PERSPECTIVE

Determining the mechanism behind yoga's effects on preventing the symptoms of Alzheimer's disease

Background on the relationship between meditation/yoga practice and its effect on Alzheimer's disease (AD): Dementia refers to a variety of conditions that affect the normal function of the brain, leading to symptoms like memory loss, issues with problem solving, difficulty in processing thoughts and disordered language (McKhann et al., 2011). AD serves as one of the major causes of dementia as it is responsible for ~80% of its cases according to the Alzheimer's Association. AD affects the ability of neurons, the major information transmitting cells of both the peripheral and central nervous system, to send and receive signals through the body, leading to the aforementioned symptoms associated with dementia. The biological hallmarks of AD include the presence of amyloid beta (Aβ) plaques as well as neurofibrillary tangles containing a protein called tau (Lane et al., 2018). These proteins disrupt the normal function of neurons through a variety of mechanisms. In particular, AD first results in a loss of a specific neuronal subtype – the cholinergic neurons found in the basal forebrain - by reducing the expression levels of choline acetyltransferase. This enzyme breaks down acetylcholine, a neurotransmitter expressed in the neuromuscular junction, resulting in the loss of normal brain function. This loss of acetylcholine in AD patients was first noted in the late 1970s, and is now known to correlate with reduced cortical choline acetyltransferase, the enzyme responsible for synthesizing acetycholine from choline, which in turn correlates with neuritic plaque numbers and reduced Mini-Mental State Exam scores (Gauthier, 2002). Currently available pharmacological treatments for AD focus on inhibiting the loss of choline acetyltransferase, but do not represent a long-term cure.

Interestingly, a wide body of work exists demonstrating the practice of meditation, where a person trains their mind to focus on a concept or idea, and yoga, a discipline where practitioners perform coordinated sets of physical, mental, and spiritual activities derived from ancient India traditions, can slow and potentially reverse the symptoms associated with AD and its progression over time (Balaji et al., 2012; Acevedo et al., 2016). Numerous studies have identified several molecular pathways targeted by yoga and meditation, including those regulating metabolic and mitochondrial function, neurotransmission and inflammation (Hassan et al., 2018). In 2014 a novel therapeutic program for treatment of AD, known as metabolic enhancement for neurodegeneration, incorporated yoga and meditation into a regimen targeting multiple aspects of early AD pathology, achieving sustained cognitive improvement in 9 out 10 patients, including unprecedented quantitative and qualitative improvements after 24 months (Hassan et al., 2018). These studies provide evidence that yoga or meditative practice contribute to slowing the progression of cognitive decline in AD. However, the molecular mechanisms behind these biological effects have not been properly elucidated, motivating our study detailed in the next section.

Developing an AD model in a dish and examining the effects of neurotransmitters on preventing cellular death: We hypothesized that the neurotransmitters secreted during the practices of meditation and yoga could potentially reverse the effects of AD using a previously described *in vitro* model of AD (Price et al., 2001). Many different ways exist to model AD *in vitro*, and we chose to derive our neuronal cultures from human induced pluripotent stem cells, as they provide a way to generate the quantities of neural tissue necessary for testing our hypothesis due to their ability to replicate continuously (**Figure 1**). Human induced pluripotent stem cells can differentiate into a variety of mature neuronal lineages found in the brain and serve as a valuable tool for studying AD (Robbins and Price, 2017).

Here we replicated a model of AD in a dish by first generating basal forebrain cholinergic neurons from neural progenitors derived from human induced pluripotent stem cells treated with a combination of fibroblast growth factor 8, purmorphamine, bone morphogenic protein 9, and nerve growth factor (Bissonnette et al., 2011). We then treated our human induced pluripotent stem cell-derived basal cholinergic neurons with varying concentrations of the oligomer $A\beta_{1-42}$ for 48 hours to replicate the conditions present in the AD affected brain. We found that our *in vitro* model replicated two of the biological effects observed in AD – the loss of choline acetyltransferase expression and a significant reduction in the expression of the receptor p75 that binds neurotrophins (Hassan et al., 2018). We then screened a number of neurotransmitters and neurotrophins secreted during the practices of meditation and yoga

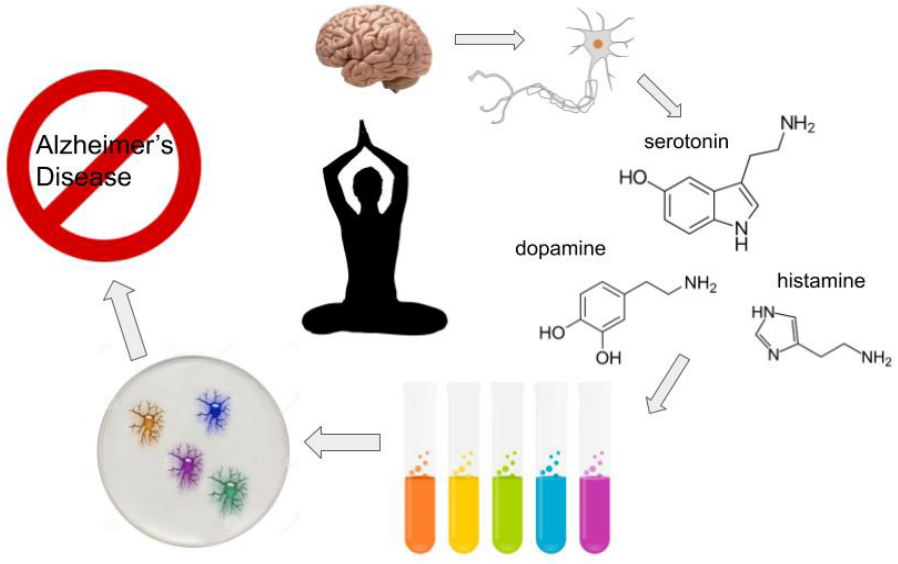

Figure 1 Graphical representation of of the human induced pluripotent stem cell-derived *in vitro* model of AD.
Human induced pluripotent stem cells are differentiated into basal forebrain cholinergic neurons, and induced to adopt an early AD pathology by addition of the oligomer $A\beta_{1-42}$. The effects of individual neurotransmitters (like serotonin, dopamine, and histamine) released during yoga and meditative practices can be studied on a cellular level in a lab setting using this model (Hassan et al., 2018). AD: Alzheimer's disease; Aβ: amyloid beta.

individually over a range of concentrations to determine where we observed effects of our *in vitro* model of AD. These screened factors included brain derived neurotrophic factor, nerve growth factor, histamine, dopamine, serotonin, and acetylcholine. Once we had determined which concentrations stimulated acetylcholine release in our model, we then tested these factors in 10 different combinations. Our work identified two combinations of factors (serotonin and dopamine, serotonin and histamine) which were sufficient to rescue cholinergic function as indicated by increased levels of choline acetyltransferase expression. This combination of factors suggests a potential mechanism for these observed neuroprotective effects through either inhibition of the synthesis of nitric oxide synthesis or by activating serotonin receptors to inhibit the production of the Aβ oligomers.

Future directions for further elucidating the effect of meditation and yoga on reversing the symptoms of AD: Our work represents a preliminary attempt at discovering the biological potential mechanisms responsible for the neuroprotective effects observed when patients suffering from AD practice mediation and/or yoga. It opens up a number of avenues for future work. In particular, it would be interesting to replicate our study by deriving basal cholinergic neurons from human induced pluripotent stem cell lines taken from AD patients to see if similar effects are observed compared to our model where healthy human induced pluripotent stem cell-derived basal cholinergic neurons are treated with exogenously applied Aβ oligomers. The variation between such patient derived cell lines may eventually lead to identification of biomarkers expressed in patient populations for whom such practices would be especially beneficial. The use of transcriptome profiling can provide a way to identify the specific molecular pathways being activated during treatment with these factors we have identified. These insights can provide a deeper understanding into the biological mechanisms of the disease and its associated pathways.

Furthermore, such an *in vitro* model would be beneficial in coordination with clinical studies to directly translate the actions of specific yoga movements and meditative practices to their subsequent neuroprotective biomechanisms. For instance, a simple 12-minute yoga technique known as Kirtan Kriya, originating from India, exemplifies how yoga can decrease stress brought on by AD. Not only does it decrease chronic stress, but it has been proven to increase blood flow to regions of the brain that regulate memory functions, helping to improve the symptoms of dementia that typically affects late onset AD patients (Khalsa and Perry, 2017). By identifying key movements, like ones supported by Kirtan Kriya (singing a specific mantra along with sequential tapping of the fingertips), yoga therapies could be more precisely prescribed to Alzheimer patients, fully realizing the therapeutic potential of yoga and meditative practices in treating AD.

Another exciting area for further exploring this mechanism would be the use of 3-dimension (3D) bioprinted models of AD. 3D bioprinting enables the generation of tissues from a design file by combining the desired cell types with a supportive bioink capable of ensuring cell survival and function (Thomas and Willerth, 2017). This technology has been rapidly evolving and the ability to bioprint 3D models of AD provides a powerful tool for both elucidating mechanisms of the disease and potential therapeutic strategies (Robbins and Price, 2017), as well as a way to screen potential drug targets (Willerth, 2018). The use of 3D bioprinting enables the culture of these neural tissues in 3D, making it a more relevant model for conducting research compared to traditional 2D culture models. Overall, this study along with these related technologies provide exciting possibilities for generating new insights and therapies for treating AD.

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