Behavioral and Brain Functions

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Neonatal maternal separation causes depressive-like behavior and potentiates memory impairment induced by amyloid-β oligomers in adult mice

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Abstract

Background Alzheimer's disease (AD) is characterized by memory decline and mood alterations. A growing body of evidence implicates stress and other social determinants of health as potential contributors to the progressive cerebral alterations that culminate in AD. In the current study, we investigated the impact of neonatal maternal separation (MS) on the susceptibility of male and female mice to AD-associated memory impairments and depressive-like behavior in adulthood, and on brain levels of pro-inflammatory cytokines and neurotransmitters.

Methodology Male and female Swiss mice were exposed to MS for 180 min daily from post-natal day 1 to 10. Seventy days post-MS, mice received an intracerebroventricular infusion of amyloid-β oligomers (AβOs), and memory and mood were evaluated. Levels of TNF-α, IL-1β, serotonin, dopamine, and related metabolites were determined in the cortex and hippocampus.

Results Previous exposure to MS alone did not cause memory impairments in adult mice. Interestingly, however, MS increased the susceptibility of adult male mice to memory impairment and depressive-like behavior induced by AβOs, and potentiated the inhibitory impact of AβOs on memory in adult females. Females were more susceptible to depressive-like behavior caused by a low dose of AβOs, regardless of MS. No changes in IL-1β were found. A decrease in TNF-α was selectively found in females exposed to MS that received an infusion of 1 pmol AβOs. MS led to an increase in serotonin (5-HT) in the hippocampus of male mice, without influencing the levels of the serotonin metabolite, 5-HIAA. Changes in serotonin turnover were predominantly observed in the cortex of female mice. No changes in dopamine or its metabolites were induced by MS or AβOs in male or female mice.

Conclusions Neonatal MS enhances the susceptibility of adult mice to AD-associated cognitive deficits and depressive-like behavior in a sex-specific manner. This suggests that early life stress may play a role in the development of AD.

Keywords Alzheimer's disease, Early life stress, Maternal separation, Sex differences

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects memory and behavior [1], and is the most common cause of dementia in the elderly. AD is characterized by the brain buildup of amyloid plaques and tangles, and by the accumulation of soluble oligomers of the amyloid-β peptide (AβOs), which cause synapse dysfunction and memory impairments [2].

Accumulating evidence suggests that stress and other social determinants of health play major roles in the progressive brain changes that lead to the development of AD [3]. Studies have shown that chronic stress can lead to changes in the brain that are similar to those seen in AD, including the formation of plaques and tangles [4, 5]. Importantly, stress affects the hippocampus, a brain region that is central for memory and is affected early in AD [6–8]. Stress can also lead to brain inflammation and changes in levels of hormones and neurotransmitters, which can affect brain function and contribute to the development of AD [3, 9–12]. Current evidence suggests that stress, including early life stress, is a risk factor for AD [4], and that there is a sexual dimorphism in the relationship between stress and AD [4, 9, 13]. Individuals who experienced neglect, stress, or trauma in childhood or early adulthood appear more likely to develop AD later in life [4, 5, 13]. Moreover, women exposed to early life stress appear at higher risk of developing AD, and experience more rapid cognitive decline than men [14]. However, the complex relationships between stress, sex, and cognitive/behavioral alterations in AD remain to be fully understood.

Previous studies indicate that stress significantly impacts cognitive function, with chronic psychosocial stress leading to accelerated cognitive decline and increased vulnerability to neurodegenerative processes. Notably, sex differences play a crucial role in how stress affects cognitive outcomes; for instance, studies have reported that females exhibit greater resilience to stress-related cognitive dysfunction compared to males [15–17]. This resilience is influenced by hormonal factors and neurobiological differences that shape stress responses and cognitive trajectories [18]. Furthermore, the relationship between stress and cognitive impairment in AD is compounded by the effects of early-life adversity, which can lead to long-term changes in behavior and brain function that differ by sex [18]. Understanding these complex relationships is essential for developing targeted interventions and improving outcomes for individuals affected by AD, and highlights the need for a sex-specific approach in research and treatment strategies. In the current study, we investigated the impact of neonatal maternal separation on the susceptibility of male and female mice to AD-associated memory impairments and depressive-like behavior in adulthood. We further examined changes in pro-inflammatory cytokines and neurotransmitters in the brains of adult mice exposed to neonatal maternal separation.

Methods

Animals

Memory and mood tests, biochemical and neurochemical determinations were performed on 2.5-month-old Swiss mice (9–10 animals per group). Mice were randomly divided into different experimental groups and were housed in groups of five per cage with free access to food and water, under a 12 h light/dark cycle with controlled room temperature (21 ± 2 °C). This study was approved by the Ethics Committee on the Use of Animals, Health Sciences Center, Federal University of Rio de Janeiro (protocol 008/23).

Maternal separation

Maternal separation was conducted as previously described [19]. In the control group, pups were left undisturbed with the dam until the 21st day of life (weaning). Dirty sawdust was carefully removed from one side of the cage, without disturbing the mother and the nest, and replaced by clean sawdust at that side by the researcher leading the study. In the group undergoing maternal separation, the dam was gently pulled to one side of the cage, the pups were removed from their home cage and were placed in a clean cage lined with clean paper towel. This cage was placed in an incubator to maintain ambient temperature at 30–32 °C. After 180 min, pups were returned to the original cases with their dams, which were in the same experimental room. This procedure was performed daily from day 1 to 10 following birth, and then pups were left undisturbed with the dam until weaning.

Preparation of AβOs

Oligomers were prepared from synthetic $A\beta_{1-42}$ peptide (Echelon) as originally described [20–22], and were routinely characterized by size exclusion high-performance liquid chromatography (HPLC-SEC) and occasionally by Western blotting [23, 24]. Briefly, the peptide was first solubilized in hexafluoroisopropanol (HFIP), the solvent was allowed to evaporate, and the dried films produced were subsequently dissolved in sterile anhydrous dimethylsulfoxide (DMSO) to make a 5 mM solution. This solution was diluted to 100 μM Aβ in ice-cold PBS and incubated for 16 h at 4 °C. Thereafter, the preparation was centrifuged at 14,000 × *g* for 10 min at 4 °C to remove insoluble aggregates (protofibrils and fibrils), and the supernatants containing soluble Aβ oligomers were stored at 4 °C and

used within 48 h of preparation. Protein concentration was determined using the BCA assay (Pierce-Thermo Scientific, Rockford, IL, USA). Characterization by HPLC-SEC and by SDS-PAGE/Western blotting using 6E10 (anti-Aβ antibody, Abcam, Cambridge, UK) or NU4 anti-Aβ oligomer antibodies (kindly provided by Prof. William L. Klein, Northwestern University) revealed that AβOs preparations were devoid of larger insoluble aggregates [20, 21, 23].

Intracerebroventricular infusion of AβOs

Seventy days after the end of the maternal separation procedure, animals were randomly assigned to two groups that received free-hand intracerebroventricular (i.c.v.) infusions of AβOs or vehicle (2% DMSO in PBS), as previously described [21, 24–26]. The free-hand i.c.v. infusion is a technique where injections are made by a trained investigator directly into the lateral ventricles of the brain without the use of a stereotaxic frame. This method relies on visual and tactile landmarks to guide the injection [27], and was implemented by our group to allow investigation of the impact of Aβ oligomers in the mouse brain [21, 24–26, 28]. Mice were anesthetized with 2.5% isoflurane (Cristália; São Paulo, Brazil) using a vaporizer system (Norwell, MA) and were gently restrained only during the injection procedure (between 30 and 60s) [22]. Using anatomical landmarks on the skull to estimate the appropriate coordinates for the injection site, a 2.5-mm-long orthodontic needle attached via plastic tubing to a 5 µL Hamilton syringe was unilaterally inserted 1 mm to the right of the midline point equidistant from each eye and 1 mm posterior to a line drawn through the anterior base of the eye [24, 27]. AβOs (1 or 10 pmol) or vehicle were injected in a final volume of 3 µL by pushing the syringe plunger slowly and carefully, and the needle was kept in place for an additional 30s to prevent backflow [22]. Mice showing any sign of hemorrhage or any signs of misplaced injections on the tissue collection (~ 5% of animals throughout the study) were excluded from further analysis.

Tissue collection

Mice were anesthetized with 1.5 ml/kg of a solution containing 10% ketamine and 2% xylazine immediately after behavioral tests. Bilateral hippocampi (HPC) and frontal cortex (CTX) were collected, immediately frozen in liquid nitrogen, and stored at – 80 °C until use for ELISA or HPLC assays.

Behavioral tests

All animals were tested in behavioral tests 62 days after the maternal separation protocol and 24h after the AβO injection. The protocols for each test are described below.

Open field

Mice were placed at the center of an open field arena (30 cm × 30 cm × 45 cm) for habituation, and their activity was recorded for five minutes. Crossing and rearing behaviors were quantified during the open field test. The arena was cleaned with 20% ethanol between trials to eliminate olfactory cues.

Novel object recognition test (NOR)

The open field arena was used to conduct the novel object recognition test, using two objects affixed to the box using tape as previously described [24, 29]. To record behavior, the test was captured on video. Animals were placed at the center of the arena during both training and test sessions, and exploratory behavior towards the objects was observed and recorded for 5 min. To avoid olfactory cues, the arena was cleaned with 20% ethanol between trials. The training session involved the use of two identical objects, and during the subsequent test session one of the objects used in the training session was replaced by a novel object. Sniffing and touching the objects were considered exploratory behavior, and a trained researcher registered the amount of time spent exploring each object [24, 29]. Results are presented as discrimination index [30]. The Discrimination Index (DI) measures the ability to distinguish between novel and familiar objects by calculating the difference in exploration time for each. It is calculated using the formula [DI = (TN − TF)/(TN + TF)], where TN represents the time spent exploring the novel object and TF represents the time spent with the familiar object. The resulting DI can range from + 1 to − 1: a positive score indicates a preference for the novel object, a negative score indicates a preference for the familiar object, and a score of zero reflects no preference between the two [30, 31].

Forced swim test (FST)

Mice were placed in a cylindrical container filled with water at a temperature of 25 °C, where they could not touch the bottom with their paws or tail. The forced swimming test involved a 6-min session for each animal. After each test, mice were gently dried and placed in a heated room. All tests were recorded by a camera and scored based on three behavioral categories: immobility, swimming, and attempting to climb. Immobility was defined as floating with only enough paw movements to

keep the head above water, swimming as the movement of the front paws horizontally with or without body displacement, and climbing as the movement of the front paws vertically near the wall or in the center, with or without body displacement [32, 33].

Neurotransmitter determinations

Reverse-phase high-performance liquid chromatography (HPLC) coupled to electrochemical detection (HPLC-ED; Shimadzu, Japan) was used to determine levels of monoamines and related metabolites (dopamine, DOPAC, serotonin, 5-HIAA) as described [34]. Samples (hippocampal or frontal cortex homogenates) were deproteinized by the addition of perchloric acid (0.1 M) for 30 min followed by centrifugation (10,000×*g* for 10 min) to remove protein pellets. Supernatants were used to determine monoamines, and standard curves were obtained using commercial reagents of the highest grade. For monoamine determination, fast isocratic separation was performed using a reverse phase LC-18 column (Supelco; 5 µm particle size, 250 mm×4.6 mm) with the following mobile phase: 20 mM sodium phosphate dibasic, 20 mM citric acid, 10% methanol, 0.12 mM Na_2EDTA, and 566 mg/liter heptanesulfonic acid, pH 2.64, as described [35].

Cytokine determinations

Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were quantified in hippocampal and cortex homogenates using mouse ELISA kits (IL-1β: Thermo Scientific, Rockford, IL; TNF-α: Biolegend, San Diego, CA) following manufacturers' instructions.

Statistical analysis

Datasets were submitted to the Shapiro–Wilk normality test and Levene's homoscedasticity test. All datasets showed normal distribution and were analyzed by Two-Way ANOVA followed by Bonferroni-Holm correction. Differences were considered statistically significant with $p < 0.05$. Data from the NOR task were analyzed by ANOVA comparing discrimination indexes of distinct experimental groups [21, 24, 25, 28]. All analyses were performed using GraphPad Prism 10 (GraphPad Software; La Jolla, CA) and JASP (JASP Team, 2024; Version 0.19.3). Results are expressed as means ± SEM, and corresponding p-values.

Results

Neonatal maternal separation potentiates the impact of AβOs on memory in male and female adult mice

Mice were either kept with their mothers or submitted to the 3-h/day maternal separation (MS) protocol between postnatal days 1–10 (see Methods). On postnatal day 70, they received an intracerebroventricular (i.c.v.) infusion of 1 or 10 pmol AβOs (or vehicle), and were assessed in the novel object recognition (NOR) memory test and forced swim test (FST) on the following day. Mice were euthanized for brain tissue collection on postnatal day 72 (Fig. 1A).

Control measurements showed no differences in crossing or rearing behaviors induced by MS or by infusion of AβOs in male ($F(2, 47) = 0.5526$; $p = 0.5792$) or female mice ($F(2, 46) = 0.6776$; $p = 0.5128$) (Supp. Figure 1A-D). We have previously shown that i.c.v. infusion of 1 pmol AβOs does not cause detectable memory impairment or mood alterations in mice, while infusion of 10 pmol AβOs causes significant and persistent memory deficits and depressive-like behavior [24, 28, 36]. While MS per se did not cause memory impairments in the NOR test in either male or female mice (Fig. 1B, C), statistically significant decreases in discrimination index were observed in males that received an infusion of AβOs ($F(1, 47) = 7.760$; $p = 0.0077$). Interestingly, male mice that had been exposed to neonatal MS showed memory impairment induced by a low dose (1 pmol) of AβOs ($F(2, 47) = 6.440$; $p = 0.0034$), which had no effect on control males not exposed to MS (Fig. 1B). In line with our previous reports [21, 24–26] infusion of 10 pmol AβOs impaired object recognition compared to mice receiving an infusion of vehicle ($p = 0.05$). MS led to a trend of further decrease in discrimination index in male mice that received an infusion of 10 pmol AβOs (Fig. 1B).

Similar to what we observed in males, MS alone or infusion of 1 pmol AβOs caused no memory impairment in females (Fig. 1C). In contrast with male mice, however, infusion of 10 pmol AβOs caused no memory impairment in females that were not exposed to MS (Fig. 1C), indicating that females are more resilient than males to the impact of AβOs on memory. Nonetheless, females submitted to MS exhibited impaired memory following the infusion of 10 pmol AβOs ($p = 0.02$) ($F(1, 46) = 6.025$; $p = 0.0180$).

Effect of neonatal maternal separation on depressive-like behavior in adult male and female mice

To assess the impact of neonatal MS on depressive-like behavior in adulthood, male and female mice were submitted to the forced swim test (FST). MS induced a statistically significant increase in immobility times in male mice ($p = 0.0014$), and a trend of increase in females (Fig. 1D, E), indicating that neonatal maternal separation led to depressive-like behavior in adulthood.

Next, we evaluated the impact of MS on the induction of depressive-like behavior by AβOs. Depressive-like behavior has been reported in transgenic models of AD

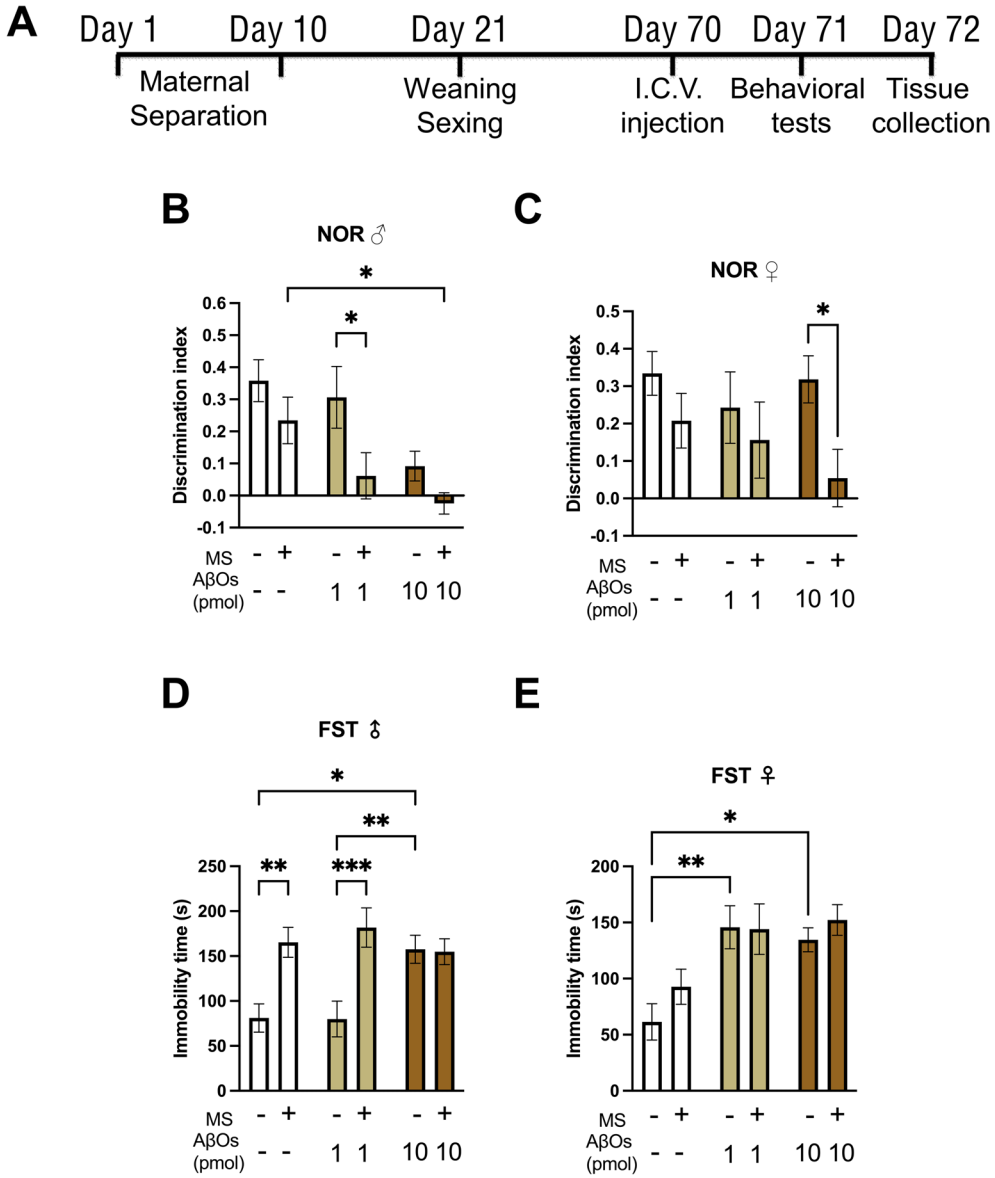

Fig. 1 Impact of neonatal maternal separation (MS) and AβOs on memory and mood in adult mice. Mice were exposed to neonatal maternal separation (MS) from postnatal day 1 to 10 or were kept under control conditions, and received an i.c.v infusion of vehicle or AβOs (1 or 10 pmol, as indicated) at postnatal day 70 (**A**). Memory performance was assessed 24 h after infusion of AβOs in males (**B**) or females (**C**) using the novel object recognition (NOR) test. Data are expressed as discrimination index ± SEM. Depressive-like behavior was also tested 1 day after infusion of AβOs using the forced swim test (FST) in males (**D**) or females (**E**). Data are expressed as immobility times, mean ± SEM. Results were analyzed by ANOVA followed by Bonferroni-Holm correction. N = 9–10 animals

[37] as well as in AD patients [38]. We have previously reported that a single i.c.v infusion of 10 pmol AβOs (but not 1 pmol) induces depressive-like behavior in male mice [26, 28]. The FST showed a significant interaction between maternal separation and AβOs ($F\ (2,\ 59) = 4.633$; $p = 0.0135$) in males. Consistent with our previous reports, infusion of 1 pmol AβOs did not cause any increase in immobility time in male mice not exposed to MS. However, previous exposure to MS induced increases in immobility times in male mice that received infusions of either vehicle or 1 pmol AβOs, indicating depressive-like behavior (Fig. 1D). Also, in line with our previous findings, infusion of 10 pmol AβOs increased immobility times in male mice, regardless of whether they had been exposed or not to the MS protocol.

Neonatal maternal separation alters proinflammatory cytokines in the hippocampus

No changes in hippocampal IL-1β were caused by exposure to MS or infusion of AβOs in either male or female mice (Fig. 2A, B). While MS alone had no effect on hippocampal TNF-α in male mice, it caused a trend of reduction ($p = 0.0728$) in males that received an infusion of 1 pmol AβOs and no effect in mice that received an infusion of 10 pmol AβOs (Fig. 2C). In contrast, MS caused reductions in hippocampal TNF-α ($F(1, 50) = 6.159$, $p = 0.0165$) in females that received infusions of vehicle ($p = 0.0669$) or 1 pmol AβOs ($p = 0.0029$) (Fig. 2D). Infusion of 10 pmol AβOs per se caused a decrease in hippocampal TNF-α in females, but no further reduction was induced by MS.

Neonatal maternal separation induces changes in brain serotonin levels

Considerable evidence implicates serotonin in stress-induced depressive behavior [39], and both preclinical and clinical data indicate that serotonergic neurotransmission is compromised in AD. This led us to hypothesize that the increased susceptibility to AβO-induced depressive-like behavior in mice exposed to neonatal MS could be related to altered serotonergic transmission.

To test this hypothesis, we measured levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA, a 5-HT metabolite) in the hippocampus (HPC) and frontal cortex (CTX) of male and female mice. An interaction between MS and AβOs was observed in HPC of male

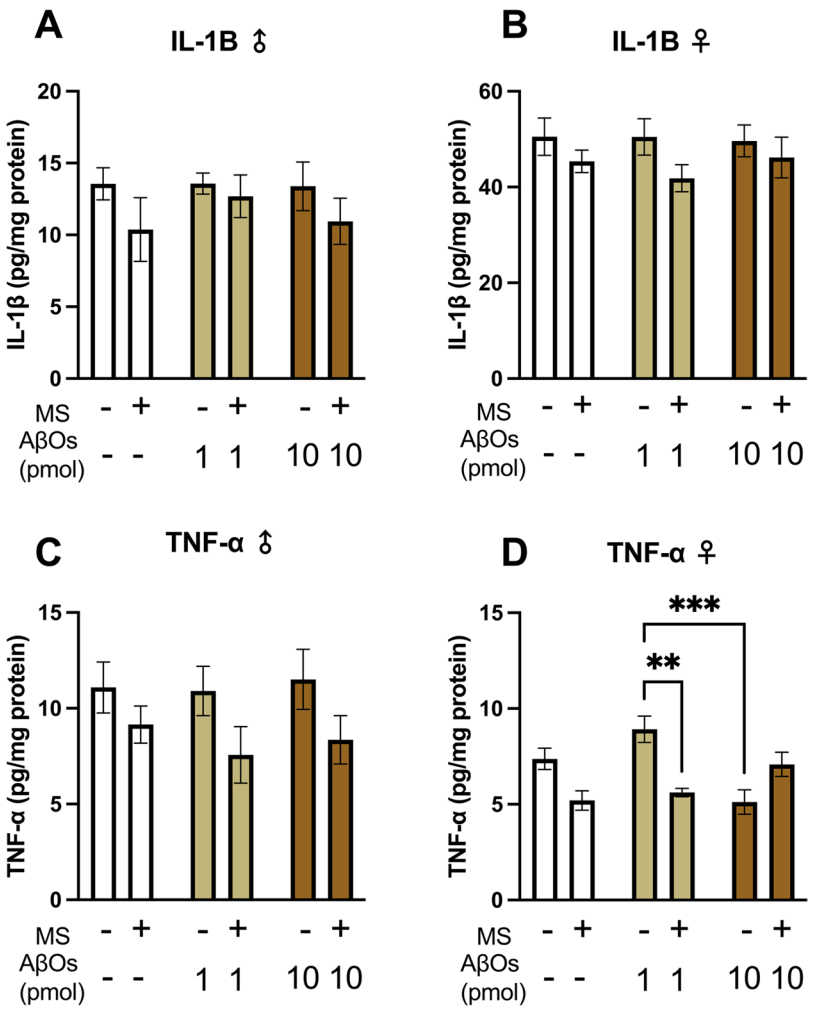

Fig. 2 Impact of neonatal MS or AβO infusion on hippocampal IL-1β and TNF-α in mice. IL-1β (**A**, **B**), and TNF-α (**C**, **D**) were determined by ELISA in hippocampal homogenates obtained 2 days after i.c.v administration of vehicle, 1 pmol or 10 pmol AβOs in males (**A**, **C**) or females (**B**, **D**) that were exposed to the MS protocol or not (as indicated). Data are expressed as means ± SEM. Results were analyzed by ANOVA followed by Bonferroni-Holm correction. N = 9–10 animals

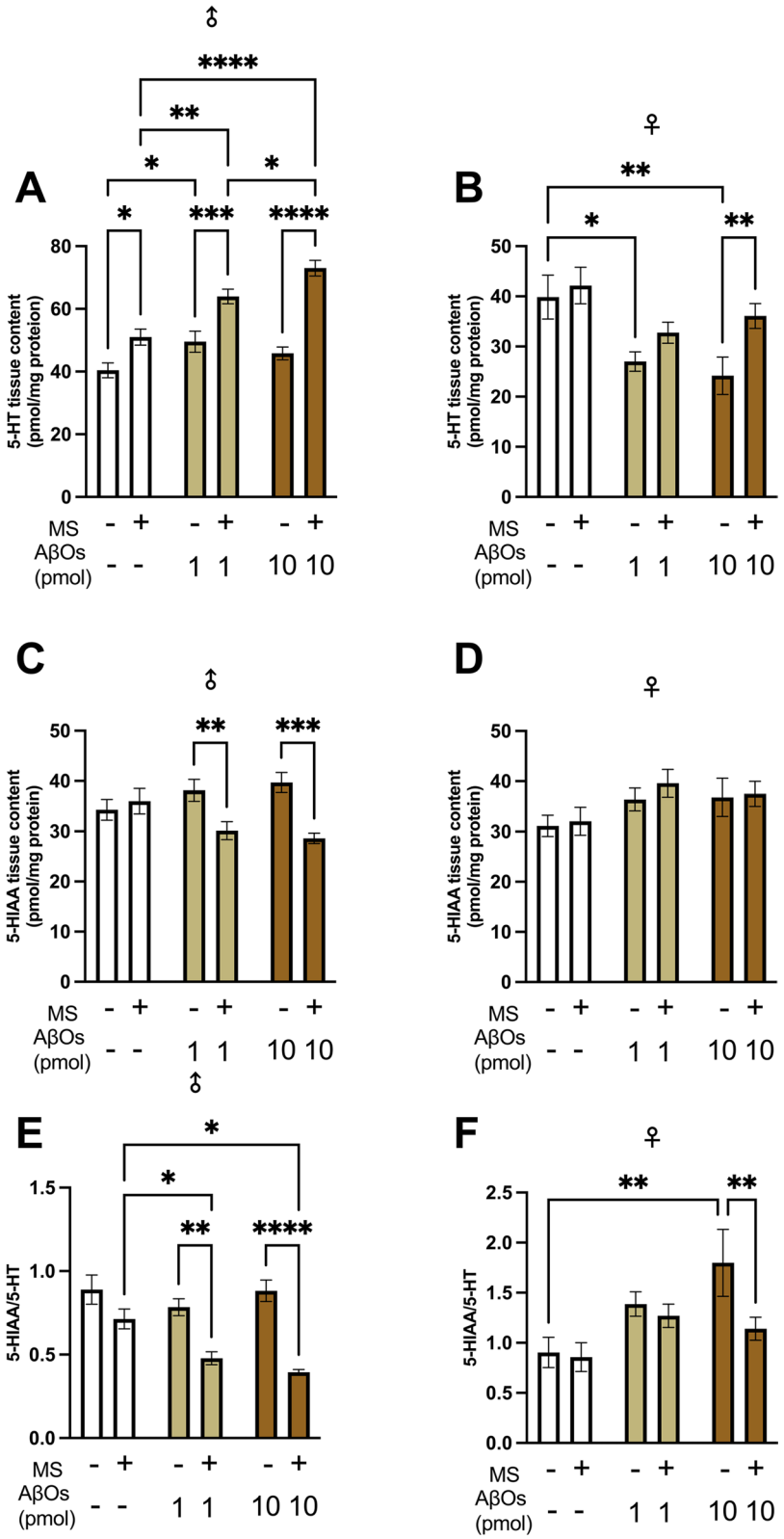

Fig. 3 Maternal separation alters serotonin levels in the hippocampus. Tissue content of 5-HT (**A**), 5-HIAA (**C**), and 5-HIAA/5-HT ratio (**E**) in CTRL or MS male mice that received infusions of vehicle or AβOs (1 or 10 pmol). Tissue content of 5-HT (**B**), 5-HIAA (**D**), and 5-HIAA/5-HT ratio (**F**) in CTRL or MS female mice that received infusions of vehicle or AβOs (1 or 10 pmol). Data are expressed as means ± SEM. ANOVA followed by Bonferroni-Holm correction. N = 9–10 animals

mice (F (2, 60) = 3.354; p = 0.0416) (Fig. 3). MS per se increased hippocampal 5-HT levels (Fig. 3A, p = 0.01). Hippocampal serotonin increased in a dose-dependent manner in AβO-infused mice subjected to the MS protocol: vehicle-infused mice had 51.0 ± 2.6 pmol 5-HT/mg protein, hile mice that received 1 pmol AβOs showed a mean increase to 64.0 ± 2.4 pmol 5-HT/mg protein (p = 0.006), and those receiving 10 pmol AβOs had 73.02 ± 2.5 5-HT/mg protein (p = 0.0001) (Fig. 3A). MS alone had no impact on hippocampal 5-HIAA in vehicle-infused mice, but it induced reductions in 5-HIAA in mice that received infusions of both 1 (p = 0.006) and 10 pmol (p = 0.004) AβOs (Fig. 3C). Calculation of the 5-HT/5-HIAA ratio as a measure of serotonin turnover revealed that MS alone induced a trend of decrease in turnover, and induced significant decreases in turnover in 1 pmol (p = 0.04) and 10 pmol (p = 0.02) AβO-infused males (Fig. 3E).

MS alone did not impact the levels of 5-HT in the hippocampus of vehicle-infused females (Fig. 3B). Interestingly, infusion of both 1 and 10 pmol AβOs induced decreases in 5-HT levels in females. Similar to the result with male mice, MS caused an increase in 5-HT in the hippocampi of females that received 10 pmol AβOs (Fig. 3B). No differences were found in hippocampal 5-HIAA levels in females submitted or not to MS and/or infused with AβOs (Fig. 3D). In contrast with males, the 5-HIAA/5-HT turnover ratio showed a trend of increase in females receiving an infusion of 1 pmol AβOs, and a significant increase induced by infusion of 10 pmol AβOs, which was not observed in females submitted to neonatal MS (Fig. 3F).

Serotonin turnover was also measured in the CTX of males and females. In males, infusion of 10 pmol (but not 1 pmol) AβOs caused a decrease in 5-HT (p = 0.01) and an increase in 5-HIAA levels, resulting in a significant increase (F (2, 40) = 7.290; p = 0.0020) in 5-HIAA/5-HT ratio (p = 0.01) (Fig. 4A, C, E). These changes were abrogated in males submitted to MS. In females, infusion of both 1 and 10 pmol AβOs induced reductions (F (2, 60) = 7.203; p = 0.0016) in 5-HT and increases in 5-HIAA/5-HT ratio (F (2, 60) = 7.058; p = 0.0018) (Fig. 4B, D, F). These changes were abrogated in females submitted to MS that received an infusion of 1 pmol AβOs (p = 0.001), but not in those infused with 10 pmol AβOs (p = 0.12).

Dopamine turnover is unaffected by neonatal maternal separation

We next asked whether MS could affect dopamine turnover in AβO-infused mice. We measured levels of dopamine (DOPA) and its metabolite dihydroxy phenylacetic acid (DOPAC) in the HPC and CTX of both male and female mice.

In males, MS caused no changes in either hippocampal (Fig. 5A, C, E) or cortical (Fig. 6A, C, E) dopamine, DOPAC, or in the DOPAC/dopamine ratio. On the other hand, infusion of 10 pmol AβOs caused reductions in dopamine content and increases in DOPAC/dopamine ratio in both hippocampus and cortex (p = 0.0007 and p = 0.02 in HPC and CTX, respectively). The increase in DOPAC/dopamine ratio induced by 10 pmol AβOs (p = 0.01) was further potentiated in the cortex of males submitted to neonatal MS (Fig. 6E).

Similar to males, in females MS caused no changes in hippocampal (Fig. 5B, D, F) or cortical (Fig. 6B, D, F) dopamine, DOPAC or DOPAC/dopamine ratio. Hippocampal DOPAC levels and DOPAC/dopamine ratio were only increased in the hippocampus of females that received an infusion of 1 pmol AβOs and did not undergo MS (Fig. 5D, F). Female cortical levels of dopamine and DOPAC, as well as DOPAC/dopamine ratio, were not impacted by AβO infusion (Fig. 6B, D, F).

Discussion

In the current study, we investigated whether neonatal MS could exacerbate the susceptibility of male or female mice to memory impairment and depressive-like behavior induced by AβOs in adulthood. To this end, we utilized an MS protocol wherein we separated pups from their mothers from postnatal day 1–10 for 180 min per day. This model of early-life stress was applied to investigate brain cytokine and neurotransmitter alterations in brain regions affected by AβOs, since stress experienced during the neonatal period has been shown to exacerbate age-related cognitive decline [40, 41].

We have previously shown that a single intracerebroventricular injection of AβOs (10 pmol) causes memory impairment [21, 24, 25] and depressive-like behavior [26, 28] in mice. Further, we have shown that increased susceptibility to AβO-induced cognitive impairments caused by neonatal infection is linked to brain inflammation [42]. We now show that MS per se did not cause locomotor or memory impairments in male or female mice. On the other hand, we observed that exposure to neonatal MS was associated with increased susceptibility to depressive-like behavior in male mice [43, 44]. We found that, while infusion of 1 pmol AβOs did not cause memory impairment or depressive-like behavior in male mice not exposed to MS, this low dose of AβOs caused both memory impairments and depressive-like behavior in males that had been exposed to neonatal MS. These results indicate that neonatal MS increased the susceptibility of male mice to memory impairment and mood alterations induced by AβOs.

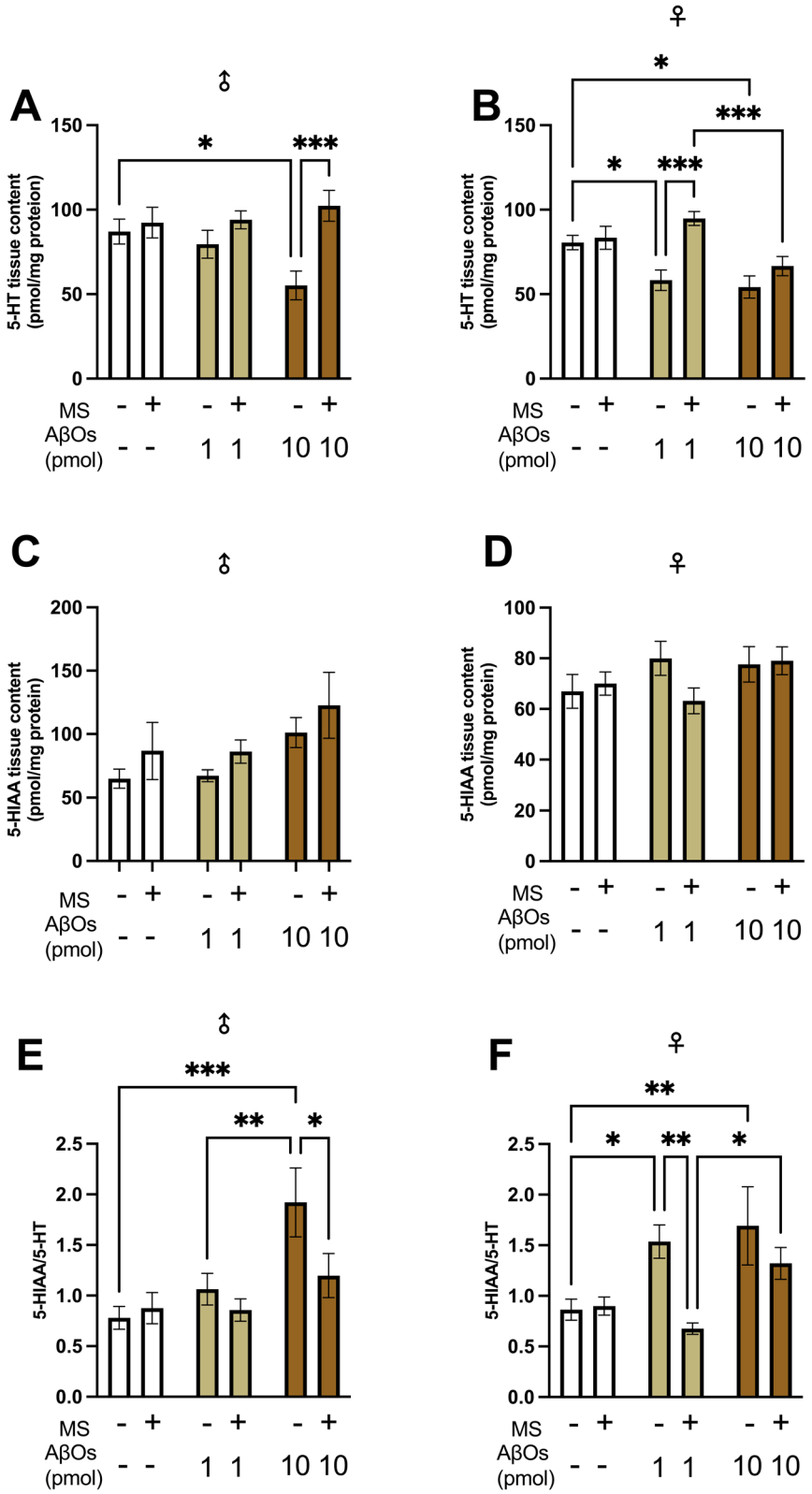

Fig. 4 Maternal separation alters serotonin levels in the cortex. Tissue content of 5-HT (**A**), 5-HIAA (**C**), and 5-HIAA/5-HT ratio (**E**) in CTRL or MS male mice that received infusions of vehicle or AβOs (1 or 10 pmol). Tissue content of 5-HT (**B**), 5-HIAA (**D**), and 5-HIAA/5-HT ratio (**F**) in CTRL or MS female mice that received infusions of vehicle or AβOs (1 or 10 pmol). Data are expressed as mean ± SEM. ANOVA followed by Bonferroni-Holm correction. N = 9–10 animals

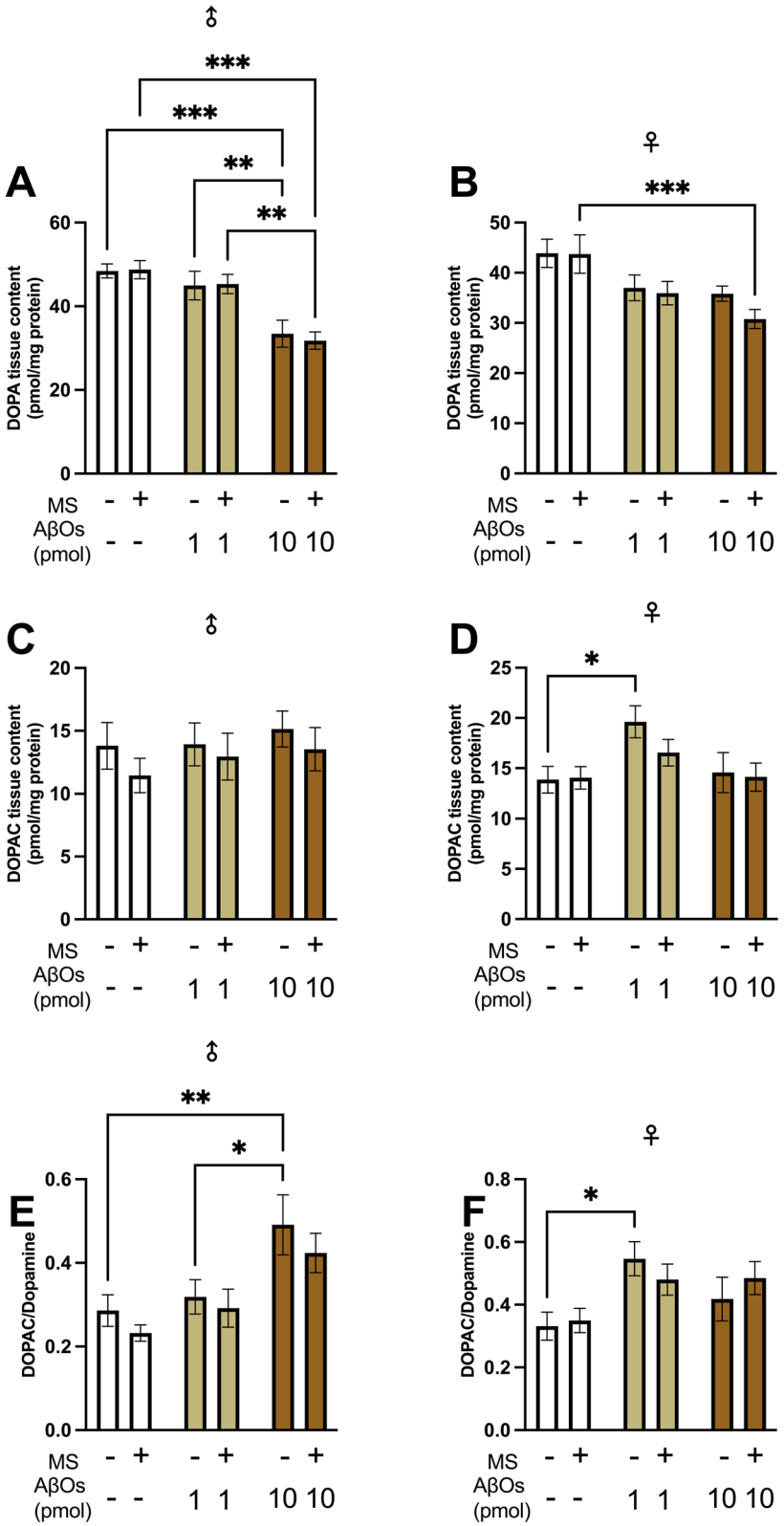

Fig. 5 Effects of maternal separation and AβOs on dopamine levels in the hippocampus. Tissue content of DOPA (**A**), DOPAC (**C**) and DOPAC/DOPA ratio (**E**) in CTRL or MS male mice that received infusions of vehicle or AβOs (1 or 10 pmol). Tissue content of DOPA (**B**), DOPAC (**D**), and DOPAC/DOPA ratio (**F**) in CTRL or MS female mice given infusions of vehicle or AβOs (1 or 10 pmol). Data are expressed as means ± SEM. ANOVA followed by Bonferroni-Holm correction. N = 9–10 animals

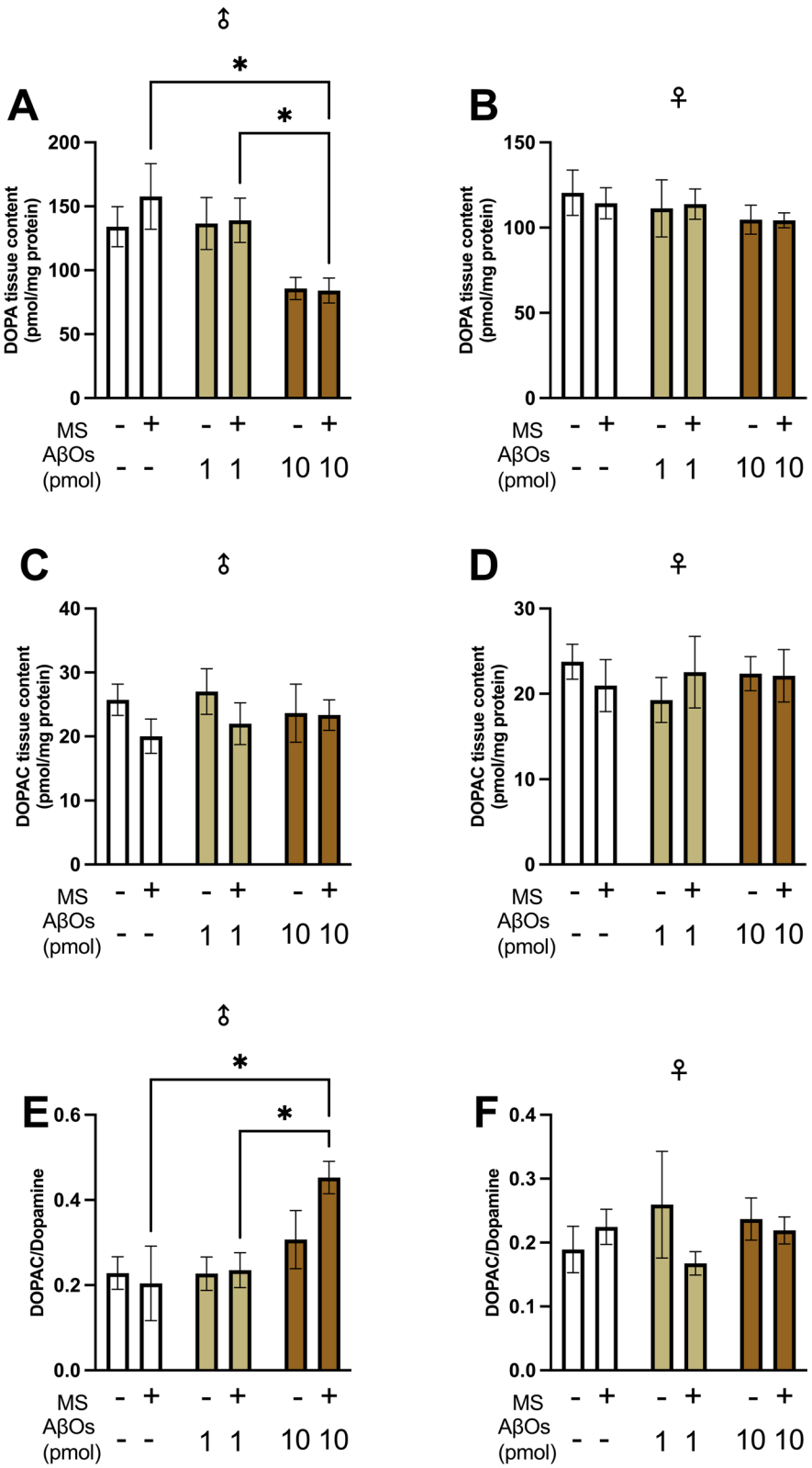

Fig. 6 Effects of maternal separation and AβOs on dopamine levels in the cortex. Tissue content of DOPA (**A**), DOPAC (**C**), and DOPAC/DOPA ratio (**E**) in CTRL or MS male mice given infusions of vehicle or AβOs (1 or 10 pmol). Tissue content of DOPA (**B**), DOPAC (**D**), and DOPAC/DOPA (**F**) ratio in CTRL or MS female mice given infusions of vehicle or AβOs (1 or 10 pmol). Data are expressed as means ± SEM. ANOVA followed by Bonferroni-Holm correction. N = 9–10 animals

Although women who experienced early life stress appear at higher risk of developing AD and experience more rapid cognitive decline than men [14], previous studies have shown that female rats are more resistant to behavioral changes induced by stress exposure, and that intermittent chronic stress impaired spatial memory in male rats but not in females [45]. We here found that female mice were more susceptible than males to depressive-like behavior caused by a low dose of AβOs (1 pmol), regardless of neonatal MS. Interestingly, infusion of both 1 and 10 pmol AβOs increased immobility times in female mice, regardless of whether they had been exposed or not to MS. These results suggest that, while female mice appear to be more resilient than males to depressive-like behavior induced by MS alone, they are more susceptible than males to the induction of depressive-like behavior by AβOs.

Taken together, these results are consistent with previous reports showing that MS alters behavior in a sex-specific manner [46, 47], and that males are more susceptible to stress-induced effects than females [43, 44]. Because the main goal of the current study was to evaluate whether and how early life stress might affect adult-life cognition and behavior in the offspring, we have not investigated possible maternal behavioral changes induced by separation. They further underscore differential sex responses to AβOs and neonatal MS, and are in accordance with studies showing that human adolescents exposed to early-life stress present sex-dependent hippocampal dysfunction with memory deficits and the development of depression [48, 49].

Brain inflammation has been identified as a key driver of AD [3, 50]. Increased levels of pro-inflammatory cytokines trigger synapse damage, affect neuroplasticity [51] and result in cognitive impairments [52, 53]. We have previously demonstrated that AβOs increase the secretion of TNF-α, which impairs brain insulin signaling and protein synthesis, driving synapse dysfunction associated with memory loss and depressive-like alterations [25, 28]. Additionally, increased brain levels of IL-1β have been linked to cognitive dysfunction in rodent models [54], and are associated with increased risk of AD [55]. Surprisingly, we did not find significant differences in IL-1β levels in the hippocampi of male or female mice, indicating that neither MS nor AβO administration had a substantial impact on this cytokine under our experimental conditions. Intriguingly, MS attenuated the increase in TNF-α caused by the infusion of 1 pmol AβOs in the hippocampus of females. Previous studies from our group have shown increases in brain IL-1β levels following i.c.v infusion of oligomers [26]. However, no significant changes were observed in hippocampal IL-1β levels across experimental conditions in both male and female mice in the current study. Although we are not completely sure what may have caused such a difference, one possible explanation is that our previous measurements of IL-1β had been performed 8 days after infusion of AβOs, versus 24h in the current study.

Impaired neurotransmission has also been implicated in AD pathogenesis [34]. 5-HT levels and serotonergic signaling are altered in AD [56, 57], and dopamine metabolites are reduced in the CSF of AD patients [34]. These alterations in neurotransmitter metabolism are recapitulated in animal models of AD [28, 58], and altered brain neurochemistry and connectivity have been reported in rodent models of early-life stress [15, 59–61]. We have previously shown that AβOs reduce 5-HT levels in the mouse brain [28]. Here, we show that neonatal MS increased 5-HT in the hippocampus of male mice, without affecting levels of 5-HIAA. Furthermore, MS potentiated the elevation of 5-HT levels caused by AβOs even when a sub-toxic dose was infused, leading to a decreased turnover (5-HIAA/5-HT) ratio in the hippocampus of male mice. Although changes in 5-HT and 5-HIAA in the cortex were observed only in male mice infused with 10 pmol AβOs, these alterations may be associated with the observation of increased immobility time in the FST.

Contrary to males, changes in serotonin turnover were found mainly in the cortex of female mice. Additionally, reduced levels of 5-HT in both the hippocampus and cortex caused by the infusion of a low dose (1 pmol) of AβOs may be responsible for the increased immobility time observed for females in the FST, indicating sex-specific susceptibility to the development of depressive-like behavior.

Dopaminergic signaling plays a central role in the connectivity of the hippocampus and brain cortex and is involved in numerous cognitive, emotional, and motor functions. We show that MS did not appear to alter DOPA or DOPAC levels in either the hippocampus or cortex, nor did it affect the DOPAC/DOPA ratio. This suggests that MS alone does not significantly impact dopamine metabolism in these brain regions of both male and female mice. On the other hand, AβOs caused noticeable reductions in dopamine and increases in DOPAC/DOPA ratio in both the hippocampus and cortex of male mice. Interestingly, these changes were further potentiated in the cortex of males that had been exposed to neonatal MS, indicating a possible synergistic effect between MS and AβOs.

While MS alone did not alter dopamine or DOPAC levels in females, infusion of 1 pmol AβOs increased hippocampal DOPAC levels and the turnover (DOPAC/DOPA) ratio. These results suggest that male mice may

be more sensitive to the impact of AβOs on dopamine metabolism in the hippocampus.

Changes in serotonergic and dopaminergic signaling reported here highlight how early life stress and AD pathogenesis in adulthood may interact, contributing to hippocampal dysfunction with deficits in memory and the development of depression in a sex-dependent manner in adulthood.

Although further research is needed to fully elucidate the mechanisms by which neonatal MS intersects the impact of AβOs on dopamine and serotonin levels and turnover, our findings provide initial insight into the complex interplay between maternal separation, AβOs, sex, and their effects on behavioral and neuroinflammatory outcomes in adulthood. Our study further highlights the importance of considering sex-specific responses involved in the neurodegenerative processes of AD.

Abbreviations

DOPAC	3,4-Dihydroxyphenylacetic acid
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine
AD	Alzheimer's disease
Aβ	Amyloid-β peptide
DMSO	Dimethylsulfoxide
DOPA	Dopamine
FST	Forced swim test
CTX	Frontal cortex
HFIP	Hexafluoroisopropanol
HPLC	High-performance liquid chromatography
HPC	Hippocamppus
IL-1β	Interleukin-1β
i.c.v.	Intracerebroventricular
MS	Maternal separation
NOR	Novel object recognition
TNF-α	Tumor necrosis factor-α

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12993-025-00266-1.

Additional file 1.

Acknowledgements
This study was suported by the following Brazilian agencies: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), National Institute for Translational Neuroscience (INNT/Brazil), and Ministério da Saúde/DECIT.

Author contributions
All authors have contributed significantly to this work. STF and FGDF supervised the study. GCK and RLF designed and performed experiments. PRS analyzed the data and wrote an initial version of the manuscript, which was subsequently revised by RLF and STF.

Funding
This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; to STF #403032/2021-0, FGDF #302702/2017-2), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; to STF, #E-26/010.0421/2019, #SEI-260003/002709/2020, #E-26/210.772/2021, #E-26/211.390/2021, #E-26/200.998/2021, FGDF, #26.201.030/2022, #26.211.354/2021 and RLF, #E-26/200.169/2023), National Institute for Translational Neuroscience (INNT/Brazil; to STF and FGDF), Ministério da Saúde/DECIT (to STF and FGDF). PRS was recipient of post-doctoral fellowships from FAPERJ and CNPq (#E-26/210.699/2023).

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All procedures involving animal research were approved by the Ethics Committee on the Use of Animals, Health Sciences Center, Federal University of Rio de Janeiro. Consent to participate is not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 2 July 2024 Accepted: 17 January 2025
Published online: 20 March 2025

References

1. Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol. 2010;9(11):1118–27.
2. Ferreira ST, Lourenco MV, Oliveira MM, De Felice FG. Soluble amyloid-beta oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. Front Cell Neurosci. 2015;9:191.
3. De Felice FG, Goncalves RA, Ferreira ST. Impaired insulin signalling and allostatic load in Alzheimer disease. Nat Rev Neurosci. 2022;23(4):215–30.
4. Huang Z, Jordan JD, Zhang Q. Early life adversity as a risk factor for cognitive impairment and Alzheimer's disease. Transl Neurodegener. 2023;12(1):25.
5. Yan Y, Dominguez S, Fisher DW, Dong H. Sex differences in chronic stress responses and Alzheimer's disease. Neurobiol Stress. 2018;8:120–6.
6. Kim JJ, Song EY, Kosten TA. Stress effects in the hippocampus: synaptic plasticity and memory. Stress. 2006;9(1):1–11.
7. Cabbia R, Consoli A, Suchecki D. Association of 24 h maternal deprivation with a saline injection in the neonatal period alters adult stress response and brain monoamines in a sex-dependent fashion. Stress. 2018;21(4):333–46.
8. Miragaia AS, de Oliveira Wertheimer GS, Consoli AC, Cabbia R, Longo BM, Girardi CEN, et al. Maternal deprivation increases anxiety- and depressive-like behaviors in an age-dependent fashion and reduces neuropeptide Y expression in the amygdala and hippocampus of male and female young adult rats. Front Behav Neurosci. 2018;12:159.
9. Sala Frigerio C, Wolfs L, Fattorelli N, Thrupp N, Voytyuk I, Schmidt I, et al. The major risk factors for Alzheimer's disease: age, sex, and genes modulate the microglia response to abeta plaques. Cell Rep. 2019;27(4):1293-306.e6.
10. Zhu D, Montagne A, Zhao Z. Alzheimer's pathogenic mechanisms and underlying sex difference. Cell Mol Life Sci. 2021;78(11):4907–20.
11. Luine V. Sex differences in chronic stress effects on memory in rats. Stress. 2002;5(3):205–16.

12. Luine VN, Spencer RL, McEwen BS. Effects of chronic corticosterone ingestion on spatial memory performance and hippocampal serotonergic function. Brain Res. 1993;616(1–2):65–70.
13. Bianco A, Antonacci Y, Liguori M. Sex and gender differences in neurodegenerative diseases: challenges for therapeutic opportunities. Int J Mol Sci. 2023;24(7):6354.
14. Fisher DW, Bennett DA, Dong H. Sexual dimorphism in predisposition to Alzheimer's disease. Neurobiol Aging. 2018;70:308–24.
15. Farrell MR, Holland FH, Shansky RM, Brenhouse HC. Sex-specific effects of early life stress on social interaction and prefrontal cortex dendritic morphology in young rats. Behav Brain Res. 2016;310:119–25.
16. Bories C, Guitton MJ, Julien C, Tremblay C, Vandal M, Msaid M, et al. Sex-dependent alterations in social behaviour and cortical synaptic activity coincide at different ages in a model of Alzheimer's disease. PLoS ONE. 2012;7(9): e46111.
17. Paolillo EW, You M, Gontrum E, Saloner R, Gaynor LS, Kramer JH, et al. Sex differences in the relationship between perceived stress and cognitive trajectories. Am J Geriatr Psychiatry. 2023;31(6):401–10.
18. Garvin MM, Bolton JL. Sex-specific behavioral outcomes of early-life adversity and emerging microglia-dependent mechanisms. Front Behav Neurosci. 2022;16:1013865.
19. Noschang CG, Krolow R, Fontella FU, Arcego DM, Diehl LA, Weis SN, et al. Neonatal handling impairs spatial memory and leads to altered nitric oxide production and DNA breaks in a sex specific manner. Neurochem Res. 2010;35(7):1083–91.
20. Brito-Moreira J, Lourenco MV, Oliveira MM, Ribeiro FC, Ledo JH, Diniz LP, et al. Interaction of amyloid-beta (Abeta) oligomers with neurexin 2alpha and neuroligin 1 mediates synapse damage and memory loss in mice. J Biol Chem. 2017;292(18):7327–37.
21. Lourenco MV, Frozza RL, de Freitas GB, Zhang H, Kincheski GC, Ribeiro FC, et al. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. Nat Med. 2019;25(1):165–75.
22. Ribeiro FC, Cozachenco D, Heimfarth L, Fortuna JTS, de Freitas GB, de Sousa JM, et al. Synaptic proteasome is inhibited in Alzheimer's disease models and associates with memory impairment in mice. Commun Biol. 2023;6(1):1127.
23. De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, et al. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem. 2007;282(15):11590–601.
24. Figueiredo CP, Clarke JR, Ledo JH, Ribeiro FC, Costa CV, Melo HM, et al. Memantine rescues transient cognitive impairment caused by high-molecular-weight abeta oligomers but not the persistent impairment induced by low-molecular-weight oligomers. J Neurosci. 2013;33(23):9626–34.
25. Lourenco MV, Clarke JR, Frozza RL, Bomfim TR, Forny-Germano L, Batista AF, et al. TNF-alpha mediates PKR-dependent memory impairment and brain IRS-1 inhibition induced by Alzheimer's beta-amyloid oligomers in mice and monkeys. Cell Metab. 2013;18(6):831–43.
26. Ledo JH, Azevedo EP, Clarke JR, Ribeiro FC, Figueiredo CP, Foguel D, et al. Amyloid-beta oligomers link depressive-like behavior and cognitive deficits in mice. Mol Psychiatry. 2013;18(10):1053–4.
27. Laursen SE, Belknap JK. Intracerebroventricular injections in mice: some methodological refinements. J Pharmacol Methods. 1986;16(4):355–7.
28. Ledo JH, Azevedo EP, Beckman D, Ribeiro FC, Santos LE, Razolli DS, et al. Cross talk between brain innate immunity and serotonin signaling underlies depressive-like behavior induced by Alzheimer's amyloid-beta oligomers in mice. J Neurosci. 2016;36(48):12106–16.
29. Suman PR, Souza LS, Kincheski GC, Melo HM, Machado MN, Carvalho GMC, et al. Lung inflammation induced by silica particles triggers hippocampal inflammation, synapse damage and memory impairment in mice. J Neuroinflamm. 2022;19(1):303.
30. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process. 2012;13(2):93–110.
31. Sivakumaran MH, Mackenzie AK, Callan IR, Ainge JA, O'Connor AR. The discrimination ratio derived from novel object recognition tasks as a measure of recognition memory sensitivity, not bias. Sci Rep. 2018;8(1):11579.
32. Suman PR, Zerbinatti N, Theindl LC, Domingues K, de Oliveira CL. Failure to detect the action of antidepressants in the forced swim test in Swiss mice. Acta Neuropsychiatr. 2018;30(3):158–67.
33. Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology. 1995;121(1):66–72.
34. Lourenco MV, Ribeiro FC, Santos LE, Beckman D, Melo HM, Sudo FK, et al. Cerebrospinal fluid neurotransmitters, cytokines, and chemokines in Alzheimer's and lewy body diseases. J Alzheimer's Dis. 2021;82(3):1067–74.
35. Beckman D, Santos LE, Americo TA, Ledo JH, de Mello FG, Linden R. Prion protein modulates monoaminergic systems and depressive-like behavior in mice. J Biol Chem. 2015;290(33):20488–98.
36. Kincheski GC, Valentim IS, Clarke JR, Cozachenco D, Castelo-Branco MTL, Ramos-Lobo AM, et al. Chronic sleep restriction promotes brain inflammation and synapse loss, and potentiates memory impairment induced by amyloid-beta oligomers in mice. Brain Behav Immun. 2017;64:140–51.
37. Tag SH, Kim B, Bae J, Chang KA, Im HI. Neuropathological and behavioral features of an APP/PS1/MAPT (6xTg) transgenic model of Alzheimer's disease. Mol Brain. 2022;15(1):51.
38. Teixeira AL, Rocha NP, Gatchel J. Behavioral or neuropsychiatric symptoms of Alzheimer's disease: from psychopathology to pharmacological management. Arq Neuropsiquiatr. 2023;81(12):1152–62.
39. Bertollo AG, Galvan ACL, Dallagnol C, Cortez AD, Ignacio ZM. Early life stress and major depressive disorder—an update on molecular mechanisms and synaptic impairments. Mol Neurobiol. 2024. https://doi.org/10.1007/s12035-024-03983-2.
40. Solas M, Aisa B, Mugueta MC, Del Rio J, Tordera RM, Ramirez MJ. Interactions between age, stress and insulin on cognition: implications for Alzheimer's disease. Neuropsychopharmacology. 2010;35(8):1664–73.
41. Chen Y, Baram TZ. Toward understanding how early-life stress reprograms cognitive and emotional brain networks. Neuropsychopharmacology. 2016;41(1):197–206.
42. Frost PS, Barros-Aragao F, da Silva RT, Venancio A, Matias I, Lyra ESNM, et al. Neonatal infection leads to increased susceptibility to Abeta oligomer-induced brain inflammation, synapse loss and cognitive impairment in mice. Cell Death Dis. 2019;10(4):323.
43. Kokras N, Dalla C. Sex differences in animal models of psychiatric disorders. Br J Pharmacol. 2014;171(20):4595–619.
44. Kokras N, Antoniou K, Mikail HG, Kafetzopoulos V, Papadopoulou-Daifoti Z, Dalla C. Forced swim test: what about females? Neuropharmacology. 2015;99:408–21.
45. Klinger K, Gomes FV, Rincon-Cortes M, Grace AA. Female rats are resistant to the long-lasting neurobehavioral changes induced by adolescent stress exposure. Eur Neuropsychopharmacol. 2019;29(10):1127–37.
46. do Prado CH, Narahari T, Holland FH, Lee HN, Murthy SK, Brenhouse HC. Effects of early adolescent environmental enrichment on cognitive dysfunction, prefrontal cortex development, and inflammatory cytokines after early life stress. Dev Psychobiol. 2016;58(4):482–91.
47. Brenhouse HC, Andersen SL. Nonsteroidal anti-inflammatory treatment prevents delayed effects of early life stress in rats. Biol Psychiatry. 2011;70(5):434–40.
48. Barch DM, Harms MP, Tillman R, Hawkey E, Luby JL. Early childhood depression, emotion regulation, episodic memory, and hippocampal development. J Abnorm Psychol. 2019;128(1):81–95.
49. Colle R, Segawa T, Chupin M, Tran Dong MN, Hardy P, Falissard B, et al. Early life adversity is associated with a smaller hippocampus in male but not female depressed in-patients: a case-control study. BMC Psychiatry. 2017;17(1):71.
50. Ferreira ST, Clarke JR, Bomfim TR, De Felice FG. Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. Alzheimers Dement. 2014;10(1 Suppl):S76-83.
51. Bobbo VC, Engel DF, Jara CP, Mendes NF, Haddad-Tovolli R, Prado TP, et al. Interleukin-6 actions in the hypothalamus protects against obesity and is involved in the regulation of neurogenesis. J Neuroinflamm. 2021;18(1):192.
52. Bauer ME, Teixeira AL. Inflammation in psychiatric disorders: what comes first? Ann N Y Acad Sci. 2019;1437(1):57–67.
53. Lyra ESNM, Goncalves RA, Pascoal TA, Lima-Filho RAS, Resende EPF, Vieira ELM, et al. Pro-inflammatory interleukin-6 signaling links cognitive impairments and peripheral metabolic alterations in Alzheimer's disease. Transl Psychiatry. 2021;11(1):251.

54. Salvador AF, de Lima KA, Kipnis J. Neuromodulation by the immune system: a focus on cytokines. Nat Rev Immunol. 2021;21(8):526–41.
55. Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med. 2015;3(10):136.
56. Cirrito JR, Disabato BM, Restivo JL, Verges DK, Goebel WD, Sathyan A, et al. Serotonin signaling is associated with lower amyloid-beta levels and plaques in transgenic mice and humans. Proc Natl Acad Sci USA. 2011;108(36):14968–73.
57. Zarros AC, Kalopita KS, Tsakiris ST. Serotoninergic impairment and aggressive behavior in Alzheimer's disease. Acta Neurobiol Exp. 2005;65(3):277–86.
58. Oh SJ, Lee N, Nam KR, Kang KJ, Han SJ, Lee KC, et al. Amyloid pathology induces dysfunction of systemic neurotransmission in aged APPswe/PS2 mice. Front Neurosci. 2022;16:930613.
59. Colich NL, Williams ES, Ho TC, King LS, Humphreys KL, Price AN, et al. The association between early life stress and prefrontal cortex activation during implicit emotion regulation is moderated by sex in early adolescence. Dev Psychopathol. 2017;29(5):1851–64.
60. Perry CJ, Campbell EJ, Drummond KD, Lum JS, Kim JH. Sex differences in the neurochemistry of frontal cortex: Impact of early life stress. J Neurochem. 2021;157(4):963–81.
61. Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, et al. Exercise induces hippocampal BDNF through a PGC-1alpha/FNDC5 pathway. Cell Metab. 2013;18(5):649–59.

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