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Abstract

Glial cells, the resident immune cells of the brain, are important for brain health; they perform critical protective functions following brain injury, during nervous system development, and to support general neuronal activity. Following brain injury, glia quickly respond by cleaning up neuronal and synaptic debris. In addition to their role as the cleaning cell of the brain, recent research has shown that during development, glia also shape and fine-tune neural connections by eliminating weak synapses fated for removal. However, whether glial cells also continue to remodel synaptic architecture in the adult brain is unclear. Moreover, synaptic loss is an early hallmark of Alzheimer's disease (AD), but whether glial cells play a role in this pathophysiology remains mysterious. In this study, we investigate the role of glial cells in AD-related synaptic loss using a *Drosophila melanogaster* model of AD. Specifically, we will explore the role of the glial engulfment receptor Draper, which is essential for glial clearance of neuronal debris after injury, and evaluate its effect on synaptic loss in the AD fly model. For these studies, we will use immunohistochemistry and western blotting techniques, as well as the power of Drosophila genetics. Through this project, the effects of amyloid beta on synaptic molecule expression are assessed and the contribution of the glial engulfment receptor Draper upon amyloid-beta induced synaptic changes are identified.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease, which is implicated by hallmark aggregations of extracellular amyloid beta protein. Mutations in the amyloid precursor protein (APP), causing a truncated version of the protein have been identified as a risk factor for Alzheimer's disease (Goate et al. 1991; Citron et al. 1992). However, attention is also shifting to the immune cells of the brain called microglia. As the resident immune cells of the brain, they are actively surveying the brain parenchyma for injury (Nimmerjahn et al. 2005). Upon injury, they quickly respond, by changing their morphology and protein expression to engulf cellular and synaptic debris through active phagocytosis (Ivacko et al. 1996). In addition, microglia are critical for proper brain development as they shape synaptic connections during development. It has been shown that developing mice deficient for one essential microglial engulfment receptor displayed unrefined synaptic connectivity due to deficits in synaptic pruning (Paolicelli et al. 2011; Zhan et al. 2014).

In Drosophila, the glial engulfment receptor Draper has been shown to be essential in glial engulfment of neuronal debris following injury (MacDonald et al. 2006). Upon axon injury, glial cells recruit their membranes at the site of injury and upregulate Draper. When Draper is knocked down or glial phagocytic activity is inhibited, axon fragments persist and are not cleared (Doherty et al. 2009). While Draper is specific to Drosophila, it is highly conserved and its mammalian homolog MEGF10 shows similar function in glial engulfment in mammals (Chung et al. 2013). This positions Draper as an essential and specific glial receptor for the interaction between glial cells and damaged/dying neurons and allows us to use the powerful Drosophila model to investigate conserved pathways.

In improving our understanding of Alzheimer's disease, recent data suggests that amyloid beta aggregation is not the very first or critical molecular symptom of Alzheimer's disease (Scheff and Price 2003). In mice, synaptic loss and dysfunction has been observed before any sign of hallmark aggregation (Yoshiyama et al. 2007), however whether glial cells are involved in this process is not clear. In addition, defects in glial cells have been shown to accumulate in the aging brain potentially leading to disease (Hanisch and Kettenmann 2007; 2010). We aim to validate a Drosophila model of Alzheimer's Disease and create a timeline of when synaptic loss occurs in relation to neurodegeneration. Using this timeline, we will then be able to examine the role of glia in this process by investigating whether synaptic loss still occurs in the AD fly model when Draper is knocked out. By investigating the very initial pathophysiology observed in Alzheimer's Disease and its associated signals, we hope to shed light on a potentially powerful mechanism to halt Alzheimer's Disease before it leads to progressive neurodegeneration and cognitive decline.

Methods

Fly Lines

w+/-; elav-Gal4/ UAS-Aβarc; +

w+/-; elav-Gal4/ w118; +

w+/-; elav-Gal4/BRP-SNAP; UAS-Aβarc

w+/-; elav-Gal4/ BRP-SNAP; +

w+/-; elav-Gal4/ BRP-SNAP; Drpr Δ5 rec 9/UAS-Aβarc Drpr Δ5 rec 9

Crosses were set up and maintained at 25° C. Flies were collected every 2 days (M, W, F) and incubated at 30° C.

Adult Fly Brain Fixation

Fix Solution 1- 1x PBS, 0.01% Triton-X, 4% Paraformaldehyde

Wash Solution 1- 1x PBS, 0.01% Triton X

Fix Solution 2- 1x PBS, 0.1% Triton-X, 4% Paraformaldehyde

Wash Solution 2- 1x PBS, 0.1% Triton X

Adult flies from chosen groups were anesthetized and heads removed. Heads were fixed in Fix Solution 1 for 20 minutes with rocking. Heads were washed in wash solution 1 3 x 2 minutes. Brains were dissected in wash solution 1 in a standard dissection plate. Newly dissected brains were transferred to new tubes with wash solution 1. Wash solution 1 was pipetted out and replaced with Fix Solution 2. Brains were fixed for 20 minutes at room temperature with rocking. Brains were washed 3 x 2 minutes in wash solution 2.

Antibody Staining

Primary antibody master mixes were prepared at the following concentrations, diluting the antibody in wash solution 2:

Mouse Anti-Draper 1:400; Guinea Pig Anti- Draper 1:400; Mouse Anti-Aβ 1:2000; Mouse Anti-Bruchpilot 1:50.

Secondary antibody master mixes were prepared at the following concentration, diluting the antibody in wash solution 2:

Mouse 488 1:400; Guinea Pig 546 1:400 (Jackson Immuno).

Brains were incubated in primary antibody with shaking at 4^{o}C overnight. Brains were washed 4 x 30 minutes in wash solution 2. Brains were then incubated in secondary antibody for 2 hours at room temperature with shaking and then washed 4 x 30 minutes in wash solution 2.

BRP-SNAP Staining

SNAP Tag mix was prepared at a concentration of 1 μM in 1x PBS with 0.3% Triton X. Brains were permeabilized for 1 x 10 min in 1x PBS with 0.3% Triton X for 10 min prior to staining. Brains were stained for 15 minutes at room temperature in a glass dissection dish with rocking. Brains were washed 1 x 5 min in 1x PBS & 0.3% Triton X and 1 x 5 min in 1x PBS.

Mounting

Brains were transferred to the glass well of a dissection dish and the wash solution was carefully removed with a pipette. A drop of Vectashield (Vector Labs) was then immediately dispensed onto the brains. Brains were incubated for 30 minutes at 4^{o}C or overnight if desired. Slide preparation required placing a strip of clear tape onto a clean microscope slide. A template was used to trace and cut 4 wells into the tape. Brains were carefully transferred from the dissection dish to their appropriate well on the microscope slide. Excess Vectashield was carefully removed and brains were aligned and oriented with antennal lobes facing up. Small drops of Vectashield were added once all brains were oriented and a coverslip was gently dropped onto the wells. The coverslip was sealed around the border with a layer of CoverGrip (Biotium).

Confocal Microscopy and Quantification

Images were taken on a Zeiss LSM 700 confocal microscope using a 40X oil immersion lens. For Draper staining, 11 slices through the Z plane were taken per brain at an interval of 1 µm. For Bruchpilot staining, 35 slices were taken through the Z plane at an interval of 0.5 µm.

Quantification was completed using the Volocity software (Perkin & Elmer). For Draper staining, the 5 slices, which depicted the same anatomical region in each sample, were quantified. Two standard circles were used to measure regions within the cortex above the antennal lobes. A threshold for background fluorescence was set in addition to a restriction of voxels 0.2 um in size or greater. For Bruchpilot staining, the 20 slices, which depicted the same anatomical region, were quantified. The antennal lobes were traced out, cropped and quantified. A threshold for background fluorescence was set in addition to a restriction of voxels 0.2 um in size or greater. Data was analyzed and graphed in GraphPad Prism 6.

Western Blot Protocol

Whole heads were pulled and collected in 1X loading buffer (10 heads per 30 uL of 1x loading buffer). Samples were homogenized using a pestle and centrifuged for 10 minutes at 4^{o}C. Supernatant was collected and boiled for 10 minutes at 95^{o}C and frozen at -80^{o}C.

10x running buffer was diluted to 1x and chilled at 4^{o}c prior to use. 12 uL of sample was loaded into each well of a 10% Tris-HCl gel. 1.5 µL of protein ladder was loaded into edge lanes. Gel was run at a constant 125V for 2 hours.

The wet transfer sandwich was prepared after the fiber pads, filter paper, and nitrocellulose membrane were soaked in transfer buffer. Gel sandwich was placed back in gel and ran at 100V for 90 minutes.

Membrane was soaked in methanol and then blocked with SEA Block (Calbiochem) for 30 min. Membrane was incubated overnight at 4^{o}C with primary antibodies of the following concentrations: Rabbit Anti-Draper 1:2000 and Sheep Anti-Tubulin 1:4000. Membrane was washed 3 x 10 min in 1xPBS-Tween. Secondary antibodies (Rabbit 790 1:2000 & Sheep 680 1:2000) were prepared in 1X PBS and applied to membrane for 2 hours at room temperature. Membrane was washed 2x5 minutes in 1xPBS-Tween and 2x 5 minutes in 1xPBS. Membrane was visualized on Li-Cor Odyssey Imager.

Results

Note:

Control: w+/-; elav-Gal4/ w118;

Experimental: w+/-; elav-Gal4/ UAS-Aβarc; +

Flies show progressive Aβ accumulation in cortex

In verification of our Drosophila model, brains were stained for Amyloid Beta which can be seen in cyan within the cortex (Figure 1). Control (w +/-; elav-Gal4/w118) flies showed no Amyloid Beta plaques. In addition, Amyloid Beta displays an increase in aggregation from the 1-3 D time point to the the later time point of 12-14 Days. Amyloid Beta was not quantified. Taken together, these initial results allow for a confirmation that our model system displays characteristic Alzheimer's disease pathology.

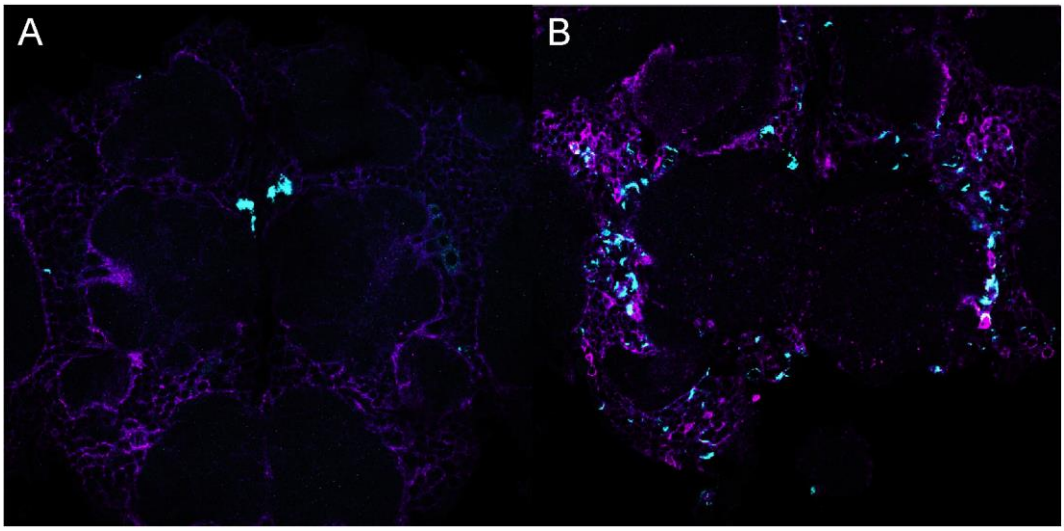

Figure 1: Flies show progressive Aβ aggregation in cortex. A) Elav-Gal4 UAS-Aβarc 1-3 Days immunostained for Draper (magenta) and Amyloid Beta (cyan). B) Elav-Gal4 UAS-Aβarc 12-14 Days immunostained for Draper (magenta) and Amyloid Beta (cyan).

Draper fluorescence is increased in young AB flies

Whole brains were stained for Draper (Figure 2) and the Draper fluorescence within the cortex was quantified (Figure 3). The Draper fluorescence was elevated, although not significantly, in flies expressing the Aβ-arc mutation. This data suggests that Draper's engulfment activity within glial cells is possibly elevated as well.

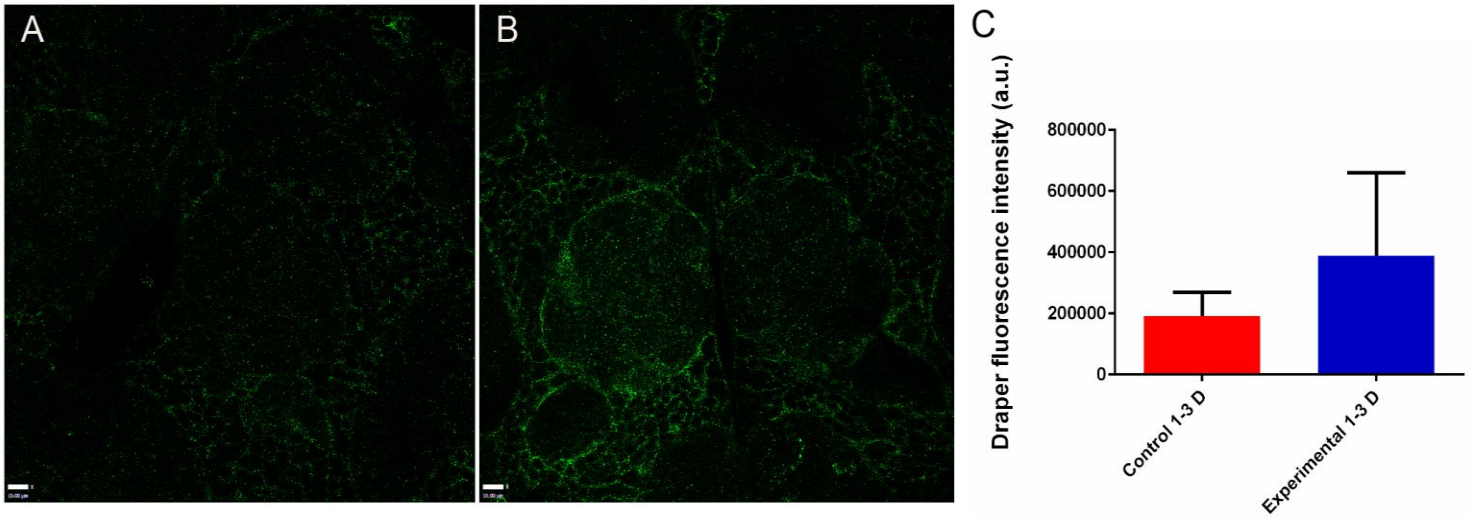

Figure 2: Draper fluorescence is increased in young Aβ flies. A) Elav-Gal4 x Wt 1-3 Days after eclosion immunostained for Draper (green). B) Elav-Gal4 UAS-Aβarc 1-3 Day flies immunostained for Draper. n= 6-15. C) Fluorescence within two standard circle regions within the cortex was quantified using Volocity software. n= 6-15.

Synaptic fluorescence and density is decreased in young Aβ flies

To further delineate glial activity, synaptic levels were assessed via immunofluroescent staining targeting the synaptic protein Bruchpilot. Control flies displayed elevated fluorescence, although not statistically significant, suggesting that Aβ-arc expressing flies have a deficit in synaptic density.

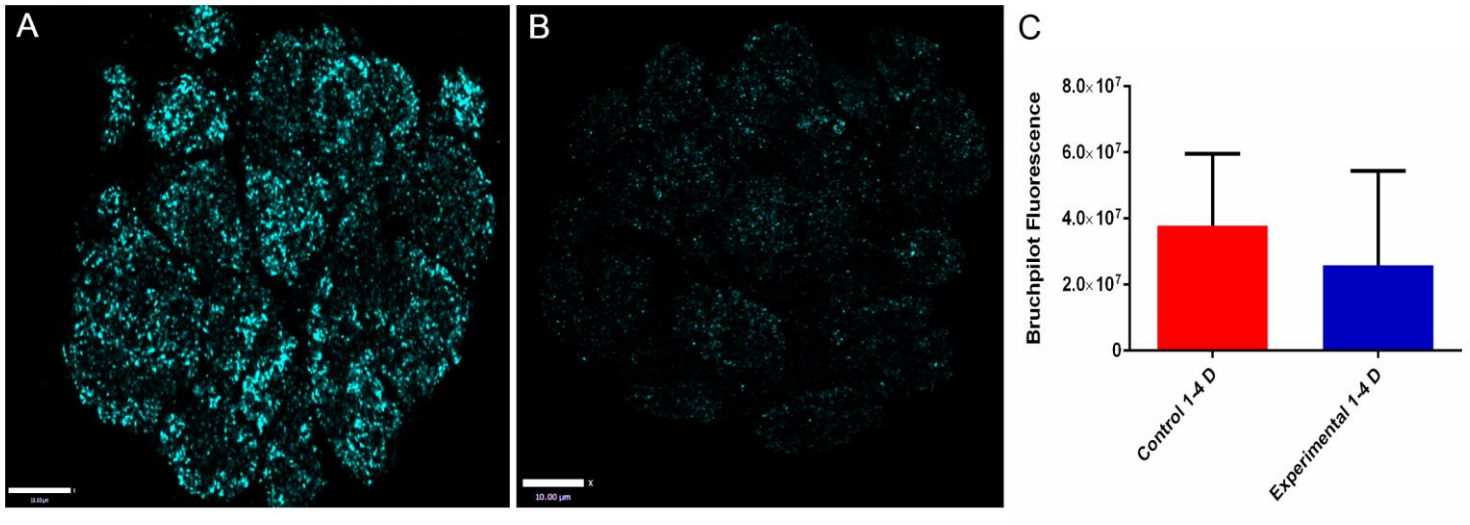

Figure 3: Synaptic density is decreased in young Aβ flies. A) Elav-Gal4 x Wt anntenal lobe aged 1-4 days stained for Bruchpilot synaptic protein. B) Elav-Gal4 UAS-Aβarc aged 1-4 Days stained for Bruchpilot synaptic protein. n= 8-13. C) Fluorescence within antennal lobes was quantified using Volocity software. n= 8-13.

Draper protein is similary expressed in whole brains of WT and AD flies

In order to more quantitatively assess Draper levels within the control and experimental groups, a Western Blot was performed to directly analyze protein levels. When quantfied and normalized against the corresponding total protein gel, Draper protein levels were similar amongst both control and experimental groups. This suggests that minimal differences exist in Draper protein expression between groups in whole brain lysates.

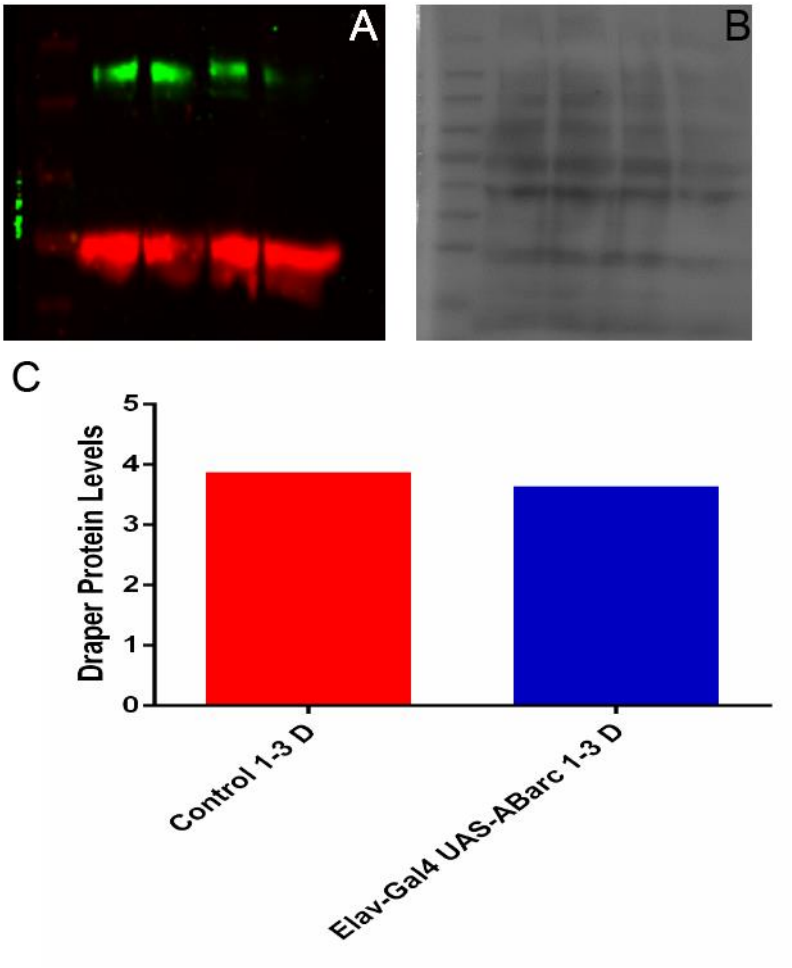

Figure 4: Western Blot of Draper and Tubulin. A) From left: Ladder, Lanes 1 & 2: Experimental 1-3 Days, Lanes 3 & 4: Control 1-3 Days. Green band refers to Draper. Red band refers to Tubulin. B) Total protein gel served as loading/normalization control. C) Draper bands were quantified and normalized against corresponding lanes in total protein gel.

Draper deficiency does not rescue synapses

To ultimately evaluate whether Draper exerts some role on synaptic loss in the earliest stages of Alzheimer's Disease, Draper null Aβarc flies were examined for Bruchpilot fluorescence. Draper null flies in an Aβarc background showed no rescue or increase in synapses in comparison to Aβarc flies.

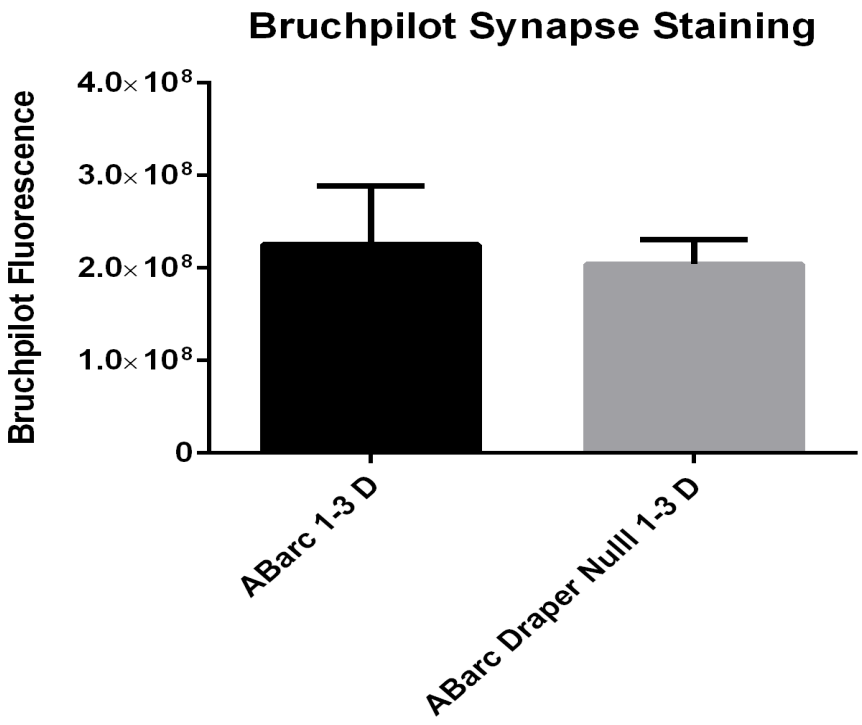

Figure 5: Draper is not essential for AD synaptic loss. w +/-; elav-Gal4/BRP-SNAP; UAS-Aβarc in left column; w +/-; elav-Gal4/ BRP-SNAP; Draper null/UAS-Aβarc Draper null in right column. Synapses were stained using a molecular probe against an endogenous SNAP-Tag attached to Bruchpilot. n= 11-16.

Discussion

Glial cells are a multi-faceted cell type playing essential roles in development, injury, and maintenance. Their significant contribution to the brain microenvironment makes them a prime candidate to investigate what effect they may have on early Alzheimer's disease (AD), which is best characterized by synaptic loss that occurs before the onset of symptoms like memory loss. As glial cells are capable of pruning and engulfing synapses during development, this study aimed to investigate how glial cells may be involved in synaptic loss in the context of Alzheimer's disease. More specifically, the highly conserved glial engulfment receptor Draper has previously been shown to be crucial for glial engulfment activity following neuronal injury (MacDonald et al. 2006; Doherty et al. 2009). Therefore, we hypothesized that Draper may also be upregulated and involved in AD related synaptic loss.

For this study, we used a Drosophila model of Alzheimer's disease, in which human Aß is expressed pan-neuronally in the fly brain. In humans and mouse models of Alzheimer's disease, this protein forms aggregates and leads to destruction of neurons. More specifically, the expressed Aß protein in our fly model contained the arctic mutation, which is known to cause severe protein aggregation in human disease (Nilsberth et al. 2001). In our model, we observed the formation of Aß-aggregates in the fly brain shortly after eclosion, and they increased in size and number with age. In addition, we observed motor deficits in the Aß-expressing flies and a reduced lifespan compared to age-matched controls. The advantages of this model system in Drosophila are the ability to selectively express human Amyloid Beta in all neurons by utilizing the Elav-Gal4 UAS

system. The limitations are that the current model does not allow for adult specific Aβ expression. Instead, Aβ is expressed throughout development thereby quickly forming plaques after eclosion. This is in stark contrast to human Alzheimer's Disease pathology which requires decades for Aβ plaques to form.

By utilizing immunohistochemistry techniques, Draper and the presynaptic protein Bruchpilot were visualized and quantified in both experimental Aß-expressing and age matched non-Aß expressing control groups. Bruchpilot staining revealed that synapses were decreased in young (1-4 Days) Aβ flies (Figure 3), while Draper staining in young (1-3 day old) Aβ flies showed an upregulation of Draper in the olfactory antennal lobe region of the adult fly brain (Figure 2). These studies suggested that Draper may play a role in glial cell mediated synaptic loss in AD.

To further investigate whether Draper is involved in early synapse loss in AD, an Aß-expressing fly line in a Draper null mutation background was created. This allowed us to explore whether the removal of Draper would result in an increase or rescue of synapses. Staining with a fluorescent molecular probe against an endogenous SNAP-Tag attached to Bruchpilot allowed for more specific and thorough staining in a control group (w+/-; elav-Gal4/BRP-SNAP; UAS-Aßarc) and experimental Draper null group (w+/-; elav-Gal4/ BRP-SNAP; Draper null/UAS-Aßarc Draper null). This study revealed that Draper removal conferred no increase or rescue of synapses (Figure 5). This preliminary data suggests that Draper is not essential in inducing synaptic loss in AD. However, this does not eliminate the possibility that glial cells contribute to synaptic loss. Alternative glial engulfment pathways such as the Crk/Mbc/dCed-12 may be implicated in synaptic loss. The Crk/Mbc/dCed-12 pathway acts in a semi non-redundant fashion downstream of Draper to actively phagocytose and uptake cellular debris (Ziegenfuss et al. 2012). While Draper is required for initial glial activation in response to injury, this study suggests that it is not the key player for AD-related synaptic loss. Other mediators in the glial engulfment pathway downstream of Draper, such as the Crk/Mbc/dCed-12 pathway, should be investigated in future research.

Although Draper was seen to be upregulated in young Aβ flies but does not rescue synaptic loss when knocked down, it may be upregulated for a myriad of other biological reasons. Glial cells are known to engulf Aβ plaques and prune synapses during development (Bolmont et al. 2008; Paolicelli et al. 2011; Chung et al. 2013) and this could be the reasons Draper upregulation was observed in our Drosophila AD model. Future work should include refining the AD model in Drosophila in order to better mimic the pathophysiology of AD. For example, in our experimental group, Aß was expressed throughout development and plaques form a few days after eclosion. In order to create a more realistic AD model, Aβ could be expressed strictly in adult flies without perturbing development. This may model the human age-related disease more closely. This may be accomplished through the use of a weaker neuronal driver than Elav-Gal4, or through genetically inhibiting Aß expression during the fly's development.

In conclusion, this study provides a preliminary depiction of the role of Draper in glial mediated synaptic loss in the context of AD. Further research would include repeating these experiments and investigating other engulfment pathways which may be implicated in the earliest symptoms of AD.

Works Cited

Bolmont T, Haiss F, Eicke D, Radde R, Mathis CA, Klunk WE, et al. Dynamics of the Microglial/Amyloid Interaction Indicate a Role in Plaque Maintenance. J Neurosci. 2008 Apr 16;28(16):4283–92.

Chung W-S, Clarke LE, Wang GX, Stafford BK, Sher A, Chakraborty C, et al. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. Nature. 2013 Dec 19;504(7480):394–400.

Citron M, Oltersdorf T, Haass C, McConlogue L, Hung AY, Seubert P, et al. Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature. 1992 Dec 17;360(6405):672–4.

Doherty J, Logan MA, Tasdemir OE, Freeman MR. Ensheathing Glia Function as Phagocytes in the Adult Drosophila Brain. J Neurosci Off J Soc Neurosci. 2009 Apr 15;29(15):4768–81.

Goate A, Chartier-Harlin M-C, Mullan M, Brown J, Crawford F, Fidani L, et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature. 1991 Feb 21;349(6311):704–6.

Hanisch U-K, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci. 2007 Nov;10(11):1387–94.

Ivacko JA, Sun R, Silverstein FS. Hypoxic-Ischemic Brain Injury Induces an Acute Microglial Reaction in Perinatal Rats1. Pediatr Res. 1996 Jan;39(1):39–47.

Lynch AM, Murphy KJ, Deighan BF, O'Reilly J-A, Gun'ko YK, Cowley TR, et al. The impact of glial activation in the aging brain. Aging Dis. 2010 Dec;1(3):262–78.

MacDonald JM, Beach MG, Porpiglia E, Sheehan AE, Watts RJ, Freeman MR. The Drosophila Cell Corpse Engulfment Receptor Draper Mediates Glial Clearance of Severed Axons. Neuron. 2006 Jun 15;50(6):869–81.

Nilsberth C, Westlind-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, et al. The "Arctic" APP mutation (E693G) causes Alzheimer's disease by enhanced Aβ protofibril formation. Nat Neurosci. 2001 Sep;4(9):887–93.

Nimmerjahn A, Kirchhoff F, Helmchen F. Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma in Vivo. Science. 2005 May 27;308(5726):1314–8.

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, et al. Synaptic Pruning by Microglia Is Necessary for Normal Brain Development. Science. 2011 Sep 9;333(6048):1456–8.

Scheff SW, Price DA. Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies. Neurobiol Aging. 2003 Dec;24(8):1029–46.

Yoshiyama Y, Higuchi M, Zhang B, Huang S-M, Iwata N, Saido TC, et al. Synapse Loss and Microglial Activation Precede Tangles in a P301S Tauopathy Mouse Model. Neuron. 2007 Feb 1;53(3):337–51.

Zhan Y, Paolicelli RC, Sforazzini F, Weinhard L, Bolasco G, Pagani F, et al. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. Nat Neurosci. 2014 Mar;17(3):400–6.

Ziegenfuss JS, Doherty J, Freeman MR. Distinct molecular pathways mediate glial activation and engulfment of axonal debris after axotomy. Nat Neurosci. 2012 Jul;15(7):979–87.