BEHAVIOURAL INVESTIGATION OF NORMAL AND MUTANT HUMAN PRESENILIN FUNCTIONS IN CAENORHABDITIS ELEGANS

by

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Abstract

Presenilins are well known as sites of mutations responsible for early-onset Alzheimer's disease. The normal functions of presenilins and the mechanisms by which presenilins cause Alzheimer disease are not yet known. Conservation of cellular and molecular functions between the *C.elegans* and human genes makes it a powerful experimental model organism to investigate cellular mechanisms of Alzheimer's disease and neurodegenerative disorders in general. Mutations in the *C.elegans* presenilin1homologue, *sel-12,* decrease Notch signaling activity, which results in an egg-laying deficit in these animals. It has been well established that pathogenic PS1 mutations impair Notch signaling; however, in the first part of this thesis we showed that a recently discovered $PS1_{\Delta s169}$ human mutation rescued the egg-laying deficit associated with Lin12/Notch pathway, suggesting that in this pathogenic PS1 mutation Notch processing remained intact.

In the second part of this thesis the behavioural phenotypes of a mutation in the *C.elegans* presenilin homologue, *sel-12,* were studied. Our results revealed that a mutation in the *sel-12* gene causes chemotaxis deficits toward volatile and water-soluble stimuli in *sel-12* mutant animals. Reintroducing the *sel-12* or the wild-type human presenilin gene decreased those behavioural phenotypes, indicating that the observed chemotaxis deficits were dependent on *sel-12* activity. However, rescuing with the human $PS1_{C410Y}$ mutation, which has a severe effect on Notch processing, did not ameliorate the chemotaxis deficit; in contrast, rescuing with $PS1_{\Delta s169}$ rescued both volatile and water-soluble chemotaxis impairments suggesting that the chemotaxis deficit causing by *sel-12* mutation depends on the Notch pathway.

Preface

The research project that I presented here was performed in the lab of Dr. Catharine Rankin, my graduate supervisor who directed me for this work. This thesis was written by me, with feedback given by Dr. Rankin and my committee members. The first part of this thesis was a collaboration between the Rankin and Song labs. The *sel-12* and human PS1 plasmids used in this study to rescue worms were given by Dr. Baumeister as a gift. The novel human $PS1_{\Delta s169}$ mutation used in this study was cloned by Dr. Fang Cai from the Song lab. I preformed all injections to make transgenic worms, ran experiments and analyzed data.

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List of Abbreviations

Aβ	Amyloid-β
AC	anchor cell
ACID	APP intracellular domain
α-CTF	α-carboxyterminal fragment
AD	Alzheimer's disease
ADAM	a disintegrin and metalloprotease
ANOVA	Analysis of variance
APH-1	anterior pharynx defective 1
ApoE	Apolipoprotein E
APLP1	APP-like protein 1
APLP2	APP-like protein 2
APP	Amyloid Precursor Protein
APPL	APP-like protein
BACE	beta-site APP cleaving enzyme
β-CTF	β-carboxyterminal fragment
CI	chemotaxis index
DNA	deoxyribonucleic acid
EGF	epidermal growth factor

Egl	egg-laying
EOAD	early-onset Alzheimer's disease
ER	endoplasmic reticulum
FAD	familial Alzheimer's disease
GDP	guanosine diphosphate
GFP	green fluorescent protein
GPCRs	G protein-coupled receptors
GTP	guanosine triphosphate
LOAD	late-onset Alzheimer's disease
MAP	microtubule-associated protein
NaAc	sodium acetate
NCT	nicastrin
NGM	nematode growth medium
NICD	Notch intracellular domain
NS	not significant
PEN-2	presenilin enhancer 2
PS1	presenilin 1
PSEN1	presenilin 1
PSEN2	presenilin 2

PTL-1	Protein with Tau-like repeats-1
TMD	transmembrane domain
VPC	vulval precursor cells
VU	ventral uterine

Acknowledgments

I would like to thank my supervisor Dr. Catharine Rankin for accepting me into her lab and helping me to gain so many valuable academic experiences while at UBC. I would like to thank Dr. Ann Rose, Dr. Weihong Song and Dr. Kurt Haas for accepting to serve on my committee and their advice and guidance for this study. A big thanks to Dr. Evan Ardiel from Rankin lab and Dr. Fang Cai from Song lab, for their help and answering all of my endless questions. I would also like to acknowledge some of the undergrad students from Rankin lab who helped me to complete my study, including Rojin Kaviani, Ravi Chalhotra and Mahraz Parvand.

To My Son

1.Introduction

Alzheimer's disease (AD) is one of the most devastating disorders with an extremely detrimental effect on the quality of life of patients and their families. Approximately 18 million people from different parts of world suffer from AD and this number is dramatically increasing with an aging population (Fuller, Stavenhagen, & Teeling, 2014). Unfortunately, the main cause(s) of the disease have yet to be determined and no treatment is available to modify disease progression. The first objective of this thesis was to use the well-characterized egg-laying phenotype associated with mutations in the *C. elegans* homologue of human presenilin to investigate the function of a novel human presenilin mutation, $PS1_{\Delta S169}$, in the Notch signaling pathway. The second objective was to assess the behavioural phenotypes related to the function of presenilin in *C.elegans*, which can be used as indicators to further investigate cellular pathways involved in Alzheimer's disease.

1.1 Alzheimer's disease

Alzheimer's disease was described for the first time by Dr. Alois Alzheimer in 1907 and named after him. He used the case of one of his patients, Auguste Deter, to characterize this disorder. The brain of Auguste was examined after her death and it showed extensive atrophy in the cortex with two unique brain deposits – one located in the nerve cells (today known as "neurofibrillary tangles") and the other in the extracellular space ("amyloid plaques" in modern terminology; O'Brien & Wong, 2011).

1.2 Familial and sporadic forms of AD

Familial EOAD (early-onset Alzheimer's disease) and sporadic LOAD (late-onset Alzheimer's disease) are the two main forms of AD. The age of onset in sporadic forms, the most frequent type of AD, is usually after 60 years, without a well-defined mode of transmission, and with only modest familial aggregation (Chouraki & Seshadri, 2014). However, some risk factors are associated to this type of AD. Apolipoprotein E (ApoE), a critical plasma protein involved in cholesterol transportation is known as one of the main risk factors associated with LOAD. Three slightly different alleles of the APOE gene are known as e2, e3, and e4. The e4 allele of the APOE gene increases an individual's chance for developing LOAD. ApoE attached to extracellular senile plaques and intracellular neurofibrillary tangles has been detected in the brains of AD patients (Strittmatter et al., 1993). Environmental risk factors are also suggested to be involved in sporadic LOAD, although such interactions are not well understood (Traynor & Singleton, 2010).

Familial forms which are representative of less than 1% of all cases of AD, are characterized by early-onset (<60 yrs of age), a strong familial aggregation, and are mostly autosomal dominant. Presenilin1 (PSEN1*)*, Presenilin2 (PSEN2*)*, or Amyloid Precursor Protein *(*APP*)* are the most common genes involved in these forms of AD (Chouraki & Seshadri, 2014). The APP gene encodes amyloid precursor protein which is a transmembrane protein of with largely unknown function. PSEN1 and PSEN2 genes encode the main components of γ-secretase, which is involved in sequential proteolytic cleavages of amyloid precursor proteins and the subsequent formation of amyloid-β peptides. Mutations in these genes change APP processing resulting in increased production of the toxic forms of the amyloid-β peptide (Delabio, Rasmussen, Mizumoto, Viani, & Chen, 2014).

1.3 Presenilin1

Presenilin1 (PSEN1) is a multi-pass transmembrane protein (Figure1;Levitan et al., 1996). Homologs of this gene have been identified in a variety of species, including *Drosophila melanogaster*, *C.elegans* and *Arabidopsis thaliana*, but not in yeast (Kopan & Goate, 2000). More than 180 mutations associated with FAD have been detected in *PSEN1 (* located on chromosome 14 in humans). In some patients with missense mutations in *PSEN1,* AD symptoms were first detected in their 40s or late 30s(Campion et al., 1999).

Presenilin is abundantly expressed in the ER and trans-Golgi network of all tissues, including the nervous system (Levitan et al., 1996; Zhang, Zhang, Cai, & Song, 2013; Ho and Shen, 2011). In neurons, presenilin is mainly expressed in the ER and also has been detected localized to plasma membrane, especially in presynaptic and postsynaptic compartments of neurons (Ho and Shen, 2011).

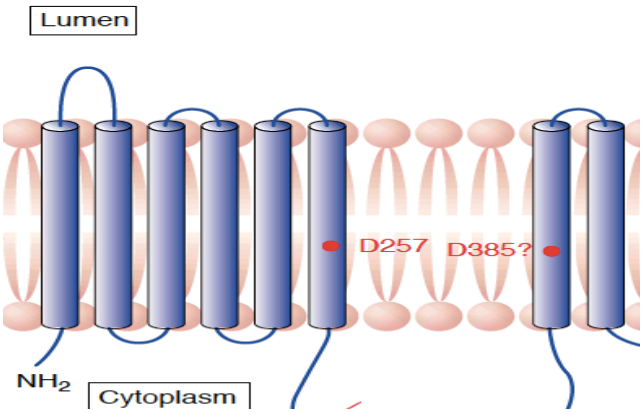

Figure 1 The transmembrane domains of presenilin (from Sisodia, Annaert, Kim, & De Strooper, 2001)

Presenilin 1, along with three other transmembrane proteins assemble a multi-subunit protease complex, termed γ-secretase (Ho and Shen, 2011;Edbauer et al., 2003). It is proposed that two transmembrane aspartate residues (257 and 385) in PSEN1 are critical for enzymatic activity of the γ-secretase including cleavage of APP (Bagyinszky, Youn, An, & Kim, 2014). Although, the majority work on presenilin has focused on its function as a catalytic subunit of the γ-secretase complex, several other presenilin functions such as calcium homeostasis, regulation of β-catenin signaling, and protein trafficking have been described. Importantly, these additional functions do not necessarily require presenilin to function as a part of γ-secretase (De strooper, Iwatsubo, & Wolfe, 2012).

1.4 γ-secretase complex

The term " γ-secretase " was first used in 1993 to describe the proteolytic activity that cleaves a single pass transmembrane protein known as amyloid precursor protein (APP; De strooper et al., 2012). γ-secretase consists of four subunits, which are all necessary to generate an active multi-subunit enzyme complex. Presenilin is synthesized in the endoplasmic reticulum and undergoes a proteolytic "maturation" cleavage before binding to the γ-secretase complex. Three other components of the complex, which are also critical for the function of γ-secretase are known as nicastrin (NCT), anterior pharynx defective 1 (APH-1), and presenilin enhancer 2 (PEN-2)(De Strooper & Annaert, 2010). The initial subcomplex, which consists of nicastrin and APH-1 forms in the ER (LaVoie et al., 2003). The fully assembled and matured γ-secretase complex is formed after interaction of the nicastrin/APH-1 subcomplex with PS and PEN-2, which then transit to post-Golgi compartments (De Strooper & Annaert, 2010). In general, NCT is necessary as a stabilizing cofactor for γ-secretase assembly, while PEN-2 and APH-1 are involved in the maturation process of presenilins (Alexander, Marfil, & Li, 2014). γ-secretase is involved in three different types of

proteolytic activities including autoproteolytic, endoproteolytic and carboxypeptidase functions all of which are important for normal function of the enzyme. The full-length presenilin undergoes an autoproteolytic cleavage in the TMD6-TMD7 loop to generate the activae form of the γ-secretase complex. This active form of the enzyme cleaves intracellular domains of different type I membrane integral protein substrates by its endoproeolytic function. Finally, the remaining fragment of the transmembrane domain in each type I single-pass protein undergoes sequential cleavages by the carboxypeptidase function of the γ-secretase, until the final fragment is completely released (Wolfe, 2013)

1.5 Amyloid precursor protein

The amyloid precursor protein (APP) is a single-pass transmembrane protein, which has been detected in many vertebrate species and belongs to an evolutionarily conserved protein family (O'Brien & Wong, 2011;Walsh et al., 2007). The APP family of proteins has three members: APP, APLP1 (APP-like protein 1) and APLP2 (APP-like protein 2). Among these three types of proteins, APP is the only one that contains an Aβ sequence and produces Aβ. APP members have critical functions during development, and these functions do not require the Aβ peptide (Alexander et al., 2014). An APP-like protein has also been detected in *Drosophila melanogaster* (APPL) and *C.elegans* (APL-1; Walsh et al., 2007).

APP undergoes several post-translational modifications such as N- and O-glycosylation, sulfation, and phosphorylation before it can be proteolytically processed by a set of proteases (Weidemann et al., 2002). To be a substrate for γ-secretase, the ectodomain segment of APP has to be removed (Figure 2). In most cells a disintegrin and metalloprotease (ADAM) are responsible for this cleavage; however, in neurons the aspartyl protease beta-site APP cleaving enzyme (BACE) is responsible for this trimming process (De Strooper & Annaert, 2010). A membrane-bound α-

carboxyterminal fragment (α-CTF) is generated as a result of the ADAM function on the Aβ sequence of the APP, which prevents generation of the Aβ. However, the BACE cleavage generates a β-CTF, which starts with the first amino acid residue of the Aβ. Both the α-CTF and the β-CTF can be substrates for γ-secretase (De Strooper & Annaert, 2010).

Further cleavage of the β-CTF by γ-secretase is accrued at the ε site (Figure 2), to release the intracellular fragment of the ACID and also forms either 48- or 49-residue of the Aβ that includes almost all the transmembrane domain. The remaining Aβ peptides of the transmembrane domain undergo sequential proteolysis to generate the 38-43-residue secreted form of the Aβ (Qi-Takahara et al., 2005). The precise initial site of ε-cleavage is critical for the two suggested pathways of the Aβ production: Aβ49 → Aβ46 → Aβ43 → Aβ40 and Aβ48 → Aβ45 → Aβ42 → Aβ39. Mutation in presenilin can change the initial site of ε-cleavage to increase formation of Aβ48 over Aβ49 which may be a reason for changing the ratio of secreted Aβ42/Aβ40 and aggregation of the peptides (Jarrett, Berger, & Lansbury, 1993). Mutations in presenilin may also decrease γ-secretase function which ultimately causes synaptic dysfunction and neurodegeneration (Chávez-Gutiérrez et al., 2012; Shen & Kelleher, 2007).

Figure 2 Cleavage sites within the transmembrane domain of APP (from Weidemann et al., 2002)

1.6 Notch pathway

Notch is a highly conserved pathway in metazoans and its function starts at a very early stage of development, when cell- cell interactions determine morphogenesis. The canonical Notch signaling pathway begins with the sequential cleavage of the Notch receptors (there are four Notch receptors in mammals, two in *C.elegans* and one in *Drosophila melanogaster*) (De Strooper & Annaert, 2010). Notch receptors are type I transmembrane proteins with an extracellular epidermal growth factor (EGF)-like repeats domain and an intracellular transcriptional activate site (Alberi, Hoey, Brai, Scotti, & Marathe, 2013). During the transportation of the Notch receptor to the cell surface, the protease furin cleaves the extracellular domain of this receptor at the S1 site (Struhl & Greenwald, 2000). Specific ligands from the Jagged and Delta serrate member families, which contain a DSL (Delta-Serrate-Lag-2) motif and are expressed on adjacent cells bind to Notch receptors in order to activate them. The second cleavage (S2) is then carried out (Figure 3) by disintegrin and metalloproteinases (ADAM-17 and ADAM-10) which makes the transmembrane domain of the receptor available for γ-secretase processing (S3) (Alberi et al., 2013). Proteolysis of the Notch receptors by γ-secretase releases the Notch intracellular domain (NICD) from the

membrane. The NICD then interacts with CSL [CBF1, Su (H), LAG1] transcription factors and reinforces coactivators to turn on expression of Notch target genes (De Strooper & Annaert, 2010).

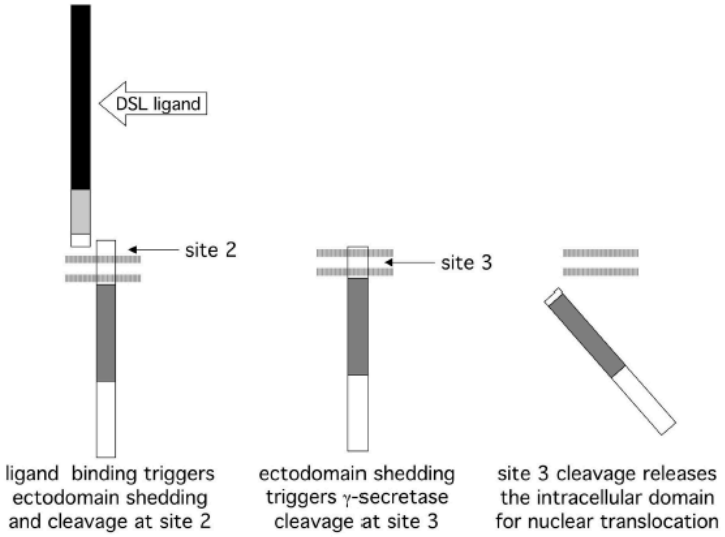

Figure 3 Mechanism of Notch signal transduction (from Greenwald, 2005)

Following cleavage at the S3 site, another endoproteolytic γ-secretase dependent cleavage (S4) occurs near the middle of the Notch transmembrane domain, 12 amino acid residues upstream of S3. Cleavage at the S4 site produces an extracellular peptide known as Nβ. PS1 mutations that are linked to the production of pathogenic forms of Aβ, also affect the generation of longer forms of Nβ (Okochi et al., 2002).

1.7 Olfactory dysfunction in AD

The olfactory system, the sensory system responsible for perception of smell and taste, is critical for the maintenance of a good quality of life in humans. Olfactory dysfunctions observed in patients with AD exceed those detected in normal aging (Djordjevic, Jones-Gotman, De Sousa, & Chertkow, 2008). These olfactory impairments occur early in the course of AD and increase as the

clinical symptoms of the disease progress. Olfactory deficits are observed at very early stages of AD and often prior to any other clinical symptoms (Ruan, Zheng, Zhang, Zhu, & Zhu, 2012). In the early stages of AD, poor performance in odor identification and recognition were found to be more prominent than alterations in odor detection threshold (Förster et al., 2010). Investigation of behavioral and olfactory systems in AβPP/PS1 transgenic mice revealed that at 3 months of age AβPP/PS1 Tg mice showed olfactory deficits but no cognitive decline. However, at 9-10 months of age olfactory dysfunction increased and cognitive dysfunction was detectable in these transgenic mice. This study showed that soluble Aβ aggregates increased and spread from the periphery to the inner olfactory centers with age, and gradually penetrated to piriform cortex, entorhinal cortex, and hippocampus (Wu, Rao, Gao, Wang, & Xu, 2013).

Recently, a large amount of research has focused on detecting a biomarker for AD disease that could lead to a diagnosis of AD prior to the development of significant neuropathology and subsequent loss of cognitive function. Olfactory loss may be a useful candidate; however, further investigation into the cellular mechanism of this loss is crucial to understand why and how the disease impacts olfaction (Wesson, Wilson, & Nixon, 2010).

1.8 *C. elegans*

Caenorhabditis elegans (Caeno, recent; rhabditis, rod; elegans, nice) is a 1 mm, free-living nematode that was introduced by Sydney Brenner in 1963 as a genetic model organism. During the last 50 years scientists have taken advantage of *C.elegans* to reveal functions of genes in cellular biology. Some of the characteristics that made *C.elegans* an amenable model for doing genetic research are its small size, short reproductive cycle, and hermaphroditic mode of reproduction. The lineage of the worm's 959 somatic cells was traced through its transparent cuticle, allowing for determination of cell fate (Sulston, Schierenberg, White, & Thomson, 1983). Furthermore,

C.elegans has a sequenced genome comprising approximately 19,000 genes, over 5000 of which have homologues in humans (Alexander et al., 2014; Bozorgmehr, Ardiel, McEwan, & Rankin, 2013). All of these characteristics contribute to the power of *C.elegans* as a valuable model system in which to investigate molecular and cellular mechanisms of human diseases (Wolozin, Gabel, Ferree & Guillily, 2011). The *C.elegans* genome contains human orthologs for presenilins, APP and *tau*, which makes it an amenable *in vivo* model for studying the molecular pathways involved in AD.

1.9 *C.elegans* and Alzheimer's disease

1.9.1 APL-1

APL-1 is the only homologue of APP identified in *C.elegans*. This single pass transmembrane protein, similar to APPL-1 and APPL-2 proteins does not contain any Aβ sequences. APL-1, like human APP, undergoes sequential cleavages; however, no BACE enzyme has been detected in *C.elegans* which means APL-1 is probably cleaved only by the α/γ-secretase pathway (Link, 2006).The α-secretase enzyme cleaves APL-1 to release an extracellular fragment of APL-1 known as sAPL-1 , followed by the cleavage of APL-1-CTFα by the γ-secretase complex to release the AICD intracellular domain (Alexander et al., 2014).

Many researchers have addressed the problem of having no Aβ peptide in *C.elegans* by generating transgenic strains in which the human Aβ sequence is expressed in all cells, in just neurons or even in a specific subset of neurons in order to investigate the effects of neurotoxic Aβ through transgenic analysis (Alexander et al., 2014). Neuronal expression of the human $A\beta_{1-42}$ led to chemotaxis impairment and an associative learning deficit toward volatile stimuli, suggesting that human $A\beta_{1-42}$ may alter some aspect of neuronal/synaptic function (Ewald & Li, 2012). Brains

of the AD patients show oxidative stress; however, it is controversial whether or not Aβ fibril accumulation is involved in the oxidative stress observed in the brains of the AD patients. In order to examine the role of Aβ accumulation in neurotoxicity, human Aβ transgenic worms were used to investigate relationship between Aβ toxicity and oxidative stress (Drake, Link, & Butterfield, 2003). Results of this study showed that oxidative stress in these transgenic worms occurs in the absence of the Aβ fibril formation suggesting that the toxicity of Aβ peptide is pre-fibrillar Aβ and not the Aβ fibril.

1.9.2 PTL-1

Structural MAPs (microtubule-associated protein) of the MAP2/tau family consist of MAP2, MAP4 and tau proteins in vertebrate, and homologs like PTL-1 (Protein with Tau-like repeats-1) in invertebrates. The only gene in *C.elegans* that encodes a *tau* related structural MAP is *ptl-1*. Investigation of this gene in *C.elegans* suggested that this protein is involved in mechanosensation and embryogenesis (Gordon et al., 2008).

1.9.3 SEL-12

In the same year in which analysis of families with inherited forms of AD implicated a mutation in the presenilin1 gene in familial forms of AD, screening for suppressors of a *lin-12* gain-of-function mutation in *C.elegans* identified a novel gene, *sel-12,* which is involved in regulation of signals mediated by *lin-12* and *glp-1* (both Notch homologs; Levitan, Greenwald, 1995).

sel-12 encodes a protein that shows about 50% amino acid sequence similarity to PS1 (Levitan, Greenwald, 1995). Mutations in *sel-12* decrease the *lin-12* activity and lead to an egg-laying defective (Egl) phenotype in *C.elegans* (Li & Greenwald, 1997). Levitan et al, 1996 found that normal human presenilin could substitute for the *C.elegans* SEL-12 protein and rescue the egg-

laying deficit, thus illustrating functional homology between SEL-12 and human PS1. However, six familial Alzheimer disease-associated mutant human presenilins showed reduced ability to rescue the Egl phenotype in *sel-12* mutant worms (Levitan et al., 1996).

A second *C.elegans* gene, *hop-1*, shows approximately 33% identity to mammalian presenilins (Cinar, Sweet, Hosemann, Earley, & Newman, 2001). Mutations in the *hop-1*gene decrease fertility in *C.elegans*. Over-expression of *hop-1* in *sel-12* mutant worms rescues the egg-laying phenotype in these animals, suggesting that HOP-1 is a functional presenilin (Li & Greenwald, 1997). Similarly, a diverged member of presenilin family in *C. elegans* called *spe-4* appears to have a very specific function in spermatogenesis as mutation in this gene leads to sterility (Gontijo, Aubert, Roelens, & Lakowski, 2009).

1.10 Lin-12/Notch activity in the development of the gonad

The egg-laying system in *C.elegans* consists of the uterus, the vulva, and associated muscles and neurons. In each larval hermaphrodite there are two cells, Z1.ppp and Z4.aaa, with equal developmental potential, that means each has an equal chance to become an anchor cell (AC) or a ventral uterine precursor cell (VU). The VU is eventually involved in ventral uterus formation; however, AC is required for vulval development. In *C. elegans*, activation of the *lin-12* gene is necessary in both Z1.ppp and Z4.aaa during development and the AC/VU decision is probably determined by a small variation in ligand and receptor activity in the *lin-12* pathway of these two cells. When *lin-12* activity is increased both Z1.ppp and Z4.aaa become VUs (the '0 AC defect'), furthermore, elimination of *lin-12* activity changes both Z1.ppp and Z4.aaa to ACs (The '2 AC defect').

The *C. elegans* hermaphrodite has six vulval precursor cells (VPCs), which eventually generate vulval cells in wild-type animals. The precise pattern of the VPCs fates depends on the graded action of the two signaling pathways known as the epidermal growth factor (EGF) and the Notch signaling pathways. An inductive signal rendered by EGF from the AC induces expression of the 1° fate while lateral signaling mediated by Notch/lin12 causes expression of the 2° fate. These signaling events during development of the vulva promote the six VPCs to adopt the same pattern of fates consisting of: 3°-3°-2°-1°-2°-3°. Lack of Notch and EGF signaling causes the VPCs to adopt the 3° fate, which generates non-specialized epidermis. All VPCs cells adopt the 2° fate and show the "Multivulva" phenotype if the *lin-12* signal is increased and no 2° fate is generated from the VPCs if the *lin-12* activity is decreased (Levitan & Greenwald, 1998; (Sternberg, 2005). AC also induces VU granddaughters to adopt the π cell fate instead of default ρ fate through the LIN-12 pathway. The uterine π cells generate the utse (uterine-seam cell) that has a thin process to separate uterine and vulval lumens until the first egg is laid. Fusion of AC with the utse is necessary for formation of a functional connection between uterine and vulval lumens. In animals lacking π cells, there is no fusion between AC and utse, which causes the passage from the uterine to the vulval lumen to be blocked (Cinar et al., 2001; Gontijo et al., 2009).

1.10.1 Egg-laying phenotype in *sel-12* mutants

sel-12 hermaphrodites show an egg-laying deficit (egl phenotype). It has been shown that *hop-1* functions redundantly with *sel-12* to promote the Notch pathway in *C.elegans*. This redundancy between the *hop-1* and *sel-12* genes covers the lack of normal function of SEL-12 necessary for the AC vs. VU decision and specification of vulval cell fates. However, HOP-1 appears to play lesser role in induction of π cells fates, which causes a significant defect in

induction of π cell fate by AC and underlies the egg-lying deficit in *sel-12* mutant animals (Cinar et al., 2001)

1.11 Olfactory system in *C.elegans*

Chemosensation is critical for a variety of functions in *C.elagans* including avoiding toxic chemical conditions, finding food, and for males finding mates (Bargmann, 2006). Among the 302 neurons in *C.elegans* 16 pairs of them have been confirmed to be chemosensory. These chemosensory neurons directly or indirectly synapse onto a set of command interneurons, which interact with motor neurons. All of these neurons control the reactions of worms to a wide variety of soluble and volatile odorants leading to forward or backward locomotion generated by body wall muscles (Hart & Chao., 2010). The four types of chemosensory organs in *C.elegans* consist of amphids, phasmids (Figure 4), inner labial and outer labial organs. Each organ consists of sensory neurons and two support cells, known as the sheath cell and the socket cell. These cells generate a pore through which dendrites of the chemosensory neurons are directly or indirectly exposed to the external milieu (Bargmann, 2006; Hart & Chao., 2010). Two amphid pores are at the end of head and each contains 11 chemosensory neurons and one thermosensory neuron (AFD). The ciliated endings of the dendrites pass through the amphid pore (ADL, ADF, ASE, ASG, ASH, ASI, ASJ, and ASK) or are embedded in the sheath cell (AWA, AWB, and AWC). The two phasmid pores near the tail contain the sensory endings of neurons (PHA, PHB), which are related to chemosensory avoidance. The inner and outer labial pores are in the area surrounding the mouth of the *C.elegans* (Hart & Chao., 2010). Chemosensory neurons directly or indirectly interact with a set of command interneurons which then synapse onto motor neurons to regulate forward and backward movement in response to different chemosensory stimuli (Hart & Chao., 2010).

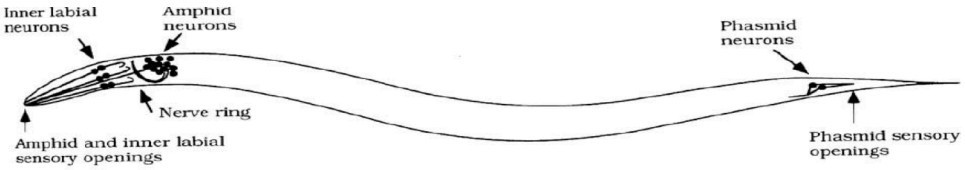

Figure 4 Scheme of the position of the amphid and phasmid neurons (Cornelia I Bargmann, 2006)

Several studies in *C. elegans* have shown that some sensory neurons (AWA, AWC and ASE) lead to attraction and the others lead to aversion (AWB, ASH and ADL; Bargmann, Hartwieg, & Horvitz, 1993; Troemel, Chou, Dwyer, Colbert, & Bargmann, 1995; Hart & Chao., 2010). In general, the AWA and AWC neurons drive responses to attractive odorants, while the ASE neurons drive responses to attractive soluble substances. The ASH neurons detect both soluble and volatile repellants, whereas the AWB and ADL neurons respond just to volatile repellants (Hart & Chao., 2010). A single olfactory neuron can be activated by several compounds, which implies that single olfactory neurons express multiple receptors, each of which binds to different odorants (Troemel, Kimmel, & Bargmann, 1997). Other amphid sensory neurons have small roles in chemotaxis. The two phasmid sensory neurons have been shown to be involved in detection of repulsive stimuli (Hart & Chao., 2010).

Olfaction in *C.elegans* depends on G protein- coupled signal transduction pathways. Binding of odorant ligands to G protein-coupled receptors (GPCRs) expressed in the olfactory sensory neurons changes the conformation of the receptors and activates the associated heterotrimeric G proteins. Gα exchanges GDP for GTP and dissociates from the GPCR complex. The Gα-GTP and Gβγ then activate downstream targets within the cell (Ezak & Ferkey, 2010).

2. Experiment 1: Investigation of Notch Activity in *C.elegans* $PS1_{\Delta S169}$Transgenic Lines

2.1 Introduction

Mutations in the human presenilin gene, the catalytic component of γ-secretase, have been shown to cause of Familial Alzheimer's disease (FAD). The mutations that have been implicated in FAD are spread throughout the entire PS1 gene and make it complicated for scientists to understand how these variously located PS1 mutations change γ-secretase activity in a way that results in similar pathologic symptoms in AD patients (Berezovska et al., 2005). Understanding how these different PS1 mutations generate similar consequences could deepen our understanding of this disorder and shed light on AD diagnosis and treatment.

Presenilin is a part of γ-secretase that has similar sequential intramembranous cleaving patterns for APP and Notch proteins. Some PS1 mutations prevent cleavage at the S3-site (similar to ε-site of APP) of Notch, which eventually impairs production of NICD in the Notch signaling pathway (Song et al., 1999). However, the pathological association of Notch dysfunction in familial forms of AD is still highly controversial.

A novel PS1 mutation known as $PS1_{\Delta S169}$, which has a deletion in serine at the site of 169, was recently discovered in a Chinese family with early-onset familial Alzheimer's disease (Guo et al., 2010). A preliminary *in vitro* study reported from the Song lab (Zhang, 2013) recently demonstrated that AD pathogenesis in $PS1_{\Delta S169}$ depends on a decrement in processing γ-secretase on APP, while the Notch pathway remained intact in this mutation. These results go against the idea that Notch processing plays a role in AD pathogenesis. To further investigate this we used *C. elegans* to examine the function of the $PS1_{\Delta S169}$ mutation on the Notch signaling pathway. Levitan

and Greenwald (1995) showed that a mutation in *sel-12* decreases the activity of *lin-12/*Notch, which is critical for cell-cell interaction during development. A decrement in *lin-12* activity in *C.elegans* causes an egg-laying defective (Egl) phenotype. Normal human presenilin can substitute for *C.elegans* SEL-12 protein to rescue the Egl phenotype. Here, we used the Egl phenotype of *sel-12* mutant worms to provide *in vivo* evidence for normal Notch pathway function in the $PS1_{\Delta S169}$ mutant.

2.2 Methods

2.2.1 *C.elegans* strain maintenance and transgenic lines

Worms were cultured on nematode growth medium (NGM) seeded with *Escherichia coli (E.coli*; OP50; Brenner, 1974). N2 Bristol and RB1672 *sel-12 (ok2078) C.elegans* were obtained from the *Caenorhabditis* Genetic Center (University of Minnesota, Minneapolis, MN). The plasmids pBY895 (containing *sel-12* rescue driven by the *sel-12* promoter), pBY140 (containing the wild-type PS1 coding region driven by the *sel-12* promoter) were a gift from Dr. Baumeister (Albert-Ludwig University in Freiburg/Breisgau, Germany). Site-directed mutagenesis was used to generate a plasmid contained $PS1_{\Delta s169}$ from the pBY140 plasmid (used as a template) by Dr. Song's lab.

Transgenic lines were established by DNA microinjection into the hermaphrodite germ line of *sel-12(ok2078)* mutant at a concentration of 10 ng/µl along with the co-injection marker *CC*::gfp. Stable F2 lines with transmission of CC::GFP> 50% were chosen for this work. DNA microinjection into the germ line of worm generates extrachromosomal arrays with some inherent variability in expression due to variable copy number of plasmids incorporated into the array and possible mosaic expression. One solution to overcome this inherent variability in expression from

different arrays is to use multiple independent lines for each construct (Levitan et al., 1996). Using multiple independent lines for each construct is a conventional method among scientists who have been using *C.elegans* as a model organism. In this work three independent lines for each construct were used except for PS1 rescue for which there were only two stable lines.

The following strains were created and used for the egg-counting assay in this work:

VG299 *sel-12 (ok2078);* CC*::GFP*

VG286 *sel-12 (ok2078); Psel-12::sel12;* CC*:: GFP*

VG287 *sel-12 (ok2078); Psel-12::sel12;* CC*:: GFP*

VG288 *sel-12 (ok2078); Psel-12::sel12;* CC*:: GFP*

VG289 *sel-12 (ok2078); Psel-12::PS1 wt* CC*:: GFP*

VG290 *sel-12 (ok2078); Psel-12::PS1 wt;* CC*:: GFP*

VG292 *sel-12 (ok2078); Psel-12::PS1*$_{\Delta s169}$*;* CC*:: GFP*

VG294 *sel-12 (ok2078); Psel-12::PS1*$_{\Delta s169}$*;* CC*:: GFP*

VG295 *sel-12 (ok2078); Psel-12::PS1*$_{\Delta s169}$*;* CC*:: GFP*

VG477 *sel-12 (ok2078); Psel-12::PS1*$_{C410Y}$*;* CC*:: GFP*

VG478 *sel-12 (ok2078); Psel-12::PS1*$_{C410Y}$*;* CC*:: GFP*

VG479 *sel-12 (ok2078); Psel-12::PS1*$_{C410Y}$*;* CC*:: GFP*

VG497 *sel-12 (ok2078); Psel-12::PS1*$_{Y115H}$*;* CC*:: GFP*

VG498 *sel-12 (ok2078); Psel-12::PS1*$_{Y115H}$*;* CC*:: GFP*

VG499 *sel-12 (ok2078); Psel-12::PS1*$_{Y115H}$*;* CC*:: GFP*

2.2.2 Quantification of egg-laying

The number of unlaid fertilized eggs that accumulated inside of the gonads of young adult worms was determined according to the method of Koelle & Horvitz (1996) with minor modifications. Animals were isolated at the late L4 stage of development when their maturing vulva appears as a large white crescent with a small black dot at its center when viewed through a dissecting microscope. Worms were allowed to develop at 20°C for 24 hr. The resulting young adult worms were individually incubated for 30 min in 96-well cell culture plates containing 50µl of 1% sodium hypochlorite. This treatment dissolves all tissues except eggs, which are protected by eggshells. Released eggs were then viewed and counted using an inverted microscope (Tritec MINJ-1000).

2.3 Statistical analysis

All data analyses were conducted using R version 3.1.0. One-way ANOVA's were conducted on all experiments. Post-hoc analyses consisted of Tukey's Honest Significant Difference test and p-values of less than 0.05 were considered significant.

2.4 Results

Young adult wild-type worms had an average of 15±3 eggs in their gonads (Figure 5), which is comparable with what previously reported (Baumeister et al., 1997). *sel-12(ok2078)* mutant worms carried on average 39±6 eggs in their gonads (Figure 5), which is significantly ($p<0.01$) more than the number of eggs in the gonads of wild-type animals (Figure 5). The GFP marker did not have any effects on egg-laying behaviour as the *sel-12(ok2078)* mutant worms in

which the GFP marker alone was expressed were not significantly different (p=0.93, NS) from the original *sel-12* mutant strain; however, the *sel-12* mutant group in which the GFP was expressed as a marker was significantly (p<0.01) different form the wild type (Figure 5). Reintroducing of the *sel-12* gene under the control of its endogenous promoter rescued the Egl phenotype (accumulation of eggs in the gonad). The number of eggs in the gonad of these transgenic lines was not significantly different from that of the wild-type animals (p=1, NS); however, all three lines were significantly different from the sel-12 mutant group (p<0.01). One out of two wild-type human PS1 transgenic lines showed rescue of the Egl phenotype (p=1, NS) while the other line showed partial rescue of the egg-laying deficit in *sel-12(ok2078)* mutant worms (p<0.01); however the two independent lines for the normal human presenilin were significantly different from the *sel-12* mutant group(p<0.01). Two out of three lines of $PS1_{\Delta s169,}$ the novel FAD-linked PS-1 mutation, were not significantly different (p=0.07,p=0.93) from the wild-type animals (Figure 5); however, all three lines were significantly different from the *sel-12* mutant group (p<0.01), suggesting that deletion of serine at the site 169 of PS1 is not required for normal function of presenilin in the egg-laying assay. Transgenic lines expressing the human presenilin with clinical mutations C410Y (3 lines) and Y115H (3 lines) were not significantly different from *sel-12(ok2078)* mutant worms in egg-laying and all six lines were significantly (p<0.01) different from the wild-type worms (Figure 5).

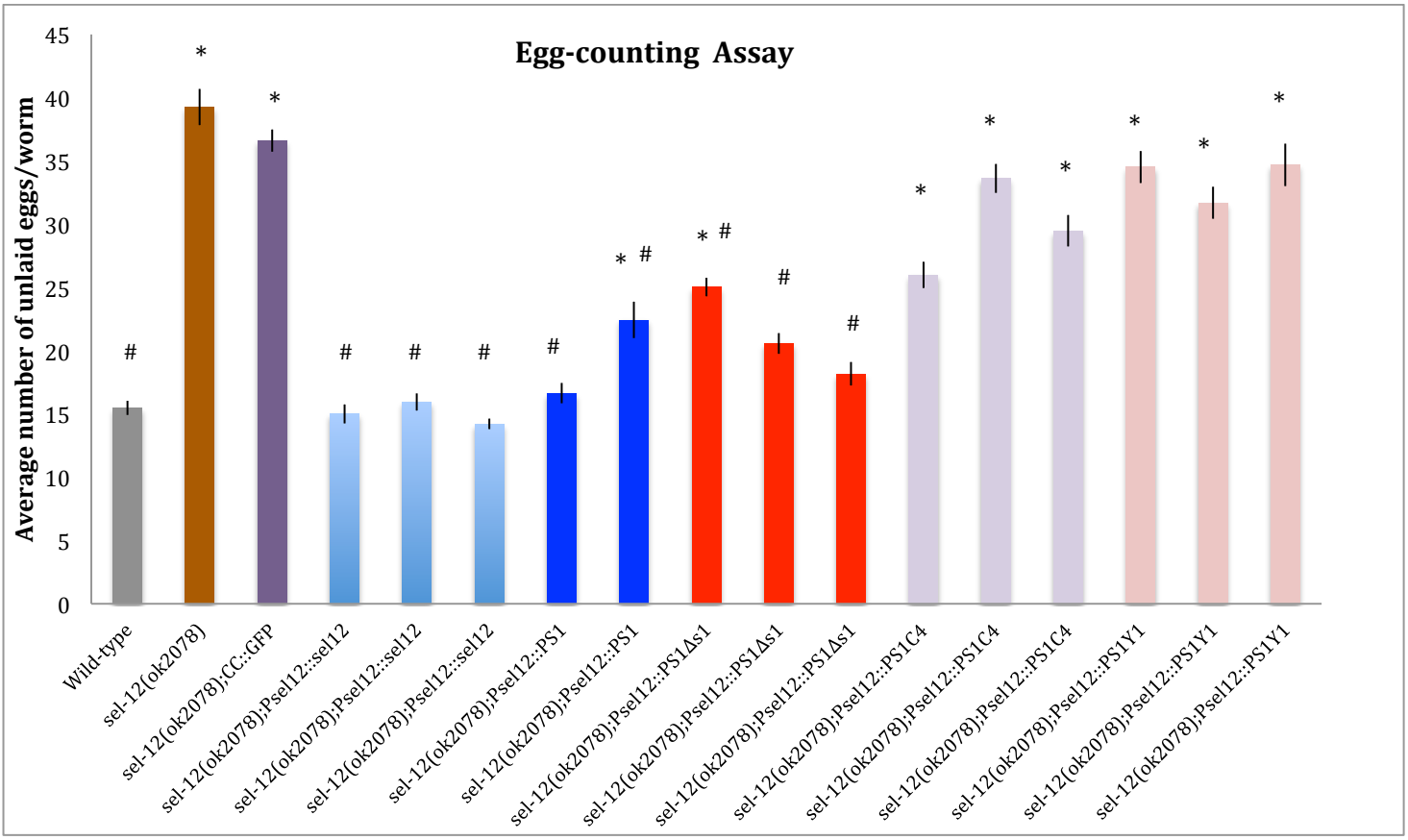

Figure 5 The average number of unlaid eggs in the gonads of worms. The * sign indicates significant differences from the wild-type level and the # sign indicates the significant differences from the *sel-12* mutant group. Values represent the means and standard errors measured from 20 to 24 young adults of each strain.

2.5 Discussion

Several human presenilin mutations linked to FAD demonstrate impairment in Notch signaling; however, involvement of this pathway in pathogenesis of AD is still broadly controversial. Alteration in expression of Notch receptors in the brain of Alzheimer's patients raised the possibility of a role for the Notch pathway in the pathogenesis of AD (Berezovaska & Hyman, 1998). A recent study on Notch conditional knock-out mice also suggested that Notch signaling is involved in synaptic plasticity (Alberi et al., 2013). However, another line of evidence using conditional knockout mice reported that Notch receptors are not targets of the catalytic components of the γ-secretase in the adult brain.

Unpublished data from the Song lab (Zhang, 2013), described the function of a recently discovered human presenilin mutation gene, $PS1_{\Delta s169}$, on Notch and APP pathways. The *in vitro* investigation of the $PS1_{\Delta s169}$ mutation from their lab revealed that the Aβ42/40 ratio, which is a pathogenic indicator of clinical PS1 mutations was increased; however, no deficit was observed in the processing of Notch by this gene. Using the advantages of the *C. elegans* egg-laying phenotype associated with the Notch pathway, we showed that the pathogenic $PS1_{\Delta s169}$ mutation can process Notch normally in non-neuronal tissues in *C. elegans* as demonstrated by the rescue of the Egl phenotype; however, the other pathogenic mutations ($PS1_{C410Y,}$ $PS1_{Y115H}$) with strong effects on Notch processing did not rescue the egg-laying deficit, suggesting that the Notch pathway may not be involved in AD pathogenesis produced by the $PS1_{\Delta s169}$ mutation. However, we cannot exclude a role for Notch signaling in AD pathogenesis. It has been shown that mutations in different domains of PS1 have different effects on Notch cleavage. Mutations in the PS1 C-terminal inhibit Notch-1

cleavage more than mutation in the N-terminal domain (Song et al., 1999). Also, various PS1 mutations could have different pathological and clinical symptoms, which may be caused by various effects of PS1mutations on Notch functions. It remains to be determined whether altered Notch contributes to the neurodegenerative process seen in AD.

3. Experiment 2: Assessment of Presenilin Function in *C. elegans'* Chemosensation

3.1 Introduction

Olfactory dysfunction, an early symptom in AD patients, has recently been considered to be a potential indicator for early diagnosis of this neurodegenerative disorder. Research indicates that there are many alterations in olfactory system function in AD patients; however, the main causes of these olfactory impairments in AD patients are poorly understood (Ruan et al., 2012).

C. elegans is a powerful model organism that enables researchers to investigate the molecular mechanisms of neurodegenerative disorders. The most efficient way to use an invertebrate model organism to address questions related to neurodegenerative disorders in human is finding a similar phenotype in both organisms. If mutations in the presenilin gene homologue in *C.elegans* show chemosensation deficits, it could potentially address some of the questions regarding cellular processes underlying the olfactory impairments observed in AD patients. The first set of experiments was designed to investigate whether any impairment in olfactory chemotaxis could be observed in *sel-12* mutant worms, and if so, probe the function of normal and mutant human presenilin in rescuing olfactory chemotaxis deficit phenotypes in *sel-12* mutant worms. The second set of experiments was designed to explore the possible gustatory impairments in *sel-12* mutant

animals and examined the role of normal and mutant human presenilin to rescue the possible taste impairment in *sel-12* mutants worms. These results can potentially be used for addressing questions related to cellular processes involved in olfactory deficit in AD patients.

3.2 Methods

3.2.1 Chemotaxis assays to volatile odorants

Chemotaxis assays to volatile odorants were carried out according to the method described by Margie et al (2013) with minor modifications. Worms were synchronized by placing 15-25 adult animals into a droplet of hypochlorite to release the eggs, after which larvae from these eggs hatched and grew on agar with a bacterial lawn at 20°C for three days. On day three, animals were washed off the growing plate with M9 into a 1.5 ml Microfuge tube and washed an additional three times with M9. The underside of each 5cm NGM plate (Figure 6) was marked to divide it into 4 equal quadrants and in each quadrant one point was selected, ensuring the point was the same distance from the two adjacent points. All 4 equidistant points were measured to be at least 2cm away from the center. A circle with a radius of 0.5 cm was also drawn around the center. Points are marked in each quadrant with either "C" for the control or "T" for the test. In test plate, two pointes were marked as "T" and the other two points were signed as "C"; however, all four points on the control plate were marked as "C". Test solutions were prepared by mixing equal volumes of the test compound and sodium azide (0.5M), which was used to immobilize worms. The M9 was mixed with equal volumes of the sodium azide (0.5M) to make the control solution. Washed worms (50-100) were pipetted to the center of plates, followed by placing 2µl of the test solution onto the "T" spots and 2µl of the control solution onto the "C" spots. The test and control solutions were allowed to soak into the agar, prior to covering the plates with lids and incubating them in a 20°C room.

After one hour incubation in 20°C, plates were transferred into the 4°C fridge to immobilize worms and only removed when the worms were being counted (usually 2 hours after incubation in 4°C fridge). The number of worms in each quadrant that completely crossed the inner circle was recorded. A chemotaxis index (Margie et al., 2013) was used to compare the responses of worms to different odors.

Chemotaxis Index = (# Worms in Both Test Quadrants - # Worms in Both Control Quadrants) / (Total # of Scored Worms).

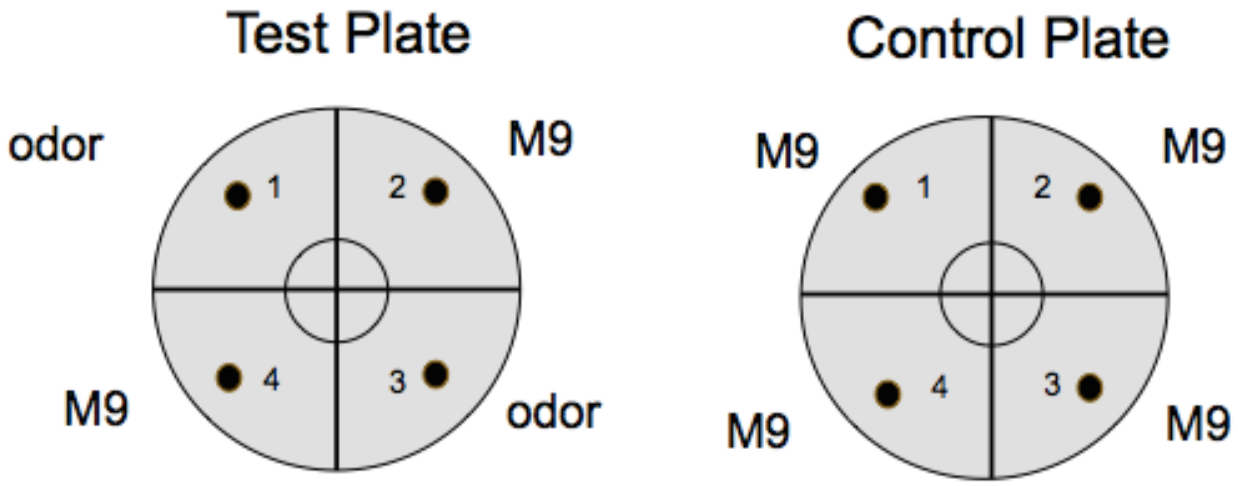

Figure 6 The chemotaxis plates prepared for odor assays (drawing by Rojin Kaviani)

3.2.2 Chemotaxis assays to water-soluble attractants

Salt assays were carried out according to method described by Ewald et al. (2012) with a minor modification. Worms were synchronized by placing 15-25 adult animals into a droplet of

hypochlorite to release the eggs after which larvae from these eggs hatched and grew on agar with a bacterial lawn at 20°C for three days. On day three, animals were washed off the growing plate with M9 into a 1.5 ml Microfuge tube and washed an additional three times with M9. Three 10 cm plates containing 10 ml of 2% agar were poured for each strain the night before the assay was done. A triangle with 4cm sides was drawn on the back of each plate (Figure 7). The three corners of the triangle were at the same distance from the side of the plate. A circular hole (test corner), 0.8 cm in diameter, was made in one corner of the triangle by removing the solid agar filled in the plate. The hole was then gently filled with 400mM Sodium acetate (NaAc), which was already dissolved in 2% liquid agar. The 2% liquid agar contained 400mM NaAc solidified after ~10 min and the sodium acetate filled in the hole, gradually dispersed in the agar and formed a gradient in the plate overnight. In the second corner of the triangle, a 0.8 cm diameter hole was made exactly the same as the test corner; however, this corner was then filled with 2% liquid agar and applied as the control corner (Figure 7). The third corner of the triangle remained untouched until the day after, when ~100 washed worms were placed on this corner and it was used as a starting point to assess worms' behaviour toward the salt. After placing worms on the site, the chemotaxis plates were transferred into a 20°C room for two hours. The chemotaxis index (CI) was scored two hours after incubation in 20°C room for each chemotaxis plate as follows:

CI= [(number of animals on the test spot - number of the animals on the control spot)/total number of animals on the plate].

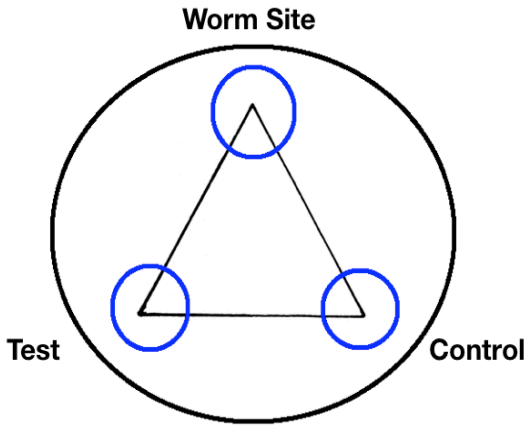

Figure 7 The salt assay plate: The test corner of the triangle was filled with 2%liquid agar contained400 mM NaAc, The control corner covered with 2% liquid agar and the worm site was used to placed worms to run the assay (drawing by Mahraz Parvand).

3.3 Statistical analysis

All data analyses were conducted using R version 3.1.0. One-way ANOVA's were conducted in all experiments, with the exception of section 3.4 in which assumption of the homogeneity of variance was violated and a Kruskal-Wallis test was conducted. Post-hoc analyses consisted of Tukey's Honest Significant Difference test and the p-values less than 0.05 were considered significant. Independent t-test was used to analyze single group experiments.

3.4 Results

C.elegans is normally attracted to or repulsed from various types of volatile and water-soluble chemicals. Wild-type animals showed negative chemotaxis responses toward the volatile odorant octanol and positive chemotaxis responses toward the volatile odorant diacetyl and the water-soluble chemical sodium acetate.

3.4.**1 Repulsive responses to octanol**

3.4.1.1 **SEL-12 is required for the avoidance response to octanol**

To determine whether the *sel-12* gene contributes in olfactory chemotaxis in *C.elegans*, we exposed *sel-12(ok2078)* mutant worms to undiluted octanol in the test quadrants, and assayed olfactory chemotaxis away from the odorant in these animals. We observed that the *sel-12(ok2078)* mutants showed significantly less avoidance of 100% octanol odor compared to the wild-type animals ($t(3.6)=-4.36$, $p<0.05$; Figure 8). Transgenic lines were generated by expression of *sel-12* cDNA under the control of *sel-12* promoter to test whether the olfactory defects were due to the *sel-12* mutation. Transgenic lines have extrachromosomal arrays with some inherent variability in expression from different arrays, due to different numbers of plasmid copies in the arrays. To overcome this variability, multiple independent lines for each construct were tested. Three independent *sel-12* rescue lines didn't show any significant differences compare to the wild type ($p=0.87$, $p=0.59$and $p=0.7$), however, two out of three *sel-12* rescue lines used in this assay showed significantly a higher avoidance response to octanol compared to the *sel-12* mutant worms (Figure 9;$F(4,15)=7.24$, $p=0.002$) indicating that the *sel-12* mutation was responsible for the decreased avoidance behaviour to octanol.

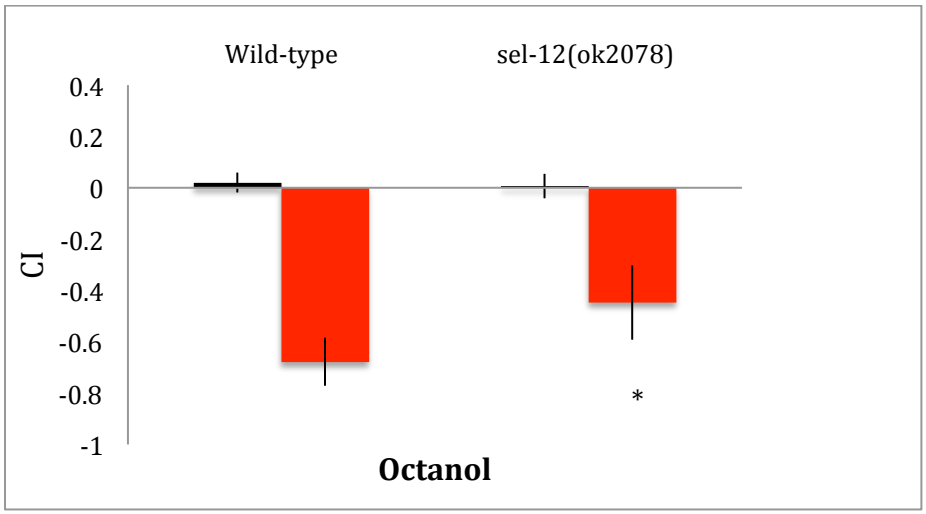

Figure 8 Octanol assay for *sel-12* mutant strain: Chemotactic indices generated from assays performed with wild-type (N2) and *sel-12* mutant worms using either octanol as a test repellant or M9 (control plates). Red bars and black bars indicate the test and the control plates, respectively. Values are averages from at least 3 independent plates (N=30-60 worms per plate) for each condition. T-test compared the wild-type and *sel-12* test plates; asterisks indicate significant differences from the wild-type (*$p<0.05$). Error bars reflect the standard error of the mean.

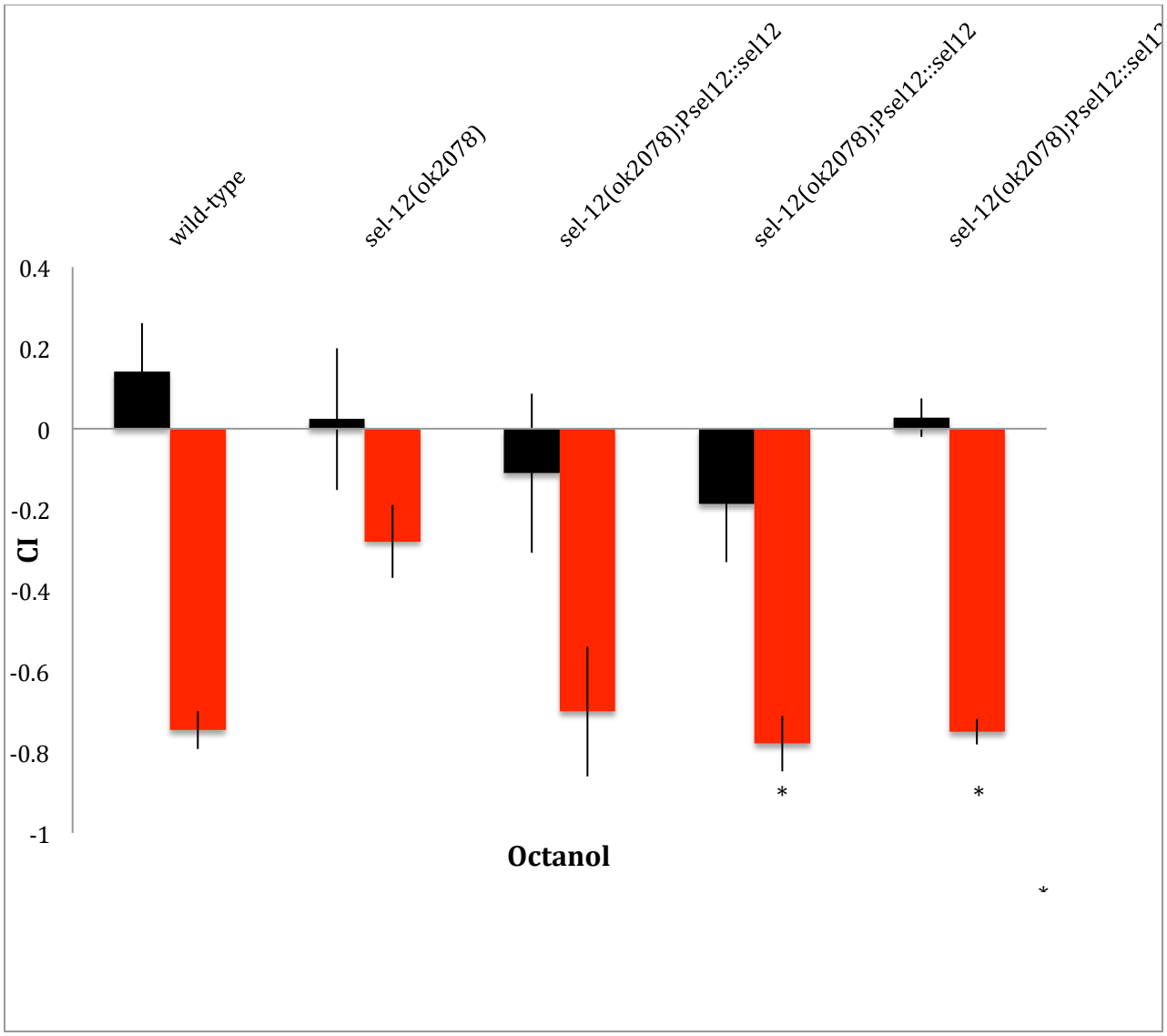

Figure 9 Octanol assay for *sel-12* rescue: Chemotactic indices generated from assays performed with wild-type (N2), *sel-12* mutant and three independent *sel-12* rescue lines using either octanol as a test repellant or M9 (control plates). Red bars and black bars indicate the test and the control plates respectively for all strains. Values are averages from at least 3 independent plates (N=20-40 worms per plate) for each condition. Asterisks indicate significant differences from the *sel-12* mutant strain (test plates; *$p<0.05$). Error bars reflect the standard error of the mean

3.4.1.2 **Olfactory deficit increases as *sel-12* mutants age**

Invertebrate model organisms, including *C.elegans*, can be considered as powerful *in vivo* tools to investigate human neurodegenerative diseases if they can help us to understand mechanisms underlying some aspects of these disorders. AD is a progressive disease, where symptoms gradually worsen over a number of years. We investigated age-dependent changes in the olfactory deficit we observed by screening *sel-12* mutant responses to octanol at 68 hours and 78hours of age respectively. Repulsive responses to octanol in the wild-type worms at 68 hours of age were not significantly different from those of 78-hour-old wild type animals (p=0.92); however, 78-hour-old *sel-12* mutant worms showed significantly less of an aversive response to octanol than did 68-hour-old *sel-12* mutant animals (p=0.03); F (3,12)=39.75, p<0.001), suggesting that the olfactory deficit in *sel-12* mutant worms increases as worms age in the same way that olfactory deficits increase with age in AD patients (Figure 10).

Because the Egl phenotype led to a gradual accumulation of eggs in the gonads of *sel-12* mutant worms, we explored whether the decrease in chemotaxis responses at 78 hours of age observed in *sel-12* mutant worms were due to a general effect on speed of movement resulting from accumulation of eggs in the gonads. To examine our hypothesis, speed of movement in *sel-12* mutants worms were measured at 68 and 78 hours of age, respectively (Figure 11). Speed of movement (Figure 11) was not significantly different between 68 and 78-hour-old worms (p=0.68), suggesting that the increase in the olfactory deficit over the time was not due to the internal accumulation of eggs

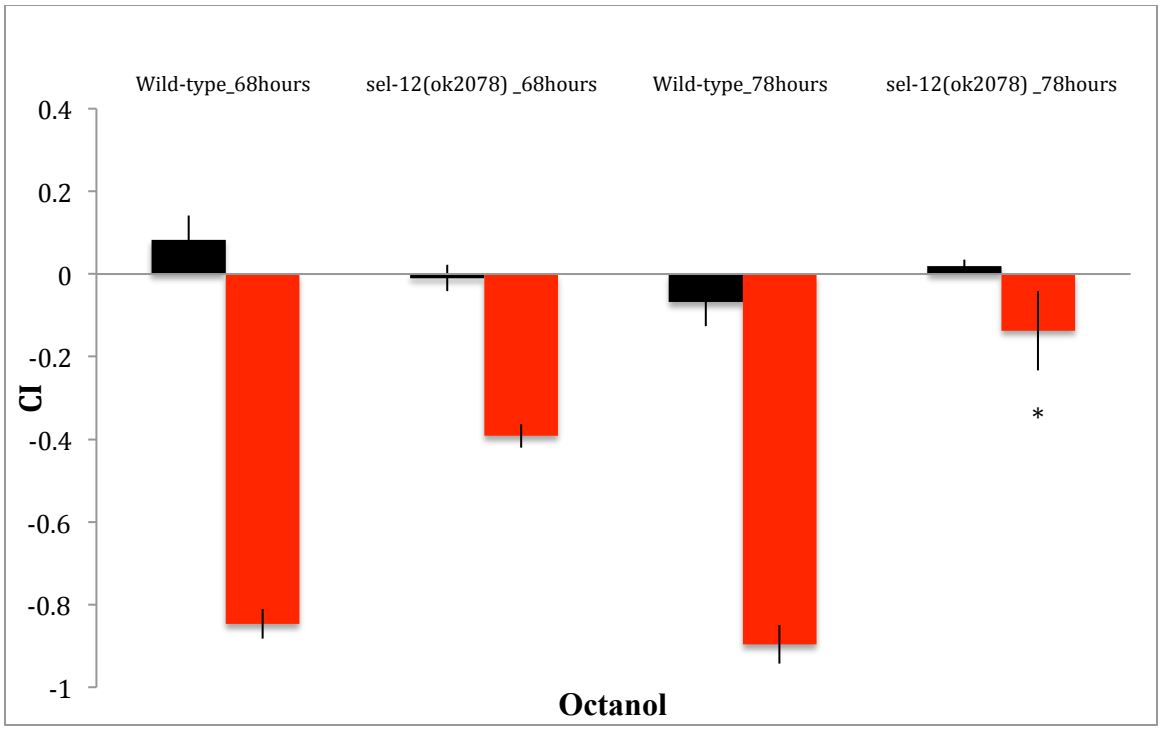

Figure 10 Octanol assay for 68 and 78 hour old worms: Chemotactic indices generated from assays performed with wild-type (N2), *sel-12* mutant at 68- or 78- hour-old worms using either octanol as a test repellant or M9 (control plates). Red bars and black bars indicate the test and the control plates respectively for all strains. Values are averages from at least 3 independent plates (N=30-60 worms per plate) for each condition. Asterisks indicate significant differences between the sel-12 mutant strains at 68-and 78-hour-old (test plates; *p<0.05). Error bars reflect the standard error of the mean.

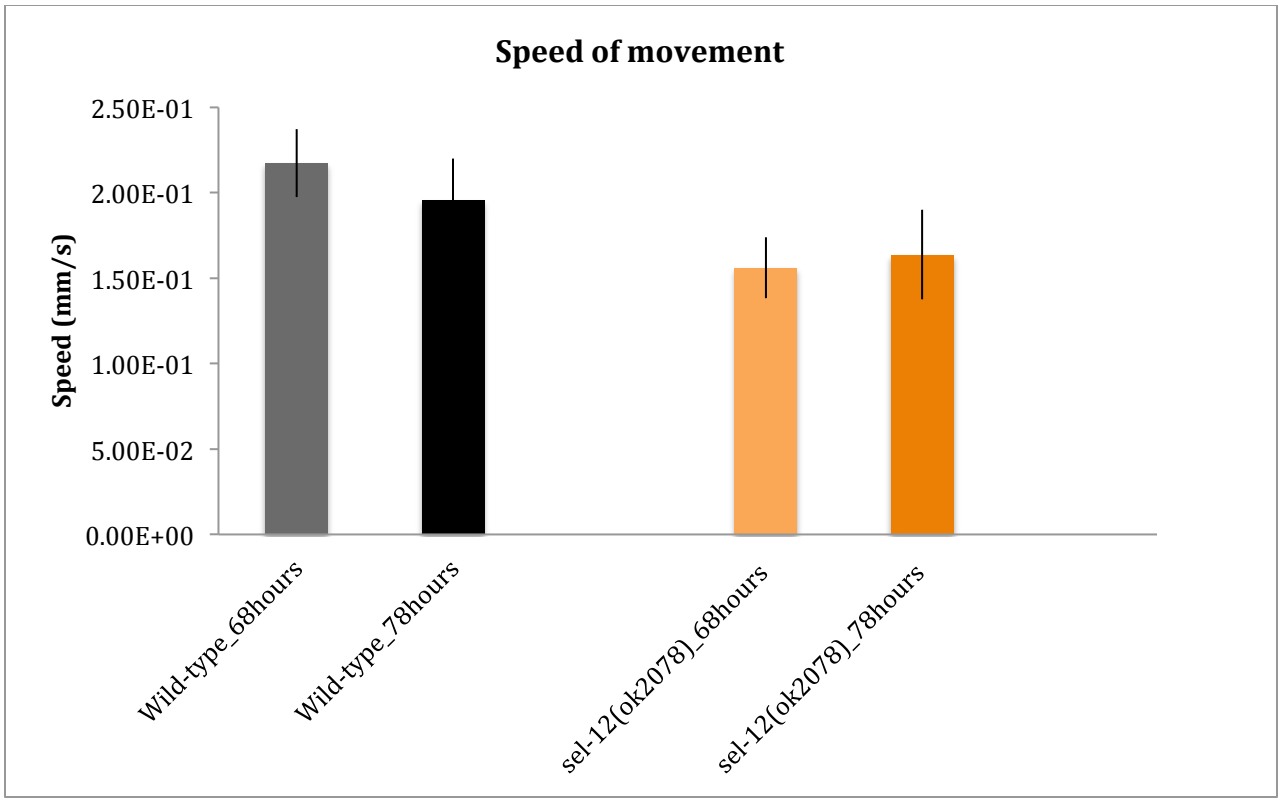

Figure 11 Speed of movement at 68 and 78 hours of age: Speed of movement at 68 and 78 hours of age for wild-type (N2) and *sel-12* mutant worms. Values are averages from individual worms (N=40 worms per plate) for each condition. There were no differences in the speed of movement for *sel-12* mutant worms or N2 at 68 and 78 hours of age. Error bars reflect the standard error of the mean.

3.4.1.3 **Normal human presenilin restores the olfactory deficit to octanol in *sel-12* mutant animals**

Because Levitan *et.al* (1996) showed that normal PS1 is functional in *C. elegans* non-neuronal tissues by demonstrating the ability of PS1 gene to rescue the Egl defect in *sel-12(ok2078)* mutant hermaphrodites, we investigated whether normal human presenilin could restore the olfactory deficit in *sel-12(ok2078)* mutant worms. The ability of wild-type human presenilin1 to rescue the escape behaviour to octanol was assessed by testing two independent PS1 rescue transgenic lines (Figure 12). We found that human presenilin1 protein can substitute for SEL-12 in rescuing the avoidance response to octanol, ($F(3,12)=3.89$, $p=0.03$). While, *sel-12* mutant worms were significantly different from the wild-type ($p=0.02$), both of the human presenilin transgenic lines showed no significant differences compared to the wild-type animals ($p=0.3$, $p=0.16$).

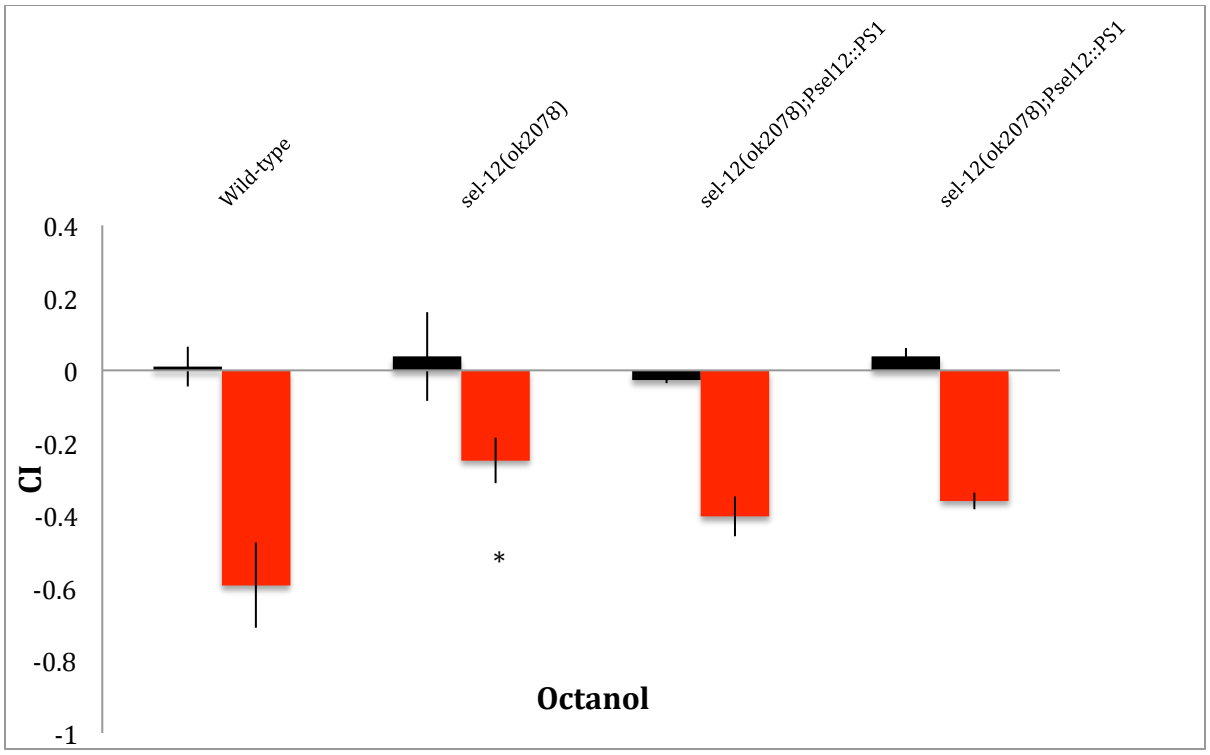

Figure 12 Octanol assay for PS1 rescue: Chemotactic indices generated from assays performed with wild-type (N2), *sel-12* mutant and two independent PS1rescue lines using either octanol as a test repellant or M9 (control plates). Red bars and black bars indicate the test and the control plates respectively for all strains. Values are averages from at least 3 independent plates (N=20-40 worms per plate) for each condition. Asterisks indicate significant differences from the wild-type (test plates; *p<0.05). Error bars reflect the standard error of the mean.

3.4.1.4 **PS1$_{\Delta s169}$, but not PS1C410Y, rescues the olfactory deficit in *sel-12* mutant animals**

Our result from the egg-counting assay (see Experiment 1) revealed that expression of PS1$_{\Delta s169,}$ the novel FAD-linked PS-1 mutation, in *sel-12(ok2078)* mutant worms suppressed the *sel-12* egg-laying deficit in a manner similar to normal human PS1 transgenic lines, suggesting that deletion of serine at the site 169 of PS1 is not required for normal function of presenilin in an egg-laying assay that depends on the Notch pathway. To investigate the possible involvement of the Notch pathway in the chemotaxis deficit in *sel-12* mutant worms, three independent transgenic lines carrying PS1$_{\Delta s169}$ gene in *sel-12(ok2078)* mutant background were screened for the avoidance response to octanol. Our results indicated that the chemotaxis deficit in *sel-12* mutant was fully rescued by human PS1$_{\Delta s169}$. The repulsive responses of three independent PS1$_{\Delta s169}$ transgenic lines and wild-type animals toward octanol were statistically indistinguishable (Figure 13; $F(5,16)=1.28$, $p=0.32$).

Notch signaling activity is reported to decrease in human presenilin mutations linked to FAD and this decrement is greatest in patients harboring the mutations PS1$_{C410Y}$ and PS1$_{Y115H}$ (Song et al., 1999). To determine whether the Notch pathway might be involved in the olfactory deficit observed in *sel-12* hermaphrodites, three PS1$_{C410Y}$ transgenic lines were tested for their repulsive response to octanol. All three lines (Figure 14) abolished the avoidance response toward octanol and none of them were significantly different from the *sel-12* mutant ($p=0.9$, $p=0.76$, $p=0.73$), while the wild-type and *sel-12* mutant were again significantly different ($p<0.01$). One out of the three independent lines was significantly different from the wild type ($p=0.03$). This finding provides more evidence to support a role for Notch signaling in the *sel-12* olfactory deficit.

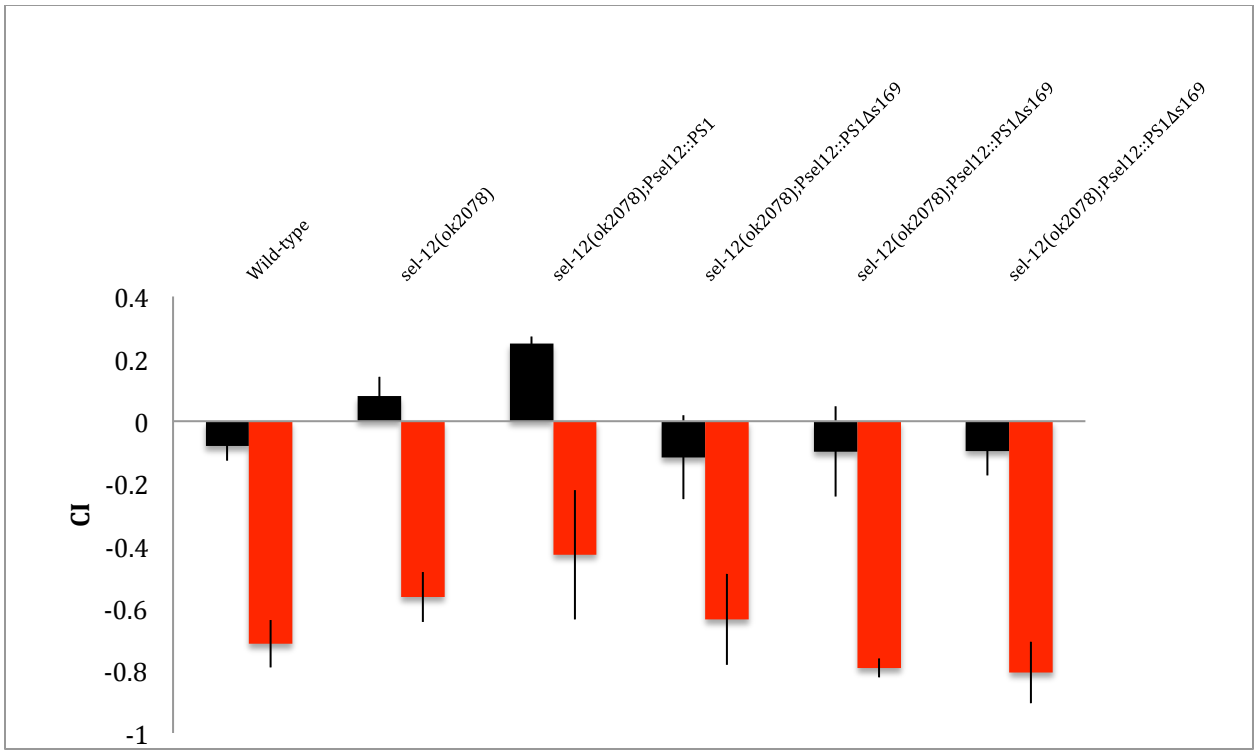

Figure 13 Octanol assay for PS1$_{\Delta s169}$ mutant: Chemotactic indices generated from assays performed with wild-type (N2), *sel-12* mutant and three independent PS1$_{\Delta s169}$ lines using either octanol as a test repellant or M9 (control plates). Red bars and black bars indicate the test and the control plates respectively for all strains. Values are averages from at least 3 independent plates (N=30-60 worms per plate) for each condition. There were no differences between the wild-type worms and three PS1$_{\Delta s169}$ transgenic lines in chemotaxis bevaviour (test plates). Error bars reflect the standard error of the mean.

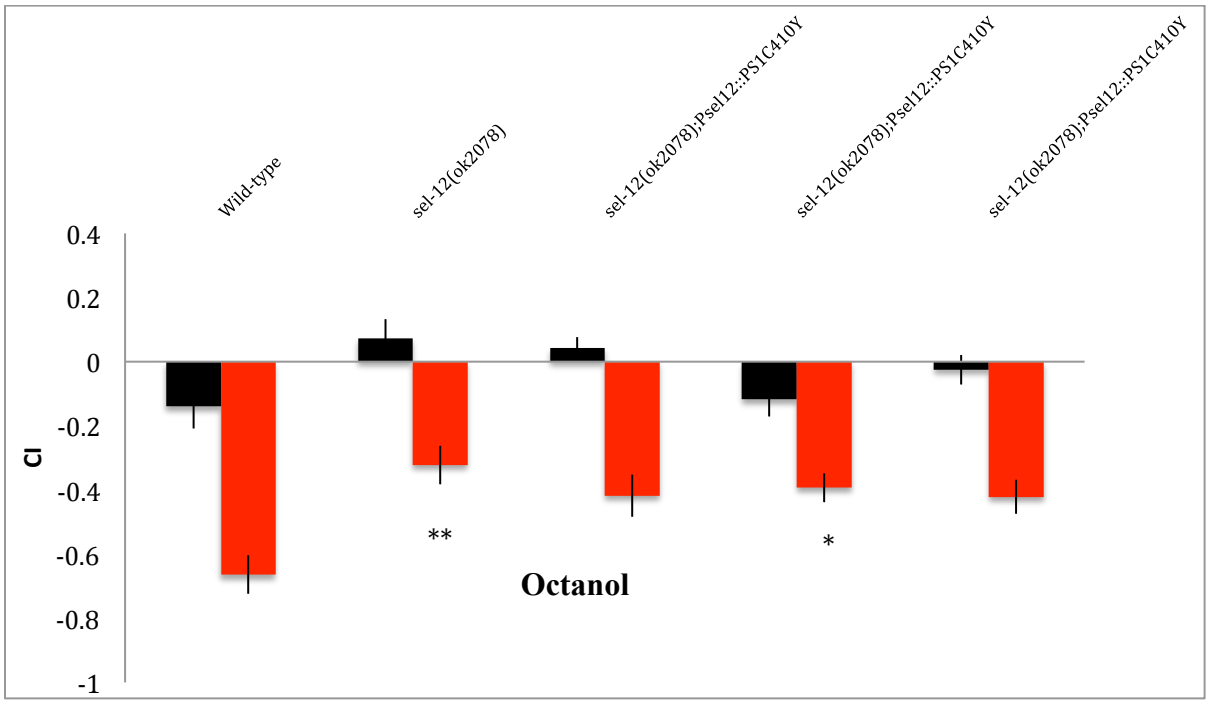

Figure 14 Octanol assay for $PS1_{C410Y}$ mutant: Chemotactic indices generated from assays performed with the wild-type (N2), *sel-12* mutant and three independent $PS1_{C410Y}$ lines using either octanol as a test repellant or M9 (control plates). Red bars and black bars indicate the test and the control plates respectively for all strains. Values are averages from at least 3 independent plates (N==30-60 worms per plate) for each condition. There were no significant differences between *sel-12* mutant worms and three $PS1_{C410Y}$ transgenic lines in chemotaxis bevaviour (test plates). Asterisks indicate significant differences from the wild-type (test plates; *p<0.5, ** p<0.01). Error bars reflect the standard error of the mean.

3.4.2 **Attractive responses to diacetyl**

3.4.2.1 ***sel-12* mutation decreases the attractive response to diacetyl**

Because *C.elegans'* attractive and repulsive responses toward volatile odors depend on different chemosensory neurons, we exposed *sel-12* mutant worms to a volatile attractant diacetyl (0.5%), which is normally detected by AWA neurons in order to investigate whether neurons mediating responses to attractive volatiles odorants are also affected by the *sel-12* mutation. Our results (Figure 15) showed that *sel-12* mutant animals exhibited less attraction toward the volatile odorant diacetyl compared to the wild-type animals; this decrement was significantly different between these two groups ($t(4.81)=3.17$, $p=0.02$), suggesting that a mutation in *sel-12* may have an effect on AWA mediated chemotaxis.

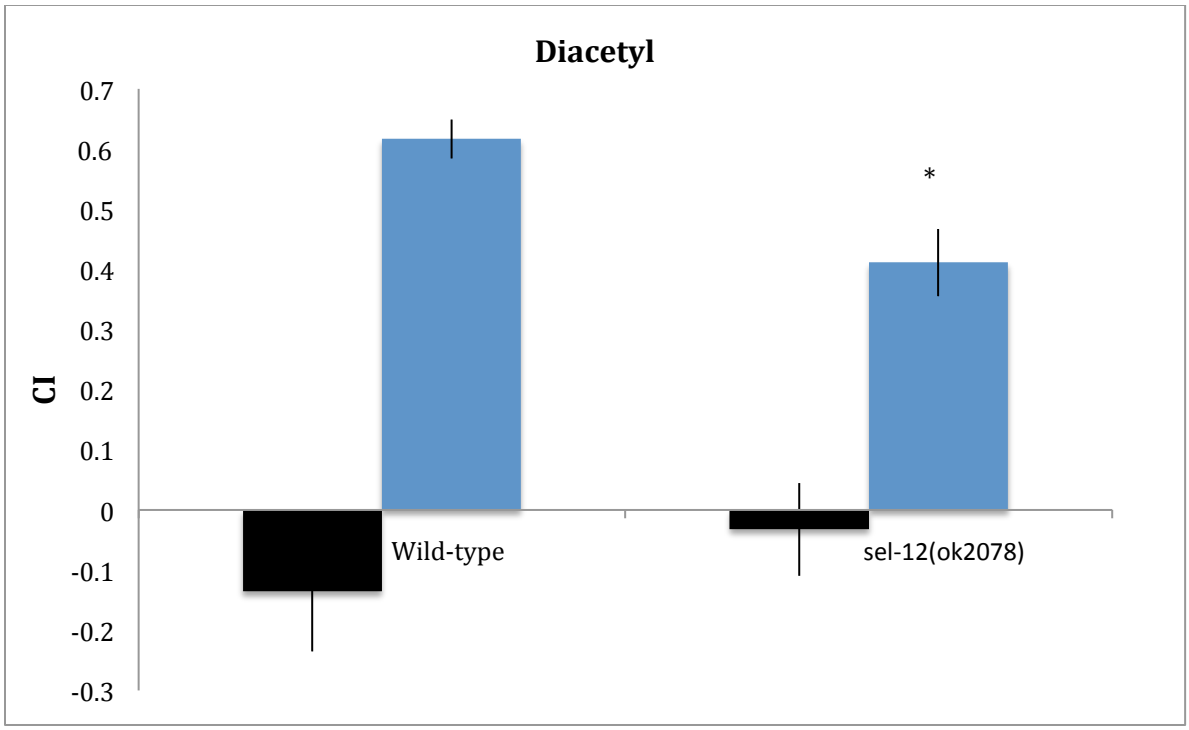

Figure 15 Diacetyl assay for *sel-12* mutant: Chemotactic indices generated from assays performed with wild-type (N2) and *sel-12* mutant worms using either diacetyl as a test attractant or ethanol (control plates). Blue bars and black bars indicate the test and the control plates, respectively. Values are averages from at least 3 independent plates (N=40-60 worms per plate) for each condition. There is a significant difference between the *sel-12* mutant and the wild-type worms (test plates) in attractive behaviour toward diacetyl (*$p<0.05$). Error bars reflect the standard error of the mean.

3.4.2.1 **Expression of the *sel-12* gene in *sel-12* mutant background improves the diacetyl decrement response**

To determine whether expression of the *sel-12* gene would improve olfactory responses to diacetyl in a *sel-12* mutant background, three rescue lines of this gene were tested using the chemotaxis assay to diacetyl. Our data showed (Figure 16) that expression of the *sel-12* gene improved the chemotaxis response to diacetyl and none of the three *sel-12* rescue lines were significantly different from the wild-type, however; the *sel-12* mutant group was significantly different form the wild-type (p=0.04), suggesting that the *sel-12* mutation was responsible for the decreased avoidance behaviour to diaceytl ($F(4,13)=3.7$, p=0.03).

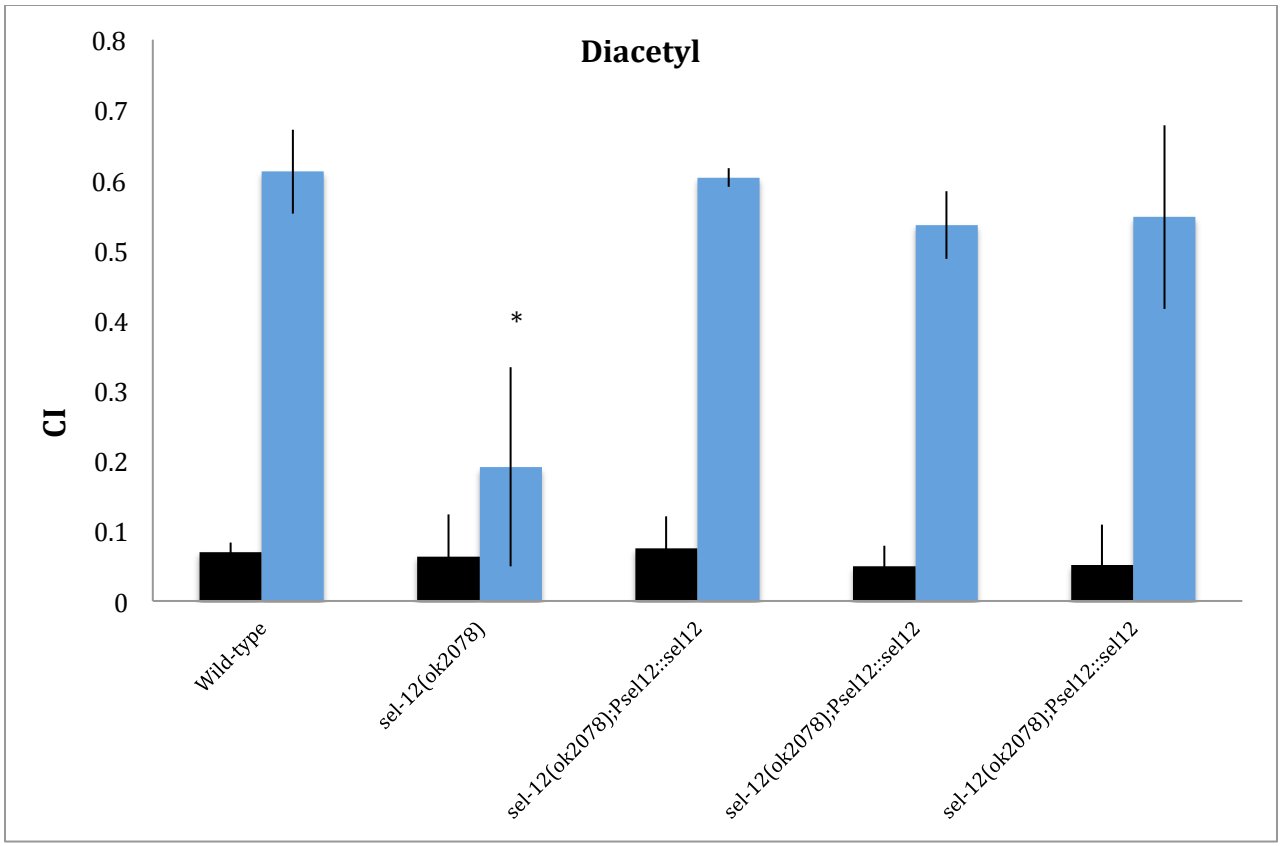

Figure 16 Diacetyl assay for *sel12* rescue: Chemotactic indices generated from assays performed with the wild-type (N2), *sel-12* mutant and three independent *sel-12* rescue lines using either diacetyl as a test attractant or ethanol (control plates). Blue bars and black bars indicate the test and the control plates respectively for all strains. Values are averages from at least 3 independent plates (N=40-60 worms per plate) for each condition. There were no significant differences between the wild-type worms and the *sel-12* rescued lines in chemotaxis behaviour (test plates). Asterisks indicate significant differences of the *sel-12* group from the wild-type (test plates; *$p<0.05$). Error bars reflect the standard error of the mean.

3.4.2.2 **Normal human presenilin decrease olfactory deficit to diacetyl in *sel-12* mutant animals**

Two human PS1 rescued lines were also screened using the chemotaxis assay to diacetyl to determine whether human presenilin is functional for chemotaxis. Results of this experiment showed that neither of the two rescued lines were significantly different from wild-type (Figure 17), indicating that human presenilin is functional in this assay and can rescue the olfactory deficit to diacetyl ($F(3,12)=2.35$, $p=0.124$). Because of high variability in this experiment, there was not a significant difference between N2 and *sel-12* ($p=0.1$), however; we found a difference between these two groups in two previous experiments and it is likely that this will become significant upon replication. This experiment is currently being replicated.

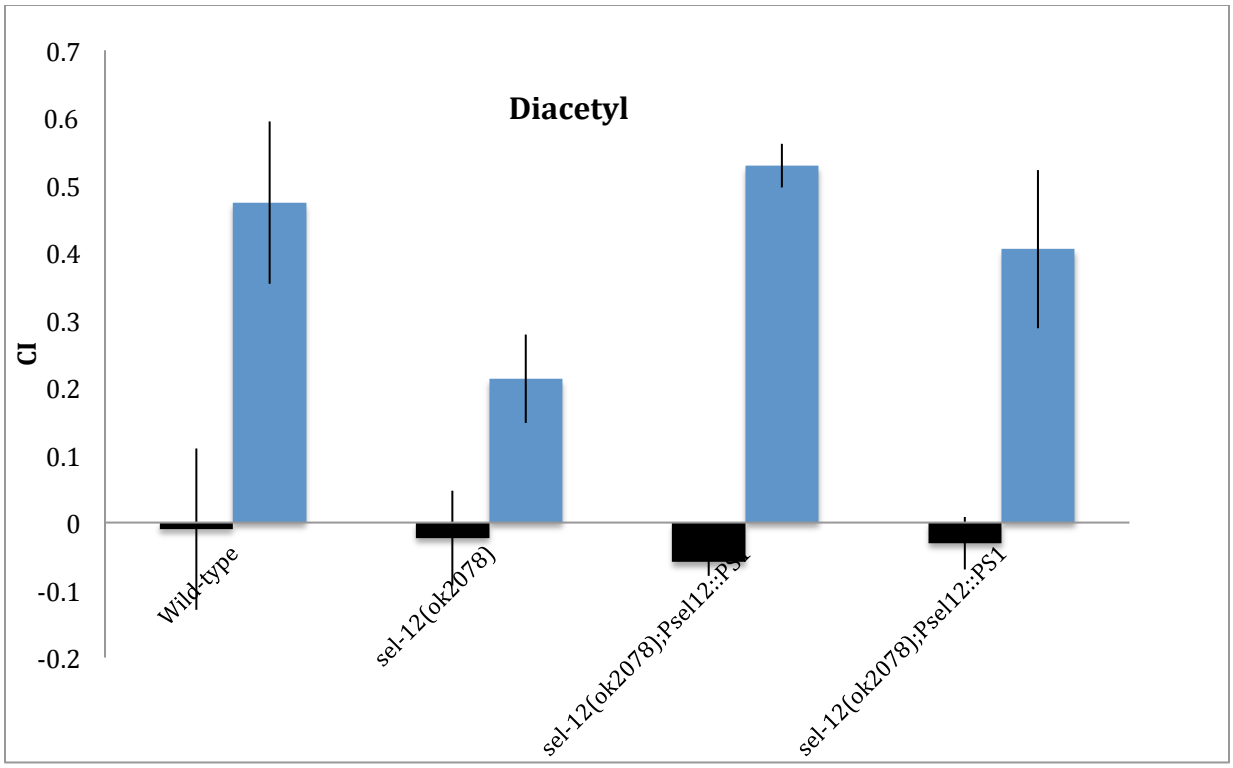

Figure 17 Diacetyl assay for PS1 rescue: Chemotactic indices generated from assays performed with wild-type (N2), *sel-12* mutant and two independent PS1rescue lines using either diacetyl as a test attractant or ethanol (control plates). Blue bars and black bars indicate the test and the control plates respectively for all strains. Values are averages from at least 3 independent plates (N=40-60 worms per plate) for each condition. No significant differences between groups were observed (test plates). Error bars reflect the standard error of the mean**.**

3.4.3 **Attractive responses to sodium acetate**

3.4.3.1 **SEL-12 is required for the gustatory response to sodium acetate**

Sodium acetate is considered a strong chemoattractant that is mainly sensed by one of the ASE neurons (ASEL) in *C. elegans*. To investigate whether the *sel-12* gene contributes to this gustatory response, positive chemotaxis responses of the *sel-12* mutant worms toward sodium acetate were tested. Our results (Figure 18) indicated that *sel-12* mutants were significantly less attracted to salt when compared with the wild-type groups, ($t(3.62)=6.96$,$p=0.003$). Three independent *sel-12* rescue lines used in this assay showed a significantly higher attractive responses toward sodium acetate compared to the *sel-12* mutant worms (Figure 19); $F(4,10)=58.59$, $p<0.001$), indicating that the SEL-12 protein is required for attraction towards the sodium acetate. However, two out of three rescue lines were also significantly different from the wild-type ($p<0.001$), suggesting that the phenotype was not fully rescued in these transgenic lines.

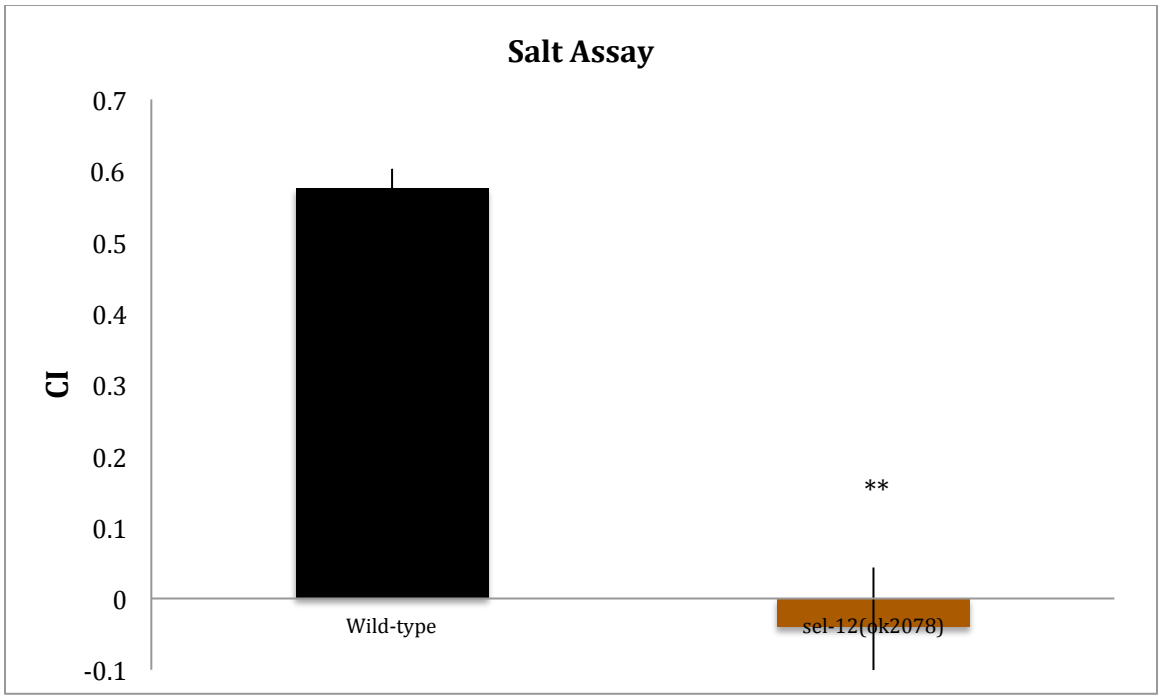

Figure 18 Salt assay for *sel-12* mutant: Chemotactic indices generated from salt assays performed with wild-type (N2) and *sel-12* mutant worms using sodium acetate as a test attractant. Values are averages from at least 3 independent plates (N=120-150 worms per plate). Asterisks indicate significant differences from the wild-type (**$p<0.01$). Error bars reflect the standard error of the mean.

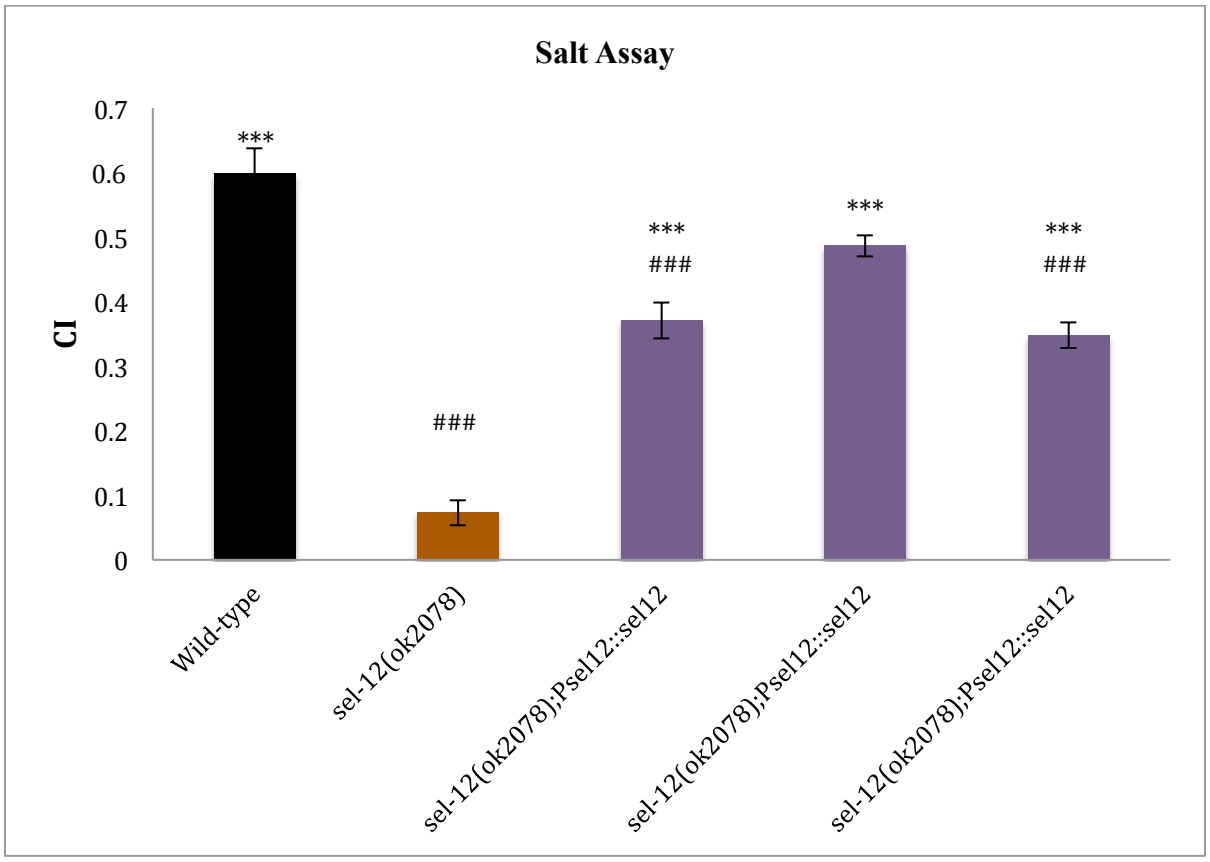

Figure 19 Salt assay for *sel-12* rescue: Chemotactic indices generated from salt assays performed with wild-type (N2) *sel-12* mutant and three independent *sel-12* rescue lines using sodium acetate as a test attractant. Values are averages from at least 3 independent plates (N=120-150 worms per plate). The *** signs indicate significant differences from the *sel-12* mutant group and the ### sign indicate significant differences from the wild-type group (***$p<0.001$, ###$p<0.001$). Error bars reflect the standard error of the mean.

3.4.3.2 Human PS1 is functional in the gustatory response to sodium acetate

The two wild-type human presenilin1 lines (Figure 20) were also tested in this assay to investigate whether human PS1can substitute for the SEL-12 protein in the gustatory response toward salt ($F(3,8)=43.9$, $p<0.001$). While *sel-12* mutant worms attraction responses were significantly lower than the wild-type ($p<0.001$), both human presenilin independent lines showed significantly greater attraction than was shown by the *sel-12* mutant animals ($p<0.001$, $p<0.001$). However, while the worms with the human presenilin transgene showed increased chemotaxis toward salt, both independent lines were still significantly lower than the wild-type ($p=0.04$,$p=0.02$), indicating that normal human presenilin is, at least, partially functional in the salt assay.

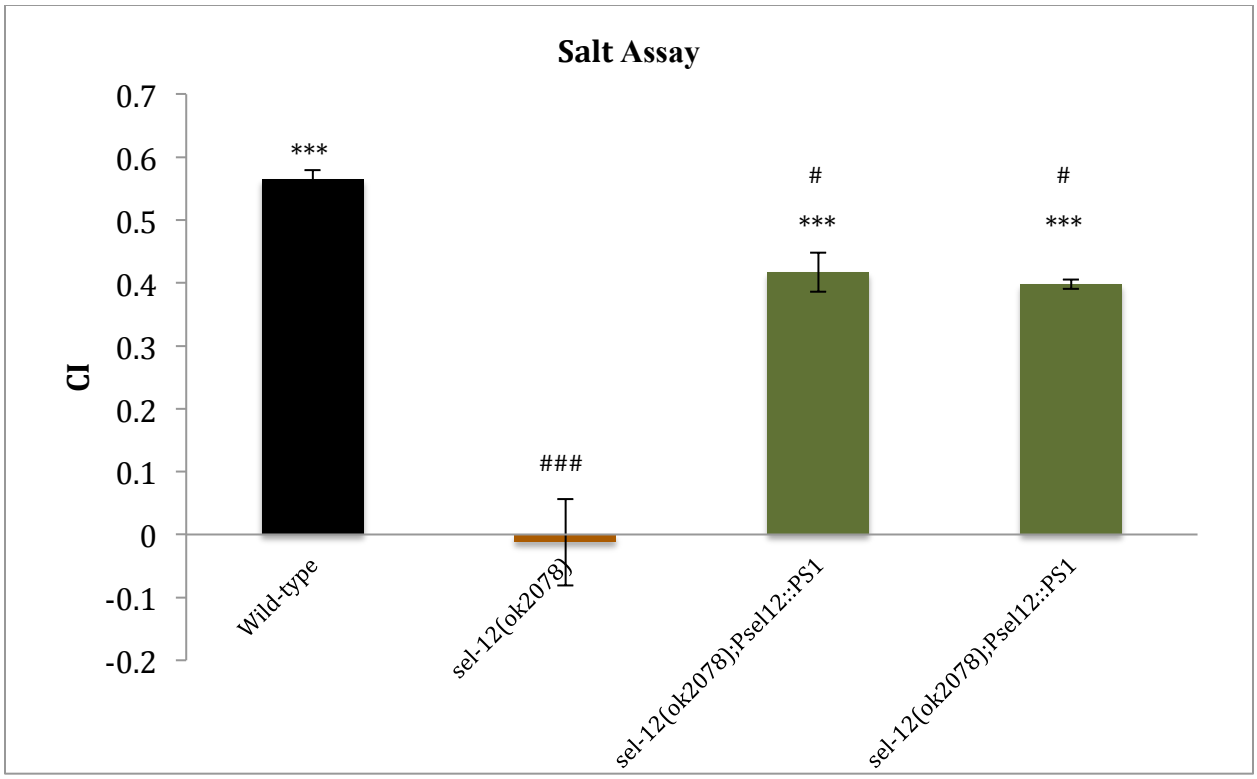

Figure 20 Salt assay for PS1 rescue: Chemotactic indices generated from salt assays performed with wild-type (N2) *sel-12* mutant and two independent *sel-12* rescue lines using sodium acetate as a test attractant. Values are averages from at least 3 independent plates (N=120-150 worms per plate). The * signs indicate significant differences from the *sel-12* mutant group and the # sign indicate significant differences from the wild-type group (*** $p<0.001$, #$p<0.5$, ### $p<0.001$)). Error bars reflect the standard error of the mean.

3.4.3.3 $PS1_{\Delta s169}$, but not $PS1_{C410Y}$, rescues the gustatory deficit in *sel-12* mutant animals

To investigate the possible role of the Notch pathway in the gustatory deficit in *sel-12* mutant worms, three independent transgenic lines carrying $PS1_{\Delta s169}$ gene in *sel-12(ok2078)* mutant background were tested for the attraction responses to the salt. Our results (Figure 21) showed that the *sel-12* mutant worms attraction responses toward salt were significantly lower than the wild-type ($p<0.001$) and the human presenilin line showed significantly greater attraction than that shown by the *sel-12* mutant animals ($p<0.001$). While the worms with the human presenilin transgene showed increased chemotaxis toward salt, this transgenic line still was significantly different from the wild-type ($p=0.002$), indicating that normal human presenilin is, at least, partially functional in salt assay. None of the three independent lines of $PS1_{\Delta s169}$ were significantly different form the normal human presenilin ($p=0.09$, $p=0.59$, $p=0.96$); however, all three lines were significantly different form the *sel-12* mutant line ($p=0.001$). One out of three lines of $PS1_{\Delta s169}$ was not significantly different from the wild-type ($p=0.30$, $p=0.03$, $p=0.001$), suggesting that the $PS1_{\Delta s169}$ mutant partially rescued the gustatory deficit in *sel-12* mutant worms.

To further investigate Notch pathway function in the gustatory deficit observed in *sel-12* hermaphrodites, three $PS1_{C410Y}$ transgenic lines were screened for their attractive response to NaAc. All three $PS1_{C410Y}$ transgenic lines (Figure 22) abolished the attractive response toward salt and none of them were significantly different from the *sel-12* mutant ($p=0.94$, $p=0.95$, $p=0.86$), while the *sel-12* mutant ($p<0.001$) and all three independent lines of $PS1_{C410Y}$ mutant ($p=0.002$, $p<0.001$, $p=0.004$) were significantly different form the wild-type. This finding provides more evidence to support a role for Notch signaling in the *sel-12* gustatory deficit.

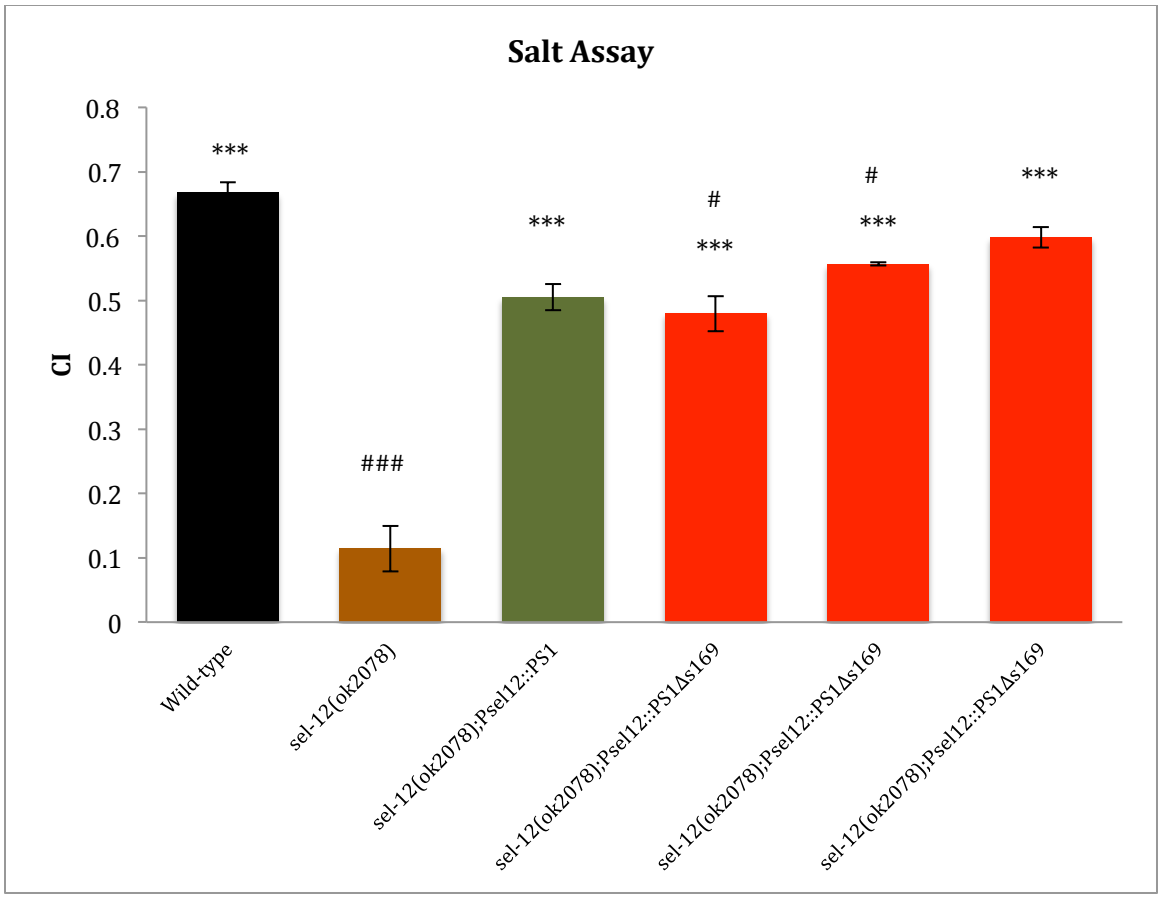

Figure 21 Salt assay for $PS1_{\Delta s169}$ mutant: Chemotactic indices generated from assays performed with the wild-type (N2), *sel-12* mutant and three independent $PS1_{\Delta s169}$ lines using sodium acetate. Values are averages from at least 3 independent plates (N=120-150 worms per plate) for each strain. The wild-type, the PS1 rescue and all three independent lines of $PS1_{\Delta s169}$ were significantly different from the *sel-12* mutant worms. The *** signs indicate significant differences from the *sel-12* mutant group and the # sign indicate significant differences from the wild-type group (*** $p<0.001$, #$p<0.5$, ### $p<0.001$)). Error bars reflect the standard error of the mean.

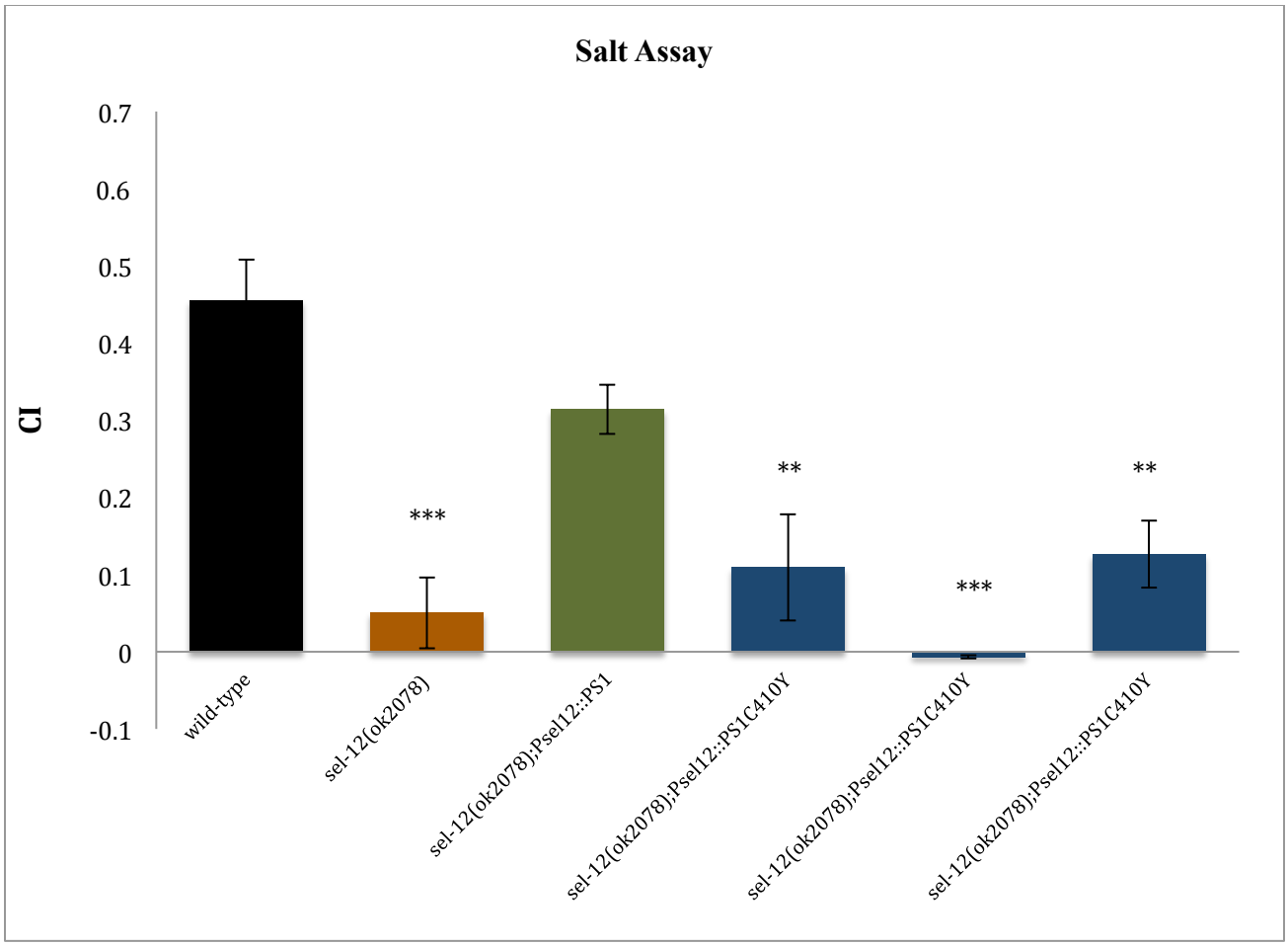

Figure 22 Salt assay for $PS1_{C410Y}$ mutant: Chemotactic indices generated from gustatory assays performed with the wild-type (N2), *sel-12* mutant, PS1 rescue and three independent $PS1_{C410Y}$ lines using NaAc. Values are averages from at least 3 independent plates (N=120-150 worms per plate) for each strain. There were no significant differences between *sel-12* mutant worms and three $PS1_{C410Y}$ transgenic lines in attractive responses toward salt. All three independent lines of $PS1_{C410Y}$ and sel-12 mutant were significantly different from the wild type. Asterisks indicate significant differences from the wild-type(** p<0.01, *** p<0.001). Error bars reflect the standard error of the mean.

3.5 Discussion

Olfactory dysfunction has been broadly considered as a potential maker for early diagnosis of AD; however, the mechanisms behind the olfactory impairment seen in AD patients remain unknown (Ruan et al., 2012). Since key elements of the biology of neurons are conserved across species, nematode models may offer systems for understanding the cellular mechanisms involved in a variety of types of neurodegenerative disorders including Alzheimer's disease(Alexander et al., 2014). Genetic mutations that cause dysfunction and disease in humans can be investigated in *C. elegans* if they show robust phenotypes related to those specific mutations.

To investigate whether the chemosensation behaviours in *C.elegans* might be associated with the *sel-12* gene, escape responses to octanol and attraction responses toward diacetyl and sodium acetate were assayed in *sel-12(ok2078)* mutant worms. Our results indicated a decrease in avoidance response to octanol, and attraction to diactyl and salt that was rescued by reintroducing the *sel-12* gene into the *sel-12* mutant animals and partially rescued by the human presenilin gene.

The normal human presenilin lines were used in this study partially rescued the odor and taste deficits detected in *sel-12* mutant worms. It is possible that these partially rescued behaviours toward smell and taste in PS1 transgenic lines were due to an overexpression of the human presenilin gene in these animals. Levitan et al (1996) showed that arrays generated at lower (10 times less) concentrations for human presenilin are still functional and able to rescue the Egl phenotype in *sel-12* mutant worms as well as the higher concentrations, suggesting that the PS1 transgenic lines generated in our lab for this study might have too many copies of the PS1 gene which might cause an over expression of the gene and decrease the normal function of the PS1 gene in these animals.

The *sel-12* gene, similar to human presenilin, is broadly expressed in neuronal and non-neuronal cells at all developmental stages from embryo to adult (Levitan et al., 1996), which encouraged Wittenburg et.al to investigate the activity of the presenilin gene in the *C.elegans* nervous system. They found that *sel-12* mutant worms showed a deficit in thermotaxis behaviour and proposed that dysfunction in the Notch signaling pathway in interneurons of thermotaxis circuit may contribute in this behaviour (Wittenburg et al., 2000). Singh et al. (2012) showed that Notch receptors are required in sensory and interneurons of the chemosensory circuit for a normal avoidance response toward octanol. From this previous research we hypothesized that a Notch pathway dysfunction in the chemosensory circuit caused by the *sel-12* mutation might contribute in the volatile odour and salt chemotaxis deficits observed in *sel-12* mutant worms.

It is intriguing that our results indicate an age-related change in the olfactory deficit in *sel-12* mutant animals. Previous research on a transgenic mice model of AD revealed that olfactory dysfunctions expand from the periphery to the inner olfactory center with age (Wu et al., 2013). In *C. elegans* it was reported that *lin-12* is probably required for octanol response in RIM, RIG, AVA interneurons; however, *glp-1*, the second homologue of Notch receptor in *C.elegans*, is broadly expressed in ciliated ASH, AWB and ADL sensory neurons and is probably responsible for octanol detection (Singh et al., 2011). These two Notch receptors function redundantly in subsets of the chemosensory circuit. Since the Wu et al. (2013) results showed that chemosensation dysfunctions start from the outer part of the olfactory system and gradually penetrates to inner olfactory centers, one hypothesis from our data is that the *sel-12* mutation affects *glp-1* receptors of the sensory neurons at earlier stage of age and gradually at the later stage of age *lin-12* receptors of the interneurons are also affected by this mutation.

Our data for the human presenilin mutation $PS1_{C410Y}$ indicate that expression of this mutation in a *sel-12* mutant background did not rescue the chemotaxis deficit to volatile and water-soluble stimuli. It was previously reported that $PS1_{C410Y}$ greatly reduced the Notch receptor cleavage in cell culture (Song et al., 1999), and in *C.elegans* expression of this mutation in the *sel-12* mutant background did not rescue the egg-laying phenotype regulated by Notch signaling pathway (Baumeister et al., 1997; Levitan et al., 1996). Our results for the $PS1_{C410Y}$ mutation in chemotaxis is consistent with those previously described by other researchers, suggesting that normal function of presenilin acting on the Notch pathway might be critical for chemosensation in *C.elegans*.

The association between Notch and olfaction has been recently identified in other organisms as well. Experiments with mice have shown that olfactory stimulation activates a subset of the mitral cells that then send signals to the piriform cortex. Activation of the subset of mitral cells is correlated with an increase in Notch activity in these cells, suggesting that the Notch pathway components expressed in specific cellular subsets of the olfactory bulb may be involved in olfactory signaling. In a transgenic mouse model in which Notch1 expression was decreased in mitral cells, the magnitude of the neuronal response to olfactory stimuli was decreased. In addition, these transgenic mice displayed reduced olfactory aversion to a non-pleasant odor as compared to the wildtype controls. These results indicated that Notch1 is involved in olfactory processing and may contribute to olfactory behaviour (Brai et al., 2014). Furthermore, Lieber et al. (2011) used an *in vivo* assay that translates Notch activity into expression of GFP to show that exposure of adult Drosophila to defined odorants increased the activity of Notch in specific olfactory receptor neurons (Lieber, Kidd &Struhl, 2012). These results are consistent with the olfactory deficit shown here for the human $PS1_{C410Y}$ mutation in which the Notch function was dramatically decreased,

suggesting that the Notch pathway might be involved in chemosensation activity in variety of species. However, our results also indicate that the normal function of presenilin acting on the Notch pathway might be critical for chemosensation in *C.elegans.*

It is intriguing that our results for the $PS1_{\Delta s169}$ mutation showed that this mutation diminished the chemotaxis deficit observed in *sel-12* mutant animals, similar to what we saw in our egg-laying experiment, suggesting that the chemosensory circuit may depend on presenilin actions on the Notch pathway for responding to chemosensory stimuli. These results provide more evidence in support of the unpublished *in vitro* data from Dr. Song who proposed that this mutation does not disturb the Notch pathway.

4.Conclusion

The overall aim of this study was to gain a better understanding of molecular mechanisms involved in AD pathogenesis this goal was accomplished as follows:

4.1 Amelioration of egg-laying deficit by pathogenic $PS1_{\Delta s169}$ mutation

Presenilin genes encode the main component of γ-secretase enzyme, which is involved in cleavages of different single pass transmembrane proteins. Among all of those proteins, APP and Notch proteins are the most well investigated substrates of γ-secretase. Mutations in the presenilin genes change APP processing, resulting in increased production of the toxic forms of amyloid-β peptide, which is a pathological hallmark of the AD (Delabio et al., 2014). The Notch pathway is well known for its involvement in cell specification during development; however, recently data from Alberi et al (2013) suggests a function for this conserved pathway in mature brain of vertebrates. This evidence increased the debate regarding the function of the Notch pathway in PS1

mutation-linked AD pathogenesis. Levitan et al. (1996) broadly described the association between *sel-12* mutation and the Notch pathway and its effect on vulval development and egg-laying in *C.elegans*. A recently discovered human presenilin mutation in a Chinese family was investigated (Zhang, 2013). Zhang's (2013) results suggested that the pathological symptoms of AD in these patients depend on APP processing and not Notch function. One of the limitations of studying Notch processing associated with PS1 mutations in transgenic mouse models is that the PS2 Knock-out mice didn't induce the typical Notch deficient phenotype. However, in *C. elegans* the egg-laying phenotype in *sel-12* mutant worms is a well-described indicator of Notch dysfunction. By using *C. elegans* we were able to investigate the role of $PS1_{\Delta s169}$ on Notch pathway function. Our results support the *in vitro* data observed by Zhang (2013) by showing that $PS1_{\Delta s169}$ ameliorates the egg-laying deficit associated with Notch signalling in *sel-12* mutant worms, suggesting that the Notch pathway is not affected by this mutation. However, decline of the importance of Notch activity in AD symptoms associated with this mutation cannot direct all attention toward APP dysfunction. Largely because a major side effect of γ-secretase inhibitors is Notch impairment, the Notch pathway is the second most studied pathway in the field of AD research; however, more than 50 other proteins are also known substrates of γ-secretase and any of them could be involved in the AD pathogenesis associated with $PS1_{\Delta s169}$ mutation.

Another limitation of this study is that the transgenic lines generated for this research have extrachromosomal arrays with different numbers of plasmid copies placed in the array, which may cause overexpression of the gene in our lines. The effect of six human presenilin mutations on egg-laying behaviour of *sel-12* mutant worms were investigated by Greenwald lab (1996) and results of their experiment indicated that one out of six tested PS1 mutated genes rescued the egg-laying phenotype. They hypothesized that overexpression of the gene might mask the small difference

between the function of normal human PS1 and the mutant one. The lower concentration of the injected DNA used in their lab rescued the egg-laying phenotype for normal human presenilin lines; however, the PS1 mutation, failed to rescue the Egl behaviour, suggesting that in their former experiment elimination of the egg-laying phenotype in PS1mutant transgenic lines might be linked to the overexpression of the inserted gene. Because we cannot control the copy number for each plasmid in each line, this likely cause over expression of inserted gene in our transgenic lines as well.

This part of my thesis provides some evidence to show that the $PS1_{\Delta s169}$ mutation changes the function of γ-secretase in a way that does not affect the Notch pathway. First of all, this result is important, as it is provides support for the hypothesis that the function of Notch pathway is not critical in the pathogenesis of AD. Secondly, as this mutation does not affect Notch, further investigation of its function on γ-secretase may shed light on discovery of a diagnosis or treatment for this devastating disorder.

4.2 **Investigation of olfactory dysfunction in *sel-12* mutant worms**

Recently, many researchers have focused on identifying a reliable biomarker to use as an early indicator for diagnosis of AD, prior the development of neuropathology and cognitive dysfunction. Olfactory loss has been established at early stages of AD and has been suggested as a potential biomarker to diagnose this disorder. Recent research has worked to elucidate the possible mechanisms of olfactory dysfunction in AD and attempted to link the prevalence of AD pathologies with olfactory function. However, several further developments in understanding of pathological changes in cellular and circuit level information processing are necessary in order to describe how the disease affects the olfactory function. *C. elegans* is a popular model organism to investigate cellular mechanism associated with neurodegenerative diseases. In this study we used *C. elegans* to

show that the SEL-12 protein is required for normal olfactory responses to odor and water-soluble stimuli. The olfactory deficit in *sel-12* mutant worms can be rescued by human presenilin, suggesting that normal human presenilin is functionally homologous to *sel-12* in this assay, which will allow us to use this assay to further investigation of cellular mechanisms affected by presenilin mutations.

Our results for the newly discovered human presenilin mutation, $PS1_{\Delta s169,}$ showed that this mutation can rescue the olfactory deficit; however, the $PS1_{C410Y}$ mutation, known for its severe impact on the Notch pathway, did not rescue the olfactory impairment. This suggests that the Notch pathway might be involved in olfactory system associated with AD.

Several FAD-associated PS1 mutations are known to lead to impaired Notch processing; however, it is controversial whether Notch signaling contributes to AD pathogenesis, and if so, how severely. Results from Zhang (2013) demonstrated that the pathological hallmarks of AD occur in patients carrying $PS1_{\Delta s169}$, while Notch is processed normally, suggesting that aberrations in Notch signaling are not essential in AD pathogenesis. However, our preliminary results suggest the possibility of involvement of Notch processing in olfactory deficits in AD; although, we cannot yet explain how Notch signaling contributes to the FAD-associated PS1 mutations.

My experiments showed that decreased chemotaxis is associated with mutations in the *C.elegans* homologue of presenilin and also suggested that the Notch pathway might also be involved in this deficit. First of all, this result is important as it supports the possibility of a role for the Notch pathway in the pathogenesis of AD, which is still highly controversial. Secondly, this chemosensation phenotype associated with PS1mutation in *C. elegans* will help us to address questions related to molecular mechanisms involved in the olfactory deficits observed in AD patients.

4.3 **Future directions**

Results from our first experiment showed that $PS1_{\Delta s169}$ ameliorates the egg-laying deficit associated with Notch signalling in *sel-12* mutant worms, suggesting that Notch pathway isn't affected by this mutation. However as it was already mentioned (see 4.1) this amelioration of the egg-laying deficit may be caused by high numbers of plasmid copies in the arrays, so it is necessary to determine the lowest concentration of the normal human presenilin gene that can rescue the Egl phenotype in *sel-12* mutant worms. This minimum concentration then can be used to make transgenic lines for $PS1_{\Delta s169}$ mutation gene. If the egg-laying phenotype is rescued by expression of the lower concentration of $PS1_{\Delta s169}$ gene, this will provide more evidence to support the notion that the Notch pathway isn't affected by $PS1_{\Delta s169}$ mutation.

Our second experiments showed that the SEL-12 protein is necessary for the normal responses to odor and water-soluble stimuli and normal human PS1is functional in chemotaxis assays. To further investigate the cellular mechanisms involved in the sel-12 related olfactory and gustatory deficit in C .elegans, it will be essential to express the human presenilin gene in the whole nervous system of the *sel-12* mutant worms to investigate whether the PS1 gene is functional in the nervous system of the worms to rescue the chemotaxis deficit associated with the *sel-12* mutant gene. To determine which part(s) of the chemosensory circuit is (are) affected by this mutation, it will be critical to express normal human PS1 gene specifically in chemosensory neurons (ASH, ASE and AWA; Bargmann, 2006) to investigate whether or not the *sel-12* gene functions in the chemosensory neurons associated with chemosensation in *C. elegans*. To determine the involvement of the *sel-12* gene in interneurons of the chemosensory circuit, we will express PS1 in different interneurons of the chemosensory circuit such as AVA, RIM, AIY and AIZ ((Hart &

Chao., 2010); (Satoh et al., 2014) to investigate the locus of defect, and whether the locus changes as worms age.

4.4 Summary

Taken together the experiments reported here offer two original findings. The first is that a novel mutation in presenilin that produces FAD leaves the Notch signaling pathway intact. This opens the door for further investigation into the actual role that Notch signaling plays in the pathogenesis of AD. The second is that mutations in the *C. elegans* homologue of presenilin produces a chemotaxis deficit that becomes more severe as worms age paralleling what is seen in human AD patients. These data suggest that *C. elegans* is a good model system in which to investigate the cellular mechanisms of presenilin associated chemosensory deficits with the hope of offering insights that will be useful in understanding the cellular mechanisms of AD.

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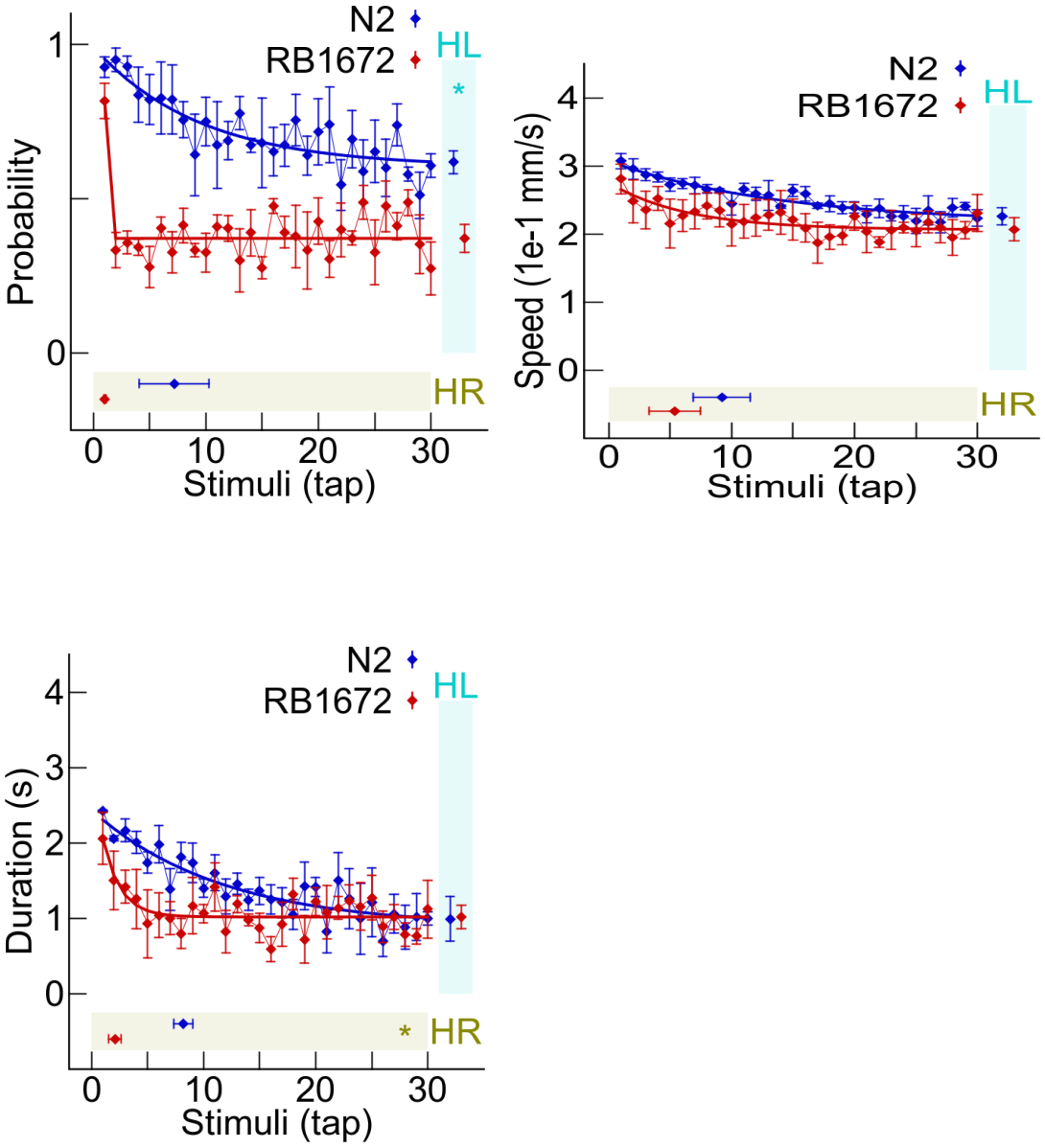
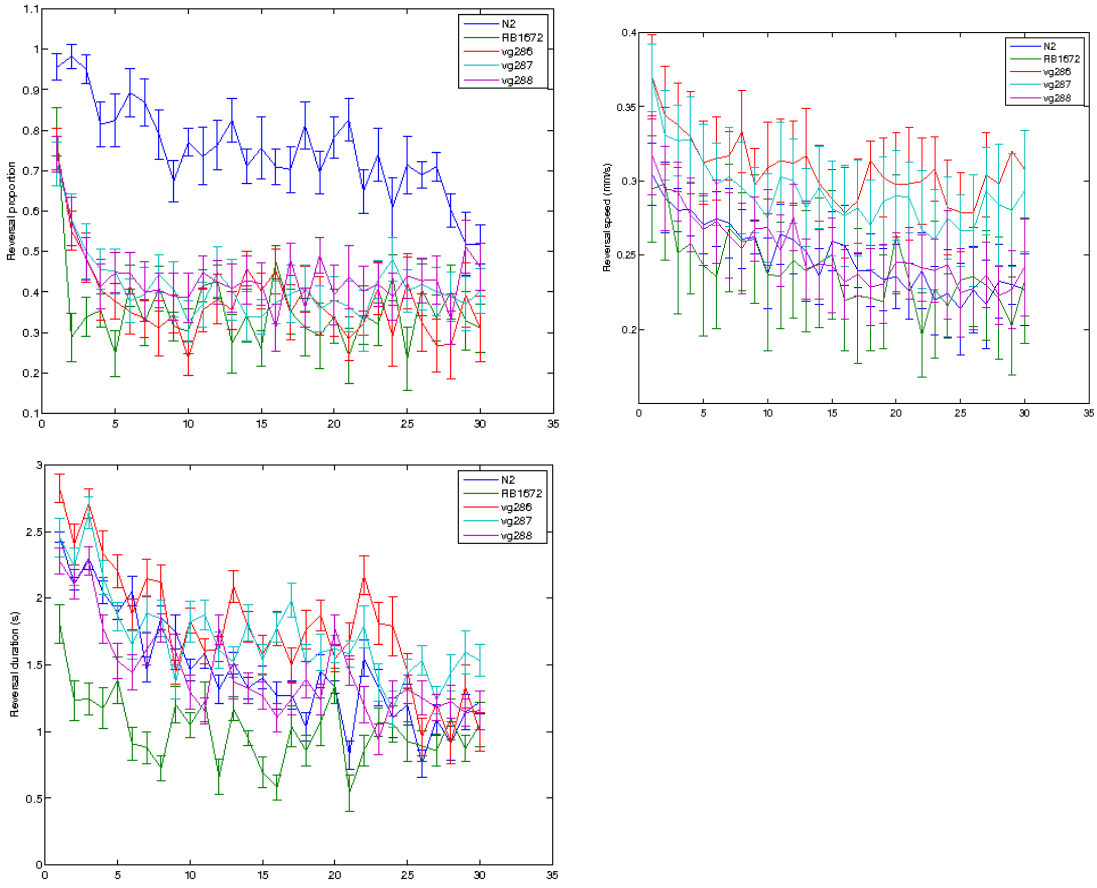
Appendices

Appendix A: Tap habituation for sel-12 mutant animals



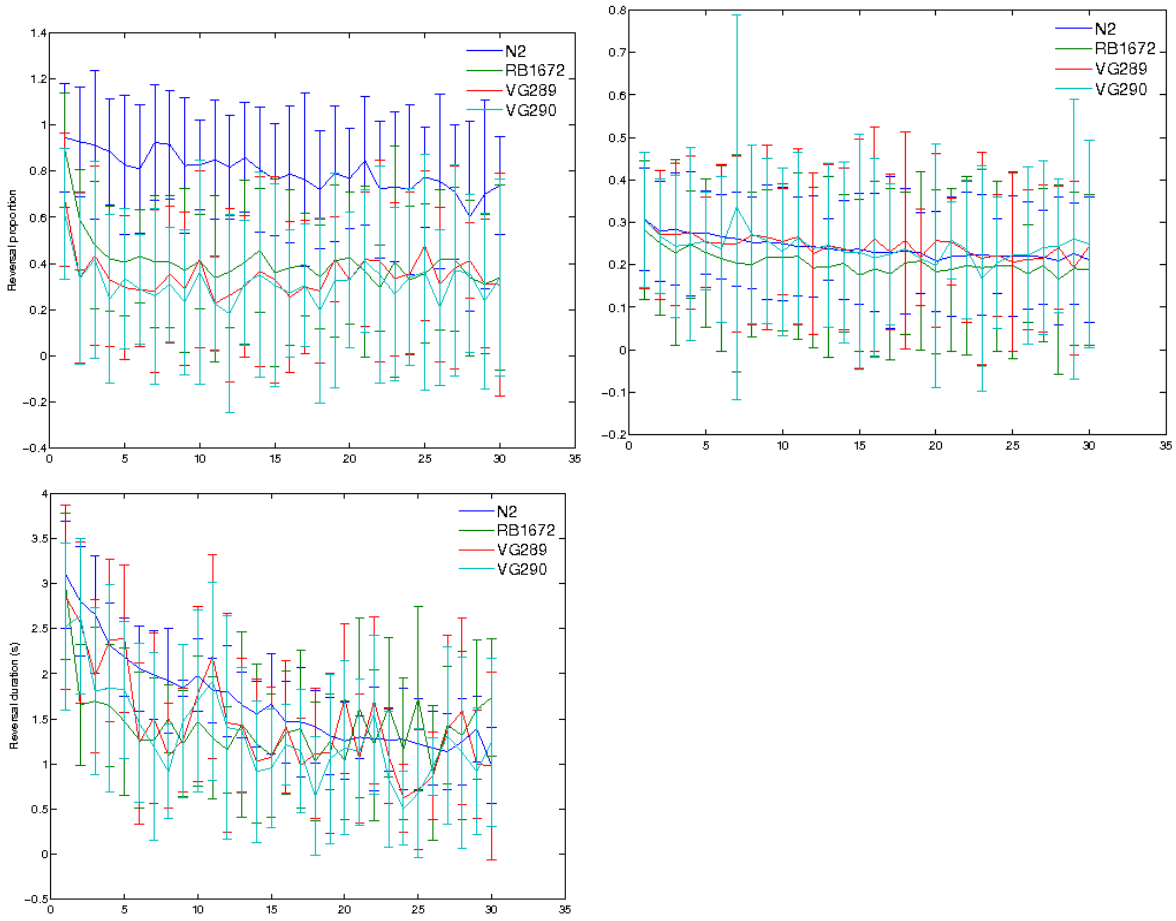
10 ISI tap habituation for *sel-12* mutation strain: worms were at three days of age and three plates were used for each strain (~50 worms/plate). Pre-plate time was 1 minute.

Appendix B: Tap habituation for sel-12 rescue strains



10 ISI tap habituation for *sel-12* rescue lines: worms were at three days of age and three plates were used for each strain (~50 worms/plate). Pre-plate time was 1 minute.

Appendix C: Tap habituation for PS1 rescue strains



10 ISI tap habituation for PS1 rescue lines: worms were at three days of age and three plates were used for each strain (~50 worms/plate). Pre-plate time was 1 minute.