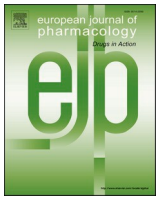
European Journal of Pharmacology 970 (2024) 176485

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar



Sex differences in luteinizing hormone aggravates Aβ deposition in APP/PS1 and $A\beta_{1-42}$-induced mouse models of Alzheimer's disease

Yongming Jia, Xinzhe Du, Yanan Wang, Qinghua Song, Ling He *

Department of Pharmacology, School of Pharmacy, China Pharmaceutical University, Nanjing, 210009, China

ARTICLE INFO

Keywords:
Alzheimer's disease
Luteinizing hormone
Neprilysin
Aβ
Sex difference

ABSTRACT

Alzheimer's disease (AD) exhibits a higher incidence rate among older women, and dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis during aging is associated with cognitive impairments and the development of dementia. luteinizing hormone (LH) has an important role in CNS function, such as mediating neuronal pregnenolone production, and modulating neuronal plasticity and cognition. The sex differences in LH and its impact on Aβ deposition in AD individuals remain unclear, with no reported specific mechanisms. Here, we show through data mining that LH-related pathways are significantly enriched in female AD patients. Additionally, LH levels are elevated in female AD patients and exhibit a negative correlation with cognitive levels but a positive correlation with AD pathology levels, and females exhibit a greater extent of AD pathology, such as Aβ deposition. *In vivo*, we observed that the exogenous injection of LH exacerbated behavioral impairments induced by $A\beta_{1-42}$ in mice. LH injection resulted in worsened neuronal damage and increased Aβ deposition. In SH-SY5Y cells, co-administration of LH with Aβ further exacerbated Aβ-induced neuronal damage. Furthermore, LH can dose-dependently decrease the levels of NEP and LHR proteins while increasing the expression of GFAP and IBA1 *in vivo* and *in vitro*. Taken together, these results indicate that LH can exacerbate cognitive impairment and neuronal damage in mice by increasing Aβ deposition. The potential mechanism may involve the reduction of NEP and LHR expression, along with the exacerbation of Aβ-induced inflammation.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with a multifactorial pathogenesis. Neuropathological hallmarks of AD commonly involve extracellular plaques containing β-amyloid (Aβ) and intracellular neurofibrillary tangles containing tau proteins (Brody, 2011). Approximately two-thirds of patients with AD are female, and significant sex differences in clinical, neuroimaging, and pathology studies were noted in AD, contributing to its complexity (Guo et al., 2022). For instance, the association between AD pathology and the clinical manifestation of the disease is more pronounced in women than in men. However, the cause of this difference remains unclear.

Distinct biological mechanisms, including menopause, and sex hormones, pregnancy, psychosocial stress responses, genetic background, inflammation, gliosis, immune modulation, and vascular disorders, contribute to the increased risk and progression of AD in women, in addition to sex differences in longevity (Zhu et al., 2021). Menopause, and sex hormones and pregnancy are linked to endocrinological factors especially estrogen, which has been associated with onset and progression of AD. However, estrogen replacement therapy has yielded conflicting results, with some results even showing adverse effect on cognition and AD risk in clinic (Saleh et al., 2023). In contrast, high serum levels of pituitary gonadotropins, including luteinizing hormone (LH), in the hypothalamic-pituitary-gonadal axis are strongly correlated with the onset of AD and may serve as possible mediators in AD sex difference. LH is increased sharply after menopause and ovariectomy and correlate with incidence of AD and other forms of age-related cognitive decline (Burnham and Thornton, 2015; Rocca et al., 2007). When age was excluded as a factor, there were no observed differences in LH and FSH levels between male AD patients and the normal control group. However, significant variations were evident in females (Short et al., 2001). The impact of LH on AD pathology requires further investigation, particularly in relation to its role in Aβ homeostasis and Tau pathogenesis.

In recent years, large-scale omics analysis offers a new avenue to improve our understanding of sex-stratified molecular mechanisms of AD. For instance, RNA-seq data were analyzed using gene co-expression networks methods and provide mechanistic insights into putative

* Corresponding author.
E-mail address: heling92@hotmail.com (L. He).

https://doi.org/10.1016/j.ejphar.2024.176485
Received 16 November 2023; Received in revised form 5 March 2024; Accepted 5 March 2024
Available online 15 March 2024
0014-2999/© 2024 Elsevier B.V. All rights reserved.

downstream signaling pathway(s) mediated by LRP10, contributing to AD pathogenesis in female (Guo et al., 2023). In this study, we attempted to investigate whether LH is a potential cause and possible molecular mechanism for the higher incidence of AD in women. We employed data mining techniques to establish the significant role of LH in the development of AD in females. Furthermore, we conducted *in vivo* experiments using an Aβ-induced AD mouse model to investigate the impact of LH on Aβ. Additionally, we examined the potential molecular mechanism using the SH-SY5Y cell model *in vitro*, hoping to clarify the difference between men and women in AD in a certain extent.

2. Materials and methods

2.1. Chemicals

LH were purchased from Shanghai Linc-Bio Science Co., Ltd. (China). Human $A\beta_{1\text{-}42}$ peptides were obtained from Shanghai Macklin Biochemical Technology Co., Ltd (China). MTT was provided by Beyotime Biotechnology Co., Ltd. (China). Antibodies were purchased from following companies: anti-GAPDH, and horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse secondary antibodies from affinity biosciences Co. Ltd (China); anti-LHCGR, anti-BDNF, anti-TrkB from Wanleibio (China). Anti-GFAP and anti-IBA1 from Cell Signaling Technology (Massachusetts, USA). All other reagents and solvents were of analytical grade and were commercially available.

2.2. Cell cultures

SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich; St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Invitrogen), 1% penicillin–streptomycin n at 37 °C with a 5% (v/v) CO_2 atmosphere and 95% relative humidity. Cell culture reagents were purchased from Gibco (Grand Island, NY). we used common DMEM medium with a decrease in serum percentage to 1%, and the supplementation with 10 μM retinoic acid for 5 days, as the differentiation agent (Shipley et al., 2016; Simões et al., 2021).

2.3. Animals

The female ICR mice weighing 20–25 g, obtained from Animal Breeding Center of QingLongShan (Nanjing, China), were allowed free access to water and chow diet. All of the animal experiments were performed according to local institutional guidelines for the care and use of laboratory animals and was approved by the Ethical Committee of China Pharmaceutical University (Approval Number: 2023-05-023).

2.4. Animal experimental design

The female mice were randomly divided into 5 groups: Control, $A\beta_{1\text{-}42}$, $A\beta_{1\text{-}42}$ plus LH (2.5 IU/per mouse), $A\beta_{1\text{-}42}$ plus LH (5.0 IU/per mouse), $A\beta_{1\text{-}42}$ plus LH (10.0 IU/per mouse). Female ICR mice underwent bilateral ovariectomy, followed by conventional housing for one month. In this study, AD mouse models were induced by lateral ventricle injection of Aβ oligomer as previously described. In brief, the experimental procedure involved anesthetizing the mice using pelltobarbitalum natricum and securing them in a stereotaxic apparatus to ensure immobilization. Subsequently, the skulls of the mice were exposed, and a microsyringe was utilized to gradually deliver Aβ oligomer or pbs (5 μl/mouse) into lateral ventricle (coordinates from bregma: anteroposterior = −0.2 mm, mediolateral = ±1.0 mm, dorsoventral = −2.4 mm). After two weeks of continuous intraperitoneal injection of different concentrations of LH, intracerebral injection of Aβ was performed, followed by two more weeks of LH administration, and then behavioral tests were conducted on mice. After conducting behavioral tests, some mice in the group (n = 3) were anesthetized and subjected to transcranial perfusion with 4% paraformaldehyde (PFA). The brain tissues were rapidly removed and immersed in 4% PFA for H&E and nissl staining. Meanwhile, Animals were killed, and their hippocampus and cortex were isolated, weighed, and then stored at −80 °C for subsequent biochemical analyses.

2.5. Open-field test

After 7 consecutive days of administration, Open-field test (OPT) was used to assess exploratory behavior, anxiety-like behavior, and general locomotor activity, following established protocols from previous studies (Cheng et al., 2020). Each female mouse was placed in the center of a square apparatus surrounded by white acrylic walls (45 × 45 × 40 cm). The total distance traveled (meters) and the time spent in the central area (seconds) were recorded. The central area was defined as the middle 20 × 20 cm section of the field. Data were collected over a 5-min period. The ANY-MAZE software was used for data analysis.

2.6. Novel object recognition test

Novel object recognition test (NORT) was employed to assess the recognition memory ability of mice as described in our previous study. In brief, after one day of habituation to the open field, during which mice were acclimatized to the environment for 5 min, two similar objects (A1/A2) were presented to each mouse. After a 24-h interval, one of these objects was replaced by a new object B. Object preference was measured as the percentage of time exploring each object, using index = EB/(EA + EB).

2.7. Y-maze procedure

Y-maze test was employed to assess the spatial working memory of mice, utilizing the cognitive parameter of spontaneous alternation behavior, as previously described. In brief, the Y-maze apparatus consists of three arms (1, 2, and 3), and the mice were randomly placed in one of the three arms for each trial. They were then allowed to explore all arms of the maze for 8 min, during which the number and order of arm entries were recorded. One alternation behavior was defined as the mouse entering three different arms in succession. Spontaneous alternations were quantified as a percentage, representing the proportion of arm choices that differed from the previous choices.

2.8. Western blot

The protein expression in tissue samples was analyzed using western blotting, following the protocol described in a previous study (Jia et al., 2015). Briefly, tissue samples from the hippocampus and cortex of mice, as well as SH-SY5Y cells, were collected and lysed using RIPA buffer containing protease and phosphatase inhibitors for 30 min at 4 °C. Protein (10 μg) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto PVDF membrane (Millipore, Bedford, MA). The blots were blocked with 5% non-fat milk at room temperature for 2 h and then incubated overnight at 4 °C with primary antibodies, specifically GAPDH (1:2000), Neprilysin (NEP, 1:1000), GFAP (1:1000), IBA1 (1:1000) and LHCGR (1:1000). Then membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies (1:5000; Proteintech). Immunoreactive bands were visualized using enhanced chemiluminescence detection reagents (Tanon, China) and quantified using a computer-assisted densitometer (Gel-Pro Analyzer).

2.9. Histopathological staining and Thioflavin-S staining

The abundance of Nissl bodies is indicative of neuronal status. In brief, paraffin-embedded brains were sliced into brain sections (5 μm thickness), followed by deparaffinized, hydrated, and subjected to Nissl staining solution at 37 °C for 30 min. Subsequently, the sections were

treated with xylene and mounted with neutral balsam. Histopathological changes were evaluated in 5 μm thick deparaffinized brain tissue sections stained with hematoxylin-eosin (H&E). The morphology of neurons in the hippocampal CA1 and CA3 regions, as well as the prefrontal cortex, was observed under a microscope.

Thioflavin-S staining as described previously (Rostagno et al., 2022). Paraffin sections were deparaffinized and hydrated using standard procedures. A 0.3% thioflavin-S solution was prepared in 50% ethanol, and the sections were incubated at room temperature for 8 min. After a 10-s wash in 80% ethanol and a subsequent rinse with pure water, the sections were stained with DAPI solution and incubated at room temperature in the dark for 10 min. Following three 5-min washes on a shaker, each with 80% ethanol, the sections were sealed with an anti-fluorescence quenching agent. Experimental results were then observed and photographed using a fluorescence microscope.

2.10. Primary cortical neuron culture

The primary cortical neuron culture followed established procedures (Sciarretta and Minichiello, 2010). In brief, pregnant female mice between the 19th and 21st day of embryogenesis were euthanized, and their brains were dissected to separate the two halves and remove the meninges. Cerebral cortex tissues were then dissociated using 0.25% trypsin and 0.05 mg/ml DNAseI at 37 °C for 10 min. Following centrifugation at 1000 rpm for 5 min, the dissociated cells were plated in a 96-well plate pre-coated with poly-L-lysine (50 μg/ml) at a density of 3.0×10^4 cells/well. The complete culture medium was replenished after 4 h with NB/B27 medium, and the neurons were maintained in culture for 7 days for subsequent experiments.

2.11. MTT assay

MTT assay was performed to evaluate the effect of LH against $A\beta_{1\text{-}42}$. SH-SY5Y cells were plated into 96-well plates (3.0×10^3 cells/well), and induced to differentiate for 5 days, and then treated with $A\beta_{1\text{-}42}$ (10 μM) in the presence or absence of LH for 24 h. For primary cortical neurons, neurons were maintained in culture for 7 days, and then exposed to $A\beta_{1\text{-}42}$ (10 μM) in the presence or absence of LH for 24 h. Following incubation with MTT (5 mg/ml, 10 μL) for another 4 h, the supernatant was removed and each well was added 100 μL/well of DMSO and the plate was incubated 15min at 37 °C. Optical density in each well was measured at 490 nm with the microplate reader.

2.12. Gene expression from microarray

Human microarray data were collected from three independent datasets (GSE15222, GSE29378 and GSE48350) (Webster et al., 2009), comparing AD cases with healthy controls using human brain samples. We collected mouse microarray data from AD transgenic mice compared to controls, specifically including APP/PS1 mice from two independent datasets (GSE168137 and GSE85162) (Forner et al., 2021). The original microarray datasets were extracted from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih. gov/geo). All raw expression data were quantile normalization and a simple log 2 transformation. Probe IDs in each dataset were assigned to corresponding NCBI Entrez Gene IDs. Differentially expressed genes (DEGs) were analyzed using the LIMMA/Bioconductor package in the R statistical processing environment. All mouse genes were converted into unique human orthologous genes using the biomaRt R package. Genes exhibiting a threshold fold change (FC) > 1.2 and FDR <0.05 using Benjamini-Hochberg's method were identified as differentially expressed and given priority as predicted AD risk genes. RankAggreg R package was used for combining the ordered DEG lists.

2.13. Enrichment analysis

DEG sets from various data sources were gathered for enrichment analysis using Fisher's exact test (Wu et al., 2021). The datasets comprised a total of 4 microarray datasets from AD patients and AD mouse model.

2.14. Statistical analysis

T-test was applied to compare two independent groups and one-way ANOVA along with Bonferroni post hoc comparisons were used for the analysis of three or more sample groups. The data are presented as means ± standard errors of the mean (S.E.M.), and differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Sex-differentially expressed genes in AD patients

We examined human gene expression from Microarray data of datasets GSE15222, including 363 samples from 190 men and 173 women post-mortem donors aged 68–102 years old (Webster et al., 2009). Gene expression data for AD brains was grouped by sex to investigate sex differences in gene expression. DEGs analysis based on sex revealed that, compared to normal females, AD females had 2020 altered genes ($|FC| > 1.5$, $p < 0.01$), including 708 upregulated genes and 1312 downregulated genes (Fig. 1A). In comparison to normal males, AD males displayed 903 altered genes ($|FC| > 1.5, p < 0.01$), with 397 upregulated genes and 506 downregulated genes (Fig. 1B). Gene Ontology (GO) enrichment analysis of DEGs showed enrichment of cognitive-related pathways in both males ($p = 7.02E\text{-}06$) and females ($p = 1.09E\text{-}16$). Furthermore, in females, DEGs were also enriched for hormone-related pathways (Fig. 1A), whereas in males, DEGs were enriched mostly metabolism-related pathways, such as adenosine cyclic enzyme metabolism process (Fig. 1B).

To further elucidate the differences in enriched pathways under sex disparities, we conducted Gene Set Enrichment Analysis (GSEA) on pathways related to memory, hormonal processes, cell death, and cellular metabolism to pinpoint specific alterations within these pathways. Within the memory-related pathways, it was observed that both male ($p = 7.02E\text{-}06$) and female ($p = 1.09E\text{-}16$) groups exhibited enrichment in processes associated with cognition and synaptic vesicle transport. In the cell death-related pathways, it was predominantly observed that both males ($p = 0.0013$) and females ($p = 9.35E\text{-}09$) were enriched in processes related to apoptosis ($p < 0.05$). In the hormone-related signaling pathways, it was found that females displayed specific enrichment in the secretion process of luteinizing hormone ($p = 0.00975$), which was not observed in males ($p = 0.0713$) (Fig. 1C and D). In the metabolic processes, both males and females were more enriched in metabolic processes such as oxidative phosphorylation and the tricarboxylic acid cycle ($p = 9.27E\text{-}08$ for male, $p = 3.28E\text{-}11$ for female).

3.2. Sex-differentially expressed genes in AD transgenic mice

To further investigate potential variations in hormone levels within the mouse model, which would contribute to a more comprehensive exploration of mechanistic pathways, we conducted sex-specific differential gene enrichment analysis in 5 × FAD mice. We observed that DEGs in both male and female mice exhibit enrichments in cognitive-related pathways ($p < 0.05$). Furthermore, in female mice, LH-related pathways and immune-related pathways were specifically enriched (Fig. 2A, $p = 0.00514$), whereas in male mice, there was specific enrichment in metabolic pathways such as glycine transport (Fig. 2B, $p = 0.00280$). To ascertain which hormone pathways were altered in both female and male mice, we performed GSEA analysis on hormone

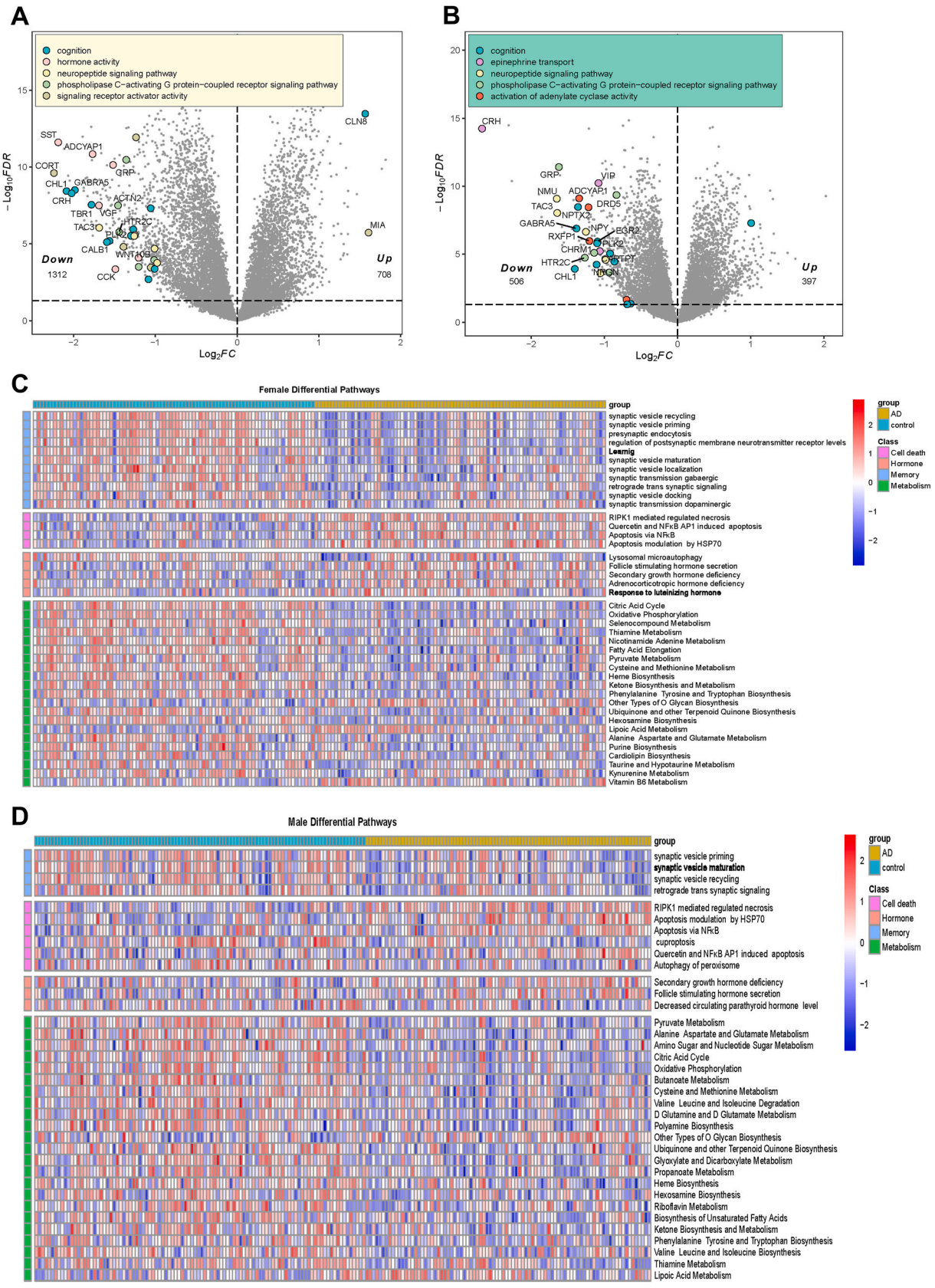


Fig. 1. Sex-differentially expressed genes in AD patients. (A): Differential gene alterations in female AD patients. (B): Differential gene alterations in male AD patients. (C) Differential enriched pathways in females in terms of cognitive, hormonal, cell death, and metabolic aspects. (D) Differential enriched pathways in males in terms of cognitive, hormonal, cell death, and metabolic aspects. Statistical analysis was performed using hypergeometric test for gene expression analysis. AD: alzheimer's disease.

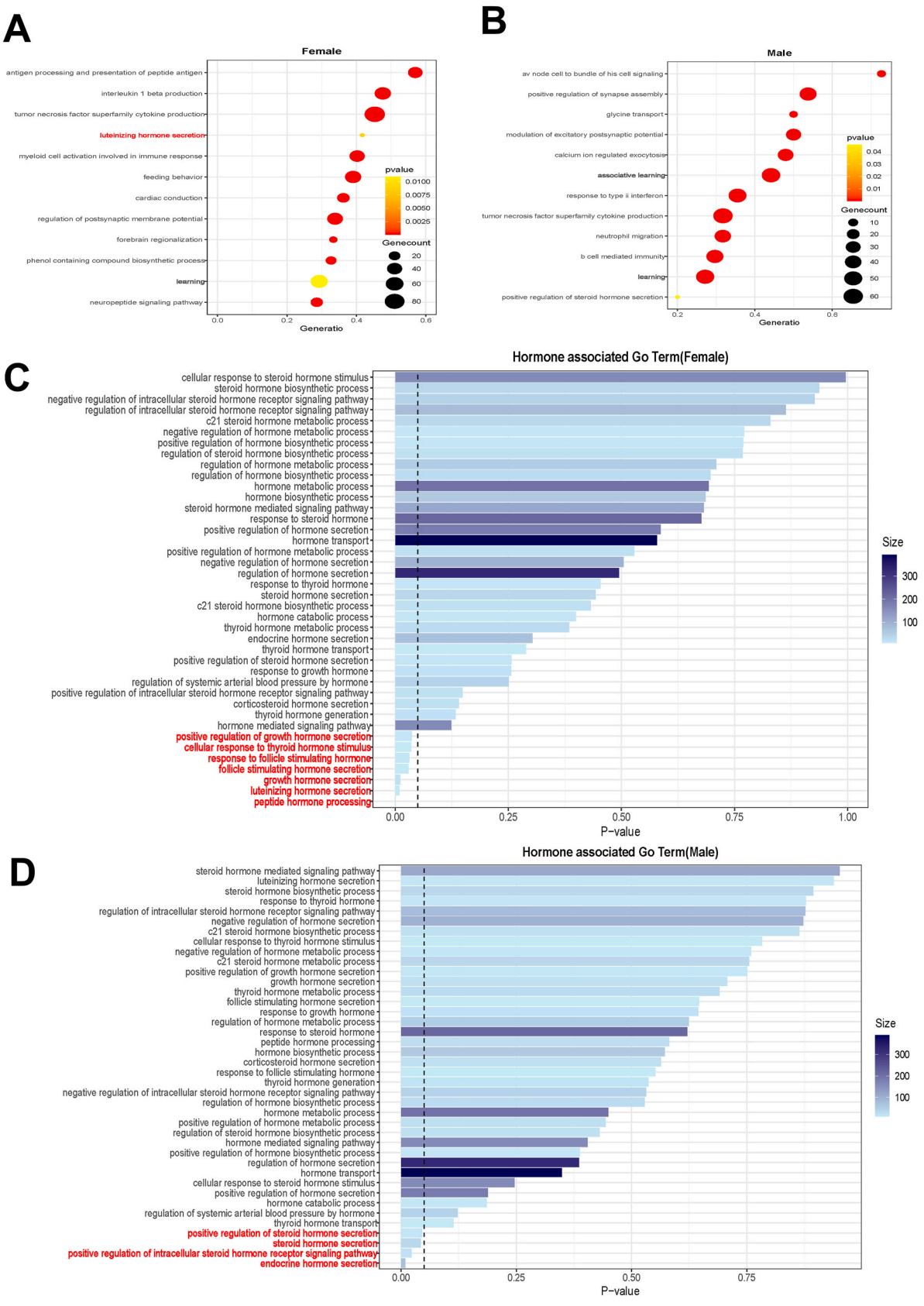


Fig. 2. Sex-differentially expressed genes in 5 × FAD mice. (A) Differential pathway enrichment of genes in female 5 × FAD mice. (B) Differential pathway enrichment of genes in male 5 × FAD mice. (C) Enrichment of hormone-related pathways in female 5 × FAD mice. (D) Enrichment of hormone-related pathways in male 5 × FAD mice. Statistical analysis was performed using hypergeometric test for gene expression analysis.

pathways. It was revealed that in females, besides enriching LH pathways, FSH pathways were also specifically enriched (Fig. 2C). In males, there was a predominant enrichment in sex hormone-related pathways, with no enrichment in gonadotropin-related pathways (Fig. 2D). These findings suggest that LH may play a role in exacerbating cognitive impairment in females.

3.3. The correlation between LH and cognitive function in AD patients

To further elucidate the correlation between LH and the cognitive status as well as AD pathology in patients, we analyzed the changes in LH levels in ADNI database and their association with AD cognition and pathology. Notably, LH levels in females were found to be 5.01-fold higher relative to males (Fig. 3B, $t = 12.62$, $df = 109$, $p < 0.0001$), while BDNF levels in females were decreased compared with male AD patients (Fig. 3C, $t = 2.080$, $df = 100$, $p = 0.0401$). We found that blood LH levels were negatively correlated with mini–mental state examination (MMSE) scores, with a correlation coefficient of −0.336, showing statistical significance (Fig. 3D, $F_{(1, 84)} = 10.71$, $p = 0.0015$). Blood LH levels showed a statistically significant positive correlation with clinical dementia rating (CDR) scores in AD patients, with a correlation coefficient of 0.3402 (Fig. 3E, $F_{(1, 101)} = 13.22$, $p = 0.0004$). But Blood LH levels did not show correlation with AD assessment scale-cog (ADAS-cog) scores (Fig. 3F, $F_{(1, 100)} = 3.122$, $p = 0.0803$). In addition, LH levels in blood were positively correlated with $A\beta_{1\text{-}42}$ ($F_{(1, 8)} = 5.838$, $p = 0.0421$), $A\beta_{1\text{-}40}$ ($F_{(1, 8)} = 2.347$, $p = 0.1640$) and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio ($F_{(1, 8)} = 3.088$, $p = 0.1170$) with the correlation being statistically significant for $A\beta_{1\text{-}42}$ levels (Fig. 3G–I). Moreover, LH in cerebrospinal fluid (CSF) was negatively correlated with MMSE scores in AD patients (Fig. 3J, $F_{(1, 47)} = 6.396$, $p = 0.0149$), while LH in CSF did not show significant correlation with CDR (Fig. 3K, $F_{(1, 53)} = 1.654$, $p = 0.2040$) and ADAS-cog (Fig. 3L, $F_{(1, 57)} = 1.044$, $p = 0.3113$). Interestingly, LH in CSF did not show correlation with CSF $A\beta_{1\text{-}42}$ levels ($F_{(1, 28)} = 0.04595$, $p = 0.8318$) and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio ($F_{(1, 28)} = 0.9683$, $p = 0.3335$). While LH in CSF was positively correlated with CSF $A\beta_{1\text{-}40}$ levels ($F_{(1, 28)} = 6.550$, $p = 0.0162$) (Fig. 3N). These results suggest that elevated LH levels are associated with decreased cognitive levels and increased Aβ pathology in AD patients.

3.4. Sex-based pathological differences in AD mice

To investigate whether there are sex differences in Aβ pathology in APP/PS1 mice, we conducted pathological assessments of 9-month-old APP/PS1 mice. In H&E staining results, we observed more nuclear condensation and tissue vacuolization in female mice (Fig. 4A). In nissl staining results, we found that female mice exhibited more neuronal loss in the CA1 ($F_{(3, 8)} = 13.62$, $p = 0.2877$ vs AD male mice), CA3 ($F_{(3, 8)} = 19.85$, $p = 0.3188$ vs AD male mice) and DG ($F_{(3, 8)} = 56.10$, $p = 0.0323$ vs AD male mice) regions. These two findings indicate that female mice exhibit more severe pathological damage (Fig. 4B and C). Immunohistochemistry was used to assess Aβ levels in male and female mice. We find that Aβ expression levels in the hippocampus and cortex regions of AD female mice were significantly higher compared to their AD male counterparts (Fig. 5A, $F_{(1, 12)} = 18.45$, $p < 0.01$ vs AD male mice in hippocampus, $F_{(1, 12)} = 33.06$, $p < 0.01$ vs AD male mice in cortex). Additionally, female APP/PS1 mice show greater accumulation of plaque pathology compared to male mice, and these plaques were also larger in size (Fig. 5B and E, $t = 6.938$, $df = 8$, $p < 0.001$ vs AD male mice). Additionally, we examined LH levels in male and female mice and observed a significant increase in LH levels in female mice (Fig. 5F, $t = 8.486$, $df = 14$, $p < 0.001$ vs AD male mice). These findings indicate that female mice exhibit increased Aβ plaque deposition, which may be significantly associated with the elevation of LH levels.

3.5. Effect of LH on Aβ-induced behavioral changes in AD female mice

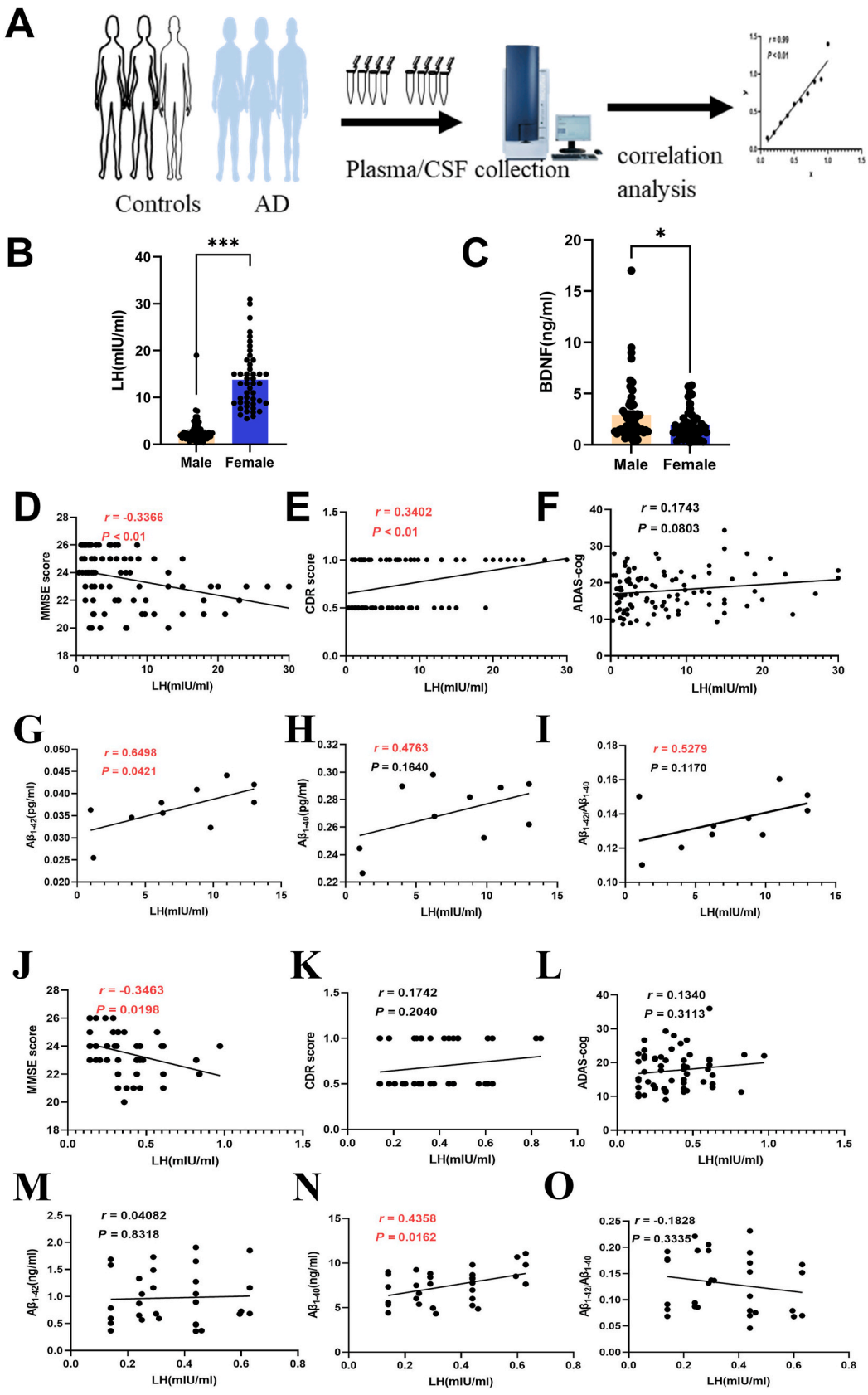
To investigate whether exogenous LH can influence Aβ-induced behavioral changes, we administered exogenous LH to female mice and observed behavioral changes following intracerebral Aβ injection (Fig. 6A). In the open field test, there were no statistical differences in total distance traveled among the groups, indicating that the exogenous injection of LH and Aβ did not affect the motor abilities of the mice (Fig. 6B, $F_{(4, 44)} = 0.8002$, $p = 0.5316$). Compared to the control group, mice in the Aβ group exhibited a significant reduction in exploration time in the central zone ($p < 0.01$). Furthermore, as LH concentration increased, there was a further decrease in exploration time in the central zone, with statistical significance observed (Fig. 6C, $F_{(4, 44)} = 80.82$, $p <$ 0.01 vs Aβ group). Results from the novel object recognition test during the training phase showed no differences in exploration of two identical objects (Fig. 6D, $p > 0.05$). However, during the testing phase, Aβ group mice exhibited a significant reduction in exploration time for the new object compared to the control group ($p < 0.01$ vs Control). Moreover, as LH levels increased, there was a further reduction in exploration time for the new object, with statistical significance observed in the high LH dose group (Fig. 6E, $F_{(4, 45)} = 36.77$, $p < 0.01$, Aβ+LH (high) vs Aβ). Y-maze results showed that Aβ group mice exhibited a significant reduction in spontaneous alternation compared to the control group (Fig. 6F, $F_{(4, 45)} = 108.8$, $p < 0.01$). Additionally, as LH concentration increased, there was a further reduction in spontaneous alternation, with statistical significance observed in the high LH dose group (Fig. 6F, $F_{(4, 45)} = 108.8$ $p < 0.01$, Aβ+LH (high) vs Aβ). These results indicate that an increase in LH concentration can lead to further cognitive impairment in $A\beta_{1\text{-}42}$-induced AD model mice.

3.6. Effect of LH on Aβ-induced pathological damage in AD female mice

To investigate whether the presence of LH leads to changes in neuronal pathology, we examined neuronal damage in the hippocampal region using H&E staining. In comparison to the control group, mice in the Aβ group exhibited nuclear condensation in the hippocampal neurons and the presence of tissue vacuoles. Importantly, as the LH concentration increased, there was a further intensify of neuronal damage (Fig. 7A). Results from nissl staining indicated that compared to the control group, the number of nissl bodies in neuronal cells of the Aβ group mice significantly decreased. Furthermore, as LH concentration increased, the reduction in the number of nissl bodies persisted (Fig. 7B). These results suggest that LH can exacerbate neuronal damage induced by Aβ.

3.7. LH accelerates Aβ deposition and related protein detection in Aβ-induced AD female mice

To clarify whether LH exacerbates Aβ pathological damage due to Aβ deposition, immunohistochemistry was used to measure Aβ levels. Compared to the control group, mice in the Aβ group exhibited a significant increase in Aβ levels ($F_{(4, 20)} = 54.62$, $p = 0.0129$ vs Control), and as LH concentration increased, there was a further increase in Aβ levels (Fig. 8A and B, $F_{(4, 20)} = 54.62$, $p = 0.0328$ Aβ+LH (M) vs Aβ, $p <$ 0.001 Aβ+LH (H) vs Aβ). To further investigate the cause of the increase in Aβ, Western blot results revealed that with increasing LH concentration, the expression level of NEP, an enzyme capable of degrading Aβ, decreased in the hippocampus ($F_{(4, 10)} = 19.18$, $p = 0.0112$ Aβ vs Control, $p = 0.0280$ LH(H) vs Aβ, Fig. 8C) and cortex ($F_{(4, 10)} = 0.1362$, $p =$ 0.0350 Aβ vs Control, $p = 0.0016$ LH(H) vs Aβ, Fig. 8H). Additionally, compared to the Aβ group, increasing LH concentration also led to an increase in the expression of GFAP ($F_{(4, 8)} = 20.02$, $p = 0.0130$, LH(L) vs Aβ, $p = 0.0163$, LH(H) vs Aβ, $p = 0.0450$, LH(H) vs Aβ) and IBA1 ($F_{(4, 10)} = 11.25$, $p = 0.0424$, LH(M) vs Aβ) proteins in the cortex (Fig. 8H). LH decreased the expression of the LH downstream target, LHR protein in the hippocampus (Fig. 8G, $F_{(4, 10)} = 25.37$, $p = 0.0063$, LH(M) vs Aβ, $p <$



(caption on next page)

Fig. 3. Correlation between LH and cognitive function in AD patients. (A) Data mining workflow. (B) Differences in LH concentration in the blood of male and female AD patients. (C) Differences in BDNF concentration in the blood of male and female AD patients. (D) Correlation analysis of blood LH concentration and MMSE scores. (E) Correlation analysis of blood LH concentration and CDR scores. (F) Correlation analysis of blood LH concentration and ADAS-cog scores. (G) Correlation analysis of blood LH concentration and Aβ1-42 concentration. (H) Correlation analysis of blood LH concentration and Aβ1-40 concentration. (I) Correlation analysis of blood LH concentration and Aβ1-42/Aβ1-40 ratio. (J) Correlation analysis of CSF LH concentration and MMSE scores. (K) Correlation analysis of CSF LH concentration and CDR scores. (L) Correlation analysis of CSF LH concentration and ADAS-cog scores. (M) Correlation analysis of CSF LH concentration and Aβ1-42 concentration. (N) Correlation analysis of CSF LH concentration and Aβ1-40 concentration. (O) (M) Correlation analysis of CSF LH concentration and Aβ1-42/Aβ1-40 ratio. Data are presented as means ± SE. *P < 0.05, *P < 0.01, ***P < 0.001. Statistical analysis was performed using two-tailed student's t-tests. AD: alzheimer's disease, BDNF: brain-derived neurotrophic factor, MMSE: mini–mental state examination, CDR: clinical dementia rating, ADAS-cog: Alzheimer disease assessment scale-cog.

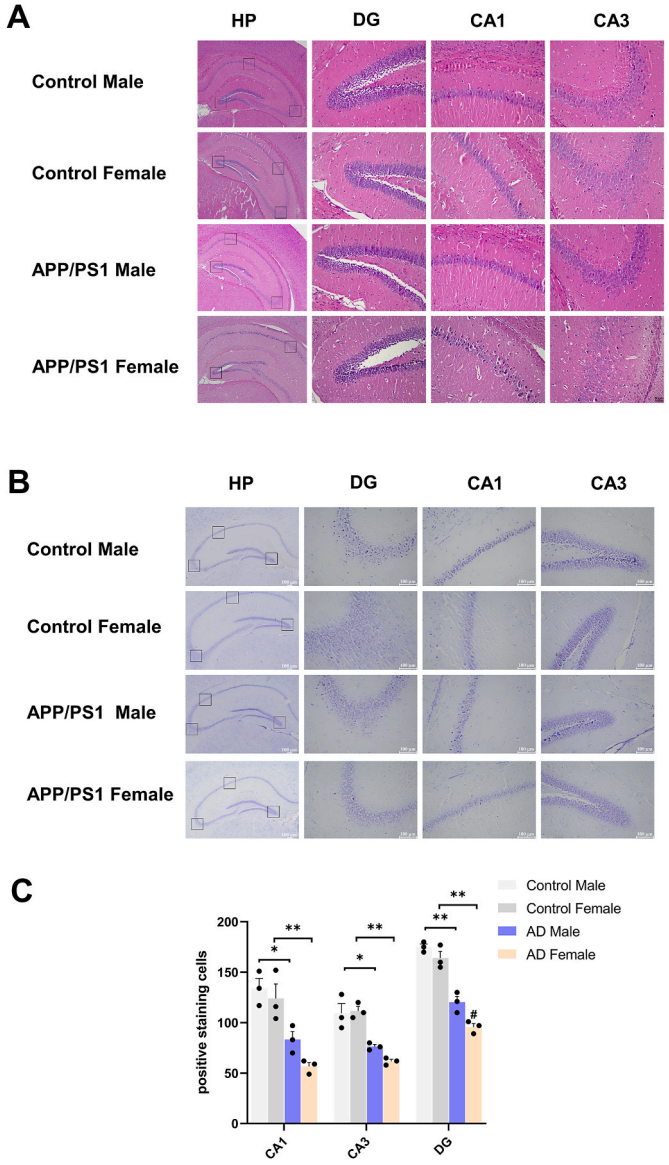


Fig. 4. Sex-based pathological differences in APP/PS1 male and female mice. (A) HE staining of the brains. Scale bar = 50 μm. (B) Nissl staining of the brains. Scale bar = 100 μm. (C) Quantitative statistics of the Nissl staining in the CA1, CA3 and DG region, showed by the number of positive staining cells (n = 3 mice per group). Data are presented as means ± SE. *P < 0.05, *P < 0.01, #P < 0.05 vs AD female. Statistical analysis was performed using two-way ANOVA. AD: alzheimer's disease.

0.01, LH(H) vs Aβ) and cortex (Fig.8L,$F_{(4,\ 10)} = 104.1$, $p < 0.001$, LH(M) vs Aβ, $p < 0.001$, LH(H) vs Aβ). These results suggest that LH may induce changes in Aβ levels by reducing Aβ-degrading enzymes, accelerating inflammation, and affecting the expression levels of their specific receptors.

3.8. Effect of LH on neuronal survival in SH-SY5Y cells and primary cortical neurons

To further investigate the impact of LH on neuronal survival, we used the MTT method to assess the effect of Aβ on neuronal survival in the presence and absence of LH. First, we examined the toxicity of LH to SH-SY5Y cells and found that LH alone did not directly cause neuronal damage (Fig. 9A, $F_{(6,\ 28)} = 2.335$, $p = 0.0592$). And Aβ in different concentrations could cause neuronal damage (Fig. 9B). However, in the presence of Aβ, Aβ was able to induce neuronal damage compared to the control group ($F_{(4,\ 20)} = 181.1$, $p < 0.0001$), and neuronal damage was further exacerbated when LH was co-administered (Fig. 9C, $F_{(4,\ 20)} = 181.1$, $p = 0.0369$ Aβ+LH (50 ng/ml) vs Aβ, $p < 0.01$ Aβ+LH (100 ng/ml) vs Aβ). We observed a similar phenomenon in primary neurons, where cell survival further decreased when Aβ was co-administered with LH compared to the Aβ group (Fig. 9D, $F_{(4,\ 20)} = 31.58$, $p = 0.001$ Aβ+LH (25 ng/ml) vs Aβ, $p = 0.001$ Aβ+LH (50 ng/ml) vs Aβ, $p = 0.0032$ Aβ+LH (100 ng/ml) vs Aβ). Furthermore, we examined changes in gene expression levels after LH treatment and found that, compared to the control group, increasing LH concentration led to a decrease in the mRNA levels of LHR ($F_{(5,\ 12)} = 10.62$, $p = 0.0049$, 3 ng/ml vs 0 ng/ml), NEP ($F_{(5,\ 12)} = 50.44$, $p < 0.001$, 3 ng/ml vs 0 ng/ml), and BDNF ($F_{(5,\ 12)} = 328.6$, $p < 0.001$, 3 ng/ml vs 0 ng/ml) (Fig. 9E–G). These results suggest that LH can reduce NEP and LHR, exacerbating Aβ deposition and subsequently reducing neuronal survival.

4. Discussion

In this study, we demonstrate that LH signaling pathway exhibits specific activation in females and shows significant sex differences between sex-based AD patients and AD model mice. In addition, female mice exhibit higher levels of Aβ deposition, and an increase in LH accelerates Aβ deposition. The mechanism by which LH accelerates Aβ deposition is closely related to the reduction in NEP levels and the levels of LHR.

Numerous studies on sex differences in AD have been observed in clinical diagnoses, neuroimaging, and pathology (Guo et al., 2022). Pathological studies revealed that females exhibited a greater extent of global AD pathology, characterized by neuritic plaques, diffuse plaques, and neurofibrillary tangles, in comparison to males. (Oveisgharan et al., 2018). The learning and memory abilities of female APP/PS1 mice were inferior to those of males, and the levels of soluble and insoluble Aβ in female APP/PS1 mouse brains were higher compared to males (Jiao et al., 2016; Li et al., 2016). Our findings indicate that female APP/PS1 mice exhibit higher levels of Aβ deposition when compared to their male counterparts (Fig. 5A and B), providing further evidence of sex differences in AD patients. Furthermore, we also observed that the serum LH concentration in female APP/PS1 mice and AD patients was higher when compared to males (Figs. 3B and 5F). Some limited research has reported the relationship between LH and AD, but it has not elucidated the specific mechanisms, particularly the direct interaction with Aβ (Bhatta et al., 2018).

Sex hormones, such as estrogens, have been widely acknowledged

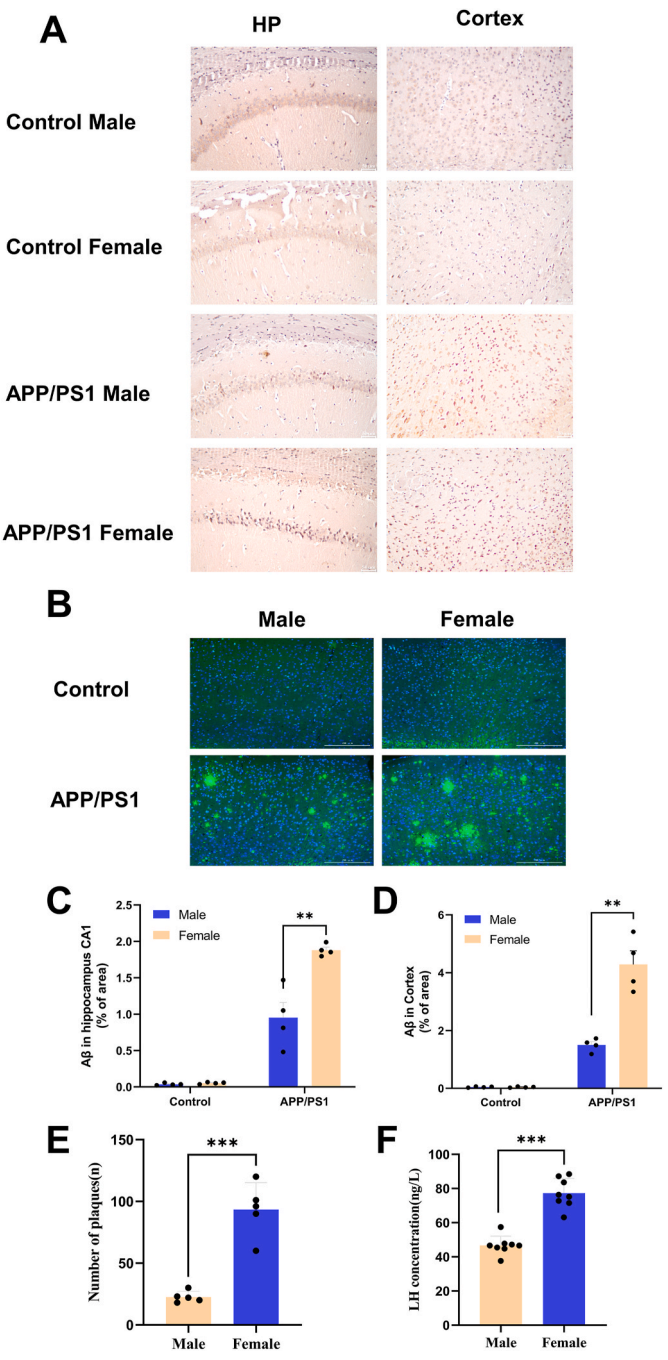


Fig. 5. Aβ levels in APP/PS1 male and APP/PS1 female mice. (A) Immunohistochemical staining of Aβ in the brain. (B) Representative image of Aβ (TS) staining (green) in brain sections of APP/PS1 male and female mice. (C–D) Quantification of Aβ levels in the hippocampus and cortex of APP/PS1 male and female mice. (E) Quantification of TS-positive Aβ plaque number in the hippocampus of APP/PS1 male and female mice (n = 4 mice per group). (F) Differences in LH levels in the serum of male and female APP/PS1 mice (n = 8 mice per group). Data are presented as means ± SE. **P < 0.01, ***P < 0.001 vs APP/PS1 male. Statistical analysis was performed using two-way ANOVA. AD: alzheimer's disease; TS: Thioflavin S.

for their role in brain development, aging, and the processes associated with AD (Gurvich et al., 2018; Rahman et al., 2019). Despite numerous neuroprotective effects attributed to estrogens, age-related declines in estrogen levels have been linked to elevated risks of cognitive decline and AD. Nevertheless, recent conflicting findings from the Women's Health Initiative (WHI) study have indicated that hormone replacement therapy (HRT) initiated in elderly post-menopausal women does not enhance cognitive performance and may potentially elevate the risk of developing AD (Shumaker et al., 2003). These observations suggest that searching for upstream pathways that influence estrogen may be a potential strategy for treating AD. LH, which stimulates estrogen production, has showed associated with AD pathology in several papers, but the specific mechanism remains unclear (Casadesus et al., 2007). We found that LH-related pathways are enriched in AD females, suggesting that LH may be a trigger for the development of AD in females (Figs. 1C and 2C). Our results indicate that in AD patients, as LH levels increase, cognitive levels decrease (Fig. 3D and J). The MMSE and CDR are common scales used to assess cognitive levels in patients, and this discovery is consistent with some existing literature (Choe et al). Similarly, in tests of mouse behavior, we observed that the exogenous LH exacerbated Aβ-induced behavioral impairments (Fig. 6B–G). In other rodent studies, the exogenous administration of human LH impaired working memory and/or increased AD pathology (Wahjoepramono et al., 2011). These findings suggest that a surge in LH can lead to cognitive impairments. However, whether LH directly damages neurons or induces cognitive impairments through other indirect pathways has not been investigated. The surge in LH levels may be a potential reason for the higher incidence of AD in females compared to males.

Aβ, produced through the cleavage of APP by β-secretase and γ-secretase, play the main role in neuronal dysfunction and AD (Graff-Radford et al., 2021; Knopman et al., 2021). Our study revealed that LH alone does not induce neuronal damage when acting on neurons. However, in the presence of Aβ, LH can further exacerbate neuronal damage (Fig. 9A and C). These findings also imply a positive correlation between Aβ pathology levels and LH concentrations (Fig. 3G and N). In blood samples, this correlation is primarily with $A\beta_{1\text{-}42}$ levels, while in cerebrospinal fluid, LH levels are positively correlated with $A\beta_{1\text{-}40}$ levels. Since insoluble $A\beta_{1\text{-}42}$ levels are mainly present in the form of plaques in the brain, this may explain why LH levels in cerebrospinal fluid are not correlated with $A\beta_{1\text{-}42}$. Simultaneously, other studies have also observed a positive correlation between plasma Aβ levels and LH levels in males (Verdile et al., 2008, 2014). In mouse models, exogenous injections of LH can exacerbate Aβ deposition in the mouse cortex. The dynamic balance of Aβ is governed by both its production and clearance processes. Any metabolic irregularities in either of these processes can result in Aβ deposition (Ullah et al., 2021). Some studies have found that direct exposure to LH can affect the APP metabolism process, thereby increasing the production of Aβ. However, there have been no reports on whether LH can influence the clearance process of Aβ (Wahjoepramono et al., 2011).

Secreted peptidases play a crucial role in the degradation of Aβ peptides. These enzymes have been reported to exhibit affinity for specific domains within the Aβ amino acid sequence, enabling them to cleave and convert these peptides into shorter, less harmful forms (Zuroff et al., 2017). NEP is regarded as the most potent Aβ-degrading enzyme, with a preference for cleaving oligomeric $A\beta_{42}$ and $A\beta_{40}$ (Iwata et al., 2000). NEP expression and activity have been observed to diminish with age and in post-mortem human AD brain tissue, which could potentially contribute to the buildup of Aβ (Kim et al., 2023; Russo et al., 2005). Our research found that LH can reduce the expression of NEP in the mouse brain (Fig. 8C), and immunohistochemistry results showed that Aβ levels increase with LH levels. These results suggest that LH may increase Aβ levels by reducing NEP expression. Additionally, LH directly acts on downstream targets, including LHR. Our findings indicate that LH can reduce the expression of LHR in the mouse brain, which to some extent suggests that LH may impair cognitive function in mice. Furthermore, LH can increase the expression of GFAP and IBA1 in the mouse brain, indicating that LH can induce inflammation in mice (Fig. 8F and K).

The precise mechanism underlying the influence of LH on neuronal function remains unclear. Some studies suggest that LH effects are likely mediated through binding to LHR. LHR is widely expressed in brain

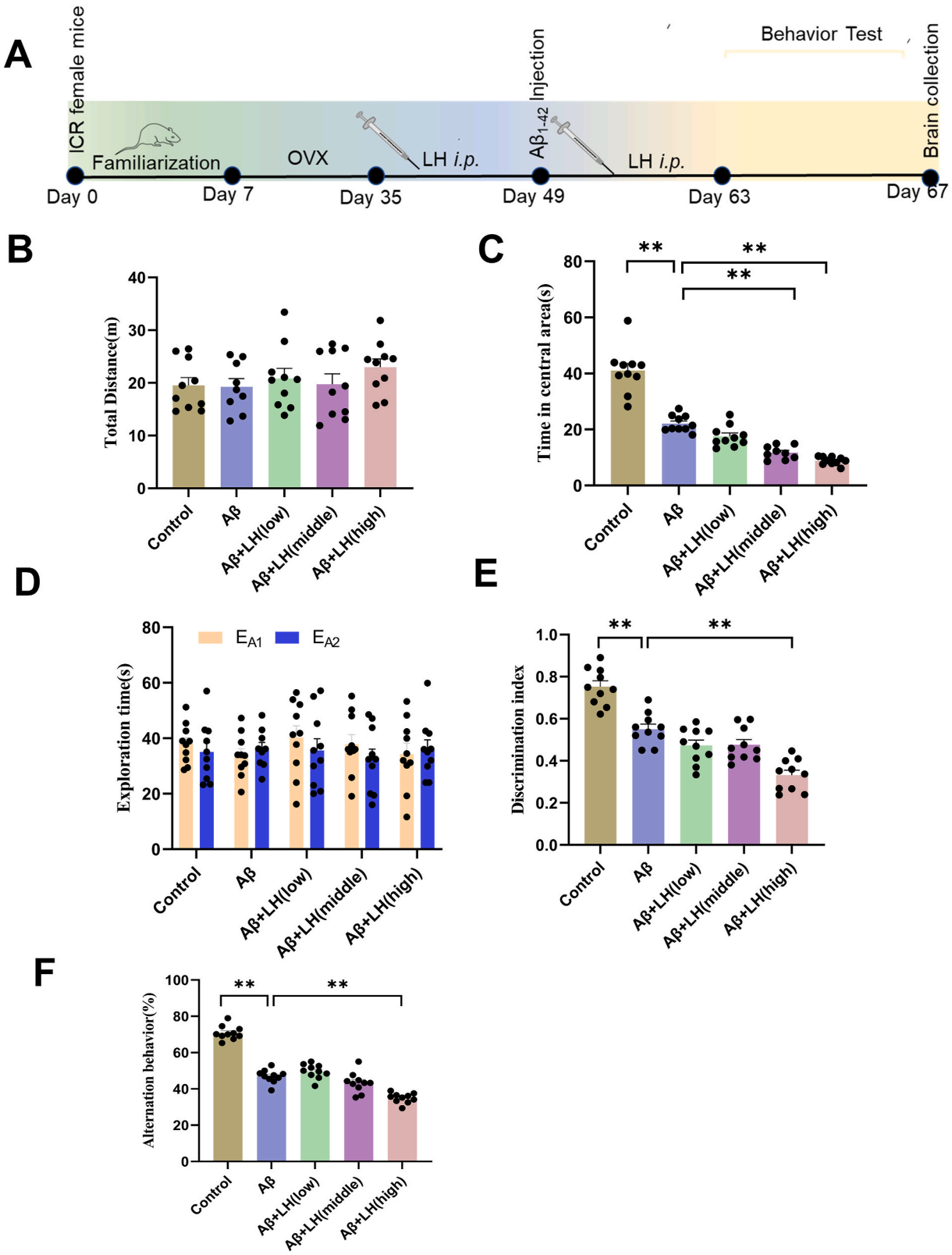


Fig. 6. Effect of LH on Aβ-induced behavioral changes in Aβ-induced AD female mice. (A): study protocol. (B)Autonomic capacity test of female mice using open field test. Effects of LH to the female mice are shown as anxiety-like behavior of mice using open field test shown as the central distance the total distance of the mice (C), (D) Exploration time for two same objects, (E) Preference index for new and old objects. (F) Spatial memory test of mice using Y-maze. n = 10 in each group. Data are presented as means ± SE, *P < 0.05, **P < 0.01. Statistical analysis was performed using one-way ANOVA. AD: alzheimer's disease.

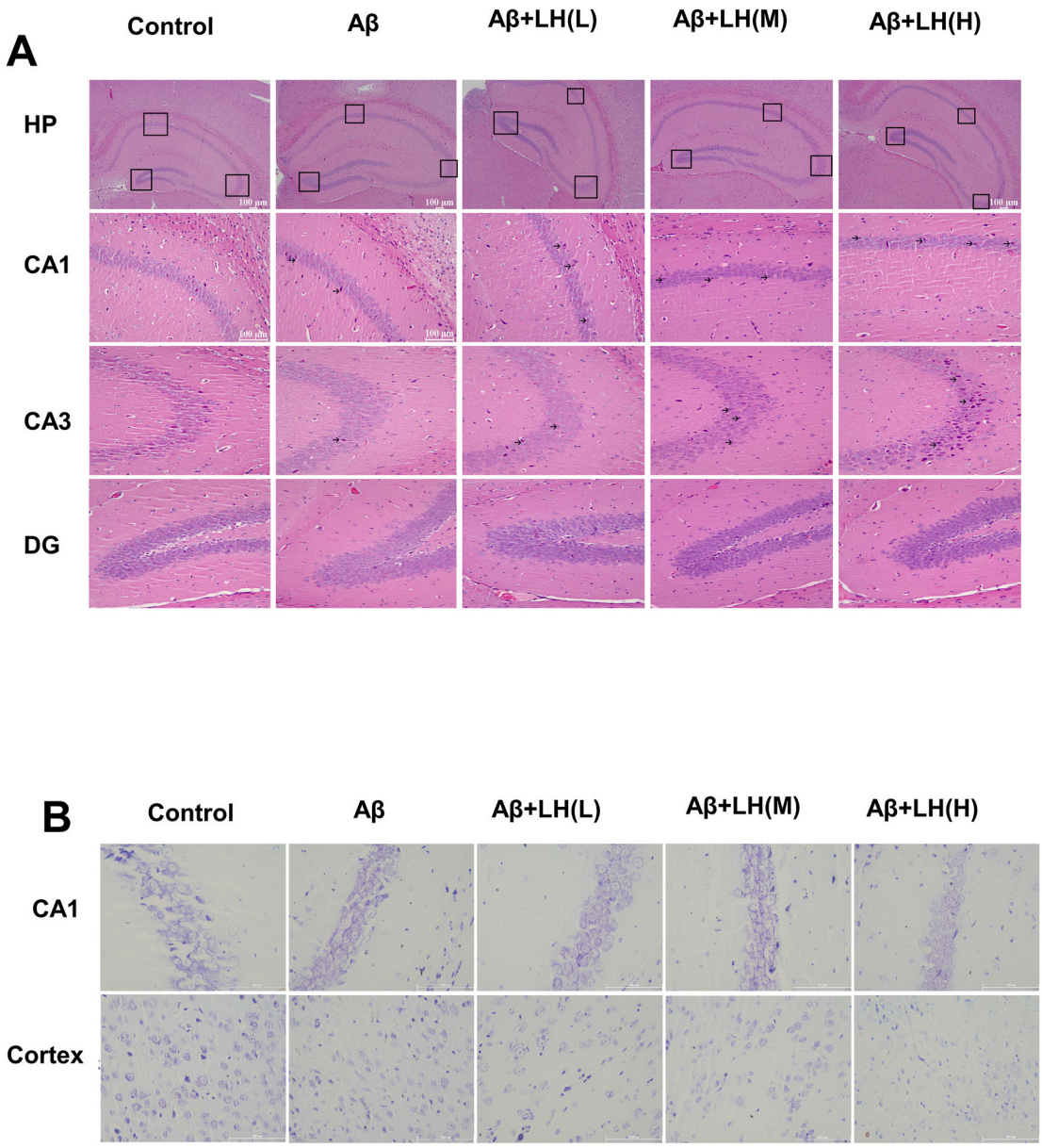


Fig. 7. Effect of LH on Aβ-induced pathological damage in Aβ-induced AD female mice. (A) HE staining of the brains. Scale bar = 100 μm. (B) Nissl staining of the brains. Scale bar = 100 μm (n = 3 mice per group).

regions, including the choroid plexus, hippocampus, cortex, and hypothalamus (Ryu et al., 2022). As a G-protein coupled receptor, LHR transmits signals via Gs, leading to elevated cAMP, CREB, and extracellular signal-related protein kinase (ERK) activation (Bhatta et al., 2018). Alternatively, LHR signals via Gq, inducing intracellular Ca^{2+} release and activating several protein kinases, including PKA and CAMKII (Burnham and Thornton, 2015). ERK and PKA are critical signaling cascades involved in long-term potentiation (LTP), memory formation, and structural plasticity. The mentioned studies suggest that the activation of LHR in the brain may be associated with the protection of neural cells.

It is well-established that LHR undergoes rapid internalization and downregulation in the presence of LH. Additionally, LH has been observed to enhance the activity of the β-secretase BACE, thereby stimulating the generation of Aβ in SH-SY5Y cells (Saberi et al., 2013). LH can also reduce the expression of NEP in the mouse brain and SH-SY5Y cells (Fig. 8C and H, Fig. 9G). Given the established canonical signaling cascades associated with LHR and the effects of LH on Aβ homeostasis, these findings indirectly suggest that LH signaling in the CNS may be detrimental.

Our study uncovered that LH can exacerbate Aβ deposition in the brain, accelerating the pathological progression of AD. This phenomenon may be closely linked to the decrease in brain NEP levels and LHR levels, suggesting the potential of LH as a candidate biomarker for AD.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81673434) and “Double First-Class” University project (CPU2018GY22).

Statement of ethics

This study protocol was reviewed and approved by Animal Care and Use Committee of China pharmaceutical university.

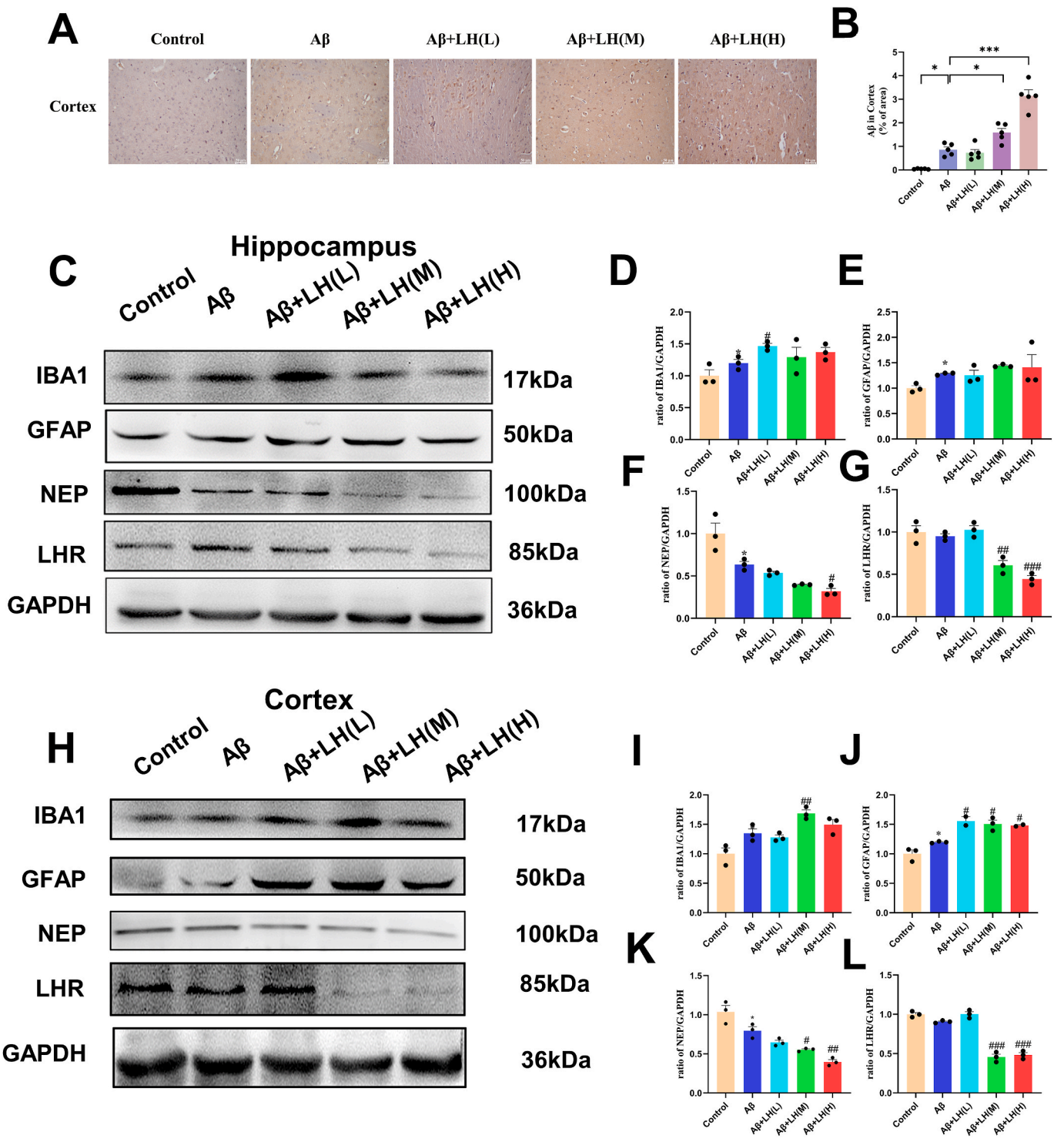


Fig. 8. LH accelerates Aβ deposition and related protein detection in Aβ-induced AD female mice. (A, B) Immunohistochemical staining of Aβ in the brain. (C–G) The protein expression levels of IBA1, GFAP, NEP, LHR, and GAPDH in the hippocampus. (H–L) The protein expression levels of IBA1, GFAP, NEP, LHR, and GAPDH in the cortex. *P < 0.05, **P < 0.01, ***P < 0.001. #P < 0.05, ##P < 0.01 vs Aβ, ##P < 0.001 vs Aβ. Data are means ± SE. (n = 3). Statistical analysis was performed using one-way ANOVA.

CRediT authorship contribution statement

Yongming Jia: Writing – review & editing, Writing – original draft, Project administration. **Xinzhe Du:** Methodology, Data curation. **Yanan Wang:** Methodology. **Qinghua Song:** Methodology. **Ling He:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

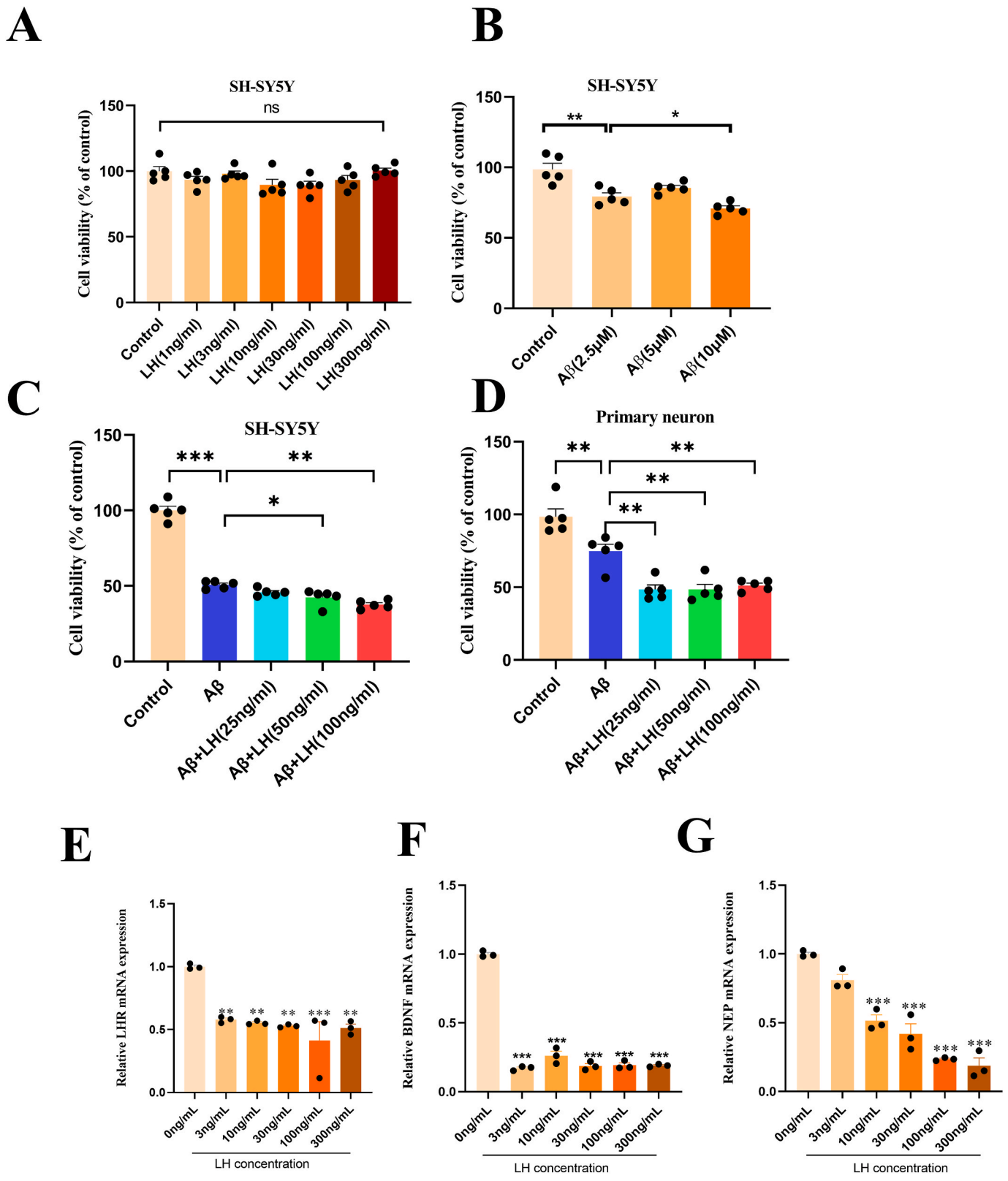


Fig. 9. Effect of LH on neuronal survival in SH-SY5Y cells and primary cortical neurons. (A) Different concentrations of LH (0–300 ng/ml) on SH-SY5Y cell survival. (B) Different concentrations of Aβ(2.5, 5, 10 μM) on SH-SY5Y cell survival. (C) Different concentrations of LH on Aβ toxicity in SH-SY5Y cells. (D) Different concentrations of LH on Aβ toxicity in primary cortical neurons. (E) Different concentrations of LH on LHR mRNA expression in SH-SY5Y cells. (F)Different concentrations of LH on BDNF mRNA expression in SH-SY5Y cells. (G) Different concentrations of LH on NEP mRNA expression in SH-SY5Y cells. Data are means ± SE, *P < 0.05, **P < 0.01, ***P < 0.001. (n = 3). Statistical analysis was performed using one-way ANOVA.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2024.176485.

References

Bhatta, S., Blair, J.A., Casadesus, G., 2018. Luteinizing hormone Involvement in aging female cognition: not all is estrogen loss. Front. Endocrinol. 9, 544. https://doi.org/10.3389/fendo.2018.00544.

Brody, H., 2011. Alzheimer's disease. Nature 475, S1. https://doi.org/10.1038/475S1a.

Burnham, V.L., Thornton, J.E., 2015. Luteinizing hormone as a key player in the cognitive decline of Alzheimer's disease. Horm. Behav. 76, 48–56. https://doi.org/10.1016/j.yhbeh.2015.05.010.

Casadesus, G., Milliken, E.L., Webber, K.M., Bowen, R.L., Lei, Z., Rao, C.V., Perry, G., Keri, R.A., Smith, M.A., 2007. Increases in luteinizing hormone are associated with declines in cognitive performance. Mol. Cell. Endocrinol. 269, 107–111. https://doi.org/10.1016/j.mce.2006.06.013.

Cheng, Z.-Y., Xia, Q.-P., Hu, Y.-H., Wang, C., He, L., 2020. Dopamine D1 receptor agonist A-68930 ameliorates Aβ1-42-induced cognitive impairment and neuroinflammation in mice. Int. Immunopharm. 88, 106963 https://doi.org/10.1016/j.intimp.2020.106963.

Choe, Y.M., Lee, B.C., Choi, I.-G., Suh, G.-H., Lee, D.Y., Kim, J.W., Alzheimer's Disease Neuroimaging Initiative, 2020. MMSE Subscale Scores as Useful Predictors of AD Conversion in Mild Cognitive Impairment. Neuropsychiatr Dis Treat 16, 1767–1775. https://doi.org/10.2147/NDT.S263702.

Forner, S., Kawauchi, S., Balderrama-Gutierrez, G., Kramár, E.A., Matheos, D.P., Phan, J., Javonillo, D.I., Tran, K.M., Hingco, E., da Cunha, C., Rezaie, N., Alcantara, J.A., Baglietto-Vargas, D., Jansen, C., Neumann, J., Wood, M.A., MacGregor, G.R., Mortazavi, A., Tenner, A.J., LaFerla, F.M., Green, K.N., 2021. Systematic phenotyping and characterization of the 5xFAD mouse model of Alzheimer's disease. Sci. Data 8, 270. https://doi.org/10.1038/s41597-021-01054-y.

Graff-Radford, J., Yong, K.X.X., Apostolova, L.G., Bouwman, F.H., Carrillo, M., Dickerson, B.C., Rabinovici, G.D., Schott, J.M., Jones, D.T., Murray, M.E., 2021. New insights into atypical Alzheimer's disease in the era of biomarkers. Lancet Neurol. 20, 222–234. https://doi.org/10.1016/S1474-4422(20)30440-3.

Guo, L., Cao, J., Hou, J., Li, Y., Huang, M., Zhu, L., Zhang, L., Lee, Y., Duarte, M.L., Zhou, X., Wang, M., Liu, C.-C., Martens, Y., Chao, M., Goate, A., Bu, G., Haroutunian, V., Cai, D., Zhang, B., 2023. Sex specific molecular networks and key drivers of Alzheimer's disease. Mol. Neurodegener. 18, 39. https://doi.org/10.1186/s13024-023-00624-5.

Guo, L., Zhong, M.B., Zhang, L., Zhang, B., Cai, D., 2022. Sex differences in alzheimer's disease: insights from the Multiomics Landscape. Biol. Psychiatr. 91, 61–71. https://doi.org/10.1016/j.biopsych.2021.02.968.

Gurvich, C., Hoy, K., Thomas, N., Kulkarni, J., 2018. Sex differences and the influence of sex hormones on cognition through Adulthood and the aging process. Brain Sci. 8, 163. https://doi.org/10.3390/brainsci8090163.

Iwata, N., Tsubuki, S., Takaki, Y., Watanabe, K., Sekiguchi, M., Hosoki, E., Kawashima-Morishima, M., Lee, H.J., Hama, E., Sekine-Aizawa, Y., Saido, T.C., 2000. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. Nat Med 6, 143–150. https://doi.org/10.1038/72237.

Jia, Y., Liu, Z., Huo, X., Wang, C., Meng, Q., Liu, Q., Sun, H., Sun, P., Yang, X., Shu, X., Liu, K., 2015. Enhancement effect of resveratrol on the intestinal absorption of bestatin by regulating PEPT1, MDR1 and MRP2 in vivo and in vitro. Int J Pharm 495, 588–598. https://doi.org/10.1016/j.ijpharm.2015.09.042.

Jiao, S.-S., Bu, X.-L., Liu, Y.-H., Zhu, C., Wang, Q.-H., Shen, L.-L., Liu, C.-H., Wang, Y.-R., Yao, X.-Q., Wang, Y.-J., 2016. Sex Dimorphism Profile of alzheimer's disease-Type Pathologies in an APP/PS1 mouse model. Neurotox. Res. 29, 256–266. https://doi.org/10.1007/s12640-015-9589-x.

Kim, E., Kim, H., Jedrychowski, M.P., Bakiasi, G., Park, J., Kruskop, J., Choi, Y., Kwak, S. S., Quinti, L., Kim, D.Y., Wrann, C.D., Spiegelman, B.M., Tanzi, R.E., Choi, S.H., 2023. Irisin reduces amyloid-β by inducing the release of neprilysin from astrocytes following downregulation of ERK-STAT3 signaling. Neuron S0896–6273 (23). https://doi.org/10.1016/j.neuron.2023.08.012, 00623–2.

Knopman, D.S., Amieva, H., Petersen, R.C., Chételat, G., Holtzman, D.M., Hyman, B.T., Nixon, R.A., Jones, D.T., 2021. Alzheimer disease. Nat. Rev. Dis. Prim. 7, 33. https://doi.org/10.1038/s41572-021-00269-y.

Li, X., Feng, Y., Wu, W., Zhao, J., Fu, C., Li, Y., Ding, Y., Wu, B., Gong, Y., Yang, G., Zhou, X., 2016. Sex differences between APPswePS1dE9 mice in A-beta accumulation and pancreatic islet function during the development of Alzheimer's disease. Lab Anim 50, 275–285. https://doi.org/10.1177/0023677215615269.

Oveisgharan, S., Arvanitakis, Z., Yu, L., Farfel, J., Schneider, J.A., Bennett, D.A., 2018. Sex differences in Alzheimer's disease and common neuropathologies of aging. Acta Neuropathol. 136, 887–900. https://doi.org/10.1007/s00401-018-1920-1.

Rahman, A., Jackson, H., Hristov, H., Isaacson, R.S., Saif, N., Shetty, T., Etingin, O., Henchcliffe, C., Brinton, R.D., Mosconi, L., 2019. Sex and Gender Driven Modifiers of alzheimer's: the role for estrogenic control across age, Race, Medical, and Lifestyle risks. Front. Aging Neurosci. 11, 315. https://doi.org/10.3389/fnagi.2019.00315.

Rocca, W.A., Bower, J.H., Maraganore, D.M., Ahlskog, J.E., Grossardt, B.R., de Andrade, M., Melton, L.J., 2007. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. Neurology 69, 1074–1083. https://doi.org/10.1212/01.wnl.0000276984.19542.e6.

Rostagno, A., Cabrera, E., Lashley, T., Ghiso, J., 2022. N-terminally truncated Aβ4-x proteoforms and their relevance for Alzheimer's pathophysiology. Transl. Neurodegener. 11, 30. https://doi.org/10.1186/s40035-022-00303-3.

Russo, R., Borghi, R., Markesbery, W., Tabaton, M., Piccini, A., 2005. Neprylisin decreases uniformly in Alzheimer's disease and in normal aging. FEBS Lett. 579, 6027–6030. https://doi.org/10.1016/j.febslet.2005.09.054.

Ryu, V., Gumerova, A.A., Korkmaz, F., Kang, S.S., Katsel, P., Miyashita, S., Kannangara, H., Cullen, L., Chan, P., Kuo, T.-C., Padilla, A., Sultana, F., Wizman, S. A., Kramskiy, N., Zaidi, S., Kim, S.-M., New, M.I., Rosen, C.J., Goosens, K.A., Frolinger, T., Haroutunian, V., Ye, K., Lizneva, D., Davies, T.F., Yuen, T., Zaidi, M., 2022. Brain atlas for glycoprotein hormone receptors at single-transcript level. Elife, LHCGR 11, e79612. https://doi.org/10.7554/eLife.79612.

Saberi, S., Du, Y.P., Christie, M., Goldsbury, C., 2013. Human chorionic gonadotropin increases β-cleavage of amyloid precursor protein in SH-SY5Y cells. Cell. Mol. Neurobiol. 33, 747–751. https://doi.org/10.1007/s10571-013-9954-3.

Saleh, R.N.M., Hornberger, M., Ritchie, C.W., Minihane, A.M., 2023. Hormone replacement therapy is associated with improved cognition and larger brain volumes in at-risk APOE4 women: results from the European Prevention of Alzheimer's Disease (EPAD) cohort. Alzheimer's Res. Ther. 15, 10. https://doi.org/10.1186/s13195-022-01121-5.

Sciarretta, C., Minichiello, L., 2010. The preparation of primary cortical neuron cultures and a practical application using immunofluorescent cytochemistry. Methods Mol. Biol. 633, 221–231. https://doi.org/10.1007/978-1-59745-019-5_16.

Shipley, M.M., Mangold, C.A., Szpara, M.L., 2016. Differentiation of the SH-SY5Y human neuroblastoma cell line. J. Vis. Exp. 53193 https://doi.org/10.3791/53193.

Short, R.A., Bowen, R.L., O'Brien, P.C., Graff-Radford, N.R., 2001. Elevated gonadotropin levels in patients with Alzheimer disease. Mayo Clin. Proc. 76, 906–909. https://doi.org/10.4065/76.9.906.

Shumaker, S.A., Legault, C., Rapp, S.R., Thal, L., Wallace, R.B., Ockene, J.K., Hendrix, S. L., Jones, B.N., Assaf, A.R., Jackson, R.D., Kotchen, J.M., Wassertheil-Smoller, S., Wactawski-Wende, J., Investigators, W.H.I.M.S., 2003. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 289, 2651–2662. https://doi.org/10.1001/jama.289.20.2651.

Simões, R.F., Ferrão, R., Silva, M.R., Pinho, S.L.C., Ferreira, L., Oliveira, P.J., Cunha-Oliveira, T., 2021. Refinement of a differentiation protocol using neuroblastoma SH-SY5Y cells for use in neurotoxicology research. Food Chem. Toxicol. 149, 111967 https://doi.org/10.1016/j.fct.2021.111967.

Ullah, R., Park, T.J., Huang, X., Kim, M.O., 2021. Abnormal amyloid beta metabolism in systemic abnormalities and Alzheimer's pathology: insights and therapeutic approaches from periphery. Ageing Res. Rev. 71, 101451 https://doi.org/10.1016/j.arr.2021.101451.

Verdile, G., Laws, S.M., Henley, D., Ames, D., Bush, A.I., Ellis, K.A., Faux, N.G., Gupta, V. B., Li, Q.-X., Masters, C.L., Pike, K.E., Rowe, C.C., Szoeke, C., Taddei, K., Villemagne, V.L., Martins, R.N., AIBL Research Group, 2014. Associations between gonadotropins, testosterone and β amyloid in men at risk of Alzheimer's disease. Mol. Psychiatr. 19, 69–75. https://doi.org/10.1038/mp.2012.147.

Verdile, G., Yeap, B.B., Clarnette, R.M., Dhaliwal, S., Burkhardt, M.S., Chubb, S.A.P., De Ruyck, K., Rodrigues, M., Mehta, P.D., Foster, J.K., Bruce, D.G., Martins, R.N., 2008. Luteinizing hormone levels are positively correlated with plasma amyloid-beta protein levels in elderly men. J Alzheimers Dis 14, 201–208. https://doi.org/10.3233/jad-2008-14208.

Wahjoepramono, E.J., Wijaya, L.K., Taddei, K., Bates, K.A., Howard, M., Martins, G., deRuyck, K., Matthews, P.M., Verdile, G., Martins, R.N., 2011. Direct exposure of Guinea pig CNS to human luteinizing hormone increases cerebrospinal fluid and cerebral beta amyloid levels. Neuroendocrinology 94, 313–322. https://doi.org/10.1159/000330812.

Webster, J.A., Gibbs, J.R., Clarke, J., Ray, M., Zhang, W., Holmans, P., Rohrer, K., Zhao, A., Marlowe, L., Kaleem, M., McCorquodale, D.S., Cuello, C., Leung, D., Bryden, L., Nath, P., Zismann, V.L., Joshipura, K., Huentelman, M.J., Hu-Lince, D., Coon, K.D., Craig, D.W., Pearson, J.V., Heward, C.B., Reiman, E.M., Stephan, D., Hardy, J., Myers, A.J., 2009. Genetic control of human brain transcript expression in Alzheimer disease. Am. J. Hum. Genet. 84, 445–458. https://doi.org/10.1016/j.ajhg.2009.03.011.

Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X., Yu, G., 2021. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. Innovation 2, 100141. https://doi.org/10.1016/j.xinn.2021.100141.

Zhu, D., Montagne, A., Zhao, Z., 2021. Alzheimer's pathogenic mechanisms and underlying sex difference. Cell. Mol. Life Sci. 78, 4907–4920. https://doi.org/10.1007/s00018-021-03830-w.

Zuroff, L., Daley, D., Black, K.L., Koronyo-Hamaoui, M., 2017. Clearance of cerebral Aβ in Alzheimer's disease: reassessing the role of microglia and monocytes. Cell. Mol. Life Sci. 74, 2167–2201. https://doi.org/10.1007/s00018-017-2463-7.