The Effects of BACE and Its Targets on Age-related Seizures in *Drosophila*

Trey Chiu, Dana Gibbons; Dr. Bonnie Bolkan, faculty advisor

Department of Biology, Linfield College, McMinnville

Abstract

The presence of Beta-Amyloid (Aβ) containing plaques in the brain is one of the histological hallmarks of Alzheimer's Disease. β-secretase (BACE) is the enzyme responsible for producing this Aβ cleavage product and has also been shown to affect myelination and general neuronal activity. Observations from geriatric medicine suggest that there may be an increase in seizure activity associated with Alzheimer's Disease. Preliminary data suggests that both over- and under-expression of BACE contributes to mechanically stimulated seizures in *Drosophila*. In vertebrates, seizure activity has been correlated with many factors including Neuregulin production and Na^+ Pump activity. Both of these proteins have also been shown to require BACE activity for proper function. However, their roles in BACE related seizures remains unknown. Here we are following up on this preliminary study and exploring the roles of Vein (the *Drosophila* homolog of Neuregulin) and Numb (a negative regulator of the Notch Pathway). We have confirmed that any perturbation in dBACE (*Drosophila BACE)* levels causes a significant increase in age related seizures, suggesting that that BACE levels must be tightly regulated. In addition an increase of Vein levels also cause an increase in seizure amounts and duration suggesting that BACE, at least in part, is acting through this signaling pathway. Understanding which BACE related signaling pathways are responsible for age related seizure activity can lead to new treatments which will hopefully slow the progression of Alzheimer's and other related neurodegenerative diseases.

Background

Alzheimer's Disease is a neurodegenerative disease marked by progressive memory loss and a lack of problem solving and judgment abilities. As of 2014, this disease was the sixth leading cause of death in the U.S. and costs $226 billion for healthcare annually [1]. The pathological features of this disease include intracellular Tau tangles and extracellular Beta-Amyloid plaques. The Beta-Amyloid peptides composing the plaques are the cleavage product of Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) activity. Many neurodegenerative mutants, including BACE, not only develop pathology in their brains but also display behavioral deficits. In fact these deficits frequently develop prior to the presence of gross degenerative abnormalities suggesting that they are indicative of specific defects rather than a loss of mass brain integrity. Previously in the lab, it was noticed that dBACE mutant flies are overly sensitive to mechanical stimulation (such as banging) resulting in seizures. Seymour Benzer first described epileptic *Drosophila* mutants that have seizures in response to banging in 1971 [2]. In the intervening decades *Drosophila* has become a well-defined and accepted model organism for seizure susceptibility studies. The Epilepsy Foundation of America reports that approximately 10% of Alzheimer's Disease patients also suffer from epilepsy compared to less than 1% prevalence in the normal population.

Here we show that alterations in levels of dBACE, the *Drosophila* homlog of BACE1, cause earlier and longer seizures in *Drosophila*. BACE has many cleavage targets including APPL, Vein and the ß-subunit of a Na^+-gated Ion Channel among others. To test which cleavage targets affect seizure susceptibility, we assayed flies overexpressing Vein or *numb*[1] mutants. We found that mutations in Numb resulted in a significant increase in seizure susceptibility.

Methods

Fly Strains: OregonR flies were used as the wildtype control and *numb*[1]/CyO flies were from Bloomington Stock Center. UAS-Vein flies were a gift of Amanda Simcox (University of Ohio), and UAS-dBACE flies were from the Kretschmar lab at OHSU. UAS-dBACE^{RNAi} were obtained from the Vienna Drosophila Resource Center.

Mechanical Stimulation of Seizures: Male flies were kept on standard food at 23°C and starved 3 hours prior to testing. To observe bang-sensitive seizure phenotypes, flies were vortexed for 8 seconds at a medium setting, after which seizure duration was recorded. Flies seizing for 3 seconds after removal from the Vortexer were counted as "seizing".

Results

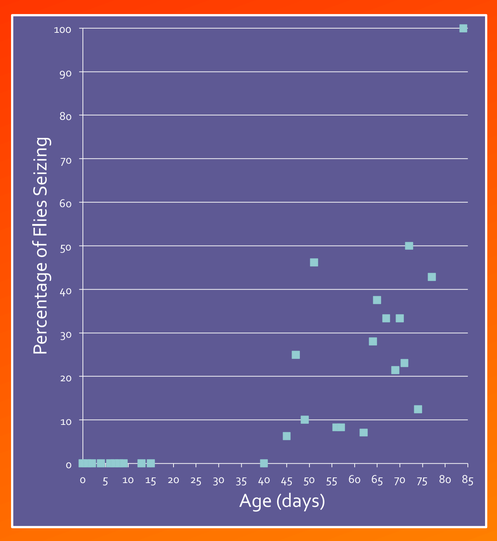

Figure 1: Proportions of UAS-dBACE^{RNAi} males seizing. Seizures became more prevalent after flies aged 45 days. Wildtype flies were completely seizure free at this age. (n = 382)

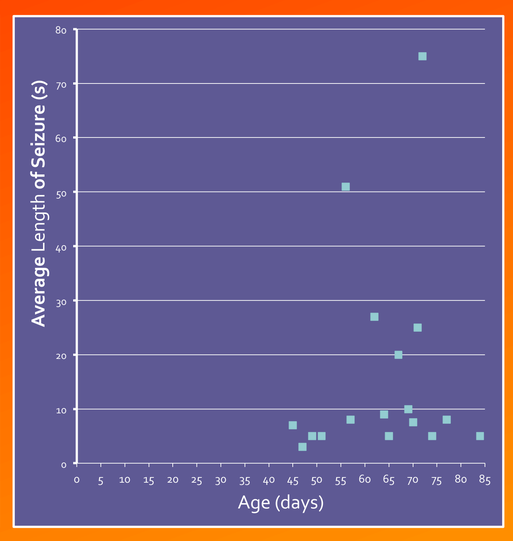

Figure 2: Average seizure lengths for male UAS-dBACE^{RNAi} flies. Seizure lengths are similar to those in flies overexpressing dBACE. (n = 382)

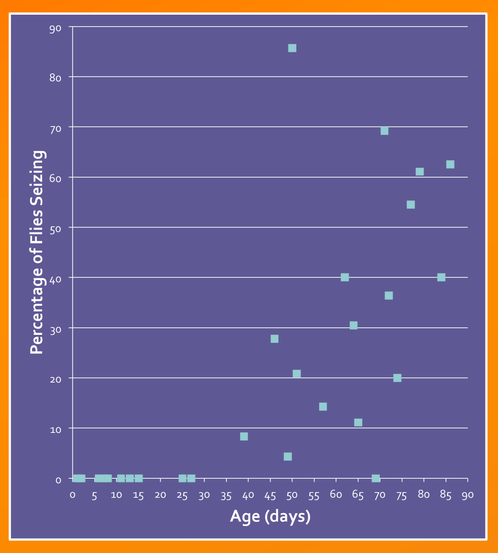

Figure 3: Proportions of UAS-dBACE males seizing. Seizures became prevalent after 39 days. (n = 414)

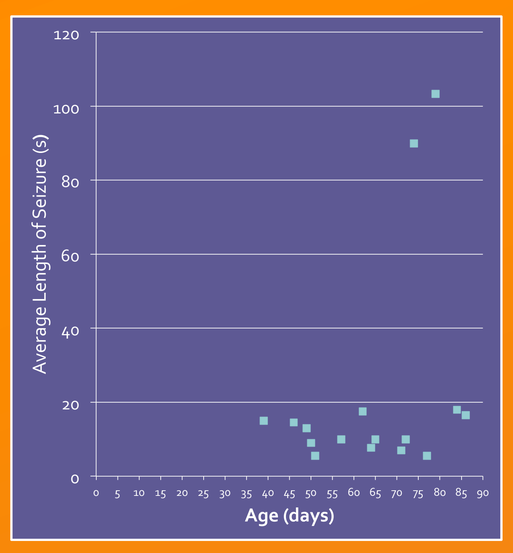

Figure 4: Average lengths of seizures for male UAS-dBACE^{RNAi} flies. (n = 414)

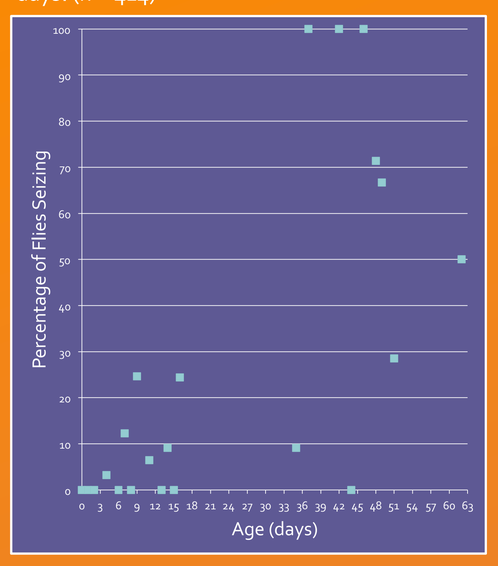

Figure 5: Proportions of UAS-Vein male flies seized. Flies began seizing at 4 days and reached 100% seizure susceptibility by 37 days. (n = 667)

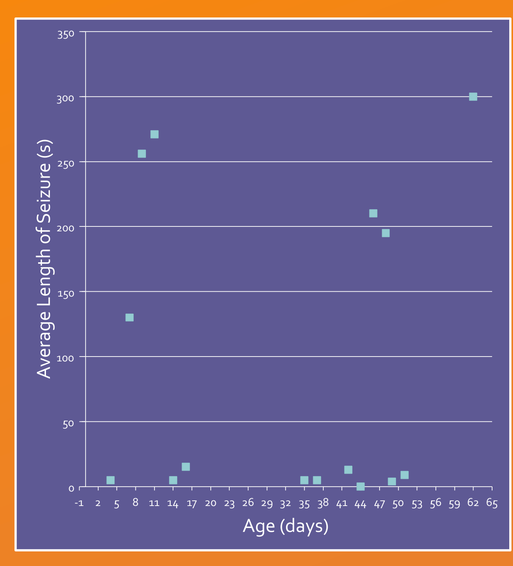

Figure 6: Average seizure lengths for male UAS-Vein flies. Seizure lengths are longer than any other observed mutant and seizure length was capped at 300s. (n = 667)

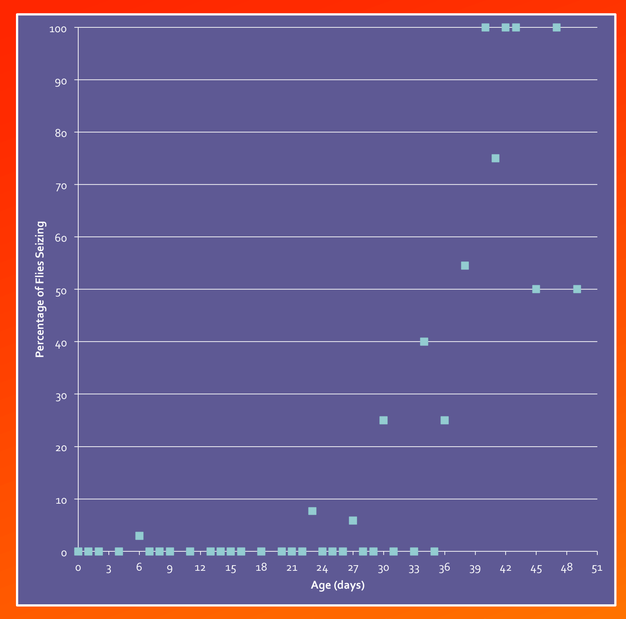

Figure 7: Proportions of *numb*[1]/CyO flies seized. Seizures became prevalent after flies aged beginning at 23 days and reached 100% seizure susceptibility beginning at 40 days. Wildtype flies, in comparison, were still completely seizure free at this age. (n = 593)

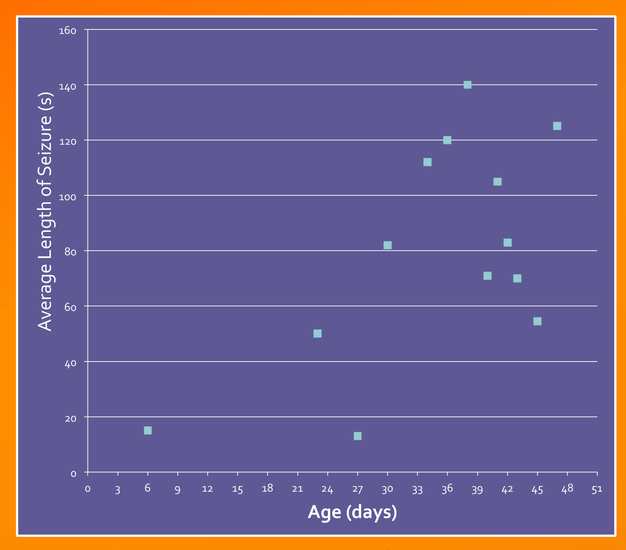

Figure 8: Average seizure lengths of male *numb*[1]/CyO flies. Of flies having seizures, the duration of seizures also increased with age. (n = 593)

Previous results using OregonR used as control completely seizure-free until 48 days old. Previous work shows that, under more stringent conditions, OregonR flies were seizure-free for 60 days.

Discussion and Future Directions

- Changing dBACE levels by overexpression or knockdown cause earlier and longer seizures.
- *numb*[1] mutants result in seizures even earlier and longer than those caused by perturbations in dBACE levels.
- Sample size for overexpression of Vein flies is too low to allow for conclusive findings. However, trends suggest that excess Vein also causes earlier and longer seizures than in wildtype flies.
- Higher n's are needed for all groups in order to determine statistical significance.
- Creation of double mutants of dBACE and Numb or Vein will allow testing of seizure susceptibility to determine if relationship exists in molecular pathway of these seizures.
- Previous results have shown all of these flies exhibit neurodegeneration phenotypes. We will determine the correlation between histology and seizure behavior.

Works Cited:

1) Alzheimer's Association. 2015 Alzheimer's Disease Facts and Figures. Alzheimer's & Dementia 2015;11(3) 3 32

2) Benzer, S. "From the gene to behavior." *JAMA* 218, no. 7 (Nov 1971): 1015-22.