A systematic review and multilevel meta-analysis of the prenatal and early life stress effects on rodent microglia, astrocyte, and oligodendrocyte density and morphology

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Abstract

Exposure to stress during early development may lead to altered neurobiological functions, thus increasing the risk for psychiatric illnesses later in life. One potential mechanism associated with those outcomes is the disruption of glial density and morphology, despite results from rodent studies have been conflicting. To address that we performed a systematic review and meta-analysis of rodent studies that investigated the effects of prenatal stress (PNS) and early life stress (ELS) on microglia, astrocyte, and oligodendrocyte density and morphology within the offspring. Our meta-analysis demonstrates that animals exposed to PNS or ELS showed significant increase in microglia density, as well as decreased oligodendrocyte density. Moreover, ELS exposure induced an increase in microglia soma size. However, we were unable to identify significant effects on astrocytes. Meta-regression indicated that experimental stress protocol, sex, age, and type of tissue analyzed are important covariates that impact those results. Importantly, PNS microglia showed higher estimates in young animals, while the ELS effects were stronger in adult animals. This set of data reinforces that alterations in glial cells could play a role in stress-induced dysfunctions throughout development.

Keywords: Prenatal stress; early life stress; microglia; astrocyte; oligodendrocyte; glia.

1. Introduction

Exposure to stress during early stages of development, such as prenatal and postnatal life may lead to long-lasting adverse consequences (Abbott et al., 2018; Teicher et al., 2016). During pregnancy, the fetus is highly vulnerable and affected by the conditions experienced by the mother. Thus, psychological, physical, or metabolic insults suffered by the mother may disrupt the intrauterine environment, which will influence the development of the fetus (Glover et al., 2018). After birth, the newborn is still sensitive to both positive and negative influences from the environment, although one key difference is that now both the mother and the newborn may be directly be exposed to a variety of stressors, such as malnutrition, abuse, trauma, and neglect (Carr et al., 2013). Disruption in both periods has been associated with an increased risk for the development of psychopathologies later in life, such as depression, anxiety, and schizophrenia (Syed & Nemeroff, 2017; Teicher et al., 2002). This may be related to alterations of brain maturation, which is more significant in vulnerable brain regions characterized by delayed developmental processes, such as the hippocampus, prefrontal cortex, and amygdala (Aleksić et al., 2016; de Azeredo et al., 2017; Teissier et al., 2020). Nevertheless, the neurobiological underpinnings of the stress effects throughout development have not yet been fully characterized.

Previous studies have shown that stress-induced alterations are not only associated with neuronal function, but also have a significant impact on glial cells, including microglia, astrocytes, and oligodendrocytes (de Pablos et al., 2014; Jauregui-Huerta et al., 2010). These cells subserve a number of important functions within the brain, including clearing unwanted debris, controlling inflammation, providing nutrients, maintaining homeostasis, and forming the myelin sheath that surrounds axons (Allen & Lyons, 2018). Moreover, these cells are particularly vulnerable to the release of glucocorticoids during hypothalamic-pituitary-adrenal

(HPA) axis's activation, which is the main regulator of the stress response (Sugama & Kakinuma, 2020).

Microglia are the primary innate immune cells of the brain. When necessary, they become activated to phagocyte dead neurons, but also release cytokines and chemokines to regulate unwanted pathogens (de Pablos et al., 2014; Lenz & Nelson, 2018). However, the sustained or chronic activation of microglia may lead to significant damage. The elevated levels of inflammatory biomarkers released by microglia in the brain can affect multiple neurobiological processes and lead to behavioral deficits later in life (Galvani et al., 2021; Stein et al., 2017). Moreover, studies have associated the impairments induced by stress during early development with a chronic inflammatory state in the brain, which could be provoked by a dysfunction in microglia (Catale et al., 2020; Gildawie et al., 2020). Nevertheless, microglia can also have anti-inflammatory functions, which indicates phenotypic heterogeneity that may shape its effects over distinct cell populations (Chen et al., 2022).

One important morphological characteristic of activated microglia is that they present increased soma size and less ramified processes, so it is important to investigate not only the density of glial cells, but also their morphological pattern (Woodburn et al., 2021). However, other factors such as disease state in the brain can influence the association between microglia morphology and function, in which specific phenotypic traits are observed in diseases, such as Alzheimer's and metastatic tissue (Lier et al., 2021). Furthermore, it is worth mentioning that microglia play an important role for maturation processes and modulation of brain plasticity, which can alter brain development and function without being strictly related to immune surveillance. For example, a recent study has shown that a microglial deficiency may lead too long-term cognitive impairments (Schalbetter et al., 2022).

Astrocytes play a pivotal role in regulating adequate brain functioning, since they regulate blood flow, synaptogenesis, glutamate recycling, as well as the release of essential neurotransmitters (Kim et al., 2019). These cells also become reactive during stressful events, and these structural and functional alterations can become irreversible, which may disrupt astrocytic function throughout life (Codeluppi et al., 2021). These effects are specially identified during periods of vulnerability, such as early brain development (Abbink et al., 2019). Oligodendrocytes are another glial cell that are mainly responsible for assembling and maintaining the myelin sheaths that surround neuronal axons. Without myelin, the impulses are severely slowed, which may lead to disruption in adequate brain functioning (Poggi et al., 2022). Previous studies have shown that stress may impair myelination, and it could be associated with the development of multiple neurological disorders (Dulamea, 2017; Teissier et al., 2020).

Even though the literature points to a disrupted glial function induced by stress during early development (Johnson & Kaffman, 2018), studies are reporting a resilience mechanism in these cells, which may indicate that exposure to a stressful event during a single period of life would not be sufficient for inducing significant long-term alterations in glial function (Calcia et al., 2016; Gildawie et al., 2020). However, exposure to stress during early development appears to prime such cells to enhance their reactivity or vulnerability to a subsequent challenge, which would eventually produce long-term functional impairments (Fonken et al., 2018). In this regard, there are conflicting results in prenatal stress (PNS) and early life stress (ELS) literature that must be clarified for a better understanding of the relationship between stress during development and alterations of glial function.

Inconsistent findings might be attributable to several variables, including the protocol of stress, the timing and length of stress exposure, the species, the brain region analyzed, as

well as sex and age differences. To the best of our knowledge this is the first systematic review and meta-analysis that investigated these outcomes in rodents. For this reason, the aim of this study was to perform a systematic review and multilevel meta-analysis compiling rodent studies that investigated the effects of PNS or ELS exposure on microglia, astrocyte, and oligodendrocyte density and morphology within the offspring. We have also explored sources of heterogeneity between studies using meta-regression models.

2. Methods

2.1 Search strategy

The search was performed on July 19th, 2022. Three online databases were utilized to localize the articles: PubMed, Embase, and Web of Science. The following terms were utilized for the search: [glia OR neuroglia OR "ependymal cells" OR oligodendrocytes OR astrocytes OR microglia OR "glial fibrillary acidic protein" OR GFAP OR "glutamate aspartate transporter" OR GLAST OR "glutamate transporter 1") OR GLT-1 OR "brain lipid-binding protein" OR BLBP OR vimentin OR S-100 OR "forkhead box protein J1" OR FoxJ1 OR "myelin basic protein" OR MBP OR "myelin oligodendrocyte glycoprotein" OR MOG OR "oligodendrocyte transcription factor" OR OLIG2 OR SOX10 OR "transmembrane protein 10" OR TMEM10 OR CD11B OR CD45 OR CD68 OR "Ionized calcium-binding adapter molecule 1" OR IBA1 OR "transmembrane protein 119" OR TMEM119] AND [rattus OR "mus musculus" OR rat OR mice OR rodent] AND ["maternal separation" OR "maternal deprivation" OR "neonatal stress" OR "postnatal stress" OR "limited bedding" OR "maternal stress" OR "early life stress" OR "early handling" OR "early life adversity" OR "prenatal stress" OR "gestational stress" OR "perinatal stress" OR "antenatal stress" OR "pregnancy stress" OR "maternal stress"]. The Cochrane recommendations for developing and performing a search strategy were followed in this study (CDIG, 2007).

2.2 Selection and eligibility

Article filtering to identify the included studies for the review was performed in three phases. In the first phase, the authors screened exclusively titles and abstracts. In the second phase, the authors read the full texts to identify possible exclusions. The following exclusion criteria were applied in the studies: (1) the study was not written in English; (2) the study was not empirical; (3) the study did not use mice or rats; (4) the study did not have a prenatal or early life stress protocol; (5) the study did not analyze glial cells in the brain; (6) the study only used transgenic or knockout animals; (7) the study did not have a baseline control group. For the third phase, the authors screened the references from the included studies to select a possible study that was not identified by the search strategy. The first and second phases were performed blindly by four authors using the Rayyan QCRI software (Ouzzani et al., 2016). Two senior authors resolved any disagreement throughout the screening process.

2.3 Data extraction

Four independent authors manually extracted the following data from the included studies: 'first author', 'publication year', 'species', 'strain', 'stress protocol', 'stress period', 'stress duration per day', 'sex', 'age of euthanasia', 'biomolecular analysis', 'analyzed tissues', 'glial cells', 'glial markers', and 'summary of results'. To perform the meta-analysis, the mean value for the outcome, the standard deviation (SD), and the number of animals per group were extracted. When necessary, standard error (SE) was utilized to calculate the SD. When the information was only presented in graphs, the data were extracted using the software WebPlotDigitizer.

2.4 Coding procedure and potential moderators

The variables and codes below were utilized as potential moderators for the meta-analysis. It is important to highlight that separate meta-analyses were performed for PNS and ELS, as well as for each type of glial cell.

- Cell type (PNS and ELS), coded as: (0) microglia; (1) astrocyte; (2) oligodendrocyte.

- Species (PNS and ELS), coded as: (0) rats; (1) mice.

- PNS protocol, coded as: (0) restraint; (1) dexamethasone; (2) lipopolysaccharide; (3) sleep deprivation; (4) unpredictable chronic stress; (5) others (cold and shock).

- ELS protocol, coded as: (0) maternal separation; (1) maternal deprivation; (2) maternal separation combined; (3) limited bedding; (4) social stress; (5) chemical manipulation (ethanol, carbon, diesel, LPS, E. coli, valproic acid); (6) early weaning.

- PNS period, coded as: (0) 1-5 days; (1) 6-10 days; (2) 11-21 days.

- ELS period, coded as: (0) 1 day; (1) 2-7 days; (2) 8-14 days; (3) 15-21 days.

- Stress duration per day (PNS and ELS), coded as: (0) Single; (1) 1-60 min; (2) 61-360 min; (3) 361-720 min; (4) 721-1440 min.

- Sex (PNS and ELS), coded as: (0) male; (1) female; (2) male and female mixed.

- Age (PNS and ELS), coded as: (0) PND1-PND21; (1) PND22-PND45; (2) PND46-PND60; (3) PND61-PND90; (4) PND91-PND120; (5) PND121 or more.

- Molecular analysis (PNS and ELS), coded as: (0) Immunohistochemistry (IHC); (1) Western blot; (2) Real-time PCR; (3) ELISA.

- Tissue (PNS and ELS), coded as: (0) hippocampus; (1) prefrontal cortex; (2) frontal cortex; (3) nucleus accumbens; (4) amygdala; (5) striatum; (6) hypothalamus; (7) cerebellum; (8) entorhinal cortex; (9) corpus callosum; (10) cortex; (11) motor cortex; (12) whole brain; (13) pons; (14) medulla; (15) cingulate cortex; (16) ventral tegmental area; (17) midbrain; (18) medial precentral cortex; (19) olfactory bulb.

- Microglia marker (PNS and ELS), coded as: (0) IBA-1; (1) CD68; (2) CD11b.

- Astrocyte marker (PNS and ELS), coded as: (0) GFAP; (1) S100B; (2) GLAST; (3) GLT-1; (4) GS.

- Oligodendrocyte marker (PNS and ELS), coded as: (0) MBP, CNPase, PLP1, CC1, APC, ASPA (mature); (1) OLIG1, OLIG2, SOX10 (whole); (2) NG2, PDGFRA, Cx29, Cx32, Cx47 (progenitor); (3) MRF (premyelination).

- ELS microglia morphology, coded as: (0) Soma size.

2.5 Data analysis

In this meta-analysis the assumption between outcomes was violated since multiple studies contributed with more than one sample. For this reason, a random-effects model (RE Model) and a multilevel approach to generate the forest plots were utilized. A 2-level hierarchical data structure was modeled, with samples within studies nested with samples between studies. The estimated effect size of PNS and ELS microglia, astrocyte, and oligodendrocyte was determined using the standardized mean difference (SMD), calculated by use of Cohen's d. Influence analysis was performed to identify possible outliers. Heterogeneity was tested using Q statistic, and the proportion of total variability due to heterogeneity was assessed with I^2. Univariate meta-regression models with potential moderators were used to explore the sources of heterogeneity of all meta-analyses. The estimated proportional reduction in the total variance for each meta-regression model was computed using the variance accounted for (VAF), and a pseudo-R-squared value. Publication bias was explored using funnel plots' asymmetry and further statistically proven by Egger's regression test. All statistical analyses for the meta-analysis were performed using the package 'metafor' (version 3.8-1) from the open-source statistical software R (version 4.2.2).

3. Results

3.1 Summary of studies included in the research

Considering all databases, we identified 846 possible studies that were extracted from PubMed (n = 212), Embase (n = 376), and Web of Science (n = 258). After the exclusion of 371 duplicate studies, we screened 475 by title and abstract, which lead to 231 exclusions. The full text analysis (n = 244) resulted in additional 149 exclusions: n = 3 studies were not written in English; n = 5 were not empirical; n = 2 did not use mice or rats; n = 40 did not have a PNS or ELS protocol; n = 75 did not analyze glial cells in the brain; n = 13 use only transgenic or knockout animals; n = 11 did not have a baseline control group. After this step, a total of 95 studies were included in this review. Moreover, the reference review did not identify extra studies. Finally, 4 studies were not added in the meta-analysis due to the impossibility to calculate their SMD. A flowchart with a detailed description of all review stages of the review can be seen in Figure 1.

3.2 Characteristics of studies

All the studies included in the systematic review are listed in Table 1. For specific results regarding the studies that performed morphological analysis, see Table 2. Regarding all the 95 studies included in the review, 25.3% utilized PNS models (n = 24), 71.6% used ELS models (n = 68), and only 3.1% performed a combination of PNS and ELS (n = 3).

Regarding the included PNS studies, 75% of them were performed on rats, while 25% used mice. Wistar was the most utilized strain in rat studies (50%), while C57BL/6 was the most utilized in mice studies (50%). Restraint stress was the most used stress protocol (45.8%), which was followed by pharmacological models utilizing LPS (12.5%) and dexamethasone (12.5%). Considering all the PNS protocols, the animals were exposed to an average of 6.8

days of stress and 6.1 hours per day. Most of the studies analyzed only males (54.1%), while 37.5% analyzed both males and females. Moreover, females or males and females mixed were utilized only in 4.2% each. There were a variety of ages (ranging from PND1 to PND640) in which the glial cells of animals were analyzed, but on average the animals were sacrificed with 84.5 days of life. The predominant brain region analyzed was the hippocampus (59.3%), followed by the prefrontal cortex (9.3%), and frontal cortex (9.3%). For the glial analysis, IHC was considerably the most utilized technique (66.6%). Regarding the specific glial cells, astrocyte was analyzed in 52% of the studies, microglia in 36%, and oligodendrocyte in 12%. The most utilized markers for microglia, astrocyte, and oligodendrocyte were IBA-1 (84.6%), GFAP (88.9%), and MBP (33.3%), respectively.

In relation to the included ELS studies, 67.6% were performed with rats, and 32.4% utilized mice. Wistar (54.3%) and C57BL/6 (50%) were the most utilized strains for rats and mice, respectively. The most frequently used stress protocols were maternal separation (52.9%), maternal deprivation (20.6%), and limited bedding (7.3%). The average of all ELS protocols was 10.1 days of stress, while the animals were exposed on average to 9.6 hours per day. Fifty percent of the studies were performed only in males, while 35.3% investigated both males and females. Males and females mixed were utilized in 11.8% of studies, while females were only utilized in 2.9%. The age for the analysis ranged from PND4 to PND540, but on average the animals were sacrificed with 67.4 days of life. Hippocampus (42.6%) and PFC (20.3%) were the most commonly investigated brain regions in the ELS studies. IHC was the most frequent technique utilized for glial analysis (68.7%). Regarding the glial cells, astrocytes (44.8%) were the most analyzed, followed by microglia (41.4%) and oligodendrocyte (13.8%). Interestingly 100% of the studies utilized IBA-1 as a marker for microglia, while 89.7% utilized GFAP for astrocyte, and MBP (58.3%) for oligodendrocyte.

3.4 Effects of prenatal stress exposure on microglia, astrocyte, and oligodendrocyte

The effects of PNS on microglia markers were reported in 8 studies with a total of 16 effect sizes. Animals exposed to PNS showed a significant increase of microglia markers in the brain compared to control animals (SMD 1.50; 95% CI 0.69, 2.31; Fig. 2). Regarding astrocytes, it was measured in 13 studies (53 effect sizes) and no significant differences between PNS and control groups were found (SMD 0.40; 95% CI -0.13, 0.93; Fig. 3). Whereas for oligodendrocytes, 3 studies reported the effects of PNS with a total of 7 effect sizes. The meta-analysis revealed that animals exposed to PNS showed reduced levels of oligodendrocyte-related markers in the brain (SMD -1.19; 95% CI -2.08, -0.31; Fig. 4).

All the meta-analyses presented significant heterogeneity between studies (microglia I^2 = 82.95%, p < 0.0001; astrocyte I^2 = 62.64%, p < 0.0001; oligodendrocyte I^2 = 72.3%, p < 0.0241). Thus, meta-regression models were performed to investigate the sources of heterogeneity using several moderators.

In relation to the microglia meta-analysis, stress period (p = 0.0092, VAF = 5.63%) was a covariate significantly associated with estimates of heterogeneity, indicating that longer stress periods (6 to 10 days) resulted in decreased estimates compared to shorter periods (1 to 5 days). Regarding the age covariate, adolescent (PND22-45) and adult (PND61-90) animals (p = 0.0243 and 0.0001, respectively, VAF = 16%) showed lower estimates compared to young animals (PND0-21), while this difference was not observed in older animals (PND91-120). About the analyzed brain region, the prefrontal cortex showed lower estimates than the hippocampus (p = 0.0005, VAF = 14.56%). By comparing the effects of different microglia markers, we found lower estimates of CD68 compared to IBA-1 (p = 0.0009; VAF = 21.25%). In addition, by using IHC as a reference of the method of analysis, western blot was associated with higher microglia estimates, while ELISA with lower (p = 0.0283, p = 0.0262, respectively,

VAF = 35.30%). Regarding astrocyte meta-analysis, the sex covariate indicated that females showed lower estimates compared to males (p = 0.0014, VAF = 14.74%). Further information on meta-regressions can be seen in Supplementary tables 1-3. Data regarding PNS Meta-analysis of microglia, astrocyte, and oligodendrocyte performed exclusively on studies that performed cell count analysis can be seen in Supplementary Figures 1-3.

Egger's regression test and funnel plots were constructed to investigate possible publication bias. Indeed, the funnel plots for microglia, astrocyte, and oligodendrocyte revealed a publication bias, shown by significant asymmetry ($z = 5.85$, $p < 0.0001$; $z = 5.18$, $p < 0.0001$; $z = -2.13$, $p = 0.0331$, respectively; Fig. 5).

3.5 Effects of early life stress exposure on microglia, astrocyte, and oligodendrocyte.

A total of 33 studies have reported the effects of ELS on microglia (172 effect sizes). Meta-analysis revealed that exposure to ELS leads to an increase in microglia-related markers (SMD 0.40; 95% CI 0.01, 0.78; Fig. 6). Moreover, the morphological meta-analysis revealed that microglia soma size was also increased after ELS exposure (SMD 0.91, 95% CI 0.19, 1.53; Fig. 7). In relation to astrocytes, 37 studies have analyzed the effects of ELS exposure (166 effect sizes). However, no significant differences were found (SMD -0.12; 95% CI -0.49, 0.24; Fig. 8). For oligodendrocytes, ELS was shown to decrease the levels of related markers in the brain (SMD -0.35; 95% CI -0.79, -0.01; Fig. 9), as shown by 12 studies (52 effect sizes).

Heterogeneity between studies was investigated for ELS studies, and the analysis detected a significant heterogeneity for all above-mentioned meta-analyses (microglia I^2 = 77.20%, $p < 0.0001$; microglia soma size I^2 = 55.94%, $p < 0.0001$; astrocyte I^2 = 85.71%, $p < 0.0001$; oligodendrocyte I^2 = 73.14%, $p < 0.0001$). Therefore, meta-regressions with a range of moderators were performed to investigate the possible sources of heterogeneity.

Regarding microglia, stress protocol was a covariate associated with the estimates of heterogeneity, indicating that the combination of maternal separation with limited bedding/early weaning or chemical manipulation (ethanol, diesel, carbon particles, etc.) leads to higher estimates compared to maternal separation alone ($p < 0.0001$ for both comparisons, VAF = 19.89%). Animals exposed to stress periods of 2-7 days or 15-21 showed higher estimates compared to animals exposed to 1 day of stress ($p = 0.0019$, $p = 0.0188$, respectively, VAF = 17.02%). Next, considering the stress duration of up to 60 min, 61-360 min and 721-1440 min per day, longer protocols were associated with lower estimates ($p = 0.0003$, $p = 0.0001$, $p = 0.0010$, respectively, VAF = 6.77%). Adult animals (PND61-90 and PND91-120) have higher estimates compared to PND0-21 animals ($p = 0.0002$, $p < 0.0001$, respectively, variance explained = 10.23%). In addition, by using IHC as a reference for the method of analysis, western blot was associated with higher microglia estimates, while real-time PCR with lower estimates ($p = 0.0247$, $p = 0.0001$, respectively, VAF = 5.55%). In relation to astrocytes, the stress protocol covariate showed that maternal deprivation, limited bedding, social stress, and chemical manipulation present higher estimates compared to maternal separation ($p < 0.0001$, $p = 0.0188$, $p = 0.0044$, $p < 0.0001$, respectively, VAF = 11.66%). Next, considering the stress duration per day, longer protocols (61-360 minutes) were associated with lower estimates ($p = 0.030$; VAF = 6.38%). Adolescent and adult animals (PND22-45, PND46-60, and PND61-90) presented higher estimates, while older animals (after PND121) showed lower estimates compared to young animals at PND0-21 ($p < 0.0001$, $p = 0.0097$, $p < 0.0001$, $p = 0.0122$, respectively, VAF = 10.97%). Regarding oligodendrocytes, stress protocol covariate indicated that maternal separation combined with limited bedding/early weaning, or limited bedding alone presented lower estimates than maternal separation ($p = 0.0016$, $p = 0.0017$, respectively, VAF = 10.3%). Longer protocols of stress (15-21 days) lead to lower estimates of

oligodendrocytes compared to protocols with one day ($p = 0.0222$, VAF = 7.65%). In addition, by using IHC as a reference for the method of analysis, real-time PCR was associated with lower estimates ($p = 0.001$; VAF = 5.87%). Detailed information on meta-regressions can be seen in Supplementary tables 4-6. Results of ELS Meta-analysis of microglia, astrocyte, and oligodendrocyte performed exclusively on studies that performed cell count analysis can be seen in Supplementary Figures 4-6.

Funnel plots and Egger's regression test were performed to evaluate publication bias. The analysis revealed a publication bias in microglia, microglia soma size, and oligodendrocyte shown by significant asymmetry ($z = 7.15$, $p < 0.0001$; $z = 6.85$, $p < 0.0001$; $z = -3.63$, $p = 0.0003$, respectively; Fig. 10). While the funnel plot of astrocyte did not present significant asymmetry ($z = 0.33$, $p = 0.7382$).

4. Discussion

Glial cells are essential players for the modulation of neuronal adequate function. Accordingly, their disruption may lead to significant impairments in the brain (Allen & Lyons, 2018). For this reason, investigating how stressful events during vulnerable periods of life can modify glial function is important to understand brain development. This study analyzed the effects of PNS and ELS exposure on microglia, astrocyte, and oligodendrocyte markers, density and morphology in the offspring. The meta-analysis revealed that exposure to PNS and ELS in rodents leads to increased microglia cell density, while the density of oligodendrocytes was decreased. Moreover, ELS exposure induced an increase in microglia soma size. Conversely, no significant alteration was observed regarding the density of astrocytes in both PNS or ELS models.

A significant increase in microglial density was observed in both PNS and ELS meta-analyses. One mechanism underlying this increase in microglia is the excessive release of glucocorticoid hormones during chronic periods of stress (Schramm & Waisman, 2022). Previous studies have associated such changes with a dysfunction of the HPA axis that may lead to an elevated production of inflammatory mediators. Although corticosterone acts as an immunosuppressor during acute stimuli, high doses during a chronic state may prime components of the immune system leading to an overactivation (Cain & Cidlowski, 2017; Silverman & Sternberg, 2012). Excessive noradrenergic signaling is another mechanism that can influence microglia function. Indeed, stimulation of β-adrenoceptor in microglia is one the mechanisms that contribute to stress-induced release of pro-inflammatory cytokines (Sugama et al., 2019). Accordingly, administration of β-adrenoceptor antagonists has been shown to promote microglia inhibition (Wohleb et al., 2011). The excessive proliferation of microglia may exacerbate the phagocytic activity on healthy neurons, thus leading to detrimental effects on the function of specific brain circuits. Furthermore, in addition to classical neuroinflammatory and immune actions, microglia play a significant role in brain development, support of neuronal function, and injury repair, which could be impaired due to alterations in the density and morphology of those cells (Colonna & Butovsky, 2017).

Our results from the morphological meta-analysis indicate an increase in soma size in the microglia of animals exposed to ELS. The increase in soma size is associated with an activated state of microglia, with higher rates of proliferation, shorter and thicker processes, and enhanced release of cytokines (Stein et al., 2017; Woodburn et al., 2021). Furthermore, it has been shown that a single session of stress may not necessarily modify microglia density, but it could prime microglia responsiveness to a secondary challenge. This would leave the cells in a sensitive state that would facilitate significant alterations in microglia morphology

and function after acute or chronic exposure to a secondary hit (Catale et al., 2021; Gildawie et al., 2020).

Our meta-analysis also revealed a decrease in oligodendrocytes in both PNS and ELS studies. This data is in line with the literature regarding the negative effects of chronic stress exposure on oligodendrocyte development and myelination (Poggi et al., 2022; Teissier et al., 2020). The first weeks of development represent a critical period for the proliferation of oligodendrocyte progenitors, and the stress-induced disruption of this process during early life could lead to adult hypomyelination and reduced oligodendrocyte density (Bordner et al., 2011). This long-term reduction in myelination has been associated with the development of multiple conditions, such as depression, schizophrenia, and bipolar disorder (Fessel, 2022; Kokkosis et al., 2022; Zhou et al., 2021).

Exposure to PNS or ELS did not produce significant changes in astrocyte density. It is possible to associate this data with the fact that astrocytes are less prone to reprograming due to the glutamatergic regulation, which leads to resilience in terms of stress-induced effects (Gleixner et al., 2016; Mahmoud et al., 2019). Moreover, astrocytes have high reparatory and survival capacities after exposure to stressful environments, which in part could also be attributed to the lack of long-term density alterations (Gallo & Deneen, 2014; Hemati-Gourabi et al., 2022). Nevertheless, stress may induce morphological alterations independently of possible changes in cell density (Aten et al., 2022; Codeluppi et al., 2021). Unfortunately, our meta-analysis could not test this possibility due to a lack of included articles that analyzed morphological changes in astrocytes. Moreover, considering the heterogeneous findings identified in our meta-analysis, it can be hypothesized that, although no significant alterations are present after an initial stress episode, such cells may show enhanced vulnerability to a secondary hit later in life (Lopez-Rodriguez et al., 2021).

Considering the possible variability between the expression of glial markers in some techniques (e.g real-time PCR, western blot, and ELISA) and cell density (IHC), we have performed an additional meta-analysis to investigate only studies that performed direct cell count analysis. For microglia and astrocytes cell count analysis, we observed the same result with similar SMDs as the general meta-analysis with all the studies included. However, when investigating oligodendrocytes, there were no significant alterations in both PNS and ELS studies. This suggests that for this cell population, an alteration on expression levels does not necessarily correlate with altered number of cells. Nevertheless, after removing the studies for this cell count analysis, we only had two studies for the PNS model, which could affect the results. Moreover, the markers from different maturation stages in the oligodendrocytes can be considered a confounding factor, it is possible that the effects of stress differ regarding each maturation stage. Due to the number of studies, it was not possible to investigate each stage separately.

In relation to the moderators for the PNS meta-analysis, we observed that multiple studies used longer periods of PNS, but the short sleep deprivation protocol resulted in the highest increase in microglia density. As previously reported, this demonstrates the robust effects of sleep deprivation in promoting immune dysfunction (Garbarino et al., 2021). Furthermore, the impact of PNS on microglia density was stronger during young life, since such alterations were not identified in older animals. This suggests that the effects are more apparent shortly after the end of the manipulation, but they may not last throughout the entire life. The increase in hippocampal microglia compared to other brain regions is in line with previous developmental studies that indicate a high vulnerability of the hippocampus during early life (Andersen, 2003). Even though there was no overall effect of the astrocyte meta-analysis, we identified a lower estimate of female astrocyte density compared to males.

This could be due to an increased vulnerability of females regarding the effects of stress (Goodwill et al., 2019; Wellman et al., 2018).

Regarding the moderators for the ELS meta-analysis, we found that the combination of maternal separation and a secondary 'challenge' leads to higher microglia estimates compared to maternal separation alone. We have recently shown that the combination of maternal separation and limited bedding has robust effects on HPA axis function, which is known to have a significant impact on microglia (Orso et al., 2020). Moreover, longer periods of stress induced more robust alterations, suggesting that stronger ELS protocols may induce more pronounced effects on microglia function. Considering the stress duration per day, we identified that longer periods induced lower microglia estimates, which could be misinterpreted. However, the single administration protocols are exclusively the inflammatory challenges (LPS, E.coli infection, and valproic acid), which are known to have a significant impact on the immune system (Lively & Schlichter, 2018).

Interestingly, maternal separation presented lower astrocyte estimates when compared to all other models, which indicates a significant disruption in astrocytic function induced by the classical maternal separation model (Banqueri et al., 2019; Tractenberg et al., 2016). Similar to the changes observed in microglia cells, estimates of astrocyte density were higher in adult animals compared to young animals. In accordance with the microglia and astrocyte ELS data, combined and long-stress models provoked a greater reduction in oligodendrocyte density, which would probably lead to a reduction in axonal myelination. In addition, for both PNS and ELS meta-regressions models, the method of analysis (IHC, real-time PCR, western blot, and ELISA) was a significant moderator, suggesting that methodological considerations regarding biochemical and molecular outcomes should be well appraised by researchers.

Considering the long-term effects observed in the moderators of the meta-analysis and the plasticity characteristics of glial cells (Pirttimaki & Parri, 2013; Robins et al., 2013), it is important to consider, especially for the ELS models that the alterations observed may not be induced by a reprogramming of the HPA axis and immune system, but they could also be led by a direct dysfunction of those cells during early development. Exposure to stress during early life may induce alterations in mechanisms related to apoptosis and survival signaling that may affect glial cell density. A recent study has shown that ELS exposure was associated with the modulation of the Tgfβ pathway, which is associated with increased microglia proliferation and survival rates (Reemst et al., 2022). On the other hand, previous studies have reported increased apoptosis of microglia after exposure to stress, which can be interpreted as a self-compensatory mechanism to control damage to nearby cells (Catale et al., 2020; Desplats et al., 2020). Moreover, epigenetic alterations during fetal development in the PNS models could play a significant role since the dams and not directly the pups are exposed to stress, while in the ELS model is a direct exposure to the pups, which could lead to a brain modulation to adapt for this new environment. Furthermore, region specific effects for the microglia analysis were only identified in the PNS models. Even though our data cannot fully explain this result, this could be in part due to the vulnerability of the hippocampus during fetal development (Jacob et al., 2011).

Limitations must be considered when interpreting the data from our study. First, the meta-analysis was carried out using data from multiple protocols, brain regions, and different cell markers, which could lead to potential heterogeneity between studies. Furthermore, the inclusion of multiple techniques in the analysis can lead to confounding results, since it is known that the expression levels in gene expression data do not necessarily correlate with protein or cell count estimations. To minimize this issue, we applied potential moderators to

follow-up the analysis. Second, funnel plots showed that studies were not symmetrically distributed, suggesting that asymmetry could be attributed to publication bias, methodological differences, or true heterogeneity, which indicates an important gap in the stress literature that should be developed. Finally, the vast majority of included studies report IBA-1 as a microglia marker. However, it is known that IBA-1 is also expressed in macrophages and brain-invading monocytes, which should be taken into consideration when interpreting the results from our analysis (Nakamura et al., 2013; Varvel et al., 2016).

In summary, our meta-analysis indicates that both PNS and ELS exposure leads to an increase in microglia density, as well as decreased oligodendrocyte density. Furthermore, animals exposed to ELS protocols showed increased microglia soma size. Nevertheless, it is important to consider that the stress protocol, sex, age, and analyzed tissue are mediators of those results. This set of data provides evidence that stress exposure during early development may disrupt glial cells, especially those involved with immune function and myelination processes. On these bases, preclinical studies represent an essential tool to characterize the contribution of these mechanisms for the susceptibility to psychiatric disorders. This will eventually allow for the development of novel strategies targeting glial cells in order to prevent or reduce the disease burden associated with early life stress exposure.

Figure Captions

Fig. 1. Flowchart of the systematic review.

Fig. 2. Forest plot demonstrating the effect sizes of microglia after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. 3. Forest plot demonstrating the effect sizes of astrocyte after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. 4. Forest plot demonstrating the effect sizes of oligodendrocyte after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. 5. Funnel plots indicating publication bias of prenatal stress studies. A) Microglia; B) Astrocyte; C) Oligodendrocyte.

Fig. 6. Forest plot demonstrating the effect sizes of microglia after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. 7. Forest plot demonstrating the effect sizes of microglia soma size after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. 8. Forest plot demonstrating the effect sizes of astrocyte after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. 9. Forest plot demonstrating the effect sizes of oligodendrocyte after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. 10. Funnel plots indicating publication bias of early life stress studies. A) Microglia; B) Microglia soma size; C) Astrocyte; D) Oligodendrocyte.

Fig. S1. Forest plot demonstrating the effect sizes of microglia cell count after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. S2. Forest plot demonstrating the effect sizes of astrocyte cell count after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. S3. Forest plot demonstrating the effect sizes of oligodendrocyte cell count after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. S4. Forest plot demonstrating the effect sizes of microglia cell count after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. S5. Forest plot demonstrating the effect sizes of astrocyte cell count after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Fig. S6. Forest plot demonstrating the effect sizes of oligodendrocyte cell count after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Tables

Table 1. Descriptive characteristics, summary, and significant findings of prenatal and early life stress studies.

Table 2. Descriptive characteristics and significant findings of morphological analysis from prenatal and early life stress studies.

Table S1. Univariate meta-regression models for microglia after prenatal stress exposure.

Table S2. Univariate meta-regression models for astrocyte after prenatal stress exposure.

Table S3. Univariate meta-regression models for oligodendrocyte after prenatal stress exposure.

Table S4. Univariate meta-regression models for microglia after early life stress exposure.

Table S5. Univariate meta-regression models for astrocyte after early life stress exposure.

Table S6. Univariate meta-regression models for oligodendrocyte after early life stress exposure.

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