Brain, Behavior, & Immunity - Health 32 (2023) 100663

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Maternal separation differentially modulates early pathology by sex in 5xFAD Alzheimer's disease-transgenic mice

M.G. Garcia [a,b], A. Paulus [a,c], S. Vázquez-Reyes [a], O. Klementieva [c], G.K. Gouras [b], S. Bachiller [a,d,e,*,1], T. Deierborg [a,**,1]

[a] Experimental Neuroinflammation Laboratory, Department of Experimental Medical Science, Lund University, 22184 Lund, Sweden
[b] Experimental Dementia Research Unit, Department of Experimental Medical Science, Lund University, 22184 Lund, Sweden
[c] Medical Microspectroscopy, Department of Experimental Medical Science, Lund University, 22184 Lund, Sweden
[d] Institute of Biomedicine of Seville, IBiS/Virgen del Rocío University Hospital/CSIC/University of Seville/Seville, Spain
[e] Department of Medical Biochemistry, Molecular Biology and Immunology, School of Medicine, University of Seville, Seville, Spain

ARTICLE INFO

Keywords:
Alzheimer's disease
Early-life stress
Maternal separation
Neuroinflammation
Amyloid-β

ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease. Most cases of AD are considered idiopathic and likely due to a combination of genetic, environmental, and lifestyle-related risk factors. Despite occurring decades before the typical age of an AD diagnosis, early-life stress (ELS) has been suggested to have long-lasting effects that may contribute to AD risk and pathogenesis. Still, the mechanisms that underlie the role of ELS on AD risk remain largely unknown. Here, we used 5xFAD transgenic mice to study relatively short-term alterations related to ELS in an AD-like susceptible mouse model at 6 weeks of age. To model ELS, we separated pups from their dams for 3 h per day from postnatal day 2–14. Around 6 weeks of age, we found that maternally separated (MS) 5xFAD mice, particularly female mice, displayed increased amyloid-β-immunoreactivity in the anterior cingulate cortex (ACC) and basolateral amygdala (BLA). In anterior cingulate cortex, we also noted significantly increased intraneuronal amyloid-β-immunoreactivity associated with MS but only in female mice. Moreover, IBA1-positive DAPI density was significantly increased in relation to MS in ACC and BLA, and microglia in BLA of MS mice had significantly different morphology compared to microglia in non-MS 5xFAD mice. Cytokine analysis showed that male MS mice, specifically, had increased levels of neuroinflammatory markers CXCL1 and IL-10 in hippocampal extracts compared to non-MS counterparts. Additionally, hippocampal extracts from both male and female MS 5xFAD mice had decreased levels of synapse- and activity-related markers *Bdnf*, *5htr6*, *Cox2*, and *Syp* in hippocampus. Lastly, we performed behavioral tests to evaluate anxiety- and depressive-like behavior and working memory but could not detect any significant differences between groups. Overall, we detected several sex-specific molecular and cellular alterations in 6-week-old adolescent 5xFAD mice associated with MS that may help explain the connection between ELS and AD risk.

1. Introduction

The majority of Alzheimer's disease (AD) cases are considered idiopathic and are likely due to a combination of genetic, environmental, and lifestyle-related risk factors. AD is the most common cause of dementia and is defined histopathologically by the presence of amyloid-beta (Aβ) plaques and tau neurofibrillary tangles in post-mortem brain. Though AD is typically diagnosed in later life, increasing evidence suggests that the pathogenesis occurs decades before notable cognitive impairment. Early alterations in AD include accumulation of intraneuronal Aβ in neurons, synapse dysfunction and loss, and altered neuronal activity and brain metabolism (Gouras et al., 2000; Mori et al., 2002; Nestor et al., 2003; Scheff et al., 2006; Takahashi et al., 2002, 2004; Terry et al., 1991). In addition, we and others have found neuroinflammatory mechanisms to be important in AD pathogenesis (Boza-Serrano et al., 2019, 2022; Jorfi et al., 2023). Therefore, risk factors that promote such molecular and cellular events, especially in vulnerable brain regions, such structures within the limbic system, may

* Corresponding author. Experimental Neuroinflammation Laboratory, Department of Experimental Medical Science, Lund University, 22184 Lund, Sweden
** Corresponding authorExperimental Neuroinflammation Laboratory, Department of Experimental Medical Science, Lund University, 22184 Lund, Sweden
E-mail addresses: sbachiller-ibis@us.es (S. Bachiller), tomas.deierborg@med.lu.se (T. Deierborg).
[1] Shared senior authorship.

https://doi.org/10.1016/j.bbih.2023.100663
Received 21 March 2023; Received in revised form 15 June 2023; Accepted 28 June 2023
Available online 11 July 2023

promote the development of AD.

Despite occurring decades before a typical AD diagnosis, early-life stress (ELS) has been associated with an increased risk of AD (Corney et al., 2022; Donley et al., 2018; Seifan et al., 2015). However, the mechanisms underlying this connection remain largely unknown. Rodent models of ELS have proven useful for understanding how ELS may promote AD pathogenesis by showing neuroinflammatory alterations, altered neuronal activity, and decreased synaptic activity (Banqueri et al., 2021; Delpech et al., 2016; Hoeijmakers et al., 2017; Wang et al., 2020). Previously, we showed that ELS elicited immediate and long-term effects in wildtype (WT) and 5xFAD AD-transgenic mice on both the peripheral and brain immune systems in a sex-specific manner (Bachiller et al., 2022). Additionally, we observed relatively short-term effects in 6-week-old WT mice exposed to ELS in the form of altered hippocampal microglia and behavior (Bachiller et al., 2020). Here, our aim was to determine the relatively short-term effects that ELS may have in an AD-vulnerable model also at 6 weeks of age.

To model ELS, we used a maternal separation (MS) protocol wherein we separated mouse pups from their dams for 3 h a day from postnatal day 2–14 and looked for alterations in Aβ pathology, neuroinflammation state, synapse- and activity-related gene expression, and behavior. In our analyses, we considered potential sex differences as female and male were impacted by AD and ELS alterations differently (Bachiller et al., 2020, 2022). By utilizing 6-week-old 5xFAD AD-transgenic mice, we could study whether MS affects early Aβ pathology, including intraneuronal Aβ levels, and related alterations, particularly neuroinflammation, changes in synapse- and activity-related markers, and behavior.

2. Methods

2.1. Animals

Hemizygous 5xFAD mice on a B6SJL background (Jackson Laboratory, MMRRC stock #34840) were used in this study. These mice overexpress human amyloid precursor protein containing three mutations (Swedish [K670N, M671L], Florida [I716V], and London [V717I]) and human presenilin-1 with two mutations (M146L and L286V) that are linked to familial Alzheimer's disease (AD) (Oakley et al., 2006). Mice were weaned at postnatal day 30 and then housed in groups of 4–5 of same sex and litter in a room with a 12 h light/dark cycle. *Ad libitum* access to food and water were provided. Cages were also provided with nesting material. Experiments were carried out in accordance with European directive 2010/63/EU and Swedish rules. Ethical permission was given by the Malmö/Lund Ethics Committee on Animal Testing (Dnr. 5.8.18–01107/2018).

Breeding was done by pairing wildtype (B6SJL) female mice with hemizygous 5xFAD male mice (9–12 weeks old). To determine which mice carried the 5xFAD transgenes, DNA from ear punches collected at postnatal day 30 was extracted using an Extract-N-Amp™ Tissue PCR Kit (Sigma-Aldrich, XNAT2). Amplification was done using PCRBIO HS Taq Mix Red (PCRBiosystems, PB10.23) with the primers listed below (Table 1).

2.2. Maternal separation

Maternal separation (MS) was performed as previously described (Bachiller et al., 2020, 2022; Teissier et al., 2020). Pups were separated from their dams daily from postnatal day 2–14, 3 h/day (during the room's light cycle, 9:00 a.m.–12:00 p.m.). MS pups were placed together into a clean cage supplemented with cotton pieces for warmth, and the cage was placed in a separate room to prevent vocalized communication between separated pups and dams. At the end of the session, pups were returned to their home cage with their dams and left undisturbed until the next session. Control (non-MS) pups were handled similarly to MS pups but not separated from their dams. At 6 weeks of age, body weight was measured and differed significantly between sexes but not non-MS/MS groups.

2.3. Transcardial perfusion and tissue retrieval

Postnatal day 43 mice (6 weeks old) were anaesthetized with 5% isoflurane (Virbac) in air and transcardially perfused with a 0.9% saline solution. Brains were split into two hemispheres for different downstream processing: One hemisphere was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4 (Histolab, HL96753.1000) overnight and then placed in a 30% sucrose solution for 48 h at 4 °C for later sectioning into 40 μm-thick coronal slices in a series of 6 using a sliding microtome (Leica Biosystems, SM2000 R). Sections were stored in −20 °C in a cryoprotective solution of 30% sucrose (Merck, 84100) and 30% ethylene glycol (Sigma-Aldrich, 102466) in 40% phosphate-buffered saline (PBS) to prevent ice crystal formation. For the remaining brain hemisphere, the hippocampus was dissected out and snap frozen at −80 °C for later analyses.

2.4. Immunofluorescence

Free-floating 40 μm-thick coronal, half-brain sections were first rinsed in 0.1 M PBS 3 times for 5 min each to wash away the cryoprotective solution. To detect intracellular Aβ, sections were incubated in 88% formic acid (Sigma-Aldrich, 33015) diluted with distilled water for 8 min before being rinsed in 0.1 M PBS with 0.3% v/v Triton X-100 (Sigma-Aldrich, T8787) (0.1 M PBS-Tx). Sections were then blocked in 0.1 M PBS-Tx for 1 h in 0.1 M PBS-Tx with 3% v/v normal donkey serum (Sigma-Aldrich, S30) (NDS). Sections were then incubated with the following primary antibodies for 48 h in 0.1 M PBS-Tx with 0.3% NDS: anti-amyloid-beta peptide 40/42 (MOAB-2, 1:500, Biosensis, M-1586, see Youmans et al., 2012), anti-NeuN (1:1000, Millipore, ABN78), and anti-IBA1 (1:1000, Novus Biologicals, NB100-1028). Sections were rinsed three times for 5 min each with 0.1 M PBS-Tx before being incubated with the respective secondary antibodies for 2 h at room temperature under dark conditions: donkey anti-mouse conjugated to Alexa Fluor 488 (1:500, Invitrogen, A21202), donkey anti-rabbit conjugated to Alexa Fluor 555 (1:500, Invitrogen, A31572), and donkey anti-goat conjugated to Alexa Fluor 647 (1:500, Invitrogen, A21447). Sections were then rinsed twice with 0.1 M PBS-Tx for 10 min each before being incubated with DAPI (1:1000, Sigma-Aldrich, D9542) in 0.1 M PBS for 10 min. Sections were finally rinsed in 0.1 M PBS twice for 10 min each before being mounted onto uncharged Superfrost glass slides (Epredia, AA00000112 E) in 0.05 M PB. SlowFade Glass

Table 1
Primer sequences used for PCR amplification.

Gene	Forward primer sequence (5' - 3')	Reverse primer sequence (5' - 3')
Mouse *App*	CTAGGCCACAGAATTGAAAGATCT	GTAGGTGGAAATTCTAGCATCATCC
Human *APP*	AGGACTGACCACTCGACCAG	CGGGGGTCTAGTTCTGCAT
Human *PSEN1*	AATAGAGAACGGCAGGAGCA	GCCATGAGGGCACTAATCAT

(Invitrogen, S36917) was then applied, and coverslips were sealed using CoverGrip coverslip sealant (Biotium, 23005).

2.5. Image acquisition, analysis, and visualization

Images for analysis of amyloid-beta-immunoreactivity and IBA1-immunoreactivity were acquired using a Nikon Eclipse 80i upright epi-fluorescence microscope using a 20 X objective (air, NA 0.75). Images for anti-IBA1 morphology analysis and representative IBA1 images were obtained using a Leica SP8 laser scanning confocal microscope with a 20 X objective (air, NA 0.75×) and 40× objective (oil, NA 1.3), respectively. The following Bregma ranges were used for the different brain regions: anterior cingulate cortex (ACC) (1.10 to −0.94 mm), basolateral amygdala (BLA) (−0.58 to −2.06), and subiculum (dorsal, −2.46 to −3.80). Image settings were kept constant for all brain regions, and the experimenter was blinded to mouse group.

Image analysis was done in (Fiji is just) ImageJ (Schindelin et al., 2012) with image import done using the Bio-Formats plugin (Linkert et al., 2010). Before applying a binarizing threshold, images were pre-processed to improve thresholding accuracy by applying, for example, subtract background, gaussian blur, gamma adjustment, smooth, despeckle, and unsharp mask. Pre-processing and thresholding was kept constant for a given channel/marker and was determined empirically as the best fit for a set of images in a given brain region and experiment. Thresholding was done using global automatic thresholding. Regions of interest (ROIs) for each brain region were drawn using the polygon tool by an experimenter blinded to the groups. The number of slices/images used for analysis per brain region per mouse were as follows: ≥3 for ACC, ≥2 for BLA, and ≥1 for subiculum. Section quality and availability differed between brain regions even within the same mouse, and fewer images/slices were available for subiculum due to how the tissue was processed. Skeleton analysis was used to investigate microglial morphology from IBA1-immunoreactivity, and the protocol was adapted from Morrison and Filosa (2013). Values obtained from multiple images for a given brain region and mouse were averaged for statistical analysis.

Representative images have the same brightness/contrast for a given marker, brain region and experiment. Note that the Aβ channel has been gamma adjusted for visualization purposes, and gamma was adjusted equally for the images of a given brain region. Original images are available upon request.

2.6. Protein extraction and multiplex immunoassay

Total hippocampal protein was extracted using TRI Reagent (Sigma-Aldrich, T9424) simultaneously with RNA following manufacturer's instructions for protein isolation. Cytokine levels were measured using a Meso Scale Discovery V-PLEX Proinflammatory Panel 1 mouse kit according to manufacturer's instructions (Meso Scale Diagnostics, Rockville, Maryland, USA). This kit works as a sensitive ELISA, and measures the protein levels of IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, KC/GRO (CXCL1), and TNF-α. IFN-γ levels were below the detection limit and not reported here. One mouse was excluded from IL-10 analysis as the IL-10 measurement was below the detection threshold.

2.7. RNA extraction and quantitative real-time PCR analysis

Total hippocampal RNA was extracted using TRI Reagent (Sigma-Aldrich, T9424) simultaneously with protein following manufacturer's instructions for RNA isolation. RNA concentration was measured using a NanoDrop 2000C (Thermo Fisher), and 1 μg of RNA underwent reverse transcription to synthesize cDNA using an iScript cDNA Synthesis Kit (Bio-Rad, 170–8891). RT-qPCR was performed at a final reaction volume of 10 μl, which contained 5 μl of SensiFAST™ SYBR No-Rox Kit (Bioline reagents), 0.4 μl of each forward and reverse primer sequence for a given gene, and 4.2 μl of cDNA. Quantitative PCR was performed in triplicate using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) using the primers listed below (Table 2). Amplification conditions were as follows: 95 °C for 2 min followed by 40 cycles of 95 °C for 5 s, 65 °C for 10s, and 72 °C for 20 s before a final step of 72 °C for 7 min. Gene expression was normalized to beta-actin and presented as ΔCT.

Table 2
Primer sequences used for RT-qPCR.

mRNA	Forward primer sequence (5' - 3')	Reverse primer sequence (5' - 3')
5ht6r	CTTCCTGCTATGCTTGGTGGT	TGTTAGGGTTGAGGTTCAGTCT
Bdnf	GCGGACCCATGGGACTCT	CTGCTGCTGTAGTGACCGA
Cox-2	CCAGCACTTCACCCATCAGTT	ACCCAGGTCCTCGCTTATGA
Gfap	TCCTGGAACAGCAAAACAAG	ATGAAACCAACCTGAGGCTG
Syp	TCTTTGTCACCGTGGCTGTGTT	TCCCTCAGTTCCTTGCATGTGT

2.8. Behavioral tests

Behavioral tests (see Fig. 1A) were performed during their light phase by the same researcher blinded to the maternal separation procedure. Mice were habituated and handled from postnatal day 37–39. Before conducting a test, mice were left in the test room for at least 1 h in order acclimate to the room. Temperature in the testing room was kept constant. Equipment was thoroughly cleaned in between mice with 70% ethanol and dried carefully. Sessions were recorded for later analysis by a researcher blinded to the groups.

2.8.1. Elevated plus maze

To evaluate *anxiety-like* behavior, we used the elevated plus maze. Postnatal day 41 mice were first placed in a closed arm facing away from the center of the maze and then allowed to explore the maze for 5 min. An anxiety index number was calculated based on the proportion of time and entries in the open arms as previously reported (Cohen et al., 2008). Results were calculated from the following equation: anxiety index = 1 – ([(Time spent in open arms/Total test time) + (Number of entries into open arms/Total number of entries)]/2).

2.8.2. Tail suspension test

To assess *depressive-like* behavior, the tail suspension test was performed. Postnatal day 42 mice were suspended by the tail 50 cm above the ground for 5 min. Adhesive tape was used to suspend the mice. Mice were scored based on time spent immobile, defined as hanging passively, lack of body movement, and motionless.

2.8.3. Y-maze

To assess spatial working memory, we used the Y-maze as previously reported (Bachiller et al., 2020). Postnatal day 40 mice were first placed at the end of an arm facing away from the center of the maze. Mice were then allowed to explore the maze for 5 min and were scored on proportion of spontaneous alterations to total alternations. A spontaneous alteration was defined as entry into each of the three arms of the maze over three consecutive alterations.

2.9. Statistics

Statistical analysis was done using GraphPad Prism Version 9 for macOS (GraphPad Software). Two-way ANOVA with an interaction term was performed with the main factors being maternal separation and sex. Multiple comparisons were then performed within the sexes (non-MS – MS) using the Šidák correction. Alpha was set at 0.05. Data is presented as mean ± SD.

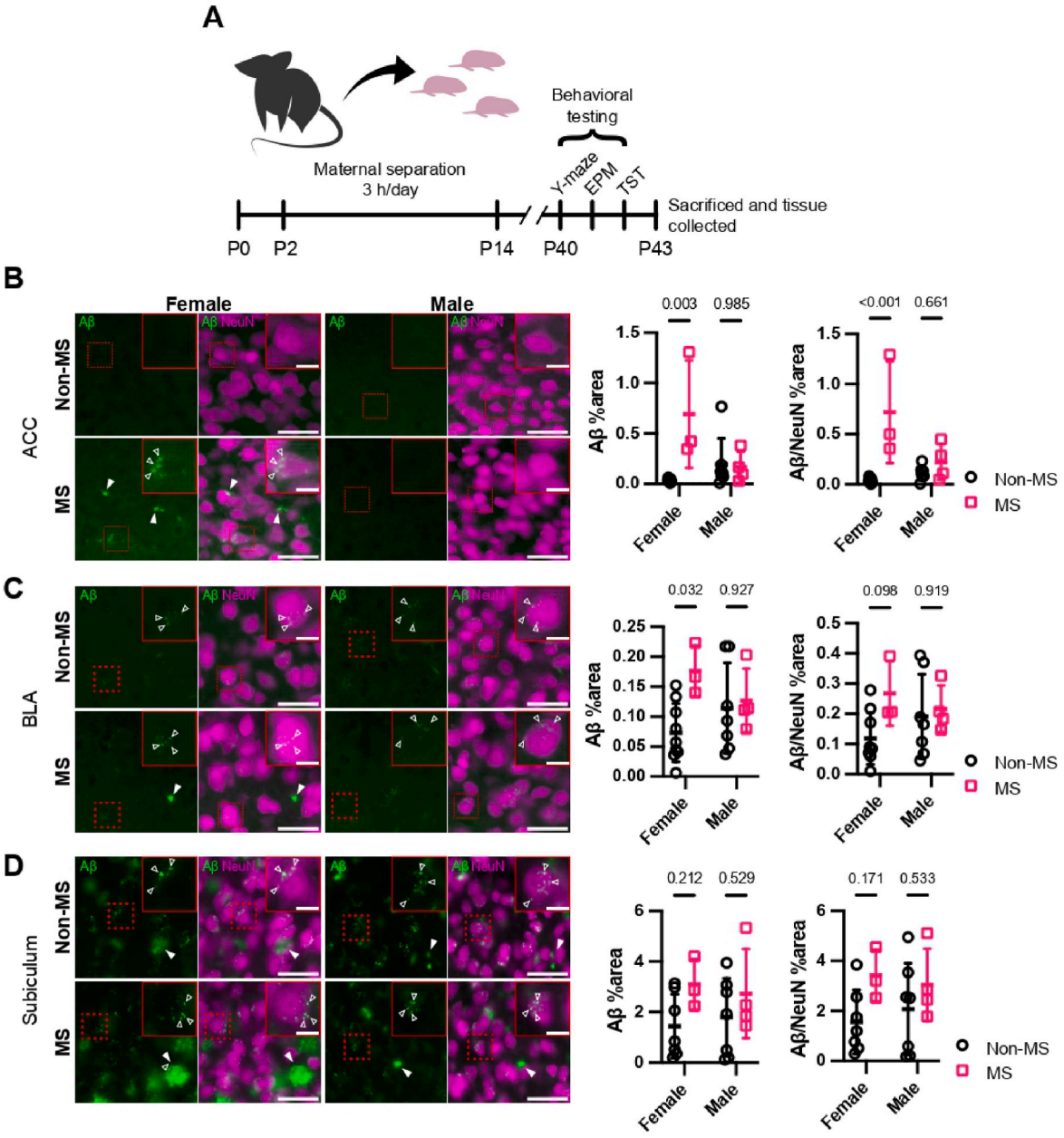

Fig. 1. Maternal separation exacerbates amyloid-β pathology in female 6-week-old 5xFAD mice. (A) Schematic with timeline pointing out maternal separation (MS) and endpoint behavioral testing and tissue collection. EPM = elevated plus maze. TST = tail suspension test. (B–D) Amyloid-β (Aβ)-immunoreactivity using anti-Aβ antibody MOAB2 was measured in anterior cingulate cortex (ACC, B), basolateral amygdala (BLA, C), and subiculum (D). Representative images (left) with inset images (upper right box, solid red line). Red dotted box indicates area blown up in the inset. Solid white arrowheads point to extra-NeuN Aβ-IR. Empty white arrowheads in inset boxes point to intraneuronal NeuN-positive Aβ. Very few/low intensity intraneuronal Aβ puncta were noted in ACC of males and non-MS females. Representative image scalebar = 40 μm. Inset scalebar = 10 μm. Aβ-IR was measured for the whole region (Aβ %area, left graph) and within a NeuN, region-specific mask (Aβ/NeuN %area, right graph). Data points represent an individual mouse. Unfilled black circles represent non-MS mice. Unfilled pink squares represent MS mice. n = 7–9 non-MS/sex/region, 3–4 MS/sex/region. P values from multiple comparisons testing are indicated at the tops of graphs. Error bars indicate mean ± SD

3. Results

3.1. Maternal separation exacerbates early amyloid-β pathology in young female 5xFAD mice

We previously observed an increase in Congo red-positive plaques in the prefrontal cortex of maternally separated (MS) four-month-old female 5xFAD mice compared to their respective controls (Bachiller et al., 2022). Here, we wondered whether alterations in amyloid-β (Aβ) in relation to MS could be detected earlier in 5xFAD mice. 5xFAD mice were subjected to MS from postnatal day (P) 2–14 for 3 h each day (Fig. 1A). At 6 weeks of age (~P42), using the anti-Aβ antibody MOAB-2, we found that the anterior cingulate cortex (ACC) and basolateral amygdala (BLA) in MS mice had increased Aβ-immunoreactivity (IR) region-wide compared to non-MS mice (ACC: $F_{1,17} = 7.42$, $P = 0.0144$; BLA: $F_{1,\ 19} = 4.70$, $P = 0.0432$; Fig. 1B–C, Supp. Table 1). Multiple comparisons testing showed that MS females had significantly increased Aβ %area in ACC ($P = 0.003$, Fig. 1B) and BLA ($P < 0.001$, Fig. 1C) compared to non-MS females, and a significant interaction effect was detected for ACC ($F_{1,\ 17} = 8.60$, $P = 0.0093$, Supp. Table 1). No statistically significant differences were detected in Aβ %area in subiculum (Fig. 1D).

We then measured Aβ-IR within an anti-NeuN-positive, region-specific mask to determine whether MS was associated with altered intraneuronal Aβ levels in 5xFAD mice. We detected significantly different Aβ/NeuN %areas in ACC between MS and non-MS mice ($F_{1, 16} = 17.2$, $P = 0.0008$) and sexes ($F_{1, 16} = 4.96$, $P = 0.0407$) as well as a statistically significant interaction effect ($F_{1, 16} = 9.04$, $P = 0.0084$) (Supp. Table 1). In particular, female MS mice had increased Aβ/NeuN %area compared to female non-MS mice ($P < 0.001$, Fig. 1B). Taken together, our data suggests that early-life stress (ELS) can increase both intraneuronal and extracellular Aβ levels in AD vulnerable models and especially in females.

3.2. Maternal separation promotes limited microglial and cytokine-related neuroinflammatory alterations in young 5xFAD mice

We next wondered whether we could detect any neuroinflammatory alterations associated with MS in these young mice. We first looked at overall IBA-IR and detected no differences in IBA %area in relation to MS in ACC, BLA, nor subiculum (Fig. 2A–C, Supp. Table 2) though we did detect a significant interaction effect ($F_{1, 21} = 6.54$, $P = 0.0183$) on IBA-IR in BLA. In addition, sex was a significant main effect on IBA % area in subiculum ($F_{1, 18} = 5.06$, $P = 0.0373$, Fig. 2C). We then looked at microglial density by counting DAPI-positive nuclei within an IBA1-IR mask and normalizing by the area of the region of interest. There we found that MS had a significant effect on microglial density in ACC ($F_{1,14} = 5.43$, $P = 0.0353$) and BLA ($F_{1,14} = 33.5$, $P < 0.0001$) but not subiculum (Supp. Table 2). Multiple comparisons testing showed that MS had a significant effect on microglial density in female ($P < 0.001$) and male ($P = 0.005$) 5xFAD mice but only in BLA (Fig. 2B). Following this, we examined whether microglial morphology was affected by MS, using IBA1-IR branch length and number of process endpoints normalized to cell counts as measures. In BLA, MS had a significant effect on branch length per cell ($F_{1,14} = 20.5$, $P < 0.001$), and both sex ($F_{1,14} = 4.74$, $P = 0.047$) and MS ($F_{1,14} = 35.9$, $P < 0.001$) were significant main effects on number of process endpoints per cell (Supp. Table 2). Furthermore, multiple comparisons testing noted that IBA1-IR microglia in ACC of female MS mice had fewer process endpoints per cell compared to non-MS female mice ($P = 0.042$, Fig. 2A). In BLA, branch length and number of endpoints per cell were significantly decreased in both female ($P = 0.002$ and $P < 0.001$, respectively) and male MS mice ($P = 0.048$ and $P = 0.033$, respectively) compared to non-MS counterparts (Fig. 2B).

To look at possible cytokine alterations, we extracted total protein from hippocampal tissue and analyzed neuroinflammatory marker levels in a multiplex immunoassay. Of the markers tested, CXCL1 had a statistically significant interaction factor ($F_{1, 15} = 7.07$, $P = 0.0179$, Supp. Table 3). Multiple comparisons showed that MS within male but not female mice had significant effects on CXCL1 ($P = 0.014$, Fig. 3) and IL-10 ($P = 0.041$, Fig. 3) levels, though no statistically differences in IL-10 levels were detected at the factor level (Supp. Table 3). In sum, these data suggest sex-specific microglial and cytokine responses to ELS with female mice altering microglial density and form and male mice exhibiting increased cytokine levels.

3.3. Maternal separation reduces expression of synapse- and activity-related markers in hippocampus

We previously observed significantly decreased *Cox2* and *Syp* gene expression with MS in both female and male 6-week-old WT mice (Bachiller et al., 2020). Additionally, we observed decreased *Bdnf* expression in MS male 4-month-old WT and 5xFAD mice (Bachiller et al., 2022). Therefore, we wondered whether these expression patterns would be similar in 6-week-old 5xFAD mice. Of the genes that we tested, *5ht6r* ($F_{1, 13} = 30.5$, $P < 0.0001$), *Bdnf* ($F_{1, 13} = 21.3$, $P = 0.0005$), *Cox2* ($F_{1, 13} = 20.1$, $P = 0.0006$), and *Syp* ($F_{1, 13} = 24.1$, $P = 0.0003$) expression but not but not *Gfap* expression were significantly decreased in MS 5xFAD mice compared to non-MS 5xFAD mice (Fig. 4A–E, Supp. Table 4). Of the markers with significantly decreased expression, mtuliple comparisons testing showed that the following markers were significantly different in both female and male MS mice compared to their non-MS counterparts: *5ht6r* (female: $P = 0.005$; male: $P = 0.002$), *Bdnf* (female: $P = 0.021$; male: $P = 0.007$), and *Syp* (female: $P = 0.023$; male: $P = 0.003$) (Fig. 4A and B,E). Only female MS mice exhibited a significant decrease of *Cox2* expression compared to non-MS female mice ($P = 0.003$) (Fig. 4C).

3.4. Maternal separation does not significantly affect behavior by 6 weeks of age

Lastly, we looked at whether the cellular and molecular alterations observed with MS could affect behavioral outcomes measured in 6-week-old 5xFAD mice. First, working memory was evaluated using the Y-maze (Fig. 5A). Contrary to what we observed in 6-week-old WT MS male mice (Bachiller et al., 2020), we did not find a significant difference in spontaneous alternations (%) in the 5xFAD mice in relation to MS nor sex (Supp. Table 5). Using the elevated plus maze, we observed no sex- nor MS-related differences in the anxiety index (%) in 5xFAD mice (Fig. 5B, Supp. Table 5). Finally, with the tail suspension test, we measured the percentage of the time spent immobile as an indicator of *depressive-like* behavior but found no significant differences between groups (Fig. 5C, Supp. Table 5). Overall, our results suggest that MS did not have a significant effect on short-term behavioral outcomes in 6-week-old 5xFAD mice.

4. Discussion

In this study, we sought to understand whether maternal separation (MS) could exacerbate pathological processes in 6-week-old Alzheimer's disease (AD)-vulnerable model mice. To that end, we utilized an MS protocol wherein we separated pups from their dams from postnatal day (P) 2–14 for 3 h per day. We applied this model for early-life stress (ELS) to 5xFAD AD-transgenic mice and probed for molecular and cellular alterations as well as behavioral differences at 6 weeks of age. Here, we measured Aβ labeling in the anterior cingulate cortex (ACC), basolateral amygdala (BLA), and subiculum of 6-week-old 5xFAD mice. ACC and BLA are vulnerable regions in ELS (Qin et al., 2021; Zhai et al., 2019), and the subiculum is a region affected particularly early in 5xFAD mice (Oakley et al., 2006). We observed that, in female mice, MS was associated with overall increased Aβ labeling in ACC and BLA. Additionally, MS mice showed an increase in intraneuronal Aβ levels in ACC neurons. We had previously seen immediate (P15 mice) and long-term (4-month-old mice) neuroinflammatory alterations in WT and 5xFAD mice with MS in a sex-dependent manner (Bachiller et al., 2020, 2022), so here we measured microglial coverage and morphology and cytokine expression in these adolescent 6-week-old 5xFAD mice. We found an effect of MS on microglial density and morphology, especially in ACC and BLA of female mice, and increased CXCL1 and IL-10 levels specifically male mice. Moreover, we found significant decreases in synapse and activity-related gene expression of *5ht6r*, *Bdnf*, *Cox2*, and *Syp*. Ultimately, though we observed molecular and cellular alterations in mice subjected to MS, the combined effect was not likely sufficient to induce changes that we could detect on the behavioral level.

5xFAD AD-transgenic mice are often considered an aggressive model in terms of pathological development as they are known to develop amyloid-β (Aβ) plaques beginning around 2 months of age (Oakley et al., 2006). Prior to plaque formation, aggregated intracellular Aβ can be detected in neurons around 1.5 months of age, especially cortical layer V and subicular pyramidal neurons. This rapid pathological development is practical when studying early Aβ pathology, especially intracellular Aβ. Plaque load is often used as a metric for degree of pathology, but plaques are a poor correlate of cognitive decline in AD (Nelson et al., 2012). In contrast, synapse loss is a better correlate of cognitive decline

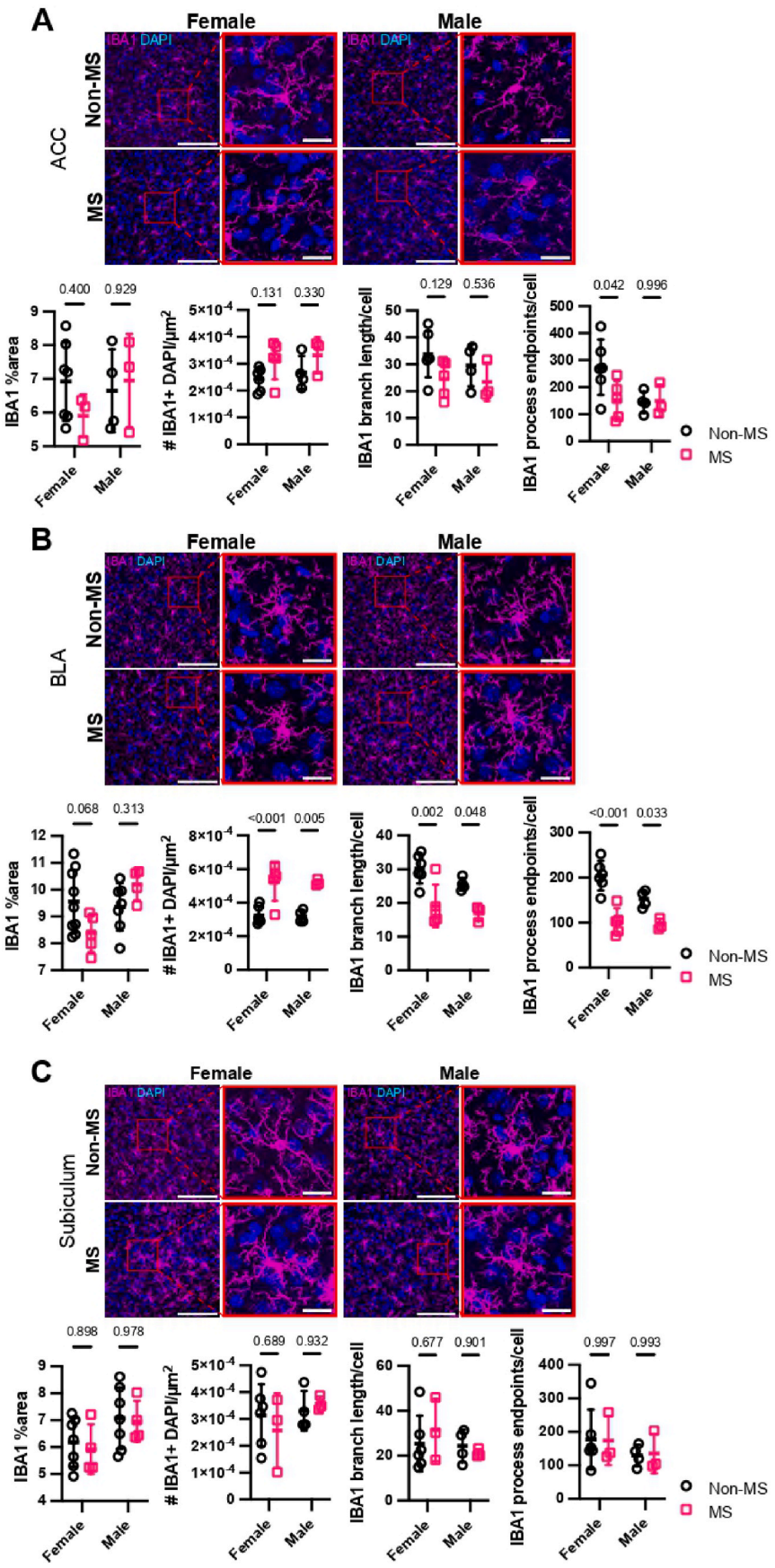

Fig. 2. Maternal separation alters IBA1 morphology in 6-week-old 5xFAD mice. (AC) IBA1-immunoreactive microglia were measured in anterior cingulate cortex (ACC, A), basolateral amygdala (BLA, B), and subiculum (C). Representative images are maximum intensity projections. Red square indicates a blow up of a representative IBA1- IR microglial cell. Lower magnification image scale bar = 100 µm. Blow up image scalebar = 20 µm. Data points represent an individual mouse. Unfilled black circles represent non-MS mice. Unfilled pink squares represent MS mice. n = 4–9 nonMS/sex/region, 3–5 MS/sex/region. P values from multiple comparisons testing are indicated at the tops of graphs. Error bars indicate mean ± SD

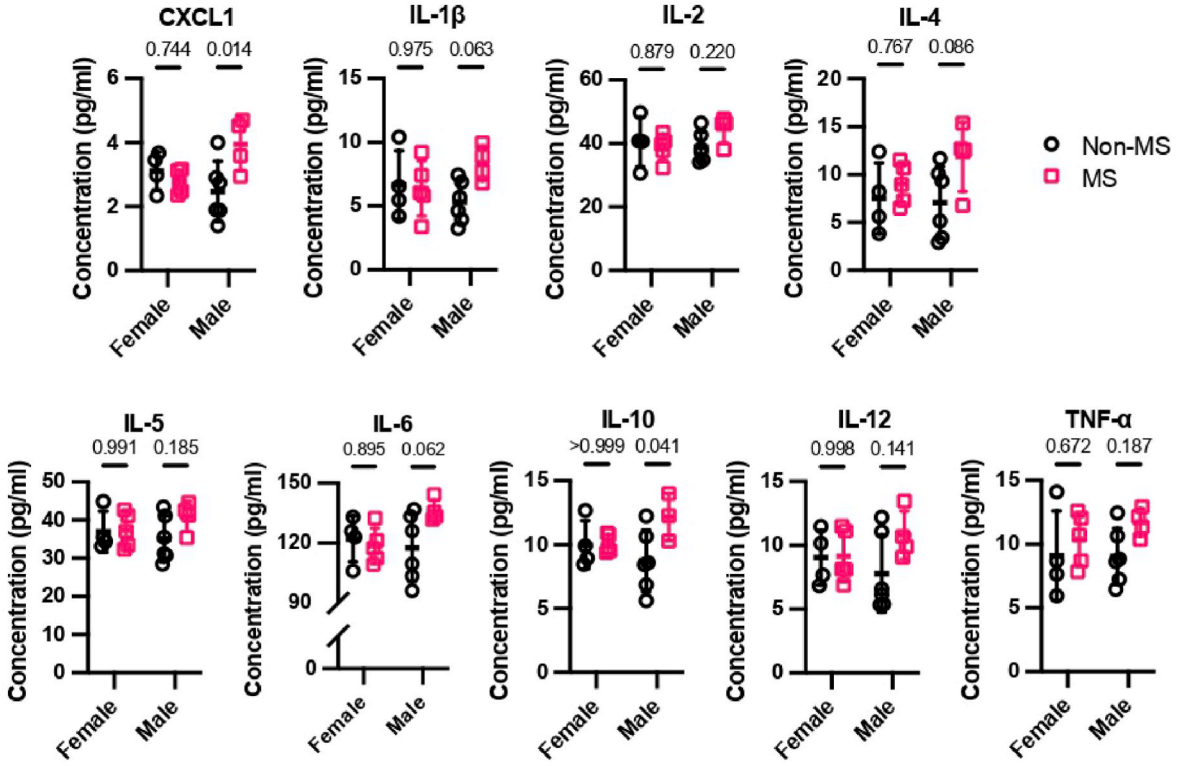

Fig. 3. Maternal separation alters cytokine levels in 6-week-old male 5xFAD mice. Multiplex immunoassay results using total protein extracted from hippocampal tissue. Data points represent an individual mouse. Unfilled black circles represent non-MS mice. Unfilled pink squares represent MS mice. n = 4–6 non-MS/sex, 3–5 MS/sex. P values from multiple comparisons testing are indicated at the tops of graphs. Error bars indicate mean ± SD

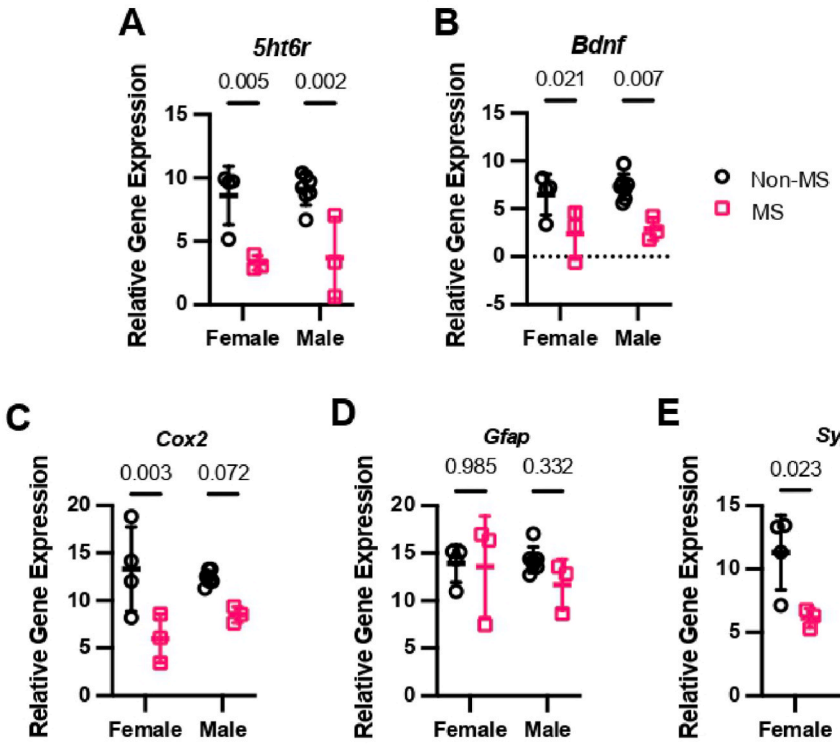

Fig. 4. Maternal separation reduces synapse and activity-related gene expression in hippocampus of 6-week-old 5xFAD mice. Gene expression levels of 5ht6r (A), Bdnf (B), Cox2 (C), Gfap (D), and Syp (E) were measured from total mRNA extracted from hippocampal tissue from 6-week-old 5xFAD mice by RT-qPCR. Data points represent an individual mouse. Unfilled black circles represent non-MS mice. Unfilled pink squares represent MS mice. n = 4–7 non-MS/sex, 3 MS/sex. P values from multiple comparisons testing are indicated at the tops of graphs. Error bars indicate mean ± SD

(Terry et al., 1991), and intraneuronal Aβ correlates with synaptic dysfunction (Bayer and Wirths, 2010; Takahashi et al., 2002). Therefore, we also measured intracellular Aβ in addition to overall Aβ and found increased Aβ in ACC and BLA in MS subjected groups. Additionally, we noted decreased gene expression of serotonin 6 receptor (*5ht6r*), brain derived neurotrophic factor (*Bdnf*), cyclooxygenase-2 (*Cox2*), and pre-synaptic protein synaptophysin (*Syp*) in hippocampal extract from MS 5xFAD mice compared to non-MS 5xFAD mice. Though we did not note

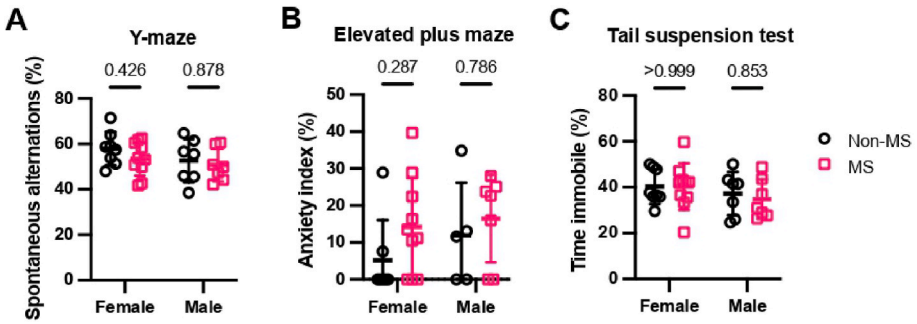

Fig. 5. Maternal separation does not affect behavioral outcomes in 6-week-old 5xFAD mice. (A) Elevated plus maze was used to assess anxiety-like behavior via anxiety index. (B) Tail suspension test was used to evaluate depressive-like behavior by measuring percent of time spent immobile. (C) Spatial working memory was assessed using Y-maze and analyzed by spontaneous alterations. Data points represent an individual mouse. Unfilled black circles represent non-MS mice. Unfilled pink squares represent MS mice. n = 6–8 non-MS/sex, 7–10 MS/sex. P values from multiple comparisons testing are indicated at the tops of graphs. Error bars indicate mean ± SD

increased intraneuronal Aβ in subiculum, a component of the hippocampal formation, a limitation in measuring intraneuronal Aβ is focusing on the cell soma. It may be more informative to measure intraneuronal Aβ levels in synaptic terminals as this is where intraneuronal Aβ may have greater pathological impact (Takahashi et al., 2002, 2004). However, measuring intraneuronal Aβ *in vivo* in the cell soma is relatively easier as detection in synapses often require more specialized or advanced techniques, such as electron microscopy (Gouras et al., 2012).

Interestingly, another study looking at the effects of ELS on APP/PS1 AD-transgenic mice also looked at intracellular Aβ, which they referred to as cell-associated amyloid, and found decreased immunoreactivity in dentate gyrus of 4-month-old ELS mice (Hoeijmakers et al., 2017). However, it is important to note that, aside from the different model, their use of the antibody 6E10 to detect Aβ confounds their results as this antibody clone cross-reacts with full-length amyloid precursor protein (APP) and β-cleaved C-terminal fragment (βCTF) (Horikoshi et al., 2004). βCTF is a product of β-processing of APP, and Aβ is produced after γ-cleavage of βCTF. Both APP and βCTF likely have their own roles to play under physiological and pathological conditions (Martinsson et al., 2019; Nixon, 2017). Considering this, interpretation of their results relative to ours would need to be done with caution.

One way that ELS could impact Aβ levels is via synaptic activity. With ELS, neural activity has been observed to be altered long-term, often leading to hyperexcitability in stress-vulnerable regions (Fóscolo et al., 2022; Lesuis et al., 2021; Qin et al., 2021). In conjunction with this, synaptic activity or the lack thereof has been known to affect APP processing and Aβ clearance (Cirrito et al., 2005, 2008; Hettinger et al., 2018; Tampellini et al., 2009, 2010). Indeed, acute and chronic stress in Tg2576 AD-transgenic mice has been observed to increase interstitial fluid levels of Aβ, and pharmacologically decreasing neuronal activity before acute stress prevents the interstitial Aβ increase (Kang et al., 2007). Here, we looked at intraneuronal Aβ in addition to extracellular Aβ. Given that we found increased intraneuronal Aβ in ACC in female mice and that APP processing to produce Aβ occurs within the endosome-lysosome system (Das et al., 2016; Koo and Squazzo, 1994), we speculate that MS increased Aβ production via altered neuronal activity to the extent that Aβ could aggregate within endosomal compartments and impair neuronal function. Such an event in the human brain may not directly cause AD but may compound with other factors to promote the disease process.

Alongside Aβ pathology, neuroinflammation is involved in AD pathology and has also been noted as being promoted by ELS. Regarding AD, large population-based studies have shed light on factors that modulate AD risk that are associated with the neuroimmune system, and experimental studies that have furthered our understanding of the mechanisms underlying the microglial role in the pathology (for a review, see Leng and Edison, 2021). Additionally, studies in rodent models of AD have suggested neuroinflammatory alterations begin even before the appearance of plaques, though the specific triggers remain largely unknown (Boza-Serrano et al., 2018; Hong et al., 2016; Welikovitch et al., 2020). Like Aβ processing, microglia are sensitive to changes in synaptic activity and have roles in regulating it (Umpierre and Wu, 2021). Moreover, experimental studies have shown that ELS can alter microglial development, promote microglial reactivity, and even affect the peripheral immune system (Bachiller et al., 2020, 2022; Delpech et al., 2016; Hoeijmakers et al., 2017; Roque et al., 2014, 2016). Taken together, microglia impacted by ELS may then have an altered capability to react to and modulate synaptic activity as well as other events within the brain. Given this, neuroinflammation may be another important link connecting ELS and AD risk.

Moreover, sex-related differences are evident in both AD and ELS as well as throughout the brain. With AD, women are disproportionately affected and have faster cognitive decline compared to men (for a review, see Ferretti et al., 2018). With ELS, both females and males are affected though in different ways. Rodent models shed light sex-specific effects in response to ELS, such as altered prefrontal cortex neurochemistry and connectivity (Colich et al., 2017; Farrell et al., 2016; Perry et al., 2021; White et al., 2020) and central and peripheral immune activation (Bachiller et al., 2020, 2022). Interestingly, in relation to sex-differences in the prefrontal cortex, BLA-projecting neurons showed increased dendritic arborization in response to stress in estrogen-treated ovariectomized female rats with no such remodeling seen in the same neurons in male rats due to stress (Shansky et al., 2009, 2010). Synaptic activity plays a large role in dendritic growth and remodeling (McAllister, 2000; Wong and Ghosh, 2002), and as mentioned previously, neuronal activity affects APP/Aβ production and metabolism. Therefore, it is tempting to speculate that the increased Aβ levels in ACC and BLA of female MS mice that we observed is consistent with the observations that Shansky et al. have made. Nevertheless, beyond ELS and AD, sex differences are prevalent throughout the brain and are important to consider in research and beyond (McEwen and Milner, 2017).

Though AD is the most common cause of dementia, reliable treatments that can prevent or modify the progression of the disease, for the most part, do not exist. Recently, the Food and Drug Administration in the US accelerated approval for lecanemab, an antibody treatment targeting soluble Aβ protofibrils, for early AD (Commissioner, 2023). In addition to reducing amyloid burden, lecanemab had a moderate effect on slowing cognitive decline, which is ultimately the goal of an AD treatment (Eisai Co., Ltd., 2022; van Dyck et al., 2023). However, given that idiopathic AD is multifactorial, understanding the factors that increase AD risk and the mechanisms underlying their impact is important in future attempts to modulate the disease. Similar to how the etiology of AD is complex, the sequalae of ELS in humans will likely vary due to a number of factors, such as genetic background, ethnicity, cultural background, and socioeconomic status (Gilsanz et al., 2019; Zuelsdorff et al., 2020). Ultimately, the mechanisms that the brain adopts in response to ELS worth understanding to advance towards a more effective AD therapy.

Funding

This study was funded by grants from the Swedish Research Council (Vetenskapsrådet), Olle Engkvists Foundation (Olle Engkvists stiftelse), the Swedish Alzheimer's Foundation (Alzheimerfonden), the Swedish Brain Foundation (Hjärnfonden), the Craaford Foundation, the Swedish Dementia Association (Demensförbundet), the Greta and Johan Kocks Foundation (Kockska stiftelserna), the Royal Physiographic Society of Lund, the Fredrik Ingrid Thurings Foundation (Thurings stiftelse), the Anna and Edwin Berger Foundation (Anna och Edwin Bergers stiftelse), the Swedish Parkinson Foundation (Parkinsonfonden), and the Strategic Research Area MultiPark at Lund University. SB was also supported by Ramón y Cajal 2021 research grant (RYC-2021-031661-I, funded by MCIN/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR).

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.

Data availability

Data will be made available on request.

Acknowledgements

We thank Bodil Israelsson for invaluable technical support, the Center for Comparative Medicine at Lund University for their animal facilities, Lund University Bioimaging Center for access to their advanced light microscopes, and the special research area MultiPark at Lund University for access to their confocal microscope and mouse behavioral platform.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2023.100663.

References

Bachiller, S., Hidalgo, I., Garcia, M.G., Boza-Serrano, A., Paulus, A., Denis, Q., Haikal, C., Manouchehrian, O., Klementieva, O., Li, J.Y., Pronk, C.J., Gouras, G.K., Deierborg, T., 2022. Early-life stress elicits peripheral and brain immune activation differently in wild type and 5xFAD mice in a sex-specific manner. J. Neuroinflammation 19, 151. https://doi.org/10.1186/s12974-022-02515-w.

Bachiller, S., Paulus, A., Vázquez-Reyes, S., García-Domínguez, I., Deierborg, T., 2020. Maternal separation leads to regional hippocampal microglial activation and alters the behavior in the adolescence in a sex-specific manner. Brain Behav. Immun. - Health 9, 100142, 10/gkddw3.

Banqueri, M., Gutiérrez-Menéndez, A., Méndez, M., Conejo, N.M., Arias, J.L., 2021. Early life stress due to repeated maternal separation alters the working memory acquisition brain functional network. Stress 24, 87–95. https://doi.org/10.1080/10253890.2020.1777974.

Bayer, T.A., Wirths, O., 2010. Intracellular accumulation of amyloid-beta – a predictor for synaptic dysfunction and neuron loss in alzheimer's disease. Front. Aging Neurosci. 2 https://doi.org/10.3389/fnagi.2010.00008.

Boza-Serrano, A., Ruiz, R., Sanchez-Varo, R., García-Revilla, J., Yang, Y., Jimenez-Ferrer, I., Paulus, A., Wennström, M., Vilalta, A., Allendorf, D., Davila, J.C., Stegmayr, J., Jiménez, S., Roca-Ceballos, M.A., Navarro-Garrido, V., Swanberg, M., Hsieh, C.L., Real, L.M., Englund, E., Linse, S., Leffler, H., Nilsson, U.J., Brown, G.C., Gutierrez, A., Vitorica, J., Venero, J.L., Deierborg, T., 2019. Galectin-3, a novel endogenous TREM2 ligand, detrimentally regulates inflammatory response in Alzheimer's disease. Acta Neuropathol. 138, 251–273, 10/gghwbg.

Boza-Serrano, A., Vrillon, A., Minta, K., Paulus, A., Camprubí-Ferrer, L., Garcia, M., Andreasson, U., Antonell, A., Wennström, M., Gouras, G., Dumurgier, J., Cognat, E., Molina-Porcel, L., Balasa, M., Vitorica, J., Sánchez-Valle, R., Paquet, C., Venero, J.L., Blennow, K., Deierborg, T., 2022. Galectin-3 is elevated in CSF and is associated with Aβ deposits and tau aggregates in brain tissue in Alzheimer's disease. Acta Neuropathol. (Berl. https://doi.org/10.1007/s00401-022-02469-6.

Boza-Serrano, A., Yang, Y., Paulus, A., Deierborg, T., 2018. Innate immune alterations are elicited in microglial cells before plaque deposition in the Alzheimer's disease mouse model 5xFAD. Sci. Rep. 8, 1550. https://doi.org/10.1038/s41598-018-19699-y.

Cirrito, J.R., Kang, J.-E., Lee, J., Stewart, F.R., Verges, D.K., Silverio, L.M., Bu, G., Mennerick, S., Holtzman, D.M., 2008. Endocytosis is required for synaptic activity-dependent release of amyloid-β in vivo. Neuron 58, 42–51. https://doi.org/10.1016/j.neuron.2008.02.003.

Cirrito, J.R., Yamada, K.A., Finn, M.B., Sloviter, R.S., Bales, K.R., May, P.C., Schoepp, D. D., Paul, S.M., Mennerick, S., Holtzman, D.M., 2005. Synaptic activity regulates interstitial fluid amyloid-β levels in vivo. Neuron 48, 913–922. https://doi.org/10.1016/j.neuron.2005.10.028.

Cohen, H., Matar, M.A., Buskila, D., Kaplan, Z., Zohar, J., 2008. Early post-stressor intervention with high-dose corticosterone attenuates posttraumatic stress response in an animal model of posttraumatic stress disorder. Biol. Psychiatr. 64, 708–717. https://doi.org/10.1016/j.biopsych.2008.05.025.

Colich, N.L., Williams, E.S., Ho, T.C., King, L.S., Humphreys, K.L., Price, A.N., Ordaz, S. J., Gotlib, I.H., 2017. The association between early life stress and prefrontal cortex activation during implicit emotion regulation is moderated by sex in early adolescence. Dev. Psychopathol. 29, 1851–1864. https://doi.org/10.1017/S0954579417001444.

Commissioner, O. Of the, 2023. FDA Grants Accelerated Approval for Alzheimer's Disease Treatment [WWW Document]. FDA. URL https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-alzheimers-disease-treatment (accessed 3.15.23).

Corney, K.B., West, E.C., Quirk, S.E., Pasco, J.A., Stuart, A.L., Manavi, B.A., Kavanagh, B. E., Williams, L.J., 2022. The relationship between adverse childhood experiences and alzheimer's disease: a systematic review. Front. Aging Neurosci. 14, 831378 https://doi.org/10.3389/fnagi.2022.831378.

Das, U., Wang, L., Ganguly, A., Saikia, J.M., Wagner, S.L., Koo, E.H., Roy, S., 2016. Visualizing APP and BACE-1 approximation in neurons yields insight into the amyloidogenic pathway. Nat. Neurosci. 19, 55–64. https://doi.org/10.1038/nn.4188.

Delpech, J.-C., Wei, L., Hao, J., Yu, X., Madore, C., Butovsky, O., Kaffman, A., 2016. Early life stress perturbs the maturation of microglia in the developing hippocampus. Brain Behav. Immun. 57, 79–93. https://doi.org/10.1016/j.bbi.2016.06.006.

Donley, G.A.R., Lönnroos, E., Tuomainen, T.-P., Kauhanen, J., 2018. Association of childhood stress with late-life dementia and Alzheimer's disease: the KIHD study. Eur. J. Publ. Health 28, 1069–1073. https://doi.org/10.1093/eurpub/cky134.

Eisai Co, Ltd, 2022. Lecanemab confirmatory phase 3 clarity ad study met primary endpoint, showing highly statistically significant reduction of clinical decline in large global clinical study of 1,795 participants with early Alzheimer's disease [WWW Document]. Eisai Co Ltd. https://www.eisai.com/news/2022/news202271.html

Farrell, M.R., Holland, F.H., Shansky, R.M., Brenhouse, H.C., 2016. Sex-specific effects of early life stress on social interaction and prefrontal cortex dendritic morphology in young rats. Behav. Brain Res. 310, 119–125. https://doi.org/10.1016/j.bbr.2016.05.009.

Ferretti, M.T., Iulita, M.F., Cavedo, E., Chiesa, P.A., Schumacher Dimech, A., Santuccione Chadha, A., Baracchi, F., Girouard, H., Misoch, S., Giacobini, E., Depypere, H., Hampel, H., 2018. Sex differences in Alzheimer disease — the gateway to precision medicine. Nat. Rev. Neurol. 14, 457–469. https://doi.org/10.1038/s41582-018-0032-9.

Fóscolo, D.R.C., Lima, P.M.A., Rodovalho, G.V., Coimbra, C.C., 2022. Early maternal separation alters the activation of stress-responsive brain areas in adulthood. Neurosci. Lett. 771, 136464 https://doi.org/10.1016/j.neulet.2022.136464.

Gilsanz, P., Mayeda, E.R., Glymour, M.M., Charles, P., Quesenberry, J., Mungas, D., DeCarli, C.S., Whitmer, R.A., 2019. Birth in high infant mortality states and dementia risk in a cohort of elderly african American and white health care members. Alzheimer Dis. Assoc. Disord. 33, 1. https://doi.org/10.1097/WAD.0000000000000270.

Gouras, G.K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., Greenfield, J.P., Haroutunian, V., Buxbaum, J.D., Xu, H., Greengard, P., Relkin, N.R., 2000. Intraneuronal Aβ42 accumulation in human brain. Am. J. Pathol. 156, 15–20. https://doi.org/10.1016/S0002-9440(10)64700-1.

Gouras, G.K., Willén, K., Tampellini, D., 2012. Critical role of intraneuronal Aβ in Alzheimer's disease: technical challenges in studying intracellular Aβ. Life Sci. 91, 1153–1158. https://doi.org/10.1016/j.lfs.2012.06.004.

Hettinger, J.C., Lee, H., Bu, G., Holtzman, D.M., Cirrito, J.R., 2018. AMPA-ergic regulation of amyloid-β levels in an Alzheimer's disease mouse model. Mol. Neurodegener. 13, 22. https://doi.org/10.1186/s13024-018-0256-6.

Hoeijmakers, L., Ruigrok, S.R., Amelianchik, A., Ivan, D., van Dam, A.-M., Lucassen, P.J., Korosi, A., 2017. Early-life stress lastingly alters the neuroinflammatory response to amyloid pathology in an Alzheimer's disease mouse model. Brain. Behav. Immun., Perinatal Inflammation 63, 160–175, 10/f9j7cq.

Hong, S., Beja-Glasser, V.F., Nfonoyim, B.M., Frouin, A., Li, S., Ramakrishnan, S., Merry, K.M., Shi, Q., Rosenthal, A., Barres, B.A., Lemere, C.A., Selkoe, D.J., Stevens, B., 2016. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science 352, 712–716. https://doi.org/10.1126/science.aad8373.

Horikoshi, Y., Sakaguchi, G., Becker, A.G., Gray, A.J., Duff, K., Aisen, P.S., Yamaguchi, H., Maeda, M., Kinoshita, N., Matsuoka, Y., 2004. Development of Aβ terminal end-specific antibodies and sensitive ELISA for Aβ variant. Biochem. Biophys. Res. Commun. 319, 733–737. https://doi.org/10.1016/j.bbrc.2004.05.051.

Jorfi, M., Maaser-Hecker, A., Tanzi, R.E., 2023. The neuroimmune axis of Alzheimer's disease. Genome Med. 15, 6. https://doi.org/10.1186/s13073-023-01155-w.

Kang, J.-E., Cirrito, J.R., Dong, H., Csernansky, J.G., Holtzman, D.M., 2007. Acute stress increases interstitial fluid amyloid-β via corticotropin-releasing factor and neuronal

activity. Proc. Natl. Acad. Sci. USA 104, 10673–10678. https://doi.org/10.1073/pnas.0700148104.
Koo, E.H., Squazzo, S.L., 1994. Evidence that production and release of amyloid beta-protein involves the endocytic pathway. J. Biol. Chem. 269, 17386–17389.
Leng, F., Edison, P., 2021. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? Nat. Rev. Neurol. 17, 157–172. https://doi.org/10.1038/s41582-020-00435-y.
Lesuis, S.L., Lucassen, P.J., Krugers, H.J., 2021. Early life stress amplifies fear responses and hippocampal synaptic potentiation in the APPswe/PS1dE9 Alzheimer mouse model. Neuroscience, Lifestyle and Brain Metaplasticity 454, 151–161. https://doi.org/10.1016/j.neuroscience.2019.07.012.
Linkert, M., Rueden, C.T., Allan, C., Burel, J.-M., Moore, W., Patterson, A., Loranger, B., Moore, J., Neves, C., MacDonald, D., Tarkowska, A., Sticco, C., Hill, E., Rossner, M., Eliceiri, K.W., Swedlow, J.R., 2010. Metadata matters: access to image data in the real world. J. Cell Biol. 189, 777–782. https://doi.org/10.1083/jcb.201004104.
Martinsson, I., Capetillo-Zarate, E., Faideau, M., Willén, K., Esteras, N., Frykman, S., Tjernberg, L.O., Gouras, G.K., 2019. APP depletion alters selective pre- and post-synaptic proteins. Mol. Cell. Neurosci. 95, 86–95, 10/ggktnr.
McAllister, A.K., 2000. Cellular and molecular mechanisms of dendrite growth. Cerebr. Cortex 10, 963–973. https://doi.org/10.1093/cercor/10.10.963.
McEwen, B.S., Milner, T.A., 2017. Understanding the broad influence of sex hormones and sex differences in the brain. J. Neurosci. Res. 95, 24–39. https://doi.org/10.1002/jnr.23809.
Mori, C., Spooner, E.T., Wisniewski, K.E., Wisniewski, T.M., Yamaguchi, H., Saido, T.C., Tolan, D.R., Selkoe, D.J., Lemere, C.A., 2002. Intraneuronal Aβ42 accumulation in Down syndrome brain. Amyloid 9, 88–102, 10/gmqgv9.
Morrison, H.W., Filosa, J.A., 2013. A quantitative spatiotemporal analysis of microglia morphology during ischemic stroke and reperfusion. J. Neuroinflammation 10, 782. https://doi.org/10.1186/1742-2094-10-4.
Nelson, P.T., Alafuzoff, I., Bigio, E.H., Bouras, C., Braak, H., Cairns, N.J., Castellani, R.J., Crain, B.J., Davies, P., Del Tredici, K., Duyckaerts, C., Frosch, M.P., Haroutunian, V., Hof, P.R., Hulette, C.M., Hyman, B.T., Iwatsubo, T., Jellinger, K.A., Jicha, G.A., Kövari, E., Kukull, W.A., Leverenz, J.B., Love, S., Mackenzie, I.R., Mann, D.M., Masliah, E., McKee, A.C., Montine, T.J., Morris, J.C., Schneider, J.A., Sonnen, J.A., Thal, D.R., Trojanowski, J.Q., Troncoso, J.C., Wisniewski, T., Woltjer, R.L., Beach, T. G., 2012. Correlation of alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J. Neuropathol. Exp. Neurol. 71, 362–381. https://doi.org/10.1097/NEN.0b013e31825018f7.
Nestor, P.J., Fryer, T.D., Smielewski, P., Hodges, J.R., 2003. Limbic hypometabolism in Alzheimer's disease and mild cognitive impairment. Ann. Neurol. 54, 343–351, 10/fgxfnq.
Nixon, R.A., 2017. Amyloid precursor protein and endosomal–lysosomal dysfunction in Alzheimer's disease: inseparable partners in a multifactorial disease. Faseb. J. 31, 2729–2743, 10/gg89g7.
Oakley, H., Cole, S.L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M., Disterhoft, J., Van Eldik, L., Berry, R., Vassar, R., 2006. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial alzheimer's disease mutations: potential factors in amyloid plaque formation. J. Neurosci. 26, 10129–10140, 10/bx3d6k.
Perry, C.J., Campbell, E.J., Drummond, K.D., Lum, J.S., Kim, J.H., 2021. Sex differences in the neurochemistry of frontal cortex: impact of early life stress. J. Neurochem. 157, 963–981. https://doi.org/10.1111/jnc.15208.
Qin, X., Liu, X.-X., Wang, Y., Wang, D., Song, Y., Zou, J.-X., Pan, H.-Q., Zhai, X.-Z., Zhang, Y.-M., Zhang, Y.-B., Hu, P., Zhang, W.-H., 2021. Early life stress induces anxiety-like behavior during adulthood through dysregulation of neuronal plasticity in the basolateral amygdala. Life Sci. 285, 119959 https://doi.org/10.1016/j.lfs.2021.119959.
Roque, A., Ochoa-Zarzosa, A., Torner, L., 2016. Maternal separation activates microglial cells and induces an inflammatory response in the hippocampus of male rat pups, independently of hypothalamic and peripheral cytokine levels. Brain. Behav. Immun., Microglia, Physiology and Behavior 55, 39–48. https://doi.org/10.1016/j.bbi.2015.09.017.
Roque, S., Mesquita, A.R., Palha, J.A., Sousa, N., Correia-Neves, M., 2014. The behavioral and immunological impact of maternal separation: a matter of timing. Front. Behav. Neurosci. 8 https://doi.org/10.3389/fnbeh.2014.00192.
Scheff, S.W., Price, D.A., Schmitt, F.A., Mufson, E.J., 2006. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol. Aging 27, 1372–1384, 10/cszjzh.
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.
Seifan, A., Schelke, M., Obeng-Aduasare, Y., Isaacson, R., 2015. Early life epidemiology of alzheimer's disease - a critical review. Neuroepidemiology 45, 237–254. https://doi.org/10.1159/000439568.
Shansky, R.M., Hamo, C., Hof, P.R., Lou, W., McEwen, B.S., Morrison, J.H., 2010. Estrogen promotes stress sensitivity in a prefrontal cortex–amygdala pathway. Cereb. Cortex N. Y. NY 20, 2560–2567. https://doi.org/10.1093/cercor/bhq003.
Shansky, R.M., Hamo, C., Hof, P.R., McEwen, B.S., Morrison, J.H., 2009. Stress-induced dendritic remodeling in the prefrontal cortex is circuit specific. Cereb. Cortex N. Y. NY 19, 2479–2484. https://doi.org/10.1093/cercor/bhp003.
Takahashi, R.H., Almeida, C.G., Kearney, P.F., Yu, F., Lin, M.T., Milner, T.A., Gouras, G. K., 2004. Oligomerization of alzheimer's β-amyloid within processes and synapses of cultured neurons and brain. J. Neurosci. 24, 3592–3599. https://doi.org/10.1523/JNEUROSCI.5167-03.2004.
Takahashi, R.H., Milner, T.A., Li, F., Nam, E.E., Edgar, M.A., Yamaguchi, H., Beal, M.F., Xu, H., Greengard, P., Gouras, G.K., 2002. Intraneuronal alzheimer Aβ42 accumulates in multivesicular bodies and is associated with synaptic pathology. Am. J. Pathol. 161, 1869–1879, 10/bbcpg5.
Tampellini, D., Capetillo-Zarate, E., Dumont, M., Huang, Z., Yu, F., Lin, M.T., Gouras, G. K., 2010. Effects of synaptic modulation on β-amyloid, synaptophysin, and memory performance in alzheimer's disease transgenic mice. J. Neurosci. 30, 14299–14304. https://doi.org/10.1523/jneurosci.3383-10.2010.
Tampellini, D., Rahman, N., Gallo, E.F., Huang, Z., Dumont, M., Capetillo-Zarate, E., Ma, T., Zheng, R., Lu, B., Nanus, D.M., Lin, M.T., Gouras, G.K., 2009. Synaptic activity reduces intraneuronal Aβ, promotes APP transport to synapses, and protects against aβ-related synaptic alterations. J. Neurosci. 29, 9704–9713. https://doi.org/10.1523/jneurosci.2292-09.2009.
Teissier, A., Le Magueresse, C., Olusakin, J., Andrade da Costa, B.L.S., De Stasi, A.M., Bacci, A., Imamura Kawasawa, Y., Vaidya, V.A., Gaspar, P., 2020. Early-life stress impairs postnatal oligodendrogenesis and adult emotional behaviour through activity-dependent mechanisms. Mol. Psychiatr. 25, 1159–1174. https://doi.org/10.1038/s41380-019-0493-2.
Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., DeTeresa, R., Hill, R., Hansen, L.A., Katzman, R., 1991. Physical basis of cognitive alterations in alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann. Neurol. 30, 572–580, 10/cz9254.
Umpierre, A.D., Wu, L.-J., 2021. How microglia sense and regulate neuronal activity. Glia 69, 1637–1653. https://doi.org/10.1002/glia.23961.
van Dyck, C.H., Swanson, C.J., Aisen, P., Bateman, R.J., Chen, C., Gee, M., Kanekiyo, M., Li, D., Reyderman, L., Cohen, S., Froelich, L., Katayama, S., Sabbagh, M., Vellas, B., Watson, D., Dhadda, S., Irizarry, M., Kramer, L.D., Iwatsubo, T., 2023. Lecanemab in early alzheimer's disease. N. Engl. J. Med. 388, 9–21. https://doi.org/10.1056/NEJMoa2212948.
Wang, A., Zou, X., Wu, J., Ma, Q., Yuan, N., Ding, F., Li, X., Chen, J., 2020. Early-life stress alters synaptic plasticity and mTOR signaling: correlation with anxiety-like and cognition-related behavior. Front. Genet. 11.
Welikovitch, L.A., Carmo, S.D., Maglóczky, Z., Malcolm, J.C., Lőke, J., Klein, W.L., Freund, T., Cuello, A.C., 2020. Early intraneuronal amyloid triggers neuron-derived inflammatory signaling in APP transgenic rats and human brain. Proc. Natl. Acad. Sci. USA 117, 6844–6854, 10/ggn4cz.
White, J.D., Arefin, T.M., Pugliese, A., Lee, C.H., Gassen, J., Zhang, J., Kaffman, A., 2020. Early life stress causes sex-specific changes in adult fronto-limbic connectivity that differentially drive learning. Elife 9, e58301. https://doi.org/10.7554/eLife.58301.
Wong, R.O.L., Ghosh, A., 2002. Activity-dependent regulation of dendritic growth and patterning. Nat. Rev. Neurosci. 3, 803–812. https://doi.org/10.1038/nrn941.
Youmans, K.L., Tai, L.M., Kanekiyo, T., Stine, W.B., Michon, S.-C., Nwabuisi-Heath, E., Manelli, A.M., Fu, Y., Riordan, S., Eimer, W.A., Binder, L., Bu, G., Yu, C., Hartley, D. M., LaDu, M.J., 2012. Intraneuronal Aβ detection in 5xFAD mice by a new Aβ-specific antibody. Mol. Neurodegener. 7, 8, 10/ggks64.
Zhai, Z.W., Yip, S.W., Lacadie, C.M., Sinha, R., Mayes, L.C., Potenza, M.N., 2019. Childhood trauma moderates inhibitory control and anterior cingulate cortex activation during stress. Neuroimage 185, 111–118. https://doi.org/10.1016/j.neuroimage.2018.10.049.
Zuelsdorff, M., Okonkwo, O.C., Norton, D., Barnes, L.L., Graham, K.L., Clark, L.R., Wyman, M.F., Benton, S.F., Gee, A., Lambrou, N., Johnson, S.C., Gleason, C.E., 2020. Stressful life events and racial disparities in cognition among middle-aged and older adults. J. Alzheimers Dis. JAD 73, 671. https://doi.org/10.3233/JAD-190439.