



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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ARTICLE INFO

Keywords:

Prenatal stress
Early life stress
Microglia
Astrocyte
Oligodendrocyte
Glia

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.

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 and 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

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<https://doi.org/10.1016/j.neubiorev.2023.105202>

Received 19 February 2023; Received in revised form 12 April 2023; Accepted 24 April 2023

Available online 26 April 2023

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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 subservise 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 and 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 and Kakinuma, 2020).

Microglia are the primary innate immune cells of the brain. When necessary, they become activated to phagocytose dead neurons, but also release cytokines and chemokines to regulate unwanted pathogens (de Pablos et al., 2014; Lenz and 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 to 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 and 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.

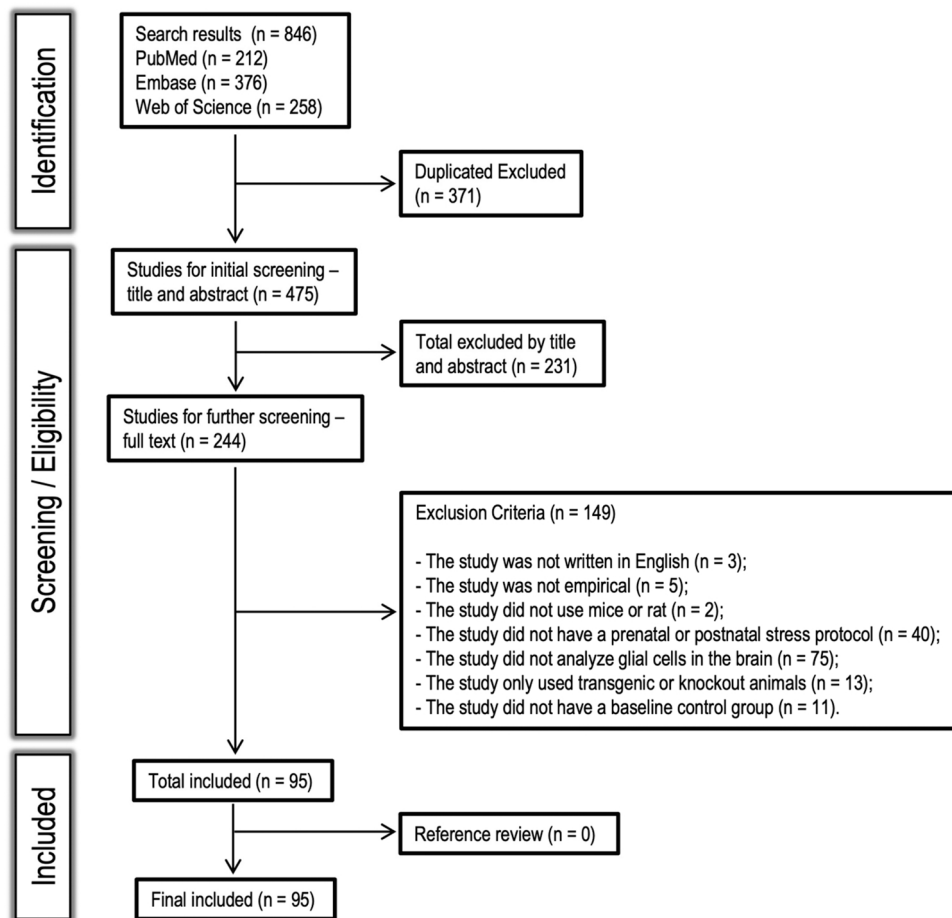


Fig. 1. Flowchart of the systematic review.

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, CCL1, 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

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

Author (year)	PNS / ELS	Rat / Mice	Strain	Stress protocol	Stress period	Stress duration per day	Sex	PND of euthanasia	Biomolecular analysis	Brain region	Glial cells	Glial marker	Results of stressed animals
Namvarpour Z (2022)	PNS	Mice	NMRI	LPS administration	GD17	Single	Male	PND56	Real-time PCR	Prefrontal Cortex	Oligodendrocyte	SOX10 / OLIG1 / MRF	No significant differences.
Verhaeghe R (2022)	PNS	Rat	Sprague-Dawley	Restraint stress	GD11-GD21	135 min	Male and female	PND630–650	Western blot	Hippocampus / Prefrontal Cortex	Astrocyte	GFAP	GFAP (hippocampus): decreased in males.
Gaspar R (2021)	PNS	Rat	Wistar Han	Dexamethasone administration	GD18-GD19	Single	Male and female	PND109	Immunohistochemistry	Hippocampus / Nucleus Accumbens	Microglia	IBA-1	See Table 2 for morphological results.
Han Y (2020)	PNS	Rat	Sprague-Dawley	Sleep deprivation	GD18-GD21	24 h	Male	PND28	Immunohistochemistry	Hippocampus	Microglia	IBA-1	IBA-1: increased in males.
Niu Y (2020)	PNS	Rat	Sprague-Dawley	Unpredictable chronic stress	GD14-GD21	24 h	Male and female	PND35 or PND70	ELISA	Prefrontal Cortex	Microglia	CD68	CD68: increased in PND35 females.
Lian S (2019)	PNS	Rat	Wistar	Cold stress	GD14-GD21	24 h	Male and female	PND21	Western blot	Hippocampus	Astrocyte	GFAP	GFAP: decreased in males and females.
Gumusoglu SB (2017)	PNS	Mice	CD1	Restraint stress	GD12-GD21	135 min	Male	PND84	Immunohistochemistry	Cortex	Microglia	IBA-1	No significant differences.
Maturana CJ (2017)	PNS	Mice	C57BL/6	Dexamethasone administration	GD14-GD21	Single	Male and female mixed	PND2	Immunohistochemistry	Hippocampus	Oligodendrocyte	Cx29 / Cx32 / Cx47	Cx32 and Cx47: decreased in males and females (mixed).
Barbie-Shoshani Y (2016)	PNS	Rat	Wistar	Unpredictable chronic stress	GD13-GD21	135 min	Male and female	PND21	Immunohistochemistry	Hippocampus	Astrocyte	GFAP / S100b	GFAP and S100B: increased in males.
Suzuki A (2016)	PNS	Mice	DDY	Restraint stress	GD12-GD21	135 min	Male	PND30	Immunohistochemistry	Hippocampus	Oligodendrocyte	MBP	MBP: decreased in males.
Adrover E (2015)	PNS	Rat	Wistar	Restraint stress	GD14-GD21	135 min	Male and female	PND60	Western blot / Real-time PCR	Hippocampus / Frontal Cortex	Astrocyte	GLT1 / GLAST	GLT1 (hippocampus): increased in males.
Bercum FM (2015)	PNS	Rat	Sprague-Dawley	Shocks	GD3-GD21	5 min	Male	PND60	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	GFAP: increased in males.
Huang CF (2015)	PNS	Rat	Sprague-Dawley	LPS administration	GD8, GD10, and GD12	Single	Male	PND90	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	GFAP: increased in males.
Shende VH (2015)	PNS	Rat	Sprague-Dawley	Dexamethasone administration	GD16-GD19	Single	Male	PND68	Immunohistochemistry	Hippocampus / Amygdala / Nucleus Accumbens	Astrocyte	GFAP	No significant differences.
Ślusarczyk J (2015)	PNS	Rat	Sprague-Dawley	Restraint stress	GD14-GD21	135 min	Male	PND92	Western blot	Hippocampus / Frontal Cortex	Microglia	IBA-1	IBA-1 (hippocampus and frontal cortex): increased in males.
Sun H (2015)	PNS	Rat	Sprague-Dawley	Restraint stress	GD14-GD21	135 min	Male and female	PND34	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	No significant differences.
Zhao QY (2015)	PNS	Rat	Wistar	Sleep deprivation	GD18-GD21	24 h	Male	PND21	Immunohistochemistry / Real-time PCR	Hippocampus	Microglia	IBA-1 / CD68	IBA-1 and CD68: increased in males.
Zhao QY (2014)	PNS	Rat	Wistar	Sleep deprivation	GD18-GD21	24 h	Male	PND21	Immunohistochemistry	Hippocampus	Microglia	IBA-1	No significant differences.

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Table 1 (continued)

Author (year)	PNS / ELS	Rat / Mice	Strain	Stress protocol	Stress period	Stress duration per day	Sex	PND of euthanasia	Biomolecular analysis	Brain region	Glial cells	Glial marker	Results of stressed animals
Diz-Chaves Y (2013)	PNS	Mice	C57BL/6	Restraint Stress	GD12-GD21	135 min	Male	PND120	Immunohistochemistry	Hippocampus	Astrocyte / Microglia	IBA-1 / GFAP	No significant differences.
Diz-Chaves Y (2012)	PNS	Mice	C57BL/6	Restraint stress	GD12-GD21	135 min	Female	PND120	Immunohistochemistry	Hippocampus	Microglia	IBA-1	IBA-1: decreased in females.
Kirsten TB (2011)	PNS	Rat	Wistar	LPS administration		Single	Male	PND60	Immunohistochemistry	Olfactory Bulb	Astrocyte	GFAP	No significant differences.
García-Cáceres C (2010)	PNS	Rat	Wistar	Restraint stress	GD14-GD21	135 min	Male and female	PND180	Western blot / Immunohistochemistry	Hypothalamus	Astrocyte	GFAP / S100b	S100b: increased in males.
Barros VG (2006)	PNS	Rat	Wistar	Restraint stress	GD14-GD21	135 min	Male	PND90	Immunohistochemistry	Hippocampus / Striatum / Frontal Cortex	Astrocyte	GFAP / S100b	GFAP and S100b (frontal cortex, striatum, and hippocampus): increased in males.
Van den Hove DL (2006)	PNS	Rat	Fisher 344	Restraint stress	GD14-GD21	135 min	Male and female	PND1	Immunohistochemistry	Hippocampus	Astrocyte	S100b	S100b: decreased in males and females.
Bachiller S (2022)	ELS	Mice	C57BL/6 J	Maternal separation	PND2-PND14	3 h	Male and female	PND15 / PND120	Immunohistochemistry	Hippocampus / Prefrontal Cortex	Microglia	IBA-1 / CD68	IBA-1 (hippocampus): increased in PND15 males. IBA-1 (prefrontal cortex): increased in PND120 males.
Mi X (2022)	ELS	Mice	C57BL/6 J	Maternal separation with Limited bedding	PND1-PND21	24 h	Male	PND93	Western blot / Immunohistochemistry	Hippocampus / Prefrontal Cortex	Microglia	IBA-1	IBA-1 (hippocampus and prefrontal cortex): increased in males.
Nicolas S (2022)	ELS	Rat	Sprague-Dawley	Maternal separation	PND2-PND12	3 h	Female	PND22	Immunohistochemistry	Hippocampus	Microglia	IBA-1	No significant differences.
Catale C (2021)	ELS	Mice	DBA2/J	Social stress	PND14-PND21	30 min	Male and female mixed	PND67	Western blot / Immunohistochemistry	Pons	Microglia / Astrocyte	IBA-1 / CD68 / GFAP	No significant differences.
Martín-Sánchez A (2021)	ELS	Mice	CD1	Maternal separation with Early weaning	PND2-PND16	4–8 h	Male	PND80	Immunohistochemistry	Hippocampus / Amygdala / Cerebellum	Microglia	IBA-1	IBA-1 (hippocampus): increased in males.
Poleksic J (2021)	ELS	Rat	Wistar Albino	Maternal deprivation	PND9	24 h	Male	PND10 / PND60	Western blot / Immunohistochemistry	Prefrontal Cortex	Microglia	IBA-1	IBA-1: increased in PND60 males.
Réus GZ (2021)	ELS	Rat	Wistar	Maternal separation	PND1-PND10	3 h	Male	PND53	Immunohistochemistry	Whole Brain	Microglia / Astrocyte	IBA-1 / GFAP	No significant differences.
Ruigrok SR (2021)	ELS	Mice	C57BL/6 J	Limited bedding	PND2-PND9	24 h	Male	PND9 / PND230	Immunohistochemistry	Hypothalamus	Microglia	IBA-1	IBA-1: increase in PND9 males.
Saavedra LM (2021)	ELS	Rat	Sprague-Dawley	Maternal separation	PND1-PND14	3 h	Male and female	PND140 / PND170	Immunohistochemistry	Hippocampus	Microglia / Astrocyte	IBA-1 / GFAP	IBA-1: decreased in males and decreased in females.
Xiong X (2021)	ELS	Rat	Sprague-Dawley	Maternal separation	PND1-PND21	6 h	Male	PND31 / PND81	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	GFAP: decreased in males.
Zetter MA (2021)	ELS	Rat	Wistar	Maternal separation	PND2-PND15	3 h	Male and female	PND15	Immunohistochemistry	Hippocampus	Microglia	IBA-1	See Table 2 for morphological results.

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Table 1 (continued)

Author (year)	PNS / ELS	Rat / Mice	Strain	Stress protocol	Stress period	Stress duration per day	Sex	PND of euthanasia	Biomolecular analysis	Brain region	Glial cells	Glial marker	Results of stressed animals
Abbink MR (2020)	ELS	Mice	C57BL/6 J	Limited bedding	PND2-PND9	24 h	Male	PND9 / PND120 / PND180 / PND300	Immunohistochemistry	Hippocampus / Entorhinal Cortex	Astrocyte	GFAP	GFAP (entorhinal cortex): increased in PND9 males. GFAP (hippocampus): decrease in PND300 males.
Bachiller S (2020)	ELS	Mice	C57BL/6 J	Maternal separation	PND2-PND14	3 h	Male and female	PND42	Immunohistochemistry	Hippocampus	Microglia	IBA-1	See Table 2 for morphological results.
Catale C (2020)	ELS	Mice	DBA	Maternal separation / Social stress	PND14-PND21	30 min	Male and female mixed	PND60	Immunohistochemistry	Hippocampus / Amygdala	Microglia	IBA-1	IBA-1 (hippocampus): increased in males and females (mixed) exposed to maternal separation and social stress. IBA-1 (amygdala): decreased in males and females (mixed) exposed to social stress.
Criado-Marrero M (2020)	ELS	Mice	Not informed	Maternal separation	PND1-PND14	3 h	Male and female mixed	PND150 / PND300	Immunohistochemistry	Hippocampus	Microglia / Astrocyte	IBA-1 / GFAP	IBA-1: increased in PND300 males and females (mixed). GFAP: decreased in PND300 males and females (mixed).
Diaz-Chávez A (2020)	ELS	Rat	Sprague-Dawley	Maternal separation	PND1-PND21	3 h	Male	PND42	Immunohistochemistry	Hippocampus	Microglia	IBA-1	No significant differences.
Gildawie KR (2020)	ELS	Rat	Sprague-Dawley	Maternal separation	PND2-PND20	4 h	Male and female	PND20 / PND40	Immunohistochemistry	Prefrontal Cortex	Microglia	IBA-1	No significant differences.
Reshetnikov V (2020)	ELS	Mice	C57BL/6 J	Maternal separation / Maternal deprivation	PND2-PND14 / PND9	3 h / 24 h	Male	PND15	Immunohistochemistry	Prefrontal Cortex / Hippocampus	Microglia	IBA-1	No significant differences.
Teissier A (2020)	ELS	Mice	BALB/cJ Rj	Maternal separation	PND2-PND14	3 h	Male	PND15 / PND100	Immunohistochemistry	Prefrontal Cortex	Oligodendrocyte	PDGFRa / CC1	PDGFRa: decreased in PND100 males. CC1: increased in PND15 males.
Wang R (2020)	ELS	Rat	Wistar	Maternal separation	PND2-PND20	4 h	Male	PND27 / PND76	Immunohistochemistry	Prefrontal Cortex / Hippocampus	Microglia	IBA-1 / CD68	IBA-1 (hippocampus and prefrontal cortex): increased in PND27 and PND76 males. CD68 (hippocampus and prefrontal cortex): increased in PND27 and PND76 males.
Zeng H (2020)	ELS	Rat	Sprague-Dawley	Maternal separation with Early weaning	PND2-PND16	4–6 h	Male	PND62	Western blot / Real-time PCR / Immunohistochemistry	Prefrontal Cortex	Astrocyte / Oligodendrocytes	GLAST / GLT-1 / GS / ASPA / OLIG2 / MBP /	MBP, ASPA, MRF, and PLP1: decreased in males. GLT-1 and GS: increased in males.

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Table 1 (continued)

Author (year)	PNS / ELS	Rat / Mice	Strain	Stress protocol	Stress period	Stress duration per day	Sex	PND of euthanasia	Biomolecular analysis	Brain region	Glial cells	Glial marker	Results of stressed animals
Banqueri M (2019)	ELS	Rat	Wistar	Maternal separation	PND1-PND21	4 h	Male	PND100	Immunohistochemistry	Prefrontal Cortex / Hippocampus / Nucleus Accumbens / Striatum	Microglia / Astrocyte	MRF / PLP1 IBA-1 / GFAP	IBA-1 (hippocampus, striatum, and nucleus accumbens): increased in males. GFAP (hippocampus, prefrontal cortex, striatum, and nucleus accumbens): decreased in males.
Chastain LG (2019)	ELS	Rat	Sprague-Dawley	Ethanol exposure	PND2-PND6	Single	Male and female mixed	PND6 / PND90	Immunohistochemistry	Hypothalamus	Microglia	IBA-1 / CD11b	IBA-1: increased in PND90 males and females (mixed). CD11b: increased in PND6 and PND90 males and females (mixed).
Giridharan VV (2019)	ELS	Rat	Wistar	Maternal separation	PND1-PND10	3 h	Male	PND10	Western blot	Prefrontal Cortex / Hippocampus	Microglia	IBA-1 / CD11b	CD11b (prefrontal cortex): increased in males.
Majcher-Mašlanka I (2019)	ELS	Rat	Wistar	Maternal separation	PND1-PND14	3 h	Male	PND35	Immunohistochemistry / Real-time PCR	Prefrontal Cortex / Corpus Callosum	Astrocyte / Oligodendrocyte / Microglia	GFAP / NG2 / CNPase / IBA-1	IBA-1 (prefrontal cortex and corpus callosum): decreased in males. GFAP and NG2: increased in males.
Morris-Schaffer K (2019) a	ELS	Mice	C57BL/6 J	Carbon particles exposure	PND4-PND7 and PND10-PND13	4 h	Male and female	PND14	Immunohistochemistry	Hippocampus / Cortex / Corpus Callosum	Astrocytes / Oligodendrocyte	GFAP / MBP	No significant differences.
Morris-Schaffer K (2019) b	ELS	Mice	C57BL/6 J	Diesel particles exposure	PND4-PND7 and PND10-PND14	4 h	Male and female	PND15	Immunohistochemistry	Hippocampus / Cortex / Corpus Callosum	Astrocytes / Oligodendrocyte	GFAP / MBP	GFAP (cortex and corpus callosum): increased in males. GFAP (hippocampus): decreased in females.
Réus GZ (2019)	ELS	Rat	Wistar	Maternal separation	PND1-PND10	3 h	Male	PND10 / PND20 / PND30 / PND40 / PND60	Immunohistochemistry / Real-time PCR	Hippocampus / Prefrontal Cortex	Microglia / Astrocyte	IBA-1 / GFAP	IBA-1 (prefrontal cortex): increased in PND10, PND20, PND30, and PND60 males. GFAP (prefrontal cortex): decreased in PND10 and PND60 males. GFAP (hippocampus): increased in PND40 and PND60 males.
Ye Y (2019)	ELS	Rat	Sprague-Dawley	Maternal separation	PND2-PND21	3 h	Female	PND51	Western blot	Prefrontal Cortex / Hippocampus	Microglia	IBA-1	No significant differences.
Baldy C (2018)	ELS	Rat	Sprague-Dawley	Maternal separation	PND3 - PND12	3 h	Male and female	PND15	Immunohistochemistry	Medulla	Microglia	IBA-1	IBA-1: increased in males and females.

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Table 1 (continued)

Author (year)	PNS / ELS	Rat / Mice	Strain	Stress protocol	Stress period	Stress duration per day	Sex	PND of euthanasia	Biomolecular analysis	Brain region	Glial cells	Glial marker	Results of stressed animals
Bukhari SHF (2018)	ELS	Rat	Sprague-Dawley	LPS administration	PND2-PND3	Single	Male and female	PND34	Immunohistochemistry	Hippocampus	Microglia	IBA-1	IBA-1: decreased in males and females.
Ganguly P (2018)	ELS	Rat	Sprague-Dawley	Maternal separation	PND2-PND20	4 h	Male and female	PND55	Immunohistochemistry	Prefrontal Cortex	Microglia	IBA-1	No significant differences.
Gong Y (2018)	ELS	Mice	DBA2/J	Maternal separation	PND14-PND21	30 min	Male and female mixed	PND68	Immunohistochemistry / Real-time PCR	Hippocampus	Microglia	IBA-1 / CD11b	IBA-1 and CD11b: decreased in males and females (mixed).
Johnson FK (2018)	ELS	Mice	BALB/cByj	Limited bedding	PND1-PND14	24 h	Male	PND14	Immunohistochemistry / Western blot	Hippocampus	Microglia	IBA-1 / MBP	IBA-1 and MBP: decreased in males.
Yamawaki Y (2018)	ELS	Rat	Sprague-Dawley	Maternal separation	PND2-PND14	3 h	Male	PND7 / PND14 / PND21 / PND70	Real-time PCR	Prefrontal Cortex	Astrocyte	GFAP	GFAP: decreased in males.
Hoeijmakers L (2017)	ELS	Mice	C57BL/6 J	Limited bedding	PND2-PND9	24 h	Male	PND9 / PND120 / PND300	Immunohistochemistry / Real-time PCR	Hippocampus / Entorhinal Cortex	Microglia	IBA-1 / CD68 / CD11b	IBA-1: decreased in PND9 males.
Saavedra LM (2017)	ELS	Rat	Sprague-Dawley	Maternal separation	PND1-PND14	3 h	Male	PND15	Immunohistochemistry	Hippocampus	Astrocytes / Microglia	IBA-1 / GFAP	IBA-1 and GFAP: decreased in males.
Yang Y (2017)	ELS	Rat	Sprague-Dawley	Maternal separation	PND3-PND21	3 h	Male	PND21	Immunohistochemistry / Western blot	Prefrontal Cortex	Oligodendrocyte	NG2 / CC1 / OLIG2 / MBP	MBP, NG2, CC1, and OLIG2: decreased in males.
∞ Bath KG (2016)	ELS	Mice	C57BL/6 N	Limited bedding	PND4-PND11	24 h	Male	PND4 / PND 8 / PND12 / PND16 / PND21 / PND28 / PND38 / PND50	Immunohistochemistry	Hippocampus	Oligodendrocyte	MBP	MBP: increased in PND12, PND16, and PND21 males.
Delpuch JC (2016)	ELS	Mice	BALB/cByj	Maternal separation	PND1-PND21	40 min	Male	PND14 / PND28	Immunohistochemistry	Hippocampus	Microglia	IBA-1	IBA-1: increased in PND14 males.
Gondré-Lewis MC (2016)	ELS	Rat	Sprague-Dawley	Maternal separation	PND2-PND21	3 h	Male and female mixed	PND80	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	No significant differences.
Gracia-Rubio I (2016)	ELS	Mice	CD1	Maternal separation with Early weaning	PND2-PND16	4–8 h	Male and female	PND30	Immunohistochemistry	Hippocampus / Prefrontal Cortex	Microglia	IBA-1	IBA-1: increased in females.
Roque A (2016)	ELS	Rat	Sprague-Dawley	Maternal separation	PND1-PND14	3 h	Male	PND15	Immunohistochemistry	Hippocampus	Astrocyte / Microglia	GFAP / IBA-1	GFAP: decreased in males.
Wang CY (2016)	ELS	Rat	Sprague-Dawley	Valproic acid administration	PND7	Single	Male	PND8	Immunohistochemistry / Real-time PCR	Hippocampus / Cortex / Amygdala	Astrocyte / Microglia / Oligodendrocyte	GFAP / IBA-1 / OLIG1 / OLIG2	IBA-1 (hippocampus and cortex): increased in males. GFAP (hippocampus): increased in males. OLIG1 and OLIG2 (hippocampus): decreased in males.
Boku S (2015)	ELS	Rat	Sprague-Dawley	Maternal separation	PND2-PND14	3 h	Male	PND56	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	No significant differences.

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Table 1 (continued)

Author (year)	PNS / ELS	Rat / Mice	Strain	Stress protocol	Stress period	Stress duration per day	Sex	PND of euthanasia	Biomolecular analysis	Brain region	Glial cells	Glial marker	Results of stressed animals
Mela V (2015)	ELS	Rat	Wistar	Maternal deprivation	PND9	24 h	Male and female	PND90	Western blot	Hippocampus / Frontal Cortex	Astrocyte / Oligodendrocyte	GFAP / GLAST / NG2	GFAP: decreased in females.
Aksifá M (2014)	ELS	Rat	Wistar	Maternal deprivation	PND9	24 h	Male	PND60	Western blot	Frontal Cortex	Astrocyte	GFAP	No significant differences.
Burke NN (2013)	ELS	Rat	Wistar Albino	Maternal deprivation	PND9	24 h	Male and female	PND103	Real-time PCR	Prefrontal Cortex / Hippocampus	Astrocyte	GFAP	No significant differences.
Lieblein-Boff JC (2013)	ELS	Mice	Balb/c	E. coli infection	PND3	Single	Male and female mixed	PND12	Immunohistochemistry	Corpus Callosum / Motor Cortex / Hippocampus	Astrocyte / Microglia / Oligodendrocyte	GFAP / IBA-1 / OLIG2	IBA-1 (corpus callosum): increased in males and females (mixed). GFAP (motor cortex): increased in males and females (mixed).
Marco EM (2013)	ELS	Rat	Wistar Albino	Maternal deprivation	PND9	24 h	Male and female	PND40	Western blot	Frontal Cortex / Hippocampus	Astrocyte / Oligodendrocyte	GFAP / NG2 / APC	GFAP (hippocampus): increased in males. NG2 (hippocampus): decreased in males and females.
Baudin A (2012)	ELS	Rat	Long-Evans	Maternal separation	PND1-PND14	3 h	Male	PND90	Immunohistochemistry	Prefrontal Cortex	Astrocyte	GFAP	No significant differences.
Bautista E (2012)	ELS	Rat	Wistar	Maternal separation	PND1-PND21	6 h	Male and female	PND60	Immunohistochemistry	Hippocampus / Prefrontal Cortex / Hypothalamus	Astrocyte	GFAP	GFAP (hippocampus and hypothalamus): decreased in males. GFAP (prefrontal cortex): increased in males and decreased in females.
López-Gallardo M (2012)	ELS	Rat	Wistar	Maternal deprivation	PND9	24 h	Male and female	PND80	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	GFAP: increased in males.
Martisova E (2012)	ELS	Rat	Wistar	Maternal separation	PND2-PND21	3 h	Male	PND90 / PND540	Western blot	Hippocampus	Astrocyte	GLAST / GLT-1	GLAST and GLT-1: increased in PND90 and PND540 males.
Mela V (2012)	ELS	Rat	Wistar	Maternal deprivation	PND9	24 h	Male and female	PND102	Real-time PCR	Hypothalamus	Astrocyte	GFAP	GFAP: decreased in males.
Mela V (2015)	ELS	Rat	Wistar	Maternal deprivation	PND9	24 h	Male and female	PND90	Western blot	Hippocampus / Frontal Cortex	Astrocyte / Oligodendrocyte	GFAP / GLAST / NG2	GFAP: decreased in females.
Sominsky L (2012)	ELS	Rat	Wistar	LPS administration	PND3 and PND5	Single	Male	PND85	Immunohistochemistry	Hippocampus	Microglia	IBA-1	IBA-1: increased in males.
Chocyk A (2011)	ELS	Rat	Wistar	Maternal separation	PND1-PND14	3 h	Male and female	PND15	Immunohistochemistry	Ventral Tegmental Area / Midbrain	Astrocyte	S100b	S100b (ventral tegmental area): decreased in males. S100b (midbrain): decreased in females.
Viveros MP (2010)	ELS	Rat	Wistar	Maternal deprivation	PND9	12 h / 24 h	Male and female	PND9 / PND10	Western blot	Hypothalamus	Astrocyte	GFAP	GFAP: decreased in males exposed to 24 h of maternal deprivation.

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Table 1 (continued)

Author (year)	PNS / ELS	Rat / Mice	Strain	Stress protocol	Stress period	Stress duration per day	Sex	PND of euthanasia	Biomolecular analysis	Brain region	Glial cells	Glial marker	Results of stressed animals
Kwak HR (2009)	ELS	Rat	Sprague-Dawley	Maternal deprivation	PND14	24 h	Male	PND43	Immunohistochemistry	Prefrontal Cortex	Astrocyte	GFAP	GFAP: increased in males.
Llorente R (2009)	ELS	Rat	Wistar Albino	Maternal deprivation	PND9	24 h	Male and female	PND13	Immunohistochemistry	Hippocampus / Cerebellum	Astrocyte	GFAP	GFAP (hippocampus): increased in males and females. GFAP (cerebellum): increased in males and decreased in females.
Musholt K (2009)	ELS	Rat	Wistar	Maternal separation	PND14	1 h / 6 h	Male	PND15	Immunohistochemistry	Cingulate Cortex / Hippocampus / Medial Precentral Cortex	Astrocyte	GFAP / S100b	GFAP and S100b (medial precentral cortex): decreased in males. S100b (cingulate cortex): decreased in males exposed to 6 h of maternal separation. No significant differences.
Oomen CA (2009)	ELS	Rat	Wistar	Maternal deprivation	PND3	24 h	Male and female	PND21	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	GFAP: increased in males and females (mixed).
Vien TN (2009)	ELS	Mice	C57BL/6	Maternal separation with hypoxia and hyperoxia	PND5-PND9	8 h	Male and female mixed	PND9	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	GFAP: increased in males.
Llorente R (2008)	ELS	Rat	Wistar Albino	Maternal deprivation	PND9	24 h	Male and female	PND13	Immunohistochemistry	Hippocampus	Astrocyte	GFAP	GFAP: increased in males.
López-Gallardo M (2008)	ELS	Rat	Wistar Albino	Maternal deprivation	PND9	24 h	Male and female	PND13	Immunohistochemistry	Cerebellum	Astrocyte	GFAP	GFAP: increased in males.
Kikusui T (2007)	ELS	Mice	ICR	Early weaning	PND14-PND21	24 h	Male and female	PND21 / PND35 / PND56	Western blot	Whole brain	Oligodendrocyte	MBP	MBP: decreased in PND21 males and increased in PND35 males.
Leventopoulos M (2007)	ELS	Rat	Fisher F344	Maternal separation	PND1-PND14	4 h	Male	PND365	Immunohistochemistry	Hippocampus / Cingulate Cortex / Prefrontal Cortex / Nucleus Accumbens / Frontal Cortex / Striatum / Amygdala / Hypothalamus	Astrocyte	GFAP	GFAP (hippocampus, prefrontal cortex, amygdala, hypothalamus, and cingulate cortex): decreased in males.
Pickering C (2006)	ELS	Rat	Wistar	Maternal separation	PND1-PND21	15 min / 6 h	Male	PND175	Real-time PCR	Hippocampus	Astrocyte	GLAST	GLAST: increased in males exposed to 6 h of maternal separation.
Diz-Chaves Y (2018)	PNS and ELS	Rat	Sprague-Dawley	Food restriction	GD12-PND21	24 h	Male and female	PND21	Immunohistochemistry	Hippocampus	Microglia / Astrocyte	IBA-1 / GFAP	IBA-1 and GFAP: increased in males.
Zelikoff JT (2018)	PNS and ELS	Mice	C57BL/6	e-cigarette exposure	GD1-PND21	3 h	Male and female	PND25	Immunohistochemistry	Hippocampus / Frontal Cortex	Microglia / Astrocyte	IBA-1 / GFAP	No significant differences.

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Table 1 (continued)

Author (year)	PNS / ELS	Rat / Mice	Strain	Stress protocol	Stress period	Stress duration per day	Sex	PND of euthanasia	Biomolecular analysis	Brain region	Glial cells	Glial marker	Results of stressed animals
Cohen S (2016)	PNS and ELS	Mice	C57BL/6	Unpredictable chronic stress with limited bedding	GD13-GD17/PND21	24 h	female mixed Male and female mixed	PND21	Immunohistochemistry / Western blot / Real-time PCR	Hippocampus	Microglia	IBA-1	IBA-1: increased in males and females (mixed).

Note: PNS = prenatal stress; ELS = Early life stress; GD = gestational day; PND = postnatal day.

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 Fig. 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 h 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 h 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

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

Author (year)	PNS / ELS	Brain region	Glial cells	Type of analysis	Results of stressed animals
Namvarpour Z (2022)	PNS	Prefrontal Cortex	Oligodendrocyte	Myelin thickness / Axons diameter	Axons diameter: decreased in males.
Gaspar R (2021)	PNS	Hippocampus / Nucleus Accumbens	Microglia	Soma size / Processes number / Processes length	Processes number (nucleus accumbens): decreased in females. Processes length (nucleus accumbens): increased in males and decreased in females.
Gumusoglu SB (2017)	PNS	Cortex	Microglia	Cell shape	Ameboid and intermediate: decreased in males. Ramified: increased in males.
Shende VH (2015)	PNS	Hippocampus / Amygdala / Nucleus Accumbens	Astrocyte	Processes number / Processes length	Processes length (hippocampus): decreased in males.
Diz-Chaves Y (2013)	PNS	Hippocampus	Microglia	Cell activation	Cell activation: increased in males.
Diz-Chaves Y (2012)	PNS	Hippocampus	Microglia	Cell shape	Intermediate: decreased in females. Ramified: increased in females.
Bachiller S (2022)	ELS	Hippocampus / Prefrontal Cortex	Microglia	Cell shape	Ameboid (PFC): increased in females. Intermediate (PFC): decreased in females.
Nicolas S (2022)	ELS	Hippocampus	Microglia	Soma size	Soma size: increased in females.
Catale C (2021)	ELS	Pons	Microglia	Soma size	Soma size: increased in males and females (mixed).
Saavedra LM (2021)	ELS	Hippocampus	Microglia / Astrocyte	Soma size / Processes number / Cell activation	Soma size and cell activation (microglia): increased in males and females. Soma size (astrocyte): increased in males and females.
Zetter MA (2021)	ELS	Hippocampus	Microglia	Processes number	Processes number: increased in males and females.
Bachiller S (2020)	ELS	Hippocampus	Microglia	Soma size	Soma size: increased in males and females.
Catale C (2020)	ELS	Striatum / Nucleus Accumbens	Microglia	Soma size	Soma size (striatum): increased in males and females (mixed) exposed to maternal separation and social stress. Soma size (nucleus accumbens): increased in males and females (mixed) exposed to maternal separation.
Gildawie KR (2020)	ELS	Prefrontal Cortex	Microglia	Soma size / Processes number / Processes length	No significant differences.
Reshetnikov V (2020)	ELS	Hippocampus / Prefrontal Cortex	Microglia	Cell shape	Ameboid (PFC): increased in males exposed to maternal separation. Intermediate (PFC): increased in males exposed to maternal deprivation.
Baldy C (2018)	ELS	Medulla	Microglia	Soma size	No significant differences.
Ganguly P (2018)	ELS	Prefrontal Cortex	Microglia	Soma size / Processes length	No significant differences.
Gong Y (2018)	ELS	Hippocampus	Microglia	Soma size / Processes length	Soma size and processes length: decreased in males and females (mixed).
Hoeijmakers L (2017)	ELS	Hippocampus / Entorhinal Cortex	Microglia	Cell shape	No significant differences.
Saavedra LM (2017)	ELS	Hippocampus	Microglia / Astrocyte	Soma size / Processes number / Cell activation	Processes number (astrocyte): decreased in males. Cell activation (microglia): increased in males.
Delpech JC (2016)	ELS	Hippocampus	Microglia	Cell shape	Ameboid and intermediate: increased in PND14 males.
Roque A (2016)	ELS	Hippocampus	Microglia / Astrocyte	Processes number / Cell activation	Processes number (astrocyte): decreased in males. Cell activation (microglia): increased in males.
Leventopoulos M (2007)	ELS	Hippocampus / Cingulate Cortex / Prefrontal Cortex / Nucleus Accumbens / Frontal Cortex / Striatum / Amygdala / Hypothalamus	Astrocyte	Processes length	No significant differences.
Diz-Chaves Y (2018)	PNS and ELS	Hippocampus	Microglia	Cell activation	Cell activation: increased in males and females (mixed).

Note: PNS = prenatal stress; ELS = Early life stress.

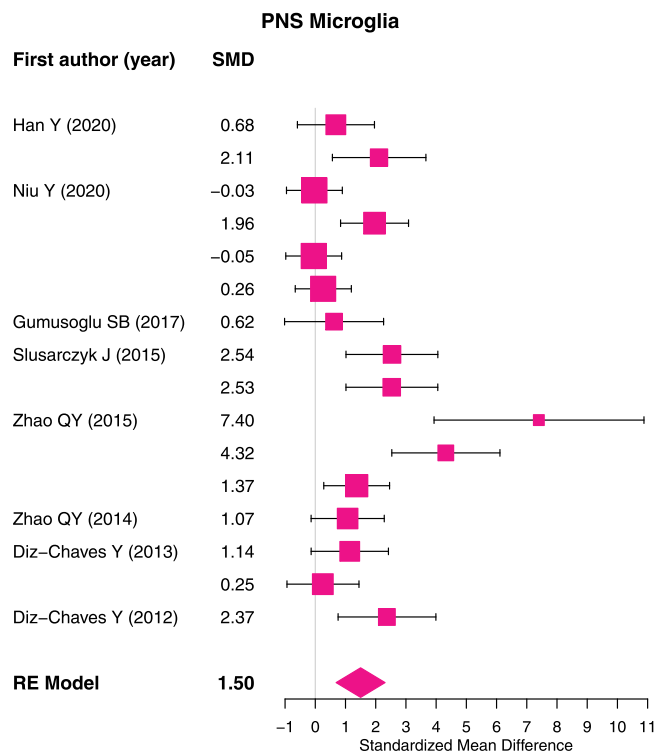


Fig. 2. Forest plot demonstrating the effect sizes of microglia after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

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.

3.3. 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 (6–10 days) resulted in decreased estimates compared to shorter periods (1–5 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).

3.4. 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 min) 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

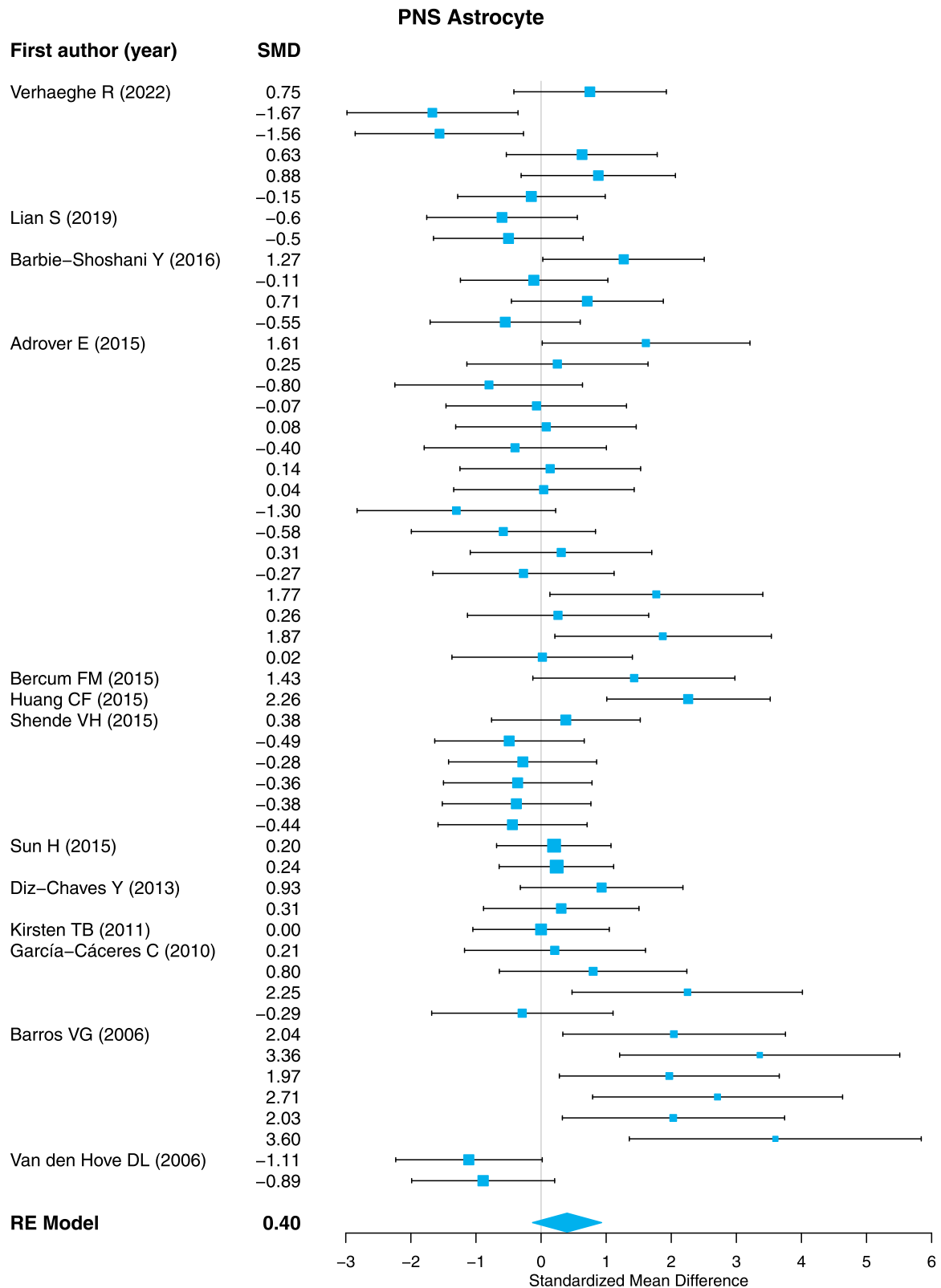


Fig. 3. Forest plot demonstrating the effect sizes of astrocyte after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

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

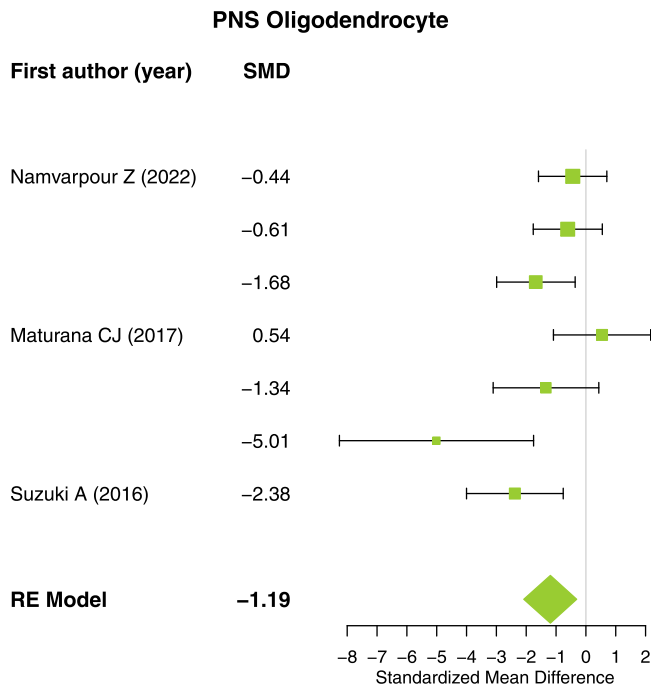


Fig. 4. Forest plot demonstrating the effect sizes of oligodendrocyte after prenatal stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

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 and 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 and 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 and Cidlowski, 2017](#); [Silverman and 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 and 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

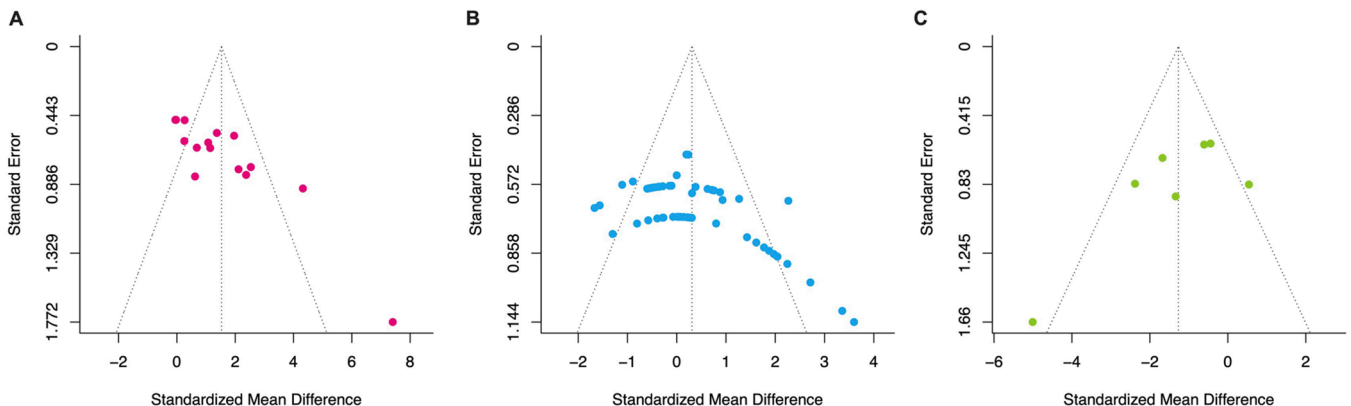


Fig. 5. Funnel plots indicating publication bias of prenatal stress studies. A) Microglia; B) Astrocyte; C) Oligodendrocyte.

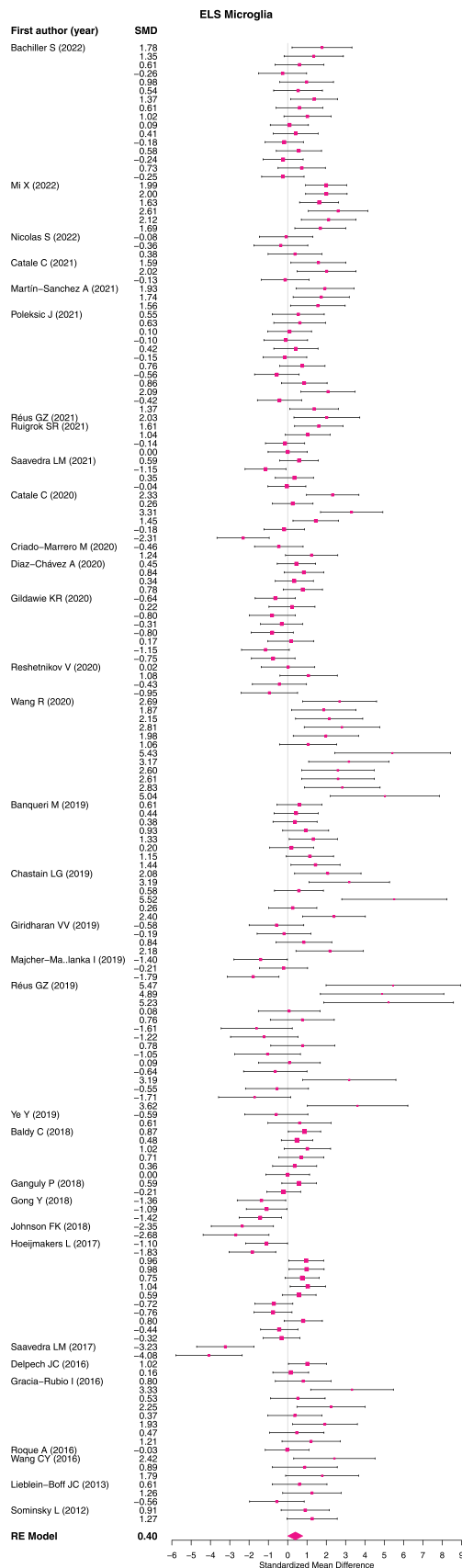


Fig. 6. Forest plot demonstrating the effect sizes of microglia after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

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 reprogramming 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 and 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

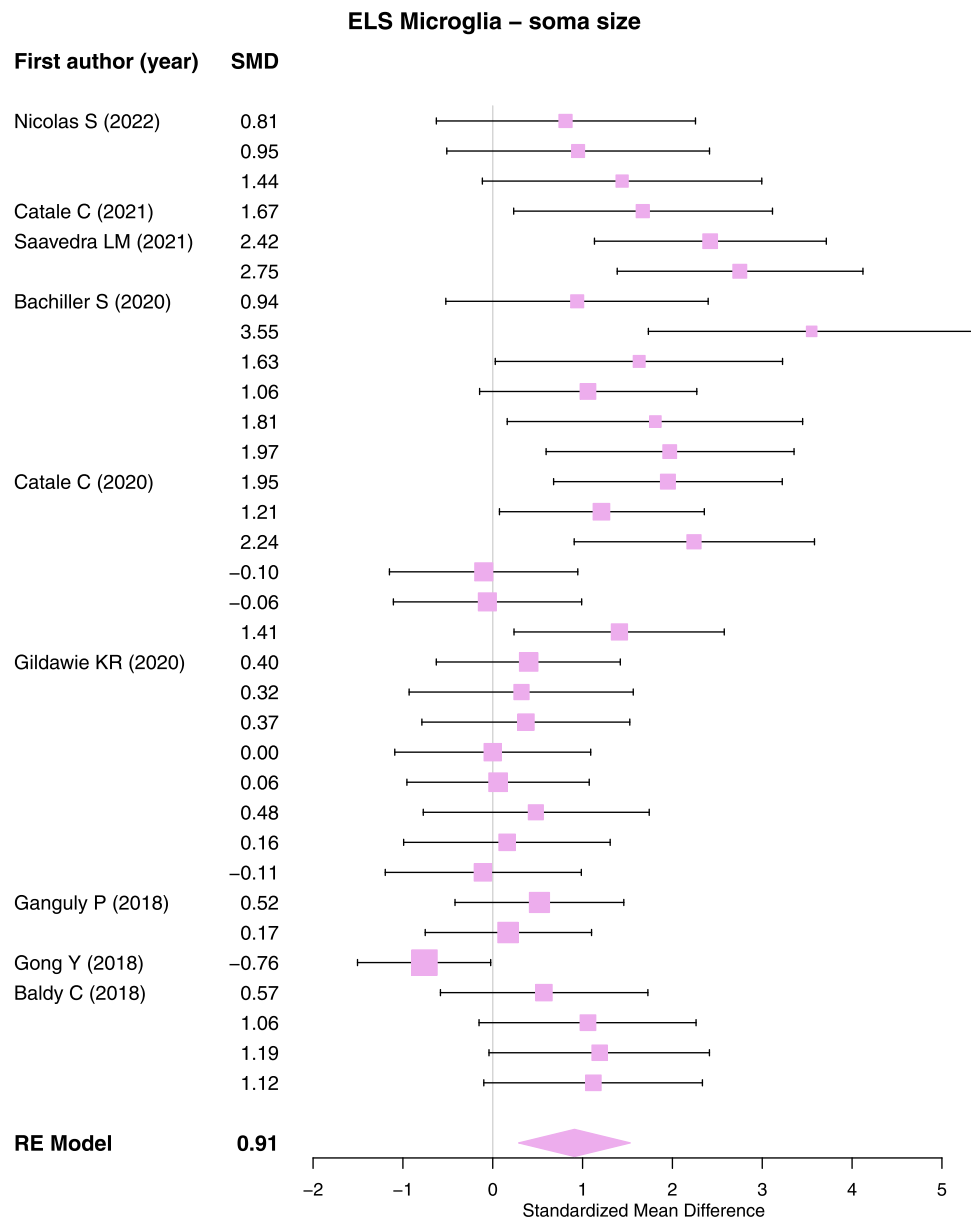


Fig. 7. Forest plot demonstrating the effect sizes of microglia soma size after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

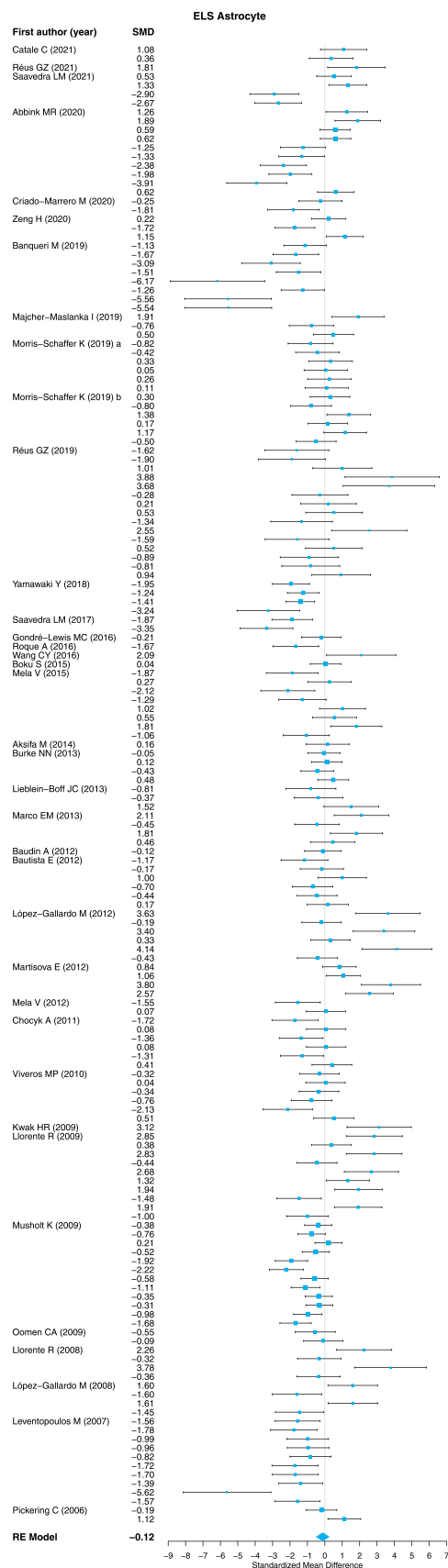


Fig. 8. Forest plot demonstrating the effect sizes of astrocyte after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

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 and 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 and 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 Tgfb 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

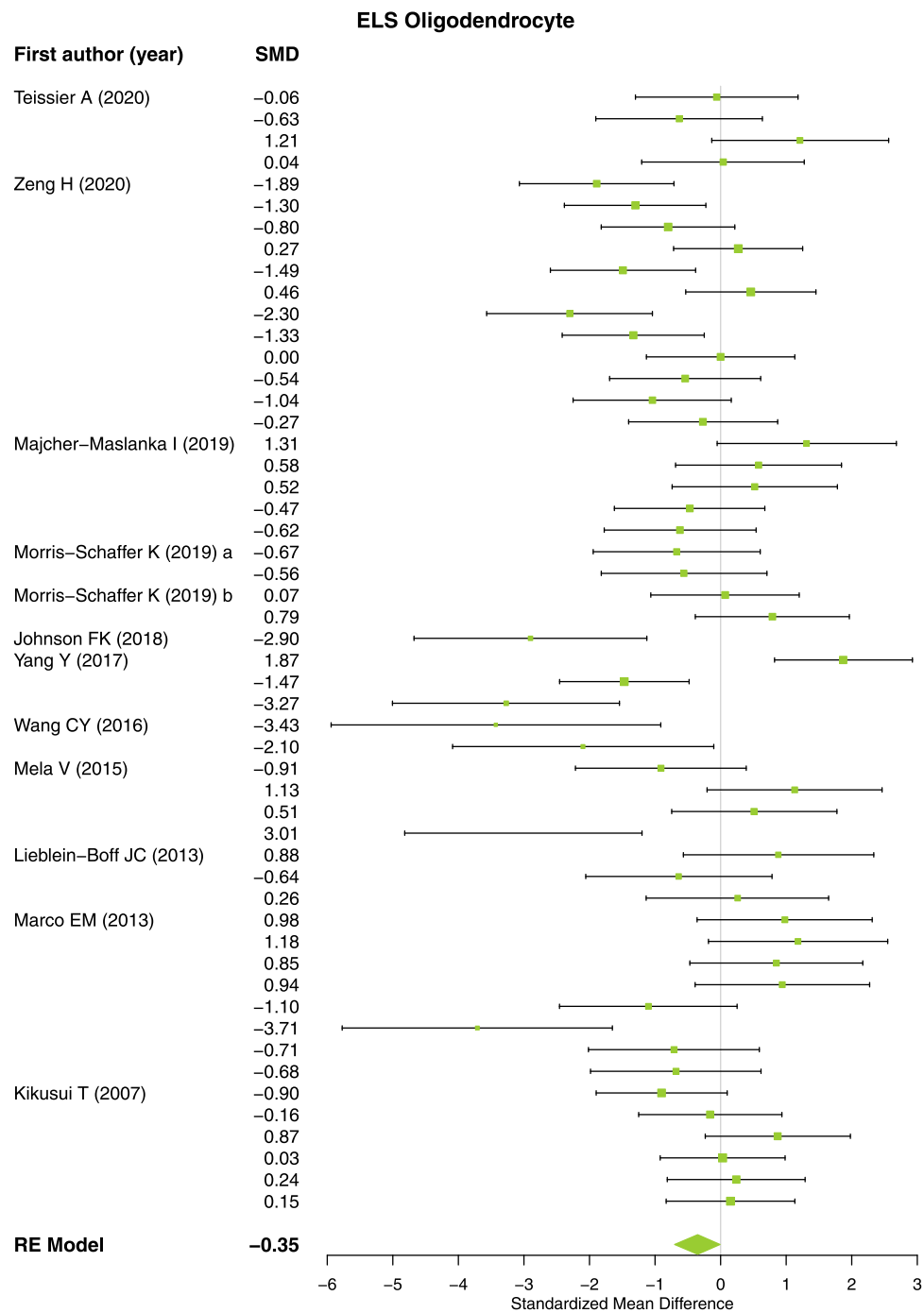


Fig. 9. Forest plot demonstrating the effect sizes of oligodendrocyte after early life stress exposure. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

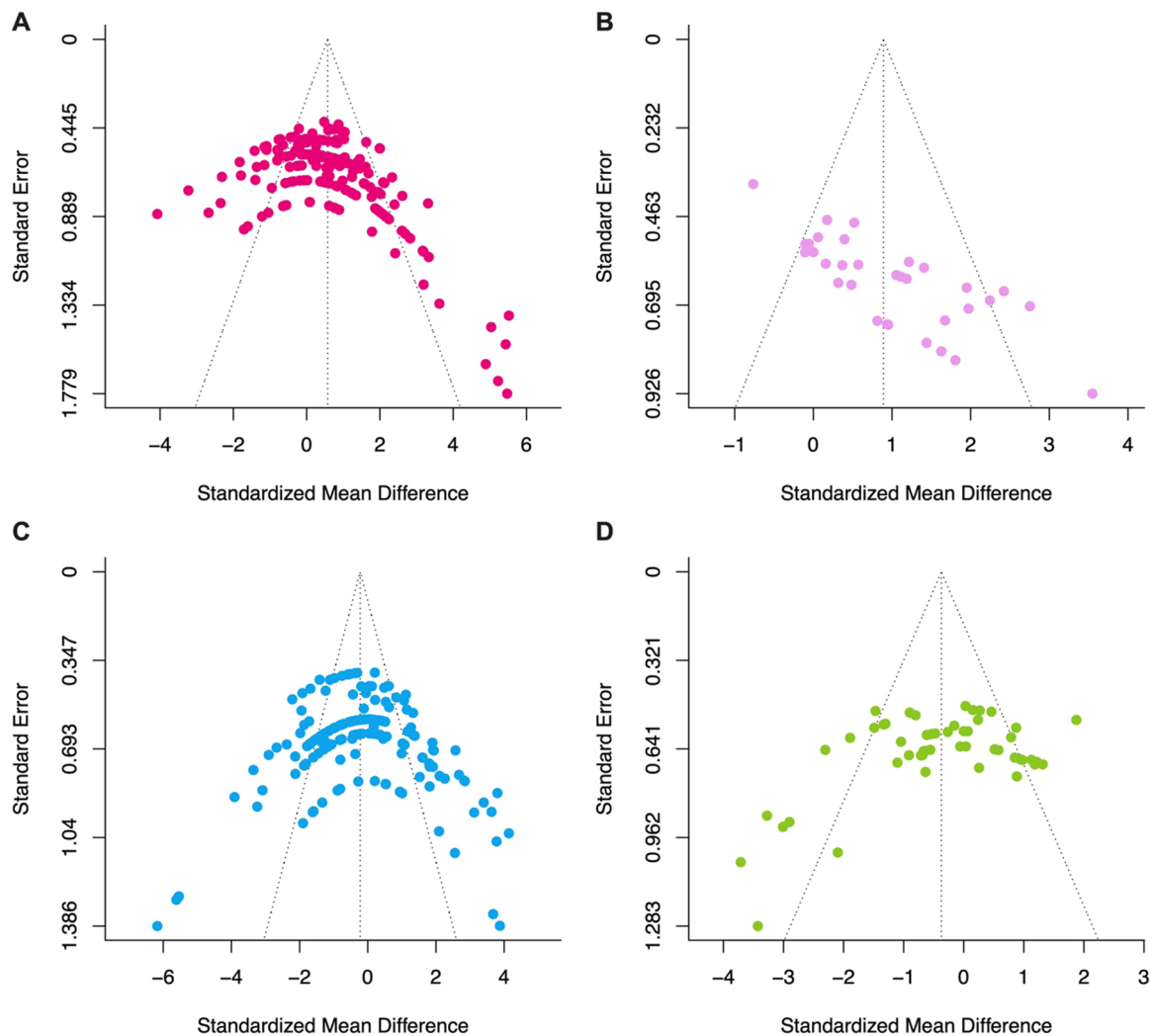


Fig. 10. Funnel plots indicating publication bias of early life stress studies. A) Microglia; B) Microglia soma size; C) Astrocyte; D) Oligodendrocyte.

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.

Financial support

This work was supported by the Italian Ministry of University and Research (grant: PRIN 2017AY8BP4 and PON "Ricerca e Innovazione" PerMedNet project ARS01_01226) to M.A.R.

Acknowledgements

Rodrigo Orso was supported by Fondazione Umberto Veronesi.

Conflict of interest

M.A.R. has received compensation as speaker/consultant from Angelini, Lundbeck, Otsuka and Sumitomo Pharma, and he has received research grants from Sumitomo Pharma. All the other authors declare no financial interests or potential conflicts of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at [doi:10.1016/j.neubiorev.2023.105202](https://doi.org/10.1016/j.neubiorev.2023.105202).

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