Alzheimer's and Related Diseases Research Award Fund

2011-2012 FINAL PROJECT REPORT SUMMARIES

The Alzheimer's and Related Diseases Research Award Fund (ARDRAF) was established by the Virginia General Assembly in 1982 t and is administered by the Virginia Center on Aging at Virginia Commonwealth University. Summaries of the final project reports submitted by investigators funded during the 2010-2011 round of competition are given below. To receive the full reports, please contact the investigators or the ARDRAF administrator, Dr. Constance Coogle (ccoogle@vcu.edu).

VCU   **Malgorzata Dukat, Ph.D. and Galia R. Abdrakhmanova, M.D., Ph.D. "Small Molecules as Negative Allosteric Modulators of α7 nAChRs"**

Both agonists and antagonists of α7 nAChRs (i.e., nicotinic acetylcholine receptors) have been shown to be of value in the treatment of AD. Agonists might desensitize the action of ACh at these receptors, thereby reducing cholinergic transmission, and antagonists block ACh transmission. An entirely novel approach is to identify negative allosteric modulators (NAMs) of α7 nAChRs that can selectively, but indirectly, block the effect of ACh at α7 nAChRs without acting at α4β2 receptors. The investigators previously identified one of the first small-molecule NAMs of α7 nAChRs, namely MD-354. Because this compound is a 5-HT$_3$ (serotonin) receptor agonist, the investigators modifed its structure to abolish that action, and thereby develop "selective" α7 nAChR allosteric modulators. They synthesized a series of MD-354 analogs and evaluated them in functional assays to determine what structural features are required, and to optimize the pharmacological actions by eliminating affinity for 5-HT$_3$ receptors. The present study delivered proof of concept that small molecules, the guanidines, represent a novel class of α7 nAChR NAMs. Furthermore, the investigators demonstrated that small structural changes to MD-354 diminished or abolished its 5-HT$_3$ receptor affinity, while retaining its α7 nAChR activity. All the NAMs examined display antagonism action at α7 nAChR with half maximal inhibitory concentration (IC$_{50}$) values ranging from 1.3 – 34.8 µM. To eliminate possible competitive antagonism or channel blocking action, MD-354 and one of the newly synthesized agents were evaluated in voltage-dependence inhibition of ACh experiments and both proved to be α7 nAChR NAMs. In addition, they were assayed for antagonistic activity at α3β4 and α4β2 nAChRs and were found to be inactive, suggesting their selective action at α7 nAChRs. The most potent NAM, was further evaluated in radioligand binding assays for its selectivity among cloned nAChRs (i.e., α2β2, α2β4, α3β2, α4β2, α3β4, α4β2, α4β4) and was found to lack binding affinity at all seven (i.e., K$_i$ > 10,000 nM). This is the first study identifying guanidine analogs as small molecule α7 nAChR NAMs. In contrast to current ACh inhibitors that are limited to symptomatic treatment of cognitive function, these new agents offer the potential for slowing the progression of AD. *(**Dr. Dukat may be contacted at 804/828-5256; mdukat@vcu.edu and Dr. Abdrakhmanova may be contacted at 804/828-1797; gabdrakhmano@vcu.edu)***

GMU   **Jane M. Flinn, Ph.D., Nathalia Peixoto, Ph.D., and Daniel N. Cox, Ph.D.** "**Behavioral and Inflammatory Changes in a Mouse Model of Late-Onset Alzheimer's Disease"**

The investigators used their mouse model of late onset AD, where the gene APOE4 is important, to examine behavioral and inflammatory changes in mice modeling early onset and late onset AD, together with controls. They examined circadian rhythms, nest building, memory, and the levels of cytokines. Surprisingly the mice carrying the APOE4 gene performed slightly better than the early onset mice, although less well than controls, on measures of circadian rhythms and nest building. (Memory scores were difficult to compare as the controls performed less well than expected.) Their measures of inflammation were also intermediate between those of the controls and the early onset mice. There was a significant correlation between measures of circadian rhythm disruption and inflammation. These were younger mice, suggesting than APOE4 may not be a risk factor at a younger age, and measures of inflammation and circadian rhythm could be useful early indicators of Alzheimer's disease. (***Dr. Flinn may be contacted at 703/ 993-4107; Dr. Peixoto may be contacted at 703/993-1567; Dr. Cox may be contacted at 703/ 993-4971)***

VCU **Aron H. Lichtman, Ph.D. and Laura E. Wise, Ph.D. "Targeting the Endogenous Cannabinoid System to Treat Alzheimer's Disease"**

In vitro studies have shown that cannabinoid receptor activation can inhibit or reduce the deposition of beta-amyloid plagues and decrease inflammation, critical features of Alzheimer's disease. While these findings indicate that cannabinoids may be beneficial in attenuating the neuropathology associated with Alzheimer's disease, very few studies have evaluated if stimulation of the endocannabinoid system can attenuate cognitive deficits and neuropatholgy in *in vivo* models of Alzheimer's disease. The goal of the funded studies was to examine whether elevating endogenous levels of the endocannabinoid anandamide via inhibition of its primary degradative enzyme fatty acid amide hydrolase (FAAH), would have beneficial effects on memory impairments and neuropathological markers in APP/PS1 transgenic mice, an *in vivo* model of Alzheimer's disease. The investigators found that repeated administration of the FAAH inhibitor PF-3845 improves memory performance in APP/PS1 transgenic mice, but not control mice, in the Morris water maze. They also found that acute treatment (i.e., drug administration only before water maze testing) with PF-3845 improves memory performance in APP/PS1 transgenic mice, but to a lesser degree than repeated PF-3845 treatment. Immunohistochemistry studies are currently underway to evaluate Aβ plaque formation and the presence of activated microglia in the dorsal hippocampus to determine whether repeated treatment with PF-3845 decreased neuropathological markers associated with Alzheimer's disease. It is apparent however, that PF-3845 improves memory performance in an *in vivo* mouse model of Alzheimer's disease. These findings suggest that FAAH inhibition may have beneficial effects on memory impairments in Alzheimer's disease. They furthermore provide proof of principle that the endogenous cannabinoid system represents a potential target for medications to treat AD..

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GMU **Robert H. Lipsky, Ph.D**. **"Functional Characterization of Promoter Polymorphisms of the Human GRIN2B Glutamate Receptor Gene Associated with Altered Memory Functioning in Older Adults"**

Mild cognitive impairment (MCI) is a clinical diagnosis that describes a small but measurable decline in an individual's cognitive abilities, including memory. A person with MCI is at greater risk of developing Alzheimer's disease. Currently, there are no methods for early detection of MCI or to predict the outcome of MCI or its progression to Alzheimer's disease. FDA-approved drugs for treating symptoms of Alzheimer's do not seem to benefit MCI patients, underscoring a need to understand the underlying mechanisms leading to MCI. Using a method that combines an understanding of human genetics and brain imaging, the investigators discovered a variant of the GRIN2B gene, a gene critical for learning and memory that may be a marker for MCI. The funded study determined how this genetic variant controls the GRIN2B gene at the biochemical and cellular level. They found a protein, Elk-1, that binds the DNA of the GRIN2B gene variant, called the A allele. The A allele also activates genes introduced into neuron-like cells maintained in the laboratory. These results support their previous observation that the A allele of the GRIN2B gene is linked to a specific pattern of brain activity seen when older adults (who were otherwise matched for age, gender, and cognitive ability) performed certain memory tasks. Taken together, these results are the first to support the role of a GRIN2B gene variant associated with human memory performance based on molecular and cellular function. They constitute the first genetic association between a functional NR2B gene variant and an endophenotype, a characteristic that is more closely related to pathophysiology of AD than diagnostic markers.

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