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The lynx1 gene and Alzheimer's Disease: A Potential Neuroprotective Mechanism

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Eckardt Thesis

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Abstract

The sociological impact of neurodegenerative diseases, such as Alzheimer's Disease (AD), places a heavy burden on the healthcare industry, patients and families, not only from a medical and financial impact, but also in terms of quality of life. The consequences to the patient, family and caregivers can be life-changing, yet quantitative studies have been sparse. Our understanding of ways to improve cognition in these patients would be highly beneficial. One such potential tool to improve cognition in these patients, the *lynx1* gene, has been identified. The purpose of this project is to compare the effects of the *lynx1* gene on Alzheimer's development in different genetic variant mice (wildtype, knockout, and heterozygous genotypes).To do this, mice underwent fear conditioned learning via the Chronic Social Defeat Stress (CSDS) paradigm. Based on the results from these behavioral tests, as well as predicted data for planned experiments, I believe that an introductory case for further studies of the relationship between the *lynx1* gene and AD has been shown.

Introduction

AD is a neurodegenerative disorder, with a distinct pathology, characterized by plaques and neurofibrillary tangles containing aggregated β-amyloid (Aβ) and hyperphosphorylated tau protein (Thomsen 2016, Alud 2002). It has been shown that soluble Aβ species are considered to be the most toxic form of Aβ and are associated with cognitive deficits in AD (Thomsen 2016), including progressive neuronal loss, inflammation, and the gradual and inevitable decline of memory and cognition[8]. AD is the most common cause of dementia and is currently estimated to affect more than 5 million people in the United States, with an expected increase to 13 million by the year 2050. The costs of care of patients with Alzheimer's disease in 2010 were estimated at more than $172 billion in the United States, an annual cost that is predicted to increase to a trillion dollars by 2050[18,19].

To date the most effective treatment for AD symptoms are Cholinesterase Inhibitors (CIs). Studies have shown that cholinergic systems in the basal forebrain are affected early in the disease process, resulting in a loss of acetylcholine neurons and loss of enzymatic function for acetylcholine synthesis and degradation. This in turn causes memory loss and deterioration of other cognitive and noncognitive functions such as neuropsychiatric symptoms[9]. Despite the initial benefit they provide, the efficacy of CIs dissipate after 6-12 months[4]. It is proposed that the *lynx1* gene could be a potential tool for improved cognition and pathology improvement in AD patients.

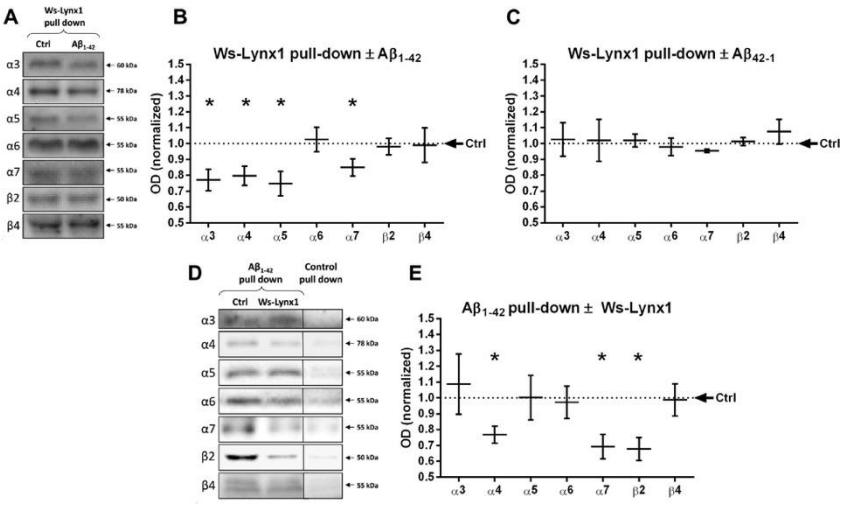
Lynx1 is a protein that is part of the Ly-6/neurotoxin family and is a glycophosphatidylinositol-anchored membrane protein. *Lynx1* has been shown to have several functions in the brain, particularly in learning and memory via inhibitory modulation on nAChR function, with high

affinity for $\alpha 7$ and $\alpha_4\beta_2$ subunit binding[7]. It has been shown that co-expression of *lynx1* results in reduced agonist sensitivity and slower recovery from nACh desensitization and that *lynx1* exhibits inhibitory function during the critical period on nAChRs in different areas of the brain[5,7]. The *lynx1* gene is highly concentrated in the Pre-Frontal Cortex (PFC) and the CA1 neurons of the hippocampus, which are the brain areas critically impacted during AD pathology[5]. In addition, studies have also shown that the *lynx1* gene competes with $A\beta_{1\text{-}42}$ plaques in binding with nicotine receptors, with both showing preferential binding to the $\alpha 7$ and $\alpha_4\beta_2$ subunits of nAChRs[2,8]. Notably, it has been shown that the knockout of the *lynx1* gene can lead to neurotoxicity and proliferation of the toxic effects of $A\beta_{1\text{-}42}$ plaques in the brain. The key figures from these studies are discussed below in greater detail.

Precedential Data

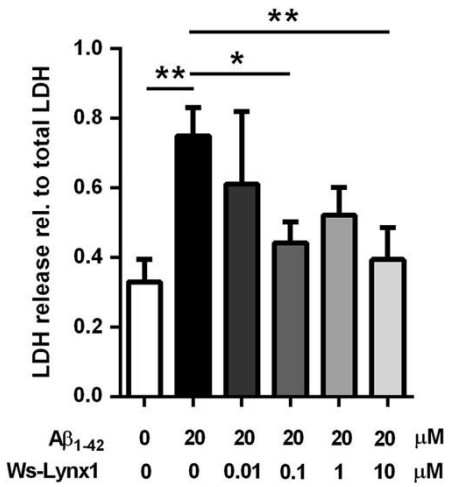
Figure 1: Ws-*lynx1* actively competes with oligomeric $A\beta_{1\text{-}42}$ plaques to bind/modulate nAChRs

Previous studies have shown that both *lynx1* and $A\beta_{1\text{-}42}$ plaques have a strong binding affinity to



the α7 subunit of the nACh receptors[2,7,11], however, it had not been determined how or if *lynx1* interacts with $A\beta_{1\text{-}42}$ plaques in regard to binding to the α7 subunit of nAChRs. Studies conducted by Thomsen et al.[20] determined that preincubation of rat cortex tissue with 200 nM of oligomeric $A\beta_{1\text{-}42}$ plaques significantly decreased the amount of nAChR subunits isolated by affinity purification with Ws-lynx1 (Figures 2A and 2B). Furthermore, preincubation of rat cortex tissues with 10 nM of Ws-*lynx1* reduced the amount of α7, α4 and β2 subunits attached to oligomeric $A\beta_{1\text{-}42}$ plaques post-affinity purification assay (Figure 2D and 2E). These data clearly demonstrate that *lynx1* competes with oligomeric $A\beta_{1\text{-}42}$ plaques to bind to nAChRs.

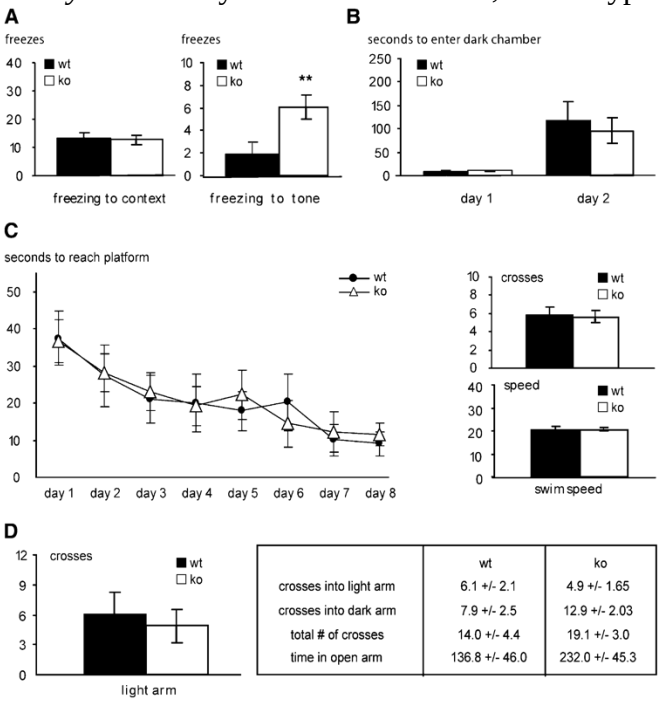
Figure 2: Ws-*lynx1* prevents $A\beta_{1\text{-}42}$ induced cytotoxicity



Another key hypothesis explored[20] was that Ws-*lynx1* prevented $A\beta_{1\text{-}42}$ induced cytotoxicity on nAChRs *in vitro*. Mouse cortical neurons were incubated with Ws-*lynx1* (0.01e10 mM) for 2 hours followed by addition of 20 mM oligomeric $A\beta_{1\text{-}42}$ for an additional 24 hours. The release of lactate dehydrogenase (LDH) to the media was used as a measure of cytotoxicity, and the overall experiment resulted in significantly decreased levels of LDH release by mouse cortical neurons where Ws-*lynx1* was expressed compared to when only oligomeric $A\beta_{1\text{-}42}$ plaques were expressed (Figure 3).

Figure 3: Enhancement of Associative Learning Ability in lynx1 Null Mutant Mice Observed in Fear-Conditioning Assays

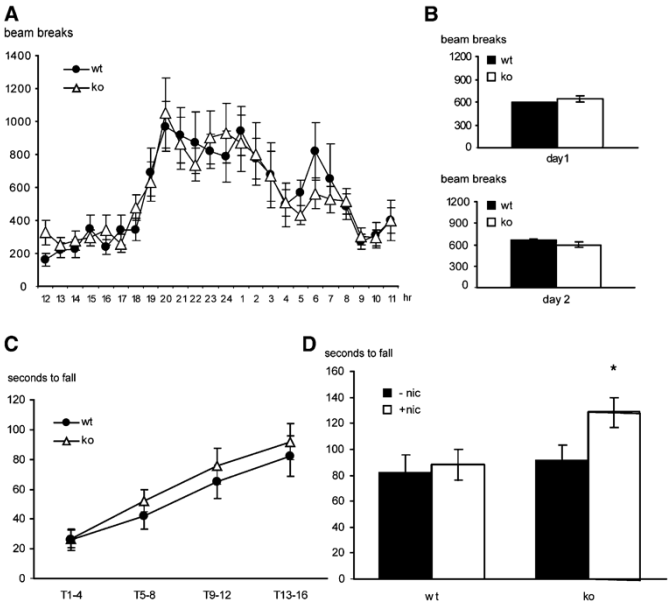
It has been determined in previous studies that nAChR activation has been shown to be an important component of specific aspects of learning and memory[3,6]. Therefore, due to the fact that *lynx1* directly modulates nAChRs, it was hypothesized that manipulation of *lynx1* would have significant effects on learning and memory. To test this, behavioral tests conducted by Miwa et al[7]. were run on *lynx1KO* mice to evaluate their learning abilities compared to *lynx1* wildtype variant mice. It was determined that both *lynx1KO* mice and *lynx1* wildtype variant mice did not behave differently in regard to novel contextual response and tone (Figure 4A), nor did *lynx1KO* mice show significant differences to *lynx1* wildtype variant mice in regard to latency to enter a light/dark box (Figure 4B) or in training and location time for the hidden platform in the Morris water maze (Figure 4C). Therefore, it was demonstrated that *lynx1* doesn't display a significant change in contextual learning between *lynx1KO* and wildtype mice.



	wt	ko
crosses into light arm	6.1 +/- 2.1	4.9 +/- 1.65
crosses into dark arm	7.9 +/- 2.5	12.9 +/- 2.03
total # of crosses	14.0 +/- 4.4	19.1 +/- 3.0
time in open arm	136.8 +/- 46.0	232.0 +/- 45.3

Figure 4: Enhancements in Nicotine-Mediated Motor Learning Performance is expressed in lynx1 Null Mutant Mice

Comparatively, when nicotine was introduced to *lynx1KO* and wildtype mice in relation to motor coordination and learning, *lynx1KO* mice showed significantly improved motor training compared to *lynx1* wildtype mice that received a placebo or nicotine and placebo (Figure 5D). This data is consistent with the hypothesis that that elimination of *lynx1* alters nAChRs toward heightened receptor sensitivity[6].



Based on these findings, the purpose of this project was to determine if manipulation of the *lynx1* gene reduces $A\beta_{1\text{-}42}$ plaque and tau protein tangle levels and improves cognition in genetically variant mice.

Hypothesis

The hypothesis for this project was that *lynx1* knockout variant mice would have increased learning but altered pathology development via heightened levels of present $A\beta_{1\text{-}42}$ plaque and tau protein tangles, *lynx1* wildtype variant mice would have decreased learning abilities but greater protection against $A\beta_{1\text{-}42}$ plaque and tau protein pathology development, and *lynx1* heterozygous variant mice would have greater learning abilities and protection against levels of $A\beta_{1\text{-}42}$ plaque and tau protein tangle pathology development.

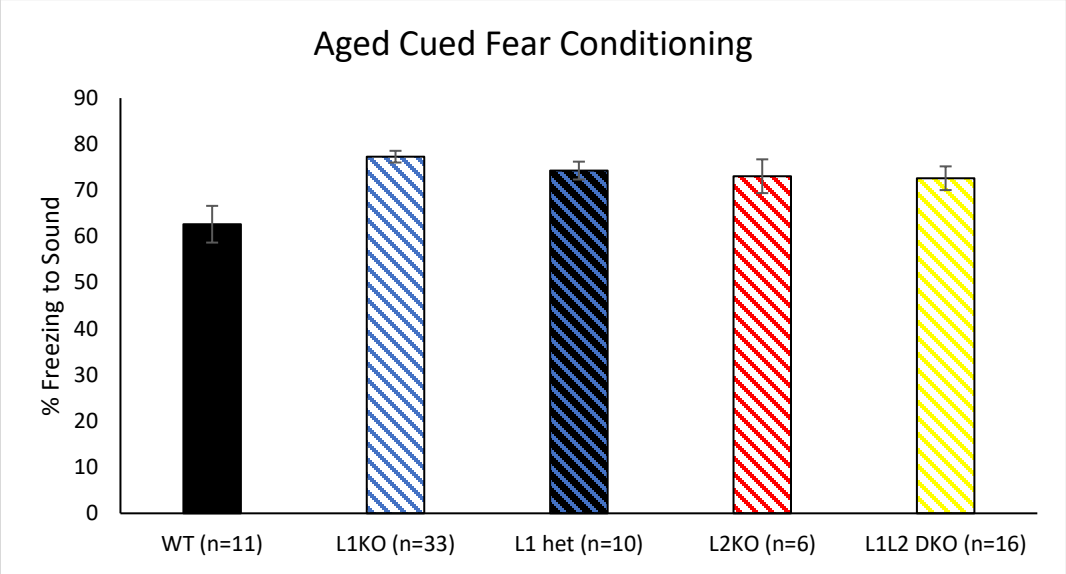
Material and Methods

Behavioral Testing Lynx1 variant mice underwent fear cue conditioning testing to examine variation in fear learning between genotypes. For the experimental setup, mice underwent an acclimation to a novel environment followed by two tone-shock pairings. The following day mice underwent a cued test which consisting of a two-minute time period without any sound followed by a two-minute sound (same sound as day 1). The context was changed between each day. Data collected was the percent freezing to sound. Any mouse that froze over 90% or below 30% was not included.

Histological Staining Histological analyses was to be carried out on a total of 25 mice, 23 test mice and two control mice (aged 6 months, both lynx1 knockout variant mice). Each genotype group was broken down into two age groups: one group 12-14 months of age at the time of histological analysis and another group 16-18 months of age. This was to compare the levels of AD pathology in the brain and to serve as a control for one another. Initially two stains were planned to be utilized, the Campbell-Switzer Silver stain to image the $A\beta_{1\text{-}42}$ plaques present in the brain, and an AT8 monoclonal antibody (mouse) to image levels of tau protein tangles. However, due to time and cost only the AT8 monoclonal antibody (mouse) was planned to be utilized. The protocol for AT8 immunochemistry staining protocol was planned to be based off of the one used by Liu et. al., 2012[15]. Both stains would have focused on the PFC and hippocampus, as these are the two brain areas immediately affected by the development of AD pathology and express the highest concentration of *lynx1* in the brain.

Data and Interpretation of Results

Lynx1KO Mice showed increased Freezing Percentage to sound compared to lynx1 wildtype and heterozygous mice



Percent freezing to sound. Lynx1KO mice displayed increased freezing to sound during the CSDS paradigm. This effect is diminished in lynx1 wildtype variant mice. L2KO and L1L2 DKO (double knock out) were used as controls. Data was collected from a previous study, none of the mice used for histology underwent this behavioral testing paradigm.

As predicted, the *lynx1KO* mice displayed increased percent freezing to sound compared to lynx1 wildtype and heterozygous mice, suggesting that the *lynx1KO* mice experienced altered cognition due to deletion of the *lynx1* gene. A possible explanation for this is that the increase in acetylcholine accessed by the nAChRs as a result of the loss of *lynx1* inhibitory modulation resulted in increased neural plasticity[6]. Interestingly, the *lynx1* heterozygous variant mice also experienced heighted levels of percent freezing to sound, implying that these mice also experienced some level of altered neural cognition. A possible explanation for this is that in these *lynx1* heterozygous variant mice acetylcholine was able to leak into the nAChRs due to the *lynx1* gene being defective as a result of misappropriate binding to the nAChRs resulting in increased neural plasticity. Further experiments will need to be done to assess these predictions.

Predicted Data and Future Experiments

Predicted Histological Data

Unfortunately due to unforeseen circumstances, the histological portion of the project could not be completed. However, based on previous studies conducted as well as the behavioral data collected for this project, it could be postulated that the following result histological results would occur: *lynx1*KO variant mice will show more expansive AD pathology development; *Lynx1* wildtype variant mice will show reduced AD pathology development. *Lynx1KO* variant mice will likely show increased AD pathology because the lynx1gene is not present to compete with $A\beta_{1\text{-}42}$ plaque to bind to nAChRs. Examples of this can been seen in Figures 1 and 2 in the Precedential Experimental Data Section. Heterozygous *lynx1* variant mice will likely show lower levels of AD pathology development similar to that of *lynx1* wildtype mice, likely because while defective, the *lynx1* gene is still present in heterozygous mice and can still express some form of binding competition with $A\beta_{1\text{-}42}$ plaques.

Future Experiments and Further Directions of Study

One potential area of future research that could be explored in relation to this project is better elucidating the binding affinity relationship between the lynx1 gene and the α7 subunit of nAChRs. Structurally, the *lynx1* gene is a protoxin variant with similar three finger fold binding mechanisms to that of α-bungarotoxin[5,8]. However unlike α-bungarotoxin, *lynx1* does not bind irreversibly to nAChRs. It has been determined that the *lynx1* gene has three functional conformational states: open, closed, and desensitized. Previous studies have determined the structure of ws-*lynx1* and have postulated that the C-loop is a key structure in relation to correct binding affinity between *lynx1* and nAChRs[17]. Specifically, the movement of the C-loop is critical for correct agonist binding from the closed to the open state. Previous studies have shown that the interactions between the C loop and that low stoichiometry binding at the α4: α4 interface on the $\alpha_4\beta_2$ subunit of nAChRs are of significant importance. Based on these studies it has been determined that the following residues on the C-loop are of particular interest for study: Arg38, Trp156 and Tyr204[16]. In addition, as previously noted, both the *lynx1* gene and $A\beta_{1\text{-}42}$ plaques demonstrate preferential binding to the α7 and $\alpha_4\beta_2$ subunits of nAChRs with similar binding affinities[2,8] . Based on these precedents, could be hypothesized that there are specific residues between the *lynx1* gene and the α7 subunit that will be critical for correct binding affinity, and lack of these residues would cause misappropriate binding. If the case, this would provide a potential mechanism for the increased cognition and protection against AD pathology predicted to be demonstrated in heterozygous *lynx1* variant mice[12,13,14]

In addition, several more iterations of the behavioral assessment/histological study done for this project should be conducted to demonstrate a more concrete relationship between the lynx1 gene and its cognitive and biological effects on AD pathology. A more robust design that could be

utilized in the future is to run behavioral assessments on the same mice that will be utilized in the histological study.

Conclusion

Overall, this project illuminated a potential relationship between the *lynx1* gene and AD pathology progression. While introductory in scope, this project has the potential to be a valuable first step for future AD research via better understanding of upstream mechanisms involved in the development of AD pathology (via exploration of the *lynx1* gene and its function within the cholinergic system) and illuminate a possible avenue for improved treatment via providing potential targets for genetic or pharmacological study in regard to AD treatment.

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