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IN VIVO GENETIC ANALYSIS OF SCHIZOPHRENIA THROUGH A NOVEL CELL DEATH PARADIGM

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by

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ABSTRACT

IN VIVO GENETIC ANALYSIS OF SCHIZOPHRENIA THROUGH A NOVEL CELL DEATH PARADIGM

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Schizophrenia is a mental disorder that affects a person's thoughts and actions. Compartmentalized Cell Elimination (CCE) is a novel cell death program, where three parts of the cell die differently. CCE can be used as a tool to address questions about psychiatric illnesses. Through a forward genetic screen looking for CCE defects, the endoplasmic reticulum (ER) network stability gene *atln-1/*atlastin and microtubule (MT) severing ATPase *spas-1*/spastin, were found to be linked to Hereditary spastic paraplegia (HSP), a neurodegenerative disease. A schizophrenic behavioral assay was performed on the CCE mutants *atln-1, spas-1, hop-1, sel-12,* and *ptl-1* to explore the question of links between developmental cell death and psychiatric illnesses. The gene *ced-3*, which is essential for CCE, did not show schizophrenic behavior. The *atln-1/*atlastin and *ptl-1/*tau mutants did show schizophrenic tendencies. This suggests novel links between schizophrenia, the ER, tau, and schizophrenia.

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CHAPTER 1

INTRODUCTION

Schizophrenia is a chronic and severe mental disorder that affects a person's thoughts, actions, expressions, emotions, and perceptions (Mayo Clinic, 2020). Approximately one percent of the world's population is reported to be diagnosed with schizophrenia and current treatments are of limited success. A better understanding of the etiology and pathogenesis is critical to develop effective therapies.

Due to ethical and moral concerns, studies related to schizophrenia *in vivo* have been limited in humans, which leaves many questions, such as whether it is rooted in early development, whether it can be a predictor of neurodegenerative diseases, and what genes are involved, unanswered. *Caenorhabditis elegans,* or *C. elegans,* provide a unique opportunity to investigate schizophrenia due to the various facets that offers as a model organism. The purpose of the study was to identify important genes related to schizophrenia and a possible link with neurodegeneration and CCE. It was hypothesized that neurodegenerative mutants will show schizophrenic behavior.

CHAPTER 2

LITERATURE REVIEW

2.1 Schizophrenia and Neurodegenerative Diseases

As previously described, schizophrenia is a chronic brain disorder that affects less than 1% of the world's population (Torres, 2020). Symptoms of schizophrenia include delusions, hallucinations, disorganized speech, lack of motivation, and trouble speaking (Torres, 2020). According to the DSM-5, the standard classification of mental disorders used by health professionals in the United States, people must present with at least two of the previously listed symptoms for at least a month in order to be diagnosed with schizophrenia (Cleveland Clinic, 2022). There is no known cure for schizophrenia though researchers have been searching for the causes and effects by studying genetics and conducting behavioral research.

Neurodegeneration is suspected to be linked to schizophrenia. Neurodegenerative disease occurs when nerve cells in the brain or peripheral nervous system lose function over time and affects millions of people across the world (NIEHS, 2022). There is no cure for neurodegenerative diseases, such as Alzheimer's disease (AD) and Hereditary spastic paraplegia; however, medication and certain treatments can relieve the symptoms (NIEHS, 2022).

Alzheimer's disease is the most common form of dementia and affects nearly 5.8 million Americans (CDC, 2020). It starts as mild memory loss and progressively gets worse leading to the possibility of losing the ability to carry on a conversation and responding to

environmental stimuli (CDC, 2020). Alzheimer's disease also affects the part of the brain that involves thought processing, memory, and language, which can lead to a person's inability to carry out daily activities (CDC, 2020). Current research has not revealed what causes AD, although scientists predict it is caused by several factors rather than just one (CDC, 2020).

Hereditary spastic paraplegia is an inherited neurodegenerative disease that is characterized by progressive weakens and stiffness of the legs and affects about 50,00 Americans (GARD, 2021). The symptoms progress to where eventually the individual with HSP relies on the assistance of a cane, walker, or wheelchair (GARD, 2021). HSP can also lead to cognitive impairment and peripheral neuropathy (GARD, 2021). The underlying cause of HSP is not known, although several genetic mutations have been identified that lead to it (GARD, 2021).

It was previously rejected that neurodegeneration plays a role in the pathophysiology of schizophrenia (Lieberman, 1999). However, evidence that came from the studies of the illness course, symptomatology, treatment effects, and neuroimaging for schizophrenia and neurodegenerative diseases suggested that schizophrenia involved a limited neurodegenerative process (Lieberman, 1999). The limited neurodegenerative process is seen by the psychotic symptoms that are most active in the early stages of schizophrenia (Lieberman, 1999). Some researchers have found evidence that schizophrenia in some patients may be caused by the buildup of abnormal proteins similar to those found in the brains of patients with neurodegenerative disorders (Johnson, 2019). These studies show that there is a possible link between schizophrenia and neurodegenerative diseases, and that more research should be pursued into the inquiry.

2.2 The Model Organism

The limitations in addressing the link between neurodegeneration and schizophrenia included modeling the cell biology of the disease *in vivo*, along with accounting for complex variability in genetics and behavior, and small sample size. These challenges were overcome by leveraging the various facets of the biology of the nematode *C. elegans*. The nematodes are hermaphrodites allowing for large invariable sample sizes. In addition, *C. elegans* and humans have about twenty thousand genes that are similar. In those similar genes, 40% are implicated in human diseases and are present in the *C. elegans* (Ghose & Shaham, 2020).

Compartmentalized Cell Elimination (CCE) is a novel cell death program found in *C.* elegans, where three compartments of the cell- soma, proximal process, and distal process- die in three different ways (Ghose & Shaham, 2020). Through a forward genetic screen looking for CCE defects, the endoplasmic reticulum (ER) network stability gene *atln-1*/atlastin and microtubule (MT) severing ATPase *spas-1*/spastin, were found to be linked to HSP, a neurodegenerative disease (Ghose & Shaham, 2020). Therefore, CCE was used as a tool to address the posed hypothesis.

2.3 Neurodegenerative Mutants

The genes *sel-12*, *hop*-1, and *ptl-1* are three more neurodegenerative genes present in *C. elegans* with human orthologs equivalents. The genes *sel-12* and *hop-1* are types of a protein coding gene with the human ortholog of presenilin 1 (Arnaboldi & Kishore, 2022). Presenilin 1 is implicated in several diseases including AD (Arnaboldi & Kishore, 2022). The gene *ptl-1* is also a type of protein coding gene with the human ortholog of tau (NCBI, 2022). Tau is also related in the ER as a stress response (Ajoolabady et al., 2022) and has

been found to play a role in AD and HSP (Zempel & Mandelkow, 2015). These neurogenerative genes were used in a schizophrenic behavioral study in the present study.

CHAPTER 3

METHODOLOGY

Psychiatric illnesses have characteristically observable behaviors called endophenotypes. One such observable behavior is the defect in sensorimotor gating known as prepulse inhibition (PPI), which is considered to be a biological marker of schizophrenia (Dwyer, 2018). PPI is the inhibition of a reflex response, such as a startle to a loud tone, then a prepulse, like a softer tone, is presented shortly before the loud tone (Dwyer, 2018). Schizophrenic patients showed less inhibition compared to individuals without schizophrenia (Dwyer, 2018); therefore, excessive startle and arousal is observed in individuals with schizophrenia (Dwyer, 2018). Psychiatric illnesses also have protophenotypes, which are endophenotypes that have been conserved during evolution (Dwyer, 2018). The protophenotype in *C. elegans* wild type (WT) gene, *N2*, is the startle inhibition of pharyngeal pumping (Dwyer, 2018). The hypothesis for the experiment states that neurodegenerative mutants will show schizophrenic behavior through observing behavioral characteristics (startle inhibition) in *C. elegans* that associate with human neurodegenerative diseases.

3.1 Behavior Assay and Data Recording

The schizophrenia behavioral assay involved placing ten WT worms on an unseeded plate and recording the number of pharyngeal pumps for twenty seconds under a microscope. Then each WT *C. elegans* was tapped near their tail and their pharyngeal pumping was recorded accordingly for another 20 seconds. The same assay was performed

on the mutants *atln-1,spas-1, ced-3, hop-1, ptl-1,* and *sel-12*. The data was inputted into PRISM, a data processing program, to find if there was a significant difference between the wild type tested and the mutant.

CHAPTER 4

DISCUSSION

Based on the degree of significance of pharyngeal pumping before and after tap, *hop*-1 and *sel-12* have the same trend as WT, meaning that there was a significant decrease in pharyngeal pumps per second before and after the *C. elegans* were tapped (Figure 4.1). *Ced-3*, which is related to CCE also had the same trend as WT (Figure 4.2). Therefore, it was concluded that CCE is not related to schizophrenia. *Atln-1* and *ptl-1* showed startle inhibition; therefore, the genes may be relevant to schizophrenia (Figure 4.3). Below are the graphs that depict the rate of pharyngeal pumping before and after the *C. elegans* was tapped compared to wildtype.

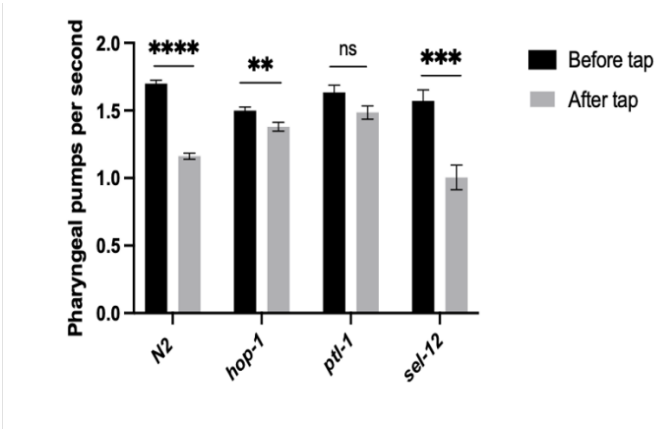

Figure 4.1: *Ptl*-1/Tau mutants may show schizophrenic like behavior

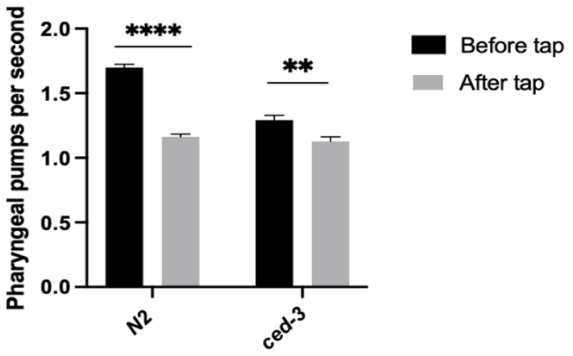

Figure 4.2: *Ced-3*/Caspase mutants do not show schizophrenic like behavior

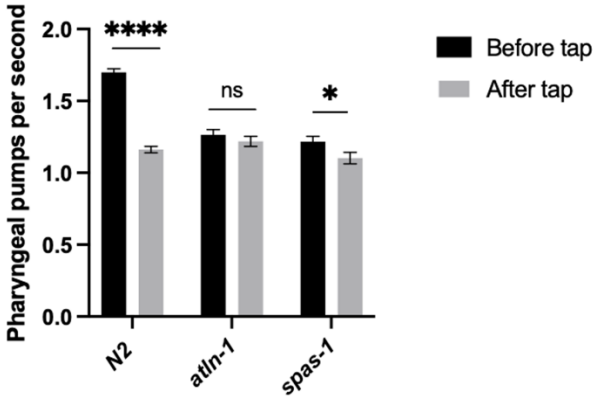

Figure 4.3: *Atln-1*/Atlastin mutants may show schizophrenic like behavior

CHAPTER 5

CONCLUSION

Using PRISM, the degree of significance for WT and the mutants was calculated based on the number of pharyngeal pumps before and after the *C. elegans* was tapped. The mutants for the genes *ced-3, hop-1, sel-12, spas-1* did not show schizophrenic like behavior. The hypothesis that CCE is related to schizophrenic behavior appears to be incorrect as *ced-3* had no defect in behavior. However, the analysis did show that two interesting genes may be involved (one of which came from the previous work done with CCE). The results showed that *atln-1/*atlastin and *ptl*-1/tau mutants, two genes that play a role in HSP and AD respectively, did show schizophrenic like tendencies. *Atln-1/*atlastin plays a role in ER stability while *ptl-1*/tau accumulates in the ER in response to an ER stressor. This suggests novel links between schizophrenia and neurogenerative diseases in relation to the ER.

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BIOGRAPHICAL INFORMATION

Hannah started her psychology undergraduate degree in 2019 and has been a part of the Honors College since her freshman year. She has been involved in research since 2020 upon joining the Ghose Lab, which studies compartmentalized cell elimination in living animals. After taking the course Abnormal Psychology, Hannah's interest in research regarding psychiatric illnesses increased, which led her to pursue her current capstone research. These opportunities have expanded her knowledge in both the psychological and biological fields. Apart from research, Hannah joined the Peer Academic Leadership position where she taught and managed a freshman class on college success. She would later use what she had learned about mentorship to become an Honors Advocate for the Honors College. Hannah aspires to enter medical school and is immensely thankful for the relevant opportunities and experiences that UTA has provided her.