to the lack of vascularization, with control of patterning axes (i.e. dorsal-ventral), and with throughput [5]. Microfluidics has been shown suitable for long-term cell culture, providing exogenous factors to induce patterning, and increasing assay reproducibility and throughput [6]. In my thesis research, I propose to **develop a method for the generation of amygdala brain organoids (AOs) via a microfluidic platform** to allow for the study of developmental and pathophysiological differences between patients with and without BD.

Objectives: The following tasks will be accomplished to develop and test the AO: Year 1-2 **(1)** Determine the exogenous factors and timing of factor exposure required to differentiate hPSCs to amygdala-like cells. **(2)** Develop a microfluidic device to aid in the development of the AO. Year 3 **(3)** Optimize the microfluidic platform by varying properties. **(4)** Verify the AO. Year 4-5 **(5)** Compare differences between AOs developed from control/healthy patients and BD patients. **(6)** Experiment and observe effects from drug treatments and differences in calcium signaling.

Research Plan: To develop the AO, I propose to integrate the protocol for creating cerebral organoids [7] and a microfluidic embryological platform developed by the lab I currently work in, the Integrated Biosystems and Biomechanics Laboratory under Dr. Jianping Fu. This microfluidic platform has been successfully utilized recently to model the human epiblast and amnion development using hPSCs [6]. Our previous work has shown that this microfluidic platform can promote the self-organization of neural progenitor cells into cystic structures. *In vivo*, the amygdala

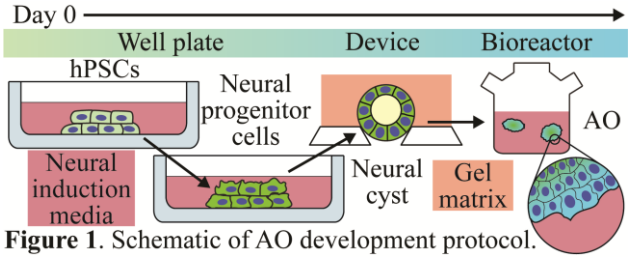

Figure 1. Schematic of AO development protocol.

is derived from the embryonic telencephalon. Thus, I will aim to first adapt published protocols used to develop telencephalon-derived organoids [5]. Since the amygdala is ventral to the hippocampus, I will examine whether adding ventralizing factors such as Sonic hedgehog (Shh) will promote more ventral telencephalon-related organoids. Based on this knowledge, our protocol will consist of (**Figure 1**): **(1)** Disassociated hPSCs are plated into a well plate and then cultured in neural induction medium supplemented with exogenous factors such as Shh to drive them towards neural progenitor cells. I plan on performing systematic assays to test different exogenous factors and the timing and dosages of these factors. **(2)** The neural progenitor cells are transferred into the microfluidic device containing a 3D gel matrix to form neural cysts. These cysts will be further developed into AOs by using additional exogenous factors. **(3)** Further growth of the AO will be carried out in a bioreactor [6, 7]. To characterize and verify the AO, I will utilize standard molecular and cellular assays, including single cell sequencing tools and immunostaining, which Dr. Fu's lab has extensive experience in [6], to observe for markers specific to the amygdala [8]. To extend my research for translational impact, I have started a collaboration with Dr. Sue O'Shea and Dr. Melvin McInnis at U of M. Dr. O'Shea researches and develops stem cell lines from BD and control patients. Dr. McInnis is a medical doctor and director of a research program focused on BD. With their help, I will compare drug response and calcium signaling dynamics between AOs developed from control/healthy and BD patients. The response to drugs for patient-derived AOs will be correlated with the patient's response to the same drugs. These important translational efforts will pave the ground for future translational applications of the AO for treating BD patients.

Intellectual Merit: The research, if successful, will yield the **first AO useful for studying BD**. It can expand our knowledge of BD, which needs new experimental tools [3, 5, 8]. The amygdala organoid, if successful, will **lay the foundation for studying the mechanisms** of BD such as calcium signaling and other cellular dysregulations [1]. This research will further add a new region-specific brain organoid model to the existing organoid toolbox and thus open new applications for future investigations of brain development and diseases.

Broader Impact: The proposed research for creating the AO will be **efficient, reproducible, and scalable**. The development of the AO will yield the first differentiation protocol for amygdala-like cells and bring us closer to patient-specific drug screening for BD. The AO made from patient-derived hPSCs could be used to test the effect of drugs and determine the optimal treatment plan, eliminating the trial-and-error method used to treat BD patients. Additional research will need to be conducted to predict patient responses. Furthermore, the AO can be used for drug discovery. AO and other organoids can provide a **quantitative approach to modern psychiatry** [3, 5, 8].

Through publications and presentations, I will communicate the importance of stem cell models and their potential use for improved understanding of mental disorders. I will try to educate the public on mental disorders, hopefully reducing the negative stigma associated with them. This research aligns with the World Health Organization mental health action plan to improve research for mental health. My overall goal is to strengthen the **bridge between medicine and engineering.**

References: [1] Kim, Y, et al. "Molecular Mechanisms…" *Front. Cell. Neurosci.* [2] Mcinnis, M, et al. "Cohort Profile…" *Int. J. of Epidemiol.* [3] Harrison, P, et al. "The Emerging Neurobiology…" *Trends Neurosci.* [4] Garrett, A, Chang K. "The Role of the Amygdala…" *Dev. and Psychopathology* [5] Gopalakrishnan, J. "The Emergence of Stem Cell-Based…" *BioEssays* [6] Zheng, Y, et al. "Controlled Modelling of Human Epiblast..." *Nat.* [7] Lancaster, M, Knoblich, J. "Generation of Cerebral Organoids…" *Nat. Protoc.* [8] Mariani, J, et al. "FOXG1-Dependent..." *Cell*