Behavioral Effects of Presenilin Mutations in Drosophila

Anastasia K. Yemelyanova and Dr. Christopher J. Jones
Department of Biological Sciences, Moravian College, 1200 Main Street, Bethlehem, PA 18018

Moravian College
1742
Department of Biological Sciences, Bethlehem, PA

Abstract

As part of an independent research project, behavioral effects of mutations in the presenilin (Psn) gene were examined in the model organism Drosophila melanogaster. Psn mutations in humans have been specifically linked to Alzheimer's disease by triggering an accumulation of amino acid polypeptides, called amyloid plaques, around neurons in the central nervous system. These plaques interfere with signal transduction along the neurites leading to the neurological disorder. Previous research has shown that certain mutations of Psn result in a range of neurological defects. Our intention was to examine the behavioral effects of these mutations, specifically on courtship, learning and memory. No statistically significant effects on courtship were observed, and learning could not be tested; however, in future, alternative experimental procedures may be explored to establish the behavioral characteristics of Psn mutants.

Introduction

The American Federation of Aging Research estimates that over 16 million people will be afflicted with Alzheimer's disease (AD) by 2050. Familial AD is an inherited disorder expressed as a decline in memory and cognitive abilities late in life. The dementia is caused by an accumulation of neurofibrillary tangles and amyloid plaques in the central nervous system (Carlsson, 2006). The buildup of plaques is caused by increased activity of secretases, such as *presenilin* (*Psn*), cleaving amyloid precursor protein into polypeptides. In AD elevated levels of amyloid β peptides result in the formation of plaques in the intercellular matrix. *Presenilin* is also responsible for cleavage of Notch protein, which is vital for cell differentiation and development (Seidner, 2006).

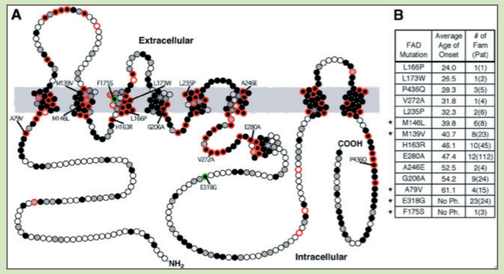

Figure 1. Mutations in Familial AD presenilin protein selected for analysis by Mark Fortini (Seidner, 2006).
A. Missense mutations in the human Psn mimicked in transgenic *Drosophila* used in Fortini's research.
B. Fourteen mutations characterized by the average age of onset and number of families and patients affected. E318G and F175S produced no phenotype (No Ph.) * Psn mutations in fly lines examined in my project.

Many mutations in psn have been identified and linked to familial AD. Mark Fortini at the National Cancer Institute (NCI) has examined the morphological effects of fourteen of these *Psn* mutations in *D. melanogaster* by silencing the normal *Psn* gene and randomly inserting a mutant copy into the genome. The severity of the neurological abnormalities seen in mutant flies was then correlated to onset age of AD associated with the corresponding mutation in humans (Figure 1). Due to variations in the location of the trangenic *Psn*, some flies with severe mutations were not viable, while others exhibited no morphological defects. Because only physical attributes were inspected in Fortini's study, I was interested in finding out if any behavioral abnormalities are caused by these mutations, including the ones that showed no signs of physical deformity. The goal of my research was to obtain morphologically normal *Psn* mutant fly stocks from the NCI and observe the effects of the mutations on courtship and on the learning and memory abilities of the flies using courtship conditioning.

Methods

Fly stocks. Wild type Canton S (CS) was obtained from the Bloomington Stock Center. Psn mutant lines 14.4-9A, E318G, F175S, A79V, G206A, H163R, M139V, and M146L were the kind gift of Mark E. Fortini at the Laboratory of Protein Dynamics and Signaling, NCI. Flies were maintained on standard cornmeal/molasses medium in vials on a L12:D12 cycle at 25 C°. Males were collected under CO_2 anesthesia and kept in isolation or in groups of 5-7 flies.

Courtship behavior. Courtship was monitored as described by Sabaliauskas (2004) with the modification that active courting time was characterized by the male being in motion toward the female. Courtship behavior of mutated males with homogenic and wild type virgins was examined and compared to the wild type behavior reported by Sabaliauskas (2004). Mann-Whitney tests comparing CS and homogenic virgin data and correlation analyses were performed using *InStat 3* software.

Figure 2. "Singing" - wing vibration of male Drosophila

Data recorded

- Copulation Latency (CL) – the time from the introduction of a virgin female into the mating chamber until the male's first wing vibration (Figure 2)
- Active Courting Time (TC) – the time the male spent in motion toward the female (Figures 2&3)
- Copulation Time (CT) (Figure 4)
- Courtship Index (CI) –the time spend actively courting (TC) divided by the total time from the introduction of the female to the start of copulation.

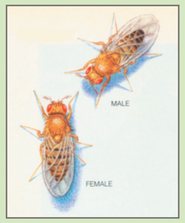

Figure 3. Male Drosophila orienting toward the female

Figure 4. "Curling" - attempted copulation

Courtship conditioning. When a male fruit fly is intro duced to a previously-mated female, she rejects him. This continued rejection eventually causes the male to not attempt to mate, even when subesquently presented with a virgin female. The duration of this phenomenon, called courtship depression, is a measure of memory. Male and mated female flies were mated once 24-48 hours prior to conditioning.

Experiments. Virgin females were allowed to mature for at least four days before testing. Male and female virgin flies tested were of the same age, between 5 and 8 days old. Courtship assays were carried out in 3.93 cm^3 chambers made out of Plexiglas and glass. The humidity in the experimental room was not controlled and varied from 38% to 62%.

Figure 5. Copulation

Results

No correlation between age of the male and CL, TC, CT, or CI was observed. CL, CT, and CI varied among mutant lines, but the variation was not significant compared to the wild type values (Figure 5). The copulation success of flies from the mutant lines A79V-1D and M139V-10A18A was lower than that of the control group 14.4-9A, which carries a wild-type Psn transgene (Figure 6). For a given male genotype, no common pattern distinguishing among courtship parameters with wild-type CS, Psn control, and mutant virgin females was observed.

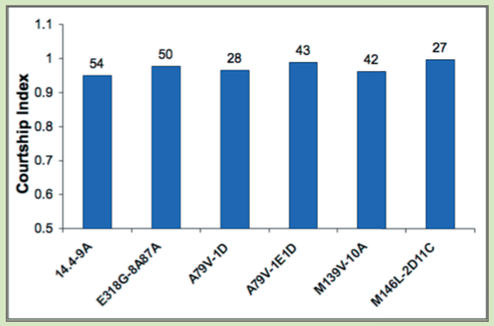

Figure 6. Copulation success. Mann-Whitney test of *InStat 3* showed significant variation in copulation success between A79V-1D and M139V-10A18A mutants and wild type *Psn* (14.4-9A). The value above the bars represents the number of mating pairs examined.

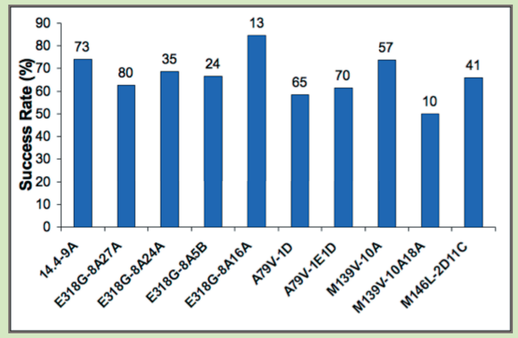

Figure 7. CI comparison. Courtship indices of successful copulations for most productive mutant lines showed no significant deviation from the control 14.4-9A. Values above the bars represent the number of successful copulations.

Discussion

The absence of a common pattern or correlation in the results of the courtship behavior data can be explained by nature of the transgenic presenilin gene and the markers used to identify the insertion of the transgene. Courtship components, such as wing vibration, are greatly affected by physical mutations. The markers used in the *Psn* lines included curly wings and tubby, which may provide a disadvantage in mating. Moreover, the *Psn* transgene was randomly inserted into the genome (Seidner, 2006), meaning that every line had the mutant *Psn* gene at a different location. Thus, any observations cannot be ascribed exclusively to the type of mutation because the position of the *Psn* gene may also affect the behavior. In order to obtain better data, the mutant fly lines to be compared should have the mutation in the *Psn* gene as the only difference in their genetic makeup.

Courtship conditioning proved to be impossible to reproduce under our laboratory conditions. The fact that neither the control nor the mutant flies showed learning is likely due to the low humidity levels in the laboratory. The conditioning processes described in the literature suggest that optimal learning occurs at humidity levels above 70%.

Conclusion

At the end of the research, several possibilities for further explorations were recognized. The knowledge gained provides a good background for a more in-depth research, such as an Honors project. Future approaches would include eliminating the genetic markers and improving memory and learning ability testing by using a different sensory modality such as vision. The next step would include inserting the transgenic *presenilin* in exactly the same locus, avoiding differences in behavior due to position effect. As new molecular technologies evolve, directly mutating the normal *Psn* gene in the fly genome would be the optimal solution; I am currently planning such experiments.

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