

The Role of Liver Soluble Epoxide Hydrolase in Modulating the Impact of Chronic Stress on Depression and Alzheimer's Disease Pathogenesis in Mice

Yueshan Chen

Guangzhou New Channel-Zengcheng Experimental Middle School, Guangzhou,
510000, China

3568760585@qq.com

Abstract. The link between depression and Alzheimer's disease (AD) is increasingly recognized, yet the underlying molecular mechanisms remain poorly understood. Chronic stress is known to contribute to both depression and AD. The *Ephx2* gene encodes soluble epoxide hydrolase (sEH), an enzyme that regulates 14,15-EET, a signaling molecule that may influence both AD pathogenesis and depressive symptoms. This research makes use of a mouse model to explore the influence of 14,15-EET on signaling pathways that are linked with depression and Alzheimer's disease (AD), with attention given to its involvement in amyloid-beta ($A\beta$) buildup and tau protein hyperphosphorylation. The study is centered on analyzing the liver sEH's part in altering how chronic stress impacts depressive behavior patterns and AD-related pathology. In the experiment, adult C57BL/6J mice, which included wild-type, sEH knockout ($sEH^{-/-}$), and those treated with an sEH inhibitor, were subjected to chronic stress and AD induction to evaluate these influences.

Keywords: *Ephx2* gene, sEH, 14,15-EET, AD, depression.

1. Introduction

Major Depressive Disorder (MDD) is seen as a leading disability cause worldwide, affecting about 322 million people in the world[1]. It happens because of a mix of personal risk factors and protective ones, although only a few progress to severe depression, even though they show some resilience to trauma and stress[2]. Alzheimer's Disease (AD), which is the most common dementia form[3], is identified by the build-up of amyloid-beta ($A\beta$) plaques[4], tangles of neurofibrillary phosphorylated tau[5], and ongoing neurodegeneration, which results in cognitive decline and dementia over time[6].

Research has recently found there to be a notable connection existing between depression and AD[7], showing the importance of the liver-brain axis[8]. The gene known as *Ephx2* is responsible for the production of sEH, an enzyme that is involved in managing the quantities of 14,15-EET within the liver[9]. This molecule, being a lipid that can cross the blood-brain barrier, is said to influence cognitive functions[10][11][12]. There is an association between higher levels of 14,15-EET and effects resembling those seen in antidepressant treatments, implying a possible protective action against depression[13]. In the situation concerning AD, the elevated levels of 14,15-EET might have an impact on the ApoE-TREM2 axis, which is associated with the activation of microglia and the improved

removal of amyloid-beta[14]. This could, in turn, lessen the symptoms connected to AD and possibly lead to better cognitive abilities[15].

Although 14,15-EET has known roles like it helps in brain protection by reducing inflammation and affecting neuron communication pathways[14], exact roles in Alzheimer's and depression development, such understanding is unclear. This study's purpose is examining liver sEH impact under long-term stress, focus on depressive behaviors and Alzheimer's condition changes. Using mouse models: normal mice, sEH-deficient mice (sEH^{-/-}), and sEH inhibitor-treated mice, we examine long-term stress effects and Alzheimer's induction. The study especially looks at 14,15-EET effects on pathways linked to both depression and Alzheimer's, emphasizing A β accumulation and tau protein over-phosphorylation. By this research, more understanding of sEH role in these processes and implications for potential treatments is aimed.

2. Previous study

Hepatic sEH activity, as age increases, becomes higher and is a key factor in the pathogenesis of AD, as mentioned in a study by Wu et al. (2023). In models of AD using mice, tauopathy, the A β accumulation in the brain, and cognitive problems are all influenced by changing the levels of hepatic sEH in both directions[14]. Specifically, the modulation of hepatic sEH affects the amounts of 14,15-EET in the blood, which crosses over the blood-brain barrier and changes A β metabolism in the brain through several pathways. The balance between the levels of 14,15-EET and A β needs to be precise to prevent A β build-up. In animals with AD, infusing 14,15-EET mimics the neuroprotective actions seen when hepatic sEH is removed, both in terms of behavior and biological effects. These results highlight the critical role played by the liver in AD pathogenesis and imply that focusing on the liver-brain axis in response to environmental cues may present a therapeutic strategy that has promise for the prevention of AD.

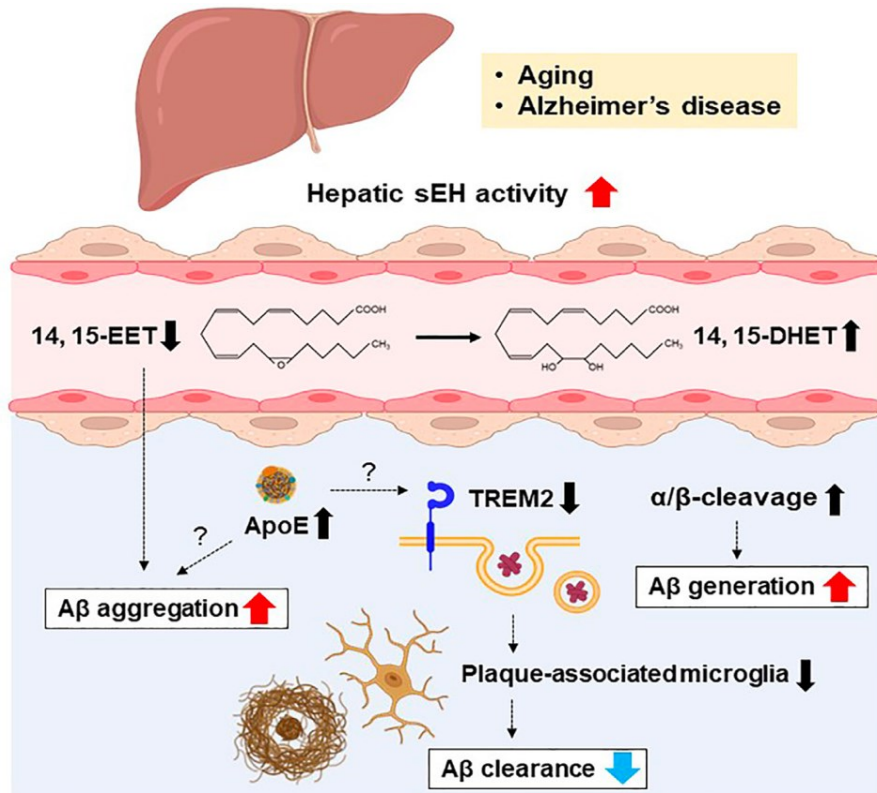


Figure 1. Mechanisms underlying hepatic sEH-related A β pathology

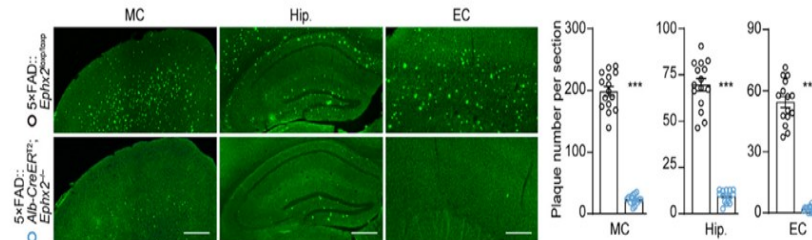


Figure 2. Representative images of Thioflavin S staining (left) and quantification (right) of plaque numbers in the MC, Hip., and EC (n = 8–9 males and 6–7 females/group) that with Hepatic Ephx2 deletion.

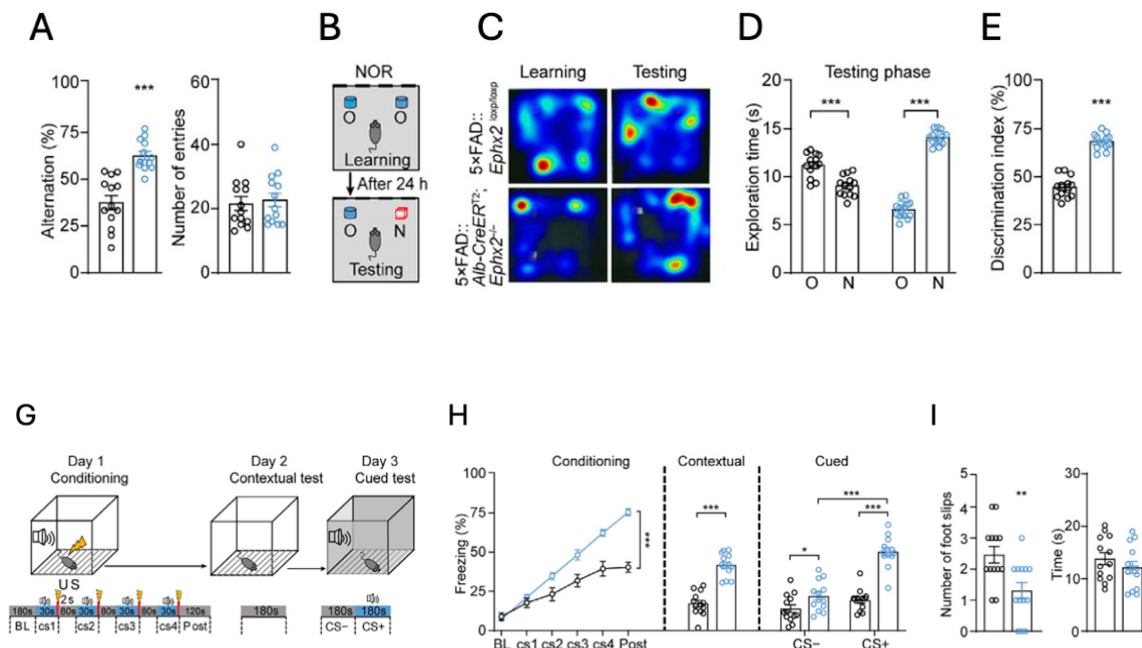


Figure 3. Y-maze, NOR, FC, and BB behavioral analyses. (A) Male 53FAD::Alb-CreERT2;Ephx2 / mice's behavioral analysis in the Y-maze test. (B–E) NOR test behavioral analysis. (G and H) Analysis of behavior in the FC exam. (G) freezing behaviors during contextual test, cued sessions, and trained conditioning (H). (I) Behavioral analysis using thirteen male mice per group in the BB.

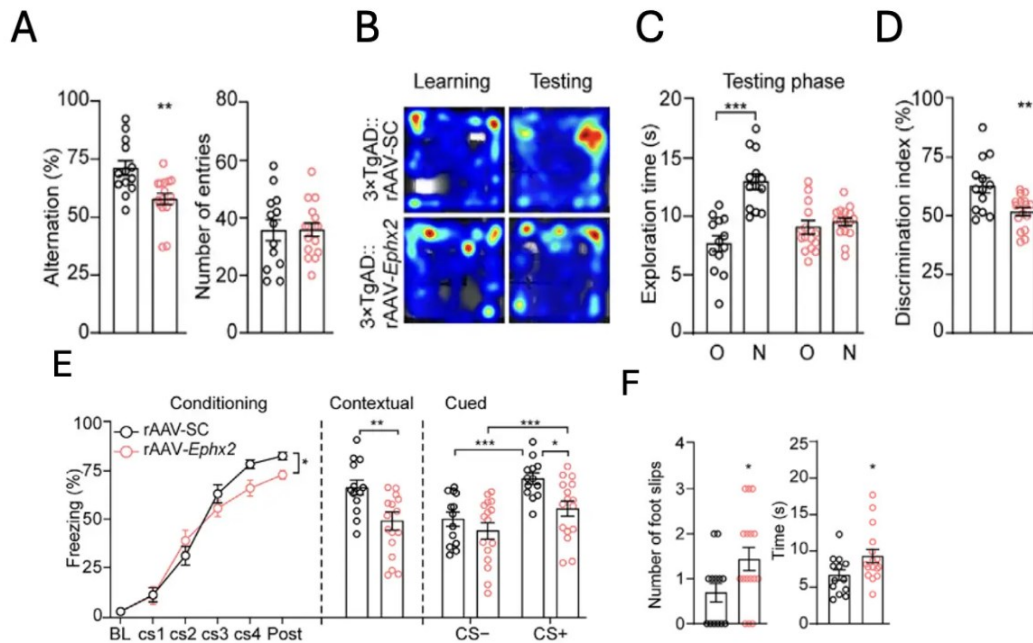


Figure 4. Effects of hepatic sEH overexpression on behavioral changes, Y-maze (A), NOR test (B-D), FC test(E), and BB test (F).

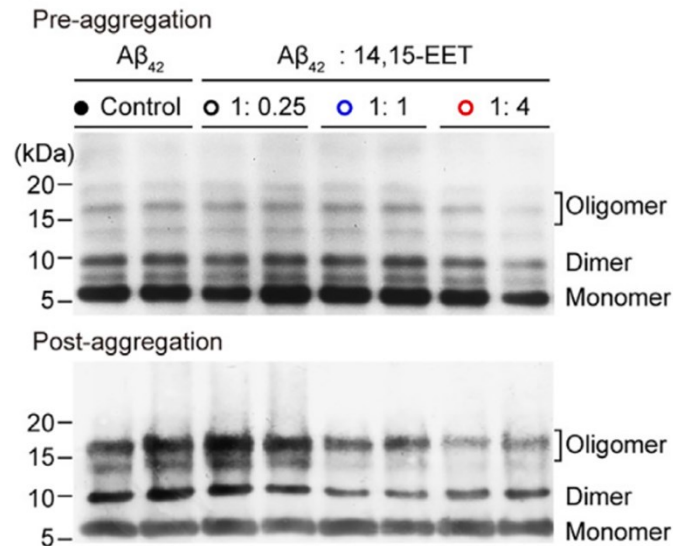


Figure 5. The effect of 14,15-EET on the pre- and post- aggregation of Aβ₄₂ at different dosages 14,15-EET.

The study by Qin et al. (2019) shows that the liver modifies depressive-like behavior by activating an internal metabolic pathway[16]. I The liver exhibits elevated protein levels of sEH, which is a crucial enzyme in EET signaling, both in monomeric and oligomeric forms during prolonged stress. Hepatic deletion of Ephx2, the gene encoding sEH, exhibits effects similar to those of an antidepressant, but hepatic overexpression of sEH causes depressive phenotypes. The effects of hepatic Ephx2 deletion are mediated through interactions between astrocytes in the medial prefrontal cortex and plasma levels of

14,15-EET, which are controlled by hepatocyte sEH activity. These findings suggest that concentrating on the mechanisms behind the hepatic response to stress may enhance depression therapy choices.

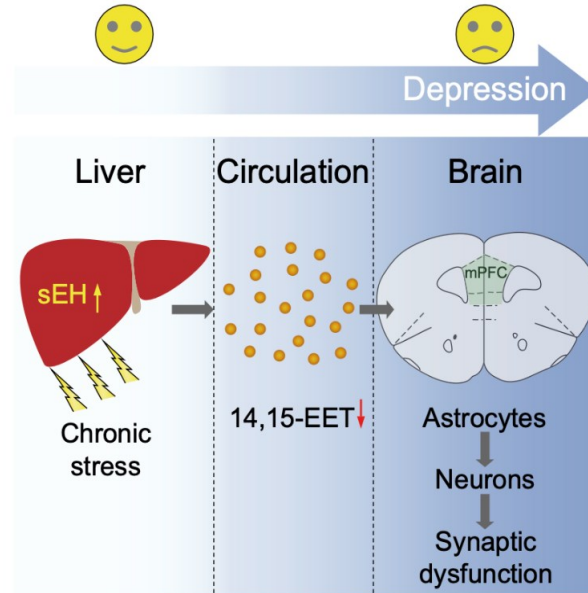


Figure 6. Hepatic sEH expression levels rise after CMS, as demonstrated by Qin et al. While hepatic Ephx2 overexpression results in depressive phenotypes, deletion of Ephx2 has effects akin to those of antidepressants. Taken together, the studies show that the liver can regulate depression.

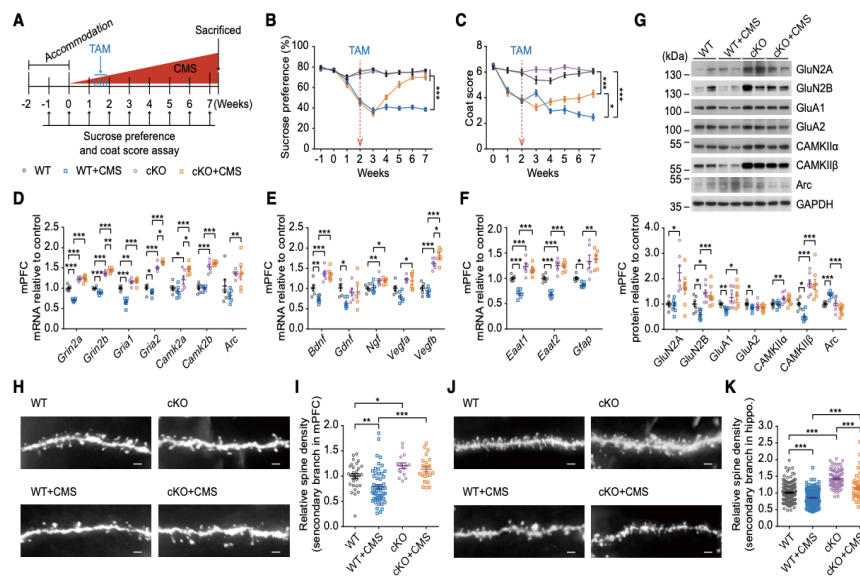


Figure 7. Experimental Design Schematic (A) (B and C) Behavioral testing revealed the reversal of reduced sucrose preference (B) and coat deterioration (C). In (D–F), the hepatic Ephx2 deletion affects the mRNA levels of neurotrophic factors following CMS. (G) Effect of hepatic Ephx2 deletion on protein expression levels in the CMS paradigm modulating synaptic activity. (H) Representative confocal images of dendritic branches of neurons in the mPFC of WT and cKO mice with or without CMS. (I) CMS-induced reductions in mPFC dendritic spine density were restored by hepatic Ephx2 deletion. Displayed are the typical confocal images (J) and the statistical analysis (K).

3. Hypothesis

We hypothesize that 14,15-EET, regulated by Ephx2 expression, modulates the PI3K/Akt/mTOR, MAPK/ERK, and NF- κ B pathways, which in turn influence amyloid-beta accumulation and tau hyperphosphorylation, thereby linking depressive symptoms to AD pathology.

4. Methods

4.1. Animal models

Species: Adult male C57BL/6J mice

Groups:

Wild-Type (WT) Group: Adult male C57BL/6J mice with normal Ephx2 expression and no genetic modifications.

Ephx2 Knockout (KO) Group: Adult male C57BL/6J mice lacking Ephx2 expression.

Ephx2 Overexpressing Group: Adult male C57BL/6J mice with overexpression of Ephx2.

sEH Inhibitor-Treated Group: WT mice treated with a selective sEH inhibitor (e.g., t-AUCB).

Sham/Vehicle Control Group: WT mice treated with a vehicle control to account for any nonspecific effects of the inhibitor.

4.2. Induction of chronic stress

Model: Chronic Unpredictable Mild Stress (CUMS)

Duration: 4-6 weeks

Mice will be exposed to a variety of mild stressors in a random order to prevent adaptation, such as: Restraint stress, Wet bedding, Social isolation, Mild tail pinch, Food and water deprivation, Reversed light/dark cycle

4.3. Behavior testing

Depressive-Like Behavior: the Sucrose Preference Test (SPT), Tail Suspension Test (TST) and the Forced Swim Test (FST) are used to evaluate depressive-like behavior.

Cognitive Function: Measured with the Novel Object Recognition Test (NORT), Y-Maze Test and the Morris Water Maze (MWM).

4.4. Tissue Collection and Analysis

sEH Activity: Measure sEH activity in liver and brain using an enzyme activity assay.

14,15-EET Quantification: Levels in brain tissue measured by mass spectrometry or ELISA.

Amyloid-Beta Accumulation: Assessed using ELISA, Western blotting, and immunohistochemistry (IHC) in hippocampal and cortical tissues.

Tau Hyperphosphorylation: Measured using Western blotting and IHC, focusing on key phosphorylation sites such as Thr231 and Ser396.

4.5. Data analysis

Behavioral Data: Use ANOVA and post-hoc testing to compare the immobility durations, learning, and memory performance of the groups.

Biochemical and Histological Data: Use t-tests or ANOVA to compare tau phosphorylation, A β plaque burden, and protein and gene expression levels between groups.

Analyze correlations: Look for relationships between depressed behaviors, sEH activity, 14,15-EET levels, and AD pathology.

5. Procedure

5.1. Animal preparation

WT mice treated with a selective sEH inhibitor (e.g., t-AUCB) to generate sEH Inhibitor-Treated mice.

Cre-LoxP system to generate Ephx2 knock out and overexpression mice.

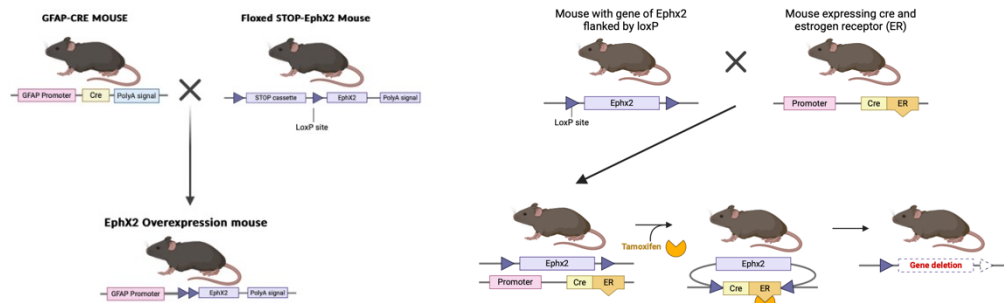


Figure 8. Cre-LoxP system. Right: the generation of conditional Ephx2 overexpression mice. Left: the generation of conditional Ephx2 knock-out mice

5.2. Pre-experiment

Days: 1 to 7

acclimatization: Give each mouse a week to become used to the lab setting.

Baseline behavioral assessment: To evaluate baseline anxiety, depressive-like behaviors, and cognitive performance, respectively, administer baseline tests such as the Morris water maze (MWM), open field test (OFT), and tail suspension test (TST)

5.3. Chronic stress induction

Days: 7 to 14

As directed by the protocol, begin exposing all groups to chronic stress (e.g., constraint stress, social defeat).

Days: 14 to 42

sEH Inhibitor-Treated Group: Give sEH inhibitors on a daily basis (t-AUCB, for example).

Group for Sham/Vehicle Control: Oversee daily vehicle control.

Maintain your ongoing chronic stress exposure.

Keep track of and document any changes in behavior.

5.4. Final Behavioral Assessments and Tissue Collection

Days: 43 to 45

Conduct final behavioral evaluations (such as the forced swim test and the sucrose preference test) to see how chronic stress and therapy affect behaviors that resemble depression.

Days: 46 to 49

Gather liver and brain tissue samples for molecular examination.

Assess the expression of sEH, 14,15-EET levels, and biomarkers associated with depression.

Perform ELISA, Western blotting, and IHC for A β and phosphorylated tau.

Quantify neuroinflammatory markers using ELISA and qPCR.

5.5. Data Analysis and Reporting

Days: 50 to 55

When required, do histological examination in addition to analyzing behavioral and molecular data.

Compare the outcomes of the various experimental groups.

Compile and edit the finished documents or reports.

Send research results to a scholarly publication or get ready to speak at a conference.

6. Predicted Results

sEH and Chronic Stress: Compared to controls, wild-type mice under chronic stress should have more depressed behaviors and worsened AD pathology.

Compared to wild-type mice, Ephx2 overexpression animals are predicted to exhibit more depressed behaviors and worsened AD pathology.

Ephx2 Knockout: decreased sEH activity may have a protective function as seen by decreased depressed symptoms and mitigated AD pathology in sEH^{-/-} animals.

Ephx2 Inhibition: It is predicted that sEH^{-/-} mice injected sEH inhibitors will resemble the protective effects, so that ensuring the potential therapeutic advantage of sEH inhibition in AD and depression caused by chronic stress.

7. Conclusion

The objective of doing this experiment is to try to figure out the role of liver soluble epoxide hydrolase (sEH) in the complicated ways that Alzheimer's disease (AD) and stress-induced depression occur. By studying what happens when sEH is active and how it interacts with a signaling molecule known as 14,15-EET, this study will provide a lot of information, though not very specific, on how these elements might be connected to both the worsening of AD and the behaviors linked with depression.

The expectation is that wild-type mice that have been under stress for a long time will likely show more depression-like behaviors and also have more severe Alzheimer's disease symptoms, which would show how continuous stress affects emotions and thinking abilities, and could speed up the progression related to AD changes.

In comparison to wild-type mice, animals that have Ephx2 overexpressed could show more intense depressive symptoms and AD-related conditions, pointing out Ephx2's potential in worsening these conditions. Conversely, knockout mice lacking Ephx2 might offer insights into how lowering Ephx2 levels influences stress responses and the initiation of AD, aiding in understanding its broader role.

However, there are several limitations worth noting. Primarily, the findings from male C57BL/6J mice might not easily apply to other mouse strains or to females, which implies that further investigations with diverse strains and female subjects could be necessary. Additionally, the study's relevance might be enhanced by employing various stress models, as the Chronic Unpredictable Mild Stress (CUMS) model may not fully capture the complexity of depression as it occurs in reality. The duration of chronic stress exposure and AD induction might not fully reflect the long-term outcomes or the early stages of the disease, suggesting that extended observation periods could provide more comprehensive insights. The importance of consistent methodologies is highlighted by the potential for technical differences in behavioral and biochemical testing to affect the reliability of results. Finally, even though ethical standards will be maintained, the invasive nature of the procedures and the stress they induce could still lead to concerns regarding animal welfare.

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