

Activity-dependent human tau release from neurons of the drosophila model of alz...

[Health & Medicine](#)



**ASSIGN
BUSTER**

Background

Aging is the process of gradual disruption and loss of important functions of the body or its parts, in particular, the ability to reproduce and regenerate (Yun, 2015). As a result of aging, the body becomes less adapted to environmental conditions, reduces and loses its ability to fight predators and resist illnesses and injuries (Yun, 2015). The phenomenon of aging in one way or another is observed in almost all living organisms. Damage at the cellular and molecular levels interact with genes and environmental factors, which determines which cells are aging naturally and which ones undergo neurodegeneration.

Accordingly, advanced age is the main risk factor for the development and progression of neurodegenerative diseases (Niccoli & Partridge, 2012). Accumulation of age-related somatic injuries, combined with a decrease in the effectiveness of compensatory mechanisms, can increase the likelihood of disease development (Johnson, 2015). In elderly people, moderate manifestations of parkinsonism are often observed, which can be caused by an age-related decrease in dopaminergic neuronal activity, dementia with Levy bodies, degenerative pathologies (early stages of Alzheimer's and Parkinson's diseases) or vascular disorders (Hindle, 2010). According to Murman (2015), neurodegenerative diseases are the most common cause of cognitive impairment in the elderly. It is also assumed that neurodegenerative diseases are also responsible for most cases of cognitive impairment (Baquero, 2015). Establishing of an accurate diagnosis of

cognitive disorders is of great importance for predicting and selecting therapeutic tactics.

Therefore, clinicians need to have an idea of the clinical features and paraclinical characteristics of neurodegenerative diseases accompanied by cognitive impairment. The number of people with Alzheimer's Disease (AD) has been increased in recent years. In 2017, approximately 5.3 million people over 65 ages or older have AD in United States (Alzheimer's Association, 2017). AD is one of the most common neurodegenerative disease and dementia is one of its features. Patients with AD have been diagnosed with several symptoms such as loss of cognitive function, difficulty swallowing, and disrupted in sleeping.

Additionally, there are different risk factors leads to develop AD which include aging and cardiovascular diseases. Also, there are some genes that associated with AD. These genes are APOE4 allele and SORL1. AD characterized by the accumulation of two proteins, which are extracellular beta amyloid plaques (A) and intracellular neurofibrillary tangles (NFTs), or aggregates of hyper-phosphorylated tau protein. A (primarily A42) is a product of amyloid precursor protein (APP) that cleaved by secretase. A has been known to be misfolded within the brain which lead to its aggregation upon itself and forms plaques. The other protein is NFTs found in neurons which are microtubule-associated protein known as tau. When tau is aggregated, it leads to destabilizing the microtubules and forms NFTs inside the neurons. It has known that tau aggregates can be defined as tauopathies (Wang & Mandelkow, 2016). In fact, cell death developed by the

accumulation of NFTs which cause cellular process dysfunction. Tau protein is monomeric with a molecular weight ranging from 45-65 kDa. It has six isoforms which can be distinguished by their binding domains which are N-terminal domain, a proline-rich region, a repeat region, and C-terminal domain.

Significance

Tau protein can be modified post translationally in both healthy and pathogenic states. It is known that hyper-phosphorylation is the most investigated by researchers because NFTs are formed by hyper-phosphorylated tau protein. Many studies suggested that tau protein hyper-phosphorylation is associated with increasing or decreasing in certain enzymes such as kinases and phosphatases (Trojanowski and Lee, 1995). Pathological features of AD depend on the site of phosphorylation on tau protein such as it phosphorylated on serine or threonine. The number of these sites reach up to seventy-nine putative sites (Buee et al. , 2000). There are many sites of phosphorylation that would be studied in this project (Figure 1).

For example, we will be focusing on the proline rich domain to investigate tau release and phosphorylation. This study will assess the activity and phosphorylation-dependent release of human Tau from *Drosophila* primary neuronal culture.

Objectives

Establish a model that can be used for transgenic study. Investigate the underlying mechanism of tau protein that cause neurodegenerative disease

<https://assignbuster.com/activity-dependent-human-tau-release-from-neurons-of-the-drosophila-model-of-alzheimers-disease/>

such as AD. Study the release, propagation, and toxicity of tau protein involved in AD. Specific Aim 1 Develop a model system to study activity-dependent release of toxic tau.

Hypotheses

Drosophila melanogaster which is known as fruit fly was used as model to study many neurodegenerative diseases such as Parkinson's disease and AD. This model was used for different purposes because it has a short time consuming when generates new line with a mutation. In addition, *Drosophila* has several genetic tools which permits researchers to develop transgenic fly easily. Fruit fly has a functional homology around 75% of human disease-causing genes. Therefore, we hypothesize that *Drosophila* could be used to study activity-dependent release of toxic tau in AD.

Methods

Fly collection: Gal4-UAS system is an upstream activating system which will be used to develop a fly line for this project. GAL4 is a yeast transcriptional activator which will be a drive line, and UAS is upstream activating sequence. These two lines (GAL4-UAS) will be crossed as shown in (Figure 2). After crossing, the offspring will be overexpressing our gene of interest. For crossing two fly lines it is important to collect virgin females. According to many factors, we can differentiate between fly age and gender. For example, females have a bigger shape than males with pointed abdomen, and a light posterior end. Where are males having smaller bodies with rounded abdomen, and darker end than females. Males are also characterized by their black sex combs which found between their legs. On the other hand,

virgin females have very pale color and characterized by the dark spot in their abdomen that called “ meconium”. We will be preparing a bottle food for flies which is composed of water, sugar, yeast, bio-agar, and 20% nipagen in ethanol. Food bottles will be changed every 7-10 days as they will be kept at room temperature (RT).

However, if the food bottles will be stored at 18C or lower, they must be changed every 3 weeks. It is known that flies are considered as virgin females before six hours old. At this time, flies are not matured and not mated yet; therefore, all females could be collected without any difference. However, if the time pass the six hours, then we will be looking only for females that have the “ meconium”. Differences between male (left), female (middle), and virgin female (right) (livegene, University of Leicester). After that, we will be collecting approximately 200 virgin females to be crossed with 50 males and will be added to a new food bottle. Then, we will start collecting larva (L2) which is going to develop into adult flies. Next, we will wait around 7 days for mating to ensure that will have enough number of eggs for primary neuronal culture. B. Fly Primary Neuronal Culture: We will use the cross bottle for spreading the egg on a plate. The lid of the bottle will be replaced by this plate and will be incubated in fly incubator. Then, after 4 hours, we will select mid-gastrula stage embryos and added with two embryos per coverslip. After that, neuro-blasts from these eggs will be used for extraction.

Specific Aim

Study cell to cell propagation and toxicity using *Drosophila* primary neuronal culture that express human tau.

<https://assignbuster.com/activity-dependent-human-tau-release-from-neurons-of-the-drosophila-model-of-alzheimers-disease/>

Hypotheses

Many studies demonstrated that patients with AD had mild cognitive impairment. These patients brain showed hyperactivity in their hippocampal area (Alicia et al. , 2015). To understand tau propagation and toxicity in neurons, we must identify the mechanism of how tau is released from neurons.

According to that, we hypothesize whether tau release will be induced by neurons stimulations using *Drosophila* primary neuronal culture expressing human tau (hTau).

Methods

1. Protein Extraction

For protein extraction, we will need fly larva and adult heads. First, we will mix a certain amount of RIPA buffer and protease inhibitor (PI) and put them on ice. Then, we will remove around 10 fly heads using razor blade. These heads will be added into homogenizer on ice, and then around 100 ul of RIPA and PI will be added to them. After that, heads will be crushed very well and we will take as much as we can with pipette, and then will be transferred into a centrifuge tube. Next, it will be incubated for 10 minutes at RT. After incubation, centrifuge will be done for 15 minutes at 12, 500 rpm, and the supernatant will be collected. Finally, store the samples at -80C.

2. Treat cells with KCL to induce membrane depolarization

After culturing, a sterile petri-dish will be prepared for adding two embryos on a glass coverslip at 0 day in vitro (DIV). Then, coverslips will be incubated at 25C and these coverslips will be checked every 2 days for cell viability. At 9 DIV, cells will be ready for KCL treatment. First, we will take the culture out of the incubator and transfer the coverslips into a 24 well-plate. Then, 150 ul of the media, will be added into each coverslip and the plate will be back again in the incubator for 1 hour. After the incubation period, plate will be taken out and media will be collected from the coverslips gently and will be added into centrifuge tube for studying protein release. To ensure that there are no cell debris, the media will be centrifuged for 15 minutes at 12, 500 rpm and then will be transferred to a column for immunoprecipitation.

3. Immunoprecipitation

After stimulation of membrane depolarization by KCL, immunoprecipitation will be done to detect hTau protein from the media. A covalent coupling of antibody will be used to ensure the concentrated binding of the protein, and then a column cross-linked with hTau antibody will be prepared. Monoclonal hTau antibody will be mixed with distilled water. Then a coupling buffer will be added to the resin in the spin column and will be incubated for cross-linking. After that, a quenching buffer will be added to end cross-linking reaction. Finally, the column could be stored in 1X coupling buffer in the refrigerator at 4C and could be reused for up to 10 times. Next, the sample that we will be collected from media then will be incubated in the prepared column. During this step, the protein of interest will be

adhered to the antibody beads. To confirm the accuracy of collecting hTau protein, an elution buffer will be added and hTau protein will be collected for further processing.

4. Western Blot Protocol

First, the protein concentration in each sample will be determined. These samples will be run on bovine serum albumin (BSA) standard curve. We will be preparing 12% of acrylamide gel and wait for its polymerization.

During this time, samples will be placed on ice and the chamber of western blot will be filled with running buffer. Samples will be heated in PCR machine for 10 minutes at 95C. Next, 5ul of Thermo Scientific PageRuler Plus prestained protein ladder will be added to the gel. Ladder will determine the molecular weight of the protein. After that, each sample will be added to each well in the gel and run western blot machine at 150 volume until the ladder shows multiple band. Then, we will turn the volume up to 200, and release the current at 100. This step lasts around 1 hour and then we will turn the apparatus off. The next step is transferring the gel onto the nitrocellulose membrane. We will be preparing 4 containers with transfer buffer.

The first container will have 5 sponges, the second will have the nitrocellulose membrane, the third will have 8 filter papers, and the last one will have the gel. Next, we will be preparing in order: 4 filter papers, the gel, the nitrocellulose membrane, the other 4 filter papers, 3 sponges at the bottom, and the other 2 sponges onto the transfer chamber. Now, the

transferring step is ready. We will turn the apparatus on at 200 current and the volume at 100 for 2 hours. However, if we will transfer 2 gels at the same time, the current will be doubled. Also, the machine will be on ice to cool it down. This step will last approximately 2-2.5 hours. After that, the membrane will be incubated in a container that has 3% BSA for 2 hours. Then, BSA will be discarded and replaced with primary antibody. The membrane will be incubated in the refrigerator at 4C overnight.

On the next day, the membrane will be washed 3 times with tris-buffered saline (TBS) solution. This step will be performed on shaker at 80 speeds for 10 minutes per each. Then, the membrane will be incubated with secondary antibody for 2 hours on shaker at low speed (less than 40). Membrane will be washed again with TBS 3 times for 15 minutes per each. Now, the membrane is ready for taking pictures.

Conclusion Recently, AD is the most common neurodegenerative disease that characterized by the accumulation of two proteins which are A and NFTs. As many studies focused on investigating A mechanism for AD, there are few researches mentioned the involvement of tau protein. In fact, the mechanism of pathogenic tau protein spreading is not well identified yet. Aggregation of hyper-phosphorylated tau represents NFTs which are microtubule-associated protein. Phosphorylated tau has been involved in microtubule polymerization. When tau is released from extracellular space, it will interact and enter other cells and lead to which is called tau spreading. This process initiates tau toxicity and the development of tauopathies. Therefore, we propose that hTau accumulation will cause neurodegeneration in Drosophila neuronal culture.

Management Plan

Ghadir (OU, graduate student) will be collecting flies, and prepare them for crossing and cell culture. Aeron (OU, lab technician) will be doing cell culture. Ghadir and Sazan (OU, graduate student) will be responsible of all experiments include: protein extraction, immunoprecipitation, and western blot. Two undergraduate students that work voluntary in lab will be responsible of preparing food bottles for flies. TimelineFly collection and prepare them for crossing (starting September 2018). Collecting eggs from crossed flies and cell culture (December 2018-February 2019). Protein extraction, KCL treatment, Immunoprecipitation, and Western Blot (March-June 2019). Taking picture of Western Blot result and data analysis (July-August 2019).