Effects of Early life stress on inflammatory cytokines: a systematic review and meta-analysis of rodent studies

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1. Introduction

Stress on early stages of life has been suggested to impact the neurodevelopment of infants through biological and behavioral changes (Provençal & Binder, 2015). Previous studies have shown that early life stress (ELS) exposure may impair brain structure and function, especially in sensitive regions, such as the prefrontal cortex, amygdala and hippocampus (Aleksić et al., 2016; de Azeredo et al., 2017; Hanson et al., 2015; Teissier et al., 2020; van Bodegom et al., 2017). Moreover, alterations in the inflammatory state and immune system are often observed in individuals exposed to ELS (Agorastos et al., 2019; Brenhouse et al., 2019; Massart et al., 2016). These alterations are considered risk factors for the emergence of psychiatric disorders, including anxiety, depression, and drug addiction (Danese & J Lewis, 2017; Lo Iacono et al., 2018; Park et al., 2021; Tannous et al., 2020). For example, chronic exposure to parental neglect, physical abuse or abandonment, induce overstimulation of the hypothalamus-pituitary-adrenal (HPA) axis, which is the main physiological system responsible for stress response, releasing stress hormones that are linked to an increase in the levels of proinflammatory cytokines (Grassi-Oliveira et al., 2016; Reed & Raison, 2016). Therefore, alterations in the levels of inflammatory cytokines and neuroimmune signaling induced by ELS play a critical role in the development of cognitive and behavioral dysfunctions later in life (Diaz-Chávez et al., 2020; Kuhlman et al., 2020).

Cytokines are signaling proteins secreted by immune cells that can trigger protective and damaging responses (Dugue et al., 2017). They contribute to the modulation of the neuroinflammatory state, neurogenesis, and synaptic processes (Pei et al., 2021). ELS may prime brain microglia to stimulate proinflammatory cytokines and chemokines release in response to chronic stress exposure (Weber et al., 2015). Higher stress reactivity induce elevation of proinflammatory cytokines, such as TNF-α, IL-1β and IL-6, which stimulate neuronal apoptosis and serotonin reuptake (Fabbri et al., 2017). Therefore, it is possible that together these inflammatory-induced changes contribute as a vulnerability factor for the development of psychiatric disorders (Bauer & Teixeira, 2019; Druzhkova et al., 2019; Pace et al., 2006).

Considering the prominent role of inflammatory cytokines on the regulation of immunological and stress systems, it is important to further understand the potential relationship of such biomarkers on the brain and on its phenotypic expression. For this reason, the use of animal models represent an essential tool to investigate the

changes promoted by ELS at different levels (Pfau & Russo, 2015). On the other hand, clinical studies face some limitation to access biological materials and provide adequately control of variables for evaluate stress effects over different periods of development. ELS animal models studies commonly reproduce adverse postnatal conditions in order to induce exposure to stressful environment. For example, in the maternal separation (MS) model, pups are separated from the dam for a period of time, increasing stress response due to an unfamiliar environment and disrupting the maternal care pattern of the dams after reuniting with the pups (Orso et al., 2019; White & Kaffman, 2019). In the limited bedding (LB) model, the dams have reduced access to bedding material and nest building resources, which are both necessary for offspring thermoregulation and adequate maternal care (McLaughlin et al., 2014; Rice et al., 2008). More recently, a combination of both models (MS and LB) was proposed in order to induce more robust ELS-induced effects and reduce the previously reported variability observed in studies that utilized MS or LB (Orso et al., 2020; Peña et al., 2017).

A recent review reported that MS exposure may indeed alter the levels of pro-inflammatory cytokines, but major modifications in the immune system were observed after a secondary hit later in life (Dutcher et al., 2020). Nevertheless, no meta-analysis compiled data regarding how ELS exposure may influence inflammatory cytokine levels in rodents. Considering that there are still inconsistencies in findings of studies utilizing MS and LB models, and that the effects of methodological variables have not been previously explored, a meta-analysis is now required to provide further statistical support. Thus, the aim of this study was to perform a systematic review and meta-analysis with findings from rodent studies that investigated the impact of ELS on inflammatory cytokines in the brain. Moreover, we explored sources of heterogeneity between studies using meta-regression models, and investigated the methodological quality of the included studies.

2. Methods

2.1 Search strategy

The search was performed on September 17th, 2019, and updated on March 5th, 2021. Three databases were used: PubMed, Web of Science, PsycInfo. The following terms were used for the search: [cytokine OR “proinflammatory cytokine” OR chemokine OR inflammation OR “tumor necrosis factor alpha” OR “interferon gamma”

OR “granulocyte-macrophage colony stimulating factor” OR “transforming growth factor” OR “C-reactive protein” OR “Macrophage Inflammatory Protein-1 alpha” OR Eotaxin-1 OR IL-1 OR IL-1β OR IL-2 OR IL-4 OR IL-5 OR IL-6 OR IL-8 OR IL-10 OR IL-12 OR IL-17 OR IL-18 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 “unpredictable stress”. This study followed the Cochrane recommendations for developing a search strategy (Cochrane Infectious Diseases Group, 2007).

2.2 Selection and eligibility

The selection of the articles was performed in two phases. For the first phase, only the titles and abstracts were screened. The second phase consisted in reading the full text for possible inclusion. The following exclusion criteria were applied to select the studies for this review: (1) the study was not written in English; (2) the study was not empirical; (3) the study did not use mice or rat; (4) the study did not have an early life stress protocol; (5) the study did not analyze cytokines in the brains of the offspring; (6) the study only used transgenic or knock-out animals. Both phases were performed blindly by two independent authors (FSL and EKF) using the Rayyan QCRI website (Ouzzani et al., 2016). Any disagreement regarding selection of studies was resolved by two senior authors (RGO and TWV).

2.3 Data extraction

Two independent authors (FSL and EKF) extracted the following data from all included studies: ‘first author’, ‘publication year’, ‘species’, ‘strain’, ‘early life stress protocol’, ‘early life stress period’, ‘sex’, ‘age’, ‘analyzed cytokines’, ‘analyzed tissues’, ‘biological material’, ‘secondary manipulations’, and ‘outcome data’. For both the stressed and control groups, the mean, the standard deviation (SD), and the number of animals per group were collected for the outcome data. When only the standard error (SE) was reported, it was used to calculate the SD. When a study reported the number of animals per group as a range, we utilized the smallest number for the meta-analysis. WebPlotDigitizer was utilized to extract the necessary information when data was only reported in graphs.

2.4 Coding procedure and potential moderators

The following variables and codes were used as potential moderators for meta-regression:

- Species, coded as: (0) rat; and (1) mice.
- Early life stress protocol, coded as: (0) maternal separation; (1) maternal separation + heat or cold stress; (2) limited bedding; (3) maternal deprivation.
- Early life stress period, coded as: (0) 1 day; (1) 2-7 days; (2) 8-14 days; (3) 15-21 days.
- Sex, coded as: (0) male, (1) female, (2) unspecified.
- Age, coded as: (0) post-natal day 0-21; (1) post-natal day 22-45; (2) post-natal day 46-60; (3) post-natal day 61-90; (4) post-natal day 91 or more.
- Tissue, coded as: (0) hippocampus; (1) cortex; (2); cerebrospinal fluid; (3) striatum; (4) nucleus accumbens; (5) brain stem; (6) hypothalamus.
- Biological material, coded as: (0) protein; (1) RNA.
- Secondary manipulations, coded as: (0) no manipulation; (1) sham surgery or vehicle injection; (2) behavior; (3) sham surgery or vehicle injection + behavior.

2.5 Analysis of methodological quality

To evaluate the methodological quality of the included studies, we utilized an adapted version of the Gold Standard Publication Checklist (GSPC) (Hooijmans et al., 2011) and the ARRIVE Guidelines for Reporting Animal Research (Kilkenny et al., 2010), which was used by Tractenberg et al. (2016). The checklist consisted of 26 items, and 0.5 points were given when a specific data was presented in the article, while 0 points were given when the information was missing. Two authors (FSL and EKF) independently performed the analysis.

2.6 Data analysis

Considering that the assumption of independence between outcomes was violated because some studies contributed with more than one sample, we conducted the meta-analysis using the random effects model (RE Model), and a multilevel approach to generate the forest plots. A 2-level hierarchical data structure was modeled, with samples within studies nested with samples between studies. The estimated effect size of each cytokine investigated on different brain regions was determined using the standardized mean difference (SMD), which was calculated

using Cohen's d. Influence analysis was performed to detect possible outliers for all targets. Q statistic was used to verify possible heterogeneity, and I^2 to assess the proportion of total variability due to heterogeneity. Univariate meta-regression models with potential moderators were used to explore the sources of heterogeneity of all meta-analyses. The existence of publication bias was identified using funnel plots' asymmetry and then statistically confirmed by Egger's regression test. All statistical analyses were performed using the package 'metafor' (version 2.4-0) from the statistical software R (version 4.0.0).

Results

The first database search yielded 828 studies, of which 244 were extracted from PUBMED, 280 from PsycInfo and 304 from Web of Science. After exclusion of 450 duplicate studies, we evaluated the title and abstract of 378 studies, which resulted in 173 studies selected for full-text screening (*n* = 205 excluded). The full-text analysis resulted in additional 153 exclusions: *n* = 6 studies were not written in English; *n* = 7 were not empirical; *n* = 81 did not use ELS protocols; *n* = 59 did not analyze cytokines in the brain. The update search provided 91 new studies for screening, in which 84 were excluded considering the criteria previous used. The final number of included studies for analysis was 27 (Amini-Khoei et al., 2017; Arabi et al., 2021; Banqueri et al., 2019; Burke et al., 2013; Ganguly et al., 2019; Giridharan et al., 2019; Hoeijmakers et al., 2017; Hohmann et al., 2017; Lajud et al., 2021; Lorigooini et al., 2021; Nouri et al., 2020; Oliveira et al., 2020; Park et al., 2014; Pinheiro et al., 2015; Romeo et al., 2004; Roque et al., 2016; Réus et al., 2013; Réus et al., 2017; Réus et al., 2015; Saavedra et al., 2017; Ströher et al., 2020; Tang et al., 2017; Viola et al., 2019; Viviani et al., 2014; Wang et al., 2020; Ye et al., 2019; Zhu et al., 2017). Flowchart information with detailed description of all stages can be viewed in Figure 1.

3.1 Studies characteristics

66.6% of eligible studies were performed with rats (*n* = 18) and 33.4% with mice (*n* = 9). Regarding ELS protocols, MS was the most used, corresponding to 85.2% of studies (*n* = 23), while both LB (*n* = 1) and MS combined with heat or cold stress (*n* = 1) corresponded to 3.7% each and finally maternal deprivation was performed in 2 studies (7.4%). Most studies utilized an ELS period of 8-14 days

(55.6%, *n* = 15), followed by 29.6% of studies using 15-21 days (*n* = 8). The use of a single day of stress (*n* = 2), or 2-7 days (*n* = 2) composed 7.4% of each. 70.4% of studies used only males (*n* = 19), only 3.7% used only females (*n* = 1), 18.5% used both male and female animals (*n* = 5) and 7.4% did not specify sex (*n* = 2). Hippocampus was the predominantly analyzed tissue (*n* = 20), corresponding to 46.5% of studies, followed by the prefrontal cortex (*n* = 12) that was analyzed in 27.9% of studies. The cerebral cortex (*n* = 3) and hypothalamus (*n* = 3) were analyzed in 7% of studies each, while 4.7% analyzed cerebrospinal fluid (*n* = 2). The striatum (*n* = 1), nucleus accumbens (*n* = 1), and Brainstem (*n* = 1), were the least analyzed regions (2.3% of studies each). 22.2% evaluated cytokines between postnatal day (PND) 0-21 (*n* = 8), 27.8% between PND 22-45 (*n* = 10), 16.7% between PND 46-60 (*n* = 6), 8.3% between PND 61-90 (*n* = 3), 16,7% from PND 91 or more (*n* = 6), and 8.3% did not report the age (*n* = 3). Furthermore, 13 studies (44.8%) were performed using RNA samples, and 16 studies (55.2%) were performed using protein samples. There is a discrepancy between the total number of studies since some of them have evaluated more than one variable.

3.2. Methodological quality assessment

Among all studies, the maximum methodological quality score obtained was 11,5, the minimum score was 7, and the average score between studies was 9,5. The methodological quality score for each study is presented in the last column of Table 1.

Considering each methodological aspects evaluated, we highlight the following features that were present in 100% of studies: housing conditions, ethical statement, light conditions, number of groups, ELS description, ELS duration, ELS time, and description of the method of biological sampling. Interestingly, only 33.3% of studies reported the total number of animals used, 25.9% of studies reported breeding procedures, 25.9% reported a description of the cages, 22.2% reported blinding procedures, and 7.4% provided information about lost samples. A detailed description of the methodological quality assessment can be viewed in Figure 2.

3.3 Impact of early-life stress on proinflammatory cytokines

The meta-analysis was performed in 26 studies previously included in the systematic review. Four cytokines (IL-1β, IL-6, TNF-α and IL-10) were used for meta-analysis due to insufficient studies analyzing the remaining cytokines. Out of these

studies, 19 analyzed IL-1β (50 effect sizes), and the results indicated that ELS exposure increases brain levels of IL-1β (SMD 0.72; 95% CI 0.27, 1.17; $p = 0.0016$) (Figure 3). Similarly, 21 studies analyzed TNF-α (51 effect sizes), and the analysis revealed increased levels of this target in the brain after ELS exposure (SMD 0.87; 95% CI 0.39, 1.36; $p = 0.0004$) (Figure 4). The anti-inflammatory cytokine IL-10 was analyzed only by 6 studies (20 effect sizes) and no significant effect of ELS was detected (SMD -0.38; 95% CI -1.46, 0.71; $p = 0.4984$) (Figure 5). Regarding IL-6, 16 studies showed data on its cytokine (43 effect sizes), and there was no statistical effect of ELS (SMD 0.68; 95% CI -0.01, 1.36) (Figure 6). However, we observed a trend ($p = 0.0524$), which indicates that ELS may lead to an increase in IL-6 levels in the brain. This increase would probably be observed with the addition of a few more studies.

The heterogeneity between studies in IL-1β, IL-6, IL-10 and TNF-α was significant ($I^2 = 83.66\%$, $p < 0.0001$; $I^2 = 88.09\%$, $p < 0.0001$; $I^2 = 80.93\%$, $p < 0.0001$; $I^2 = 79.09\%$, $p < 0.0001$, respectively). Therefore, we explored sources of heterogeneity using meta-regression analysis, including the following eight potential moderators: (1) species, (2) early life stress protocol, (3) early life stress period, (4) sex, (5) age, (6) tissue, (7) biological material, and (8) secondary manipulations. Unfortunately, it was not possible to perform a comparison of the early life stress protocols due to insufficient *n*.

The first applied moderator (species) was significantly associated with the estimates of heterogeneity only in IL-6 meta-analysis ($p < 0,0001$; variance explained = 13.65%), indicating that mice had lower levels of IL-6 estimates following ELS when compared to rats. While the ELS period, was significantly associated with the estimates of heterogeneity of IL-1β, IL-6 and TNF-α meta-analysis. In detail, animals that were exposed to 8 to 14 days or 15 to 21 days had higher IL-1β and IL-6 estimates following ELS when compared to estimates of animals exposed to only 1 day of ELS (IL-1β: $p = 0.0025$ and $p = 0.0007$, respectively; variance explained = 7.43%; IL-6: $p < 0.0001$ for both periods; variance explained = 12.32%). Moreover, animals that were exposed from 8 to 14 days also had higher TNF-α estimates following ELS ($p = 0.001$; variance explained = 3.77%). The sex of the animals was significantly associated with the estimates of heterogeneity only in TNF-α meta-analysis ($p = 0.001$; variance explained = 5.19%), indicating that female animals had lower estimates of this cytokine following ELS when compared to male animals.

Regarding the age of the animals, this moderator was significantly associated with the estimates of heterogeneity of IL-1β, IL-6 and TNF-α meta-analysis. In detail, animals analyzed from PND 46 to 60 had higher IL-1β estimates following ELS when compared to estimates of animals analyzed between PND 0 and 21 (p = 0.007; variance explained = 5.16%). Moreover, animals analyzed from PND 61 to 90 had higher IL-6 estimates while animals analyzed on PND 91 or later had lower estimates of this cytokine (p = 0.001 and p = 0.028, respectively; variance explained = 19.73%). Furthermore, animals analyzed from PND 22 to 45 and PND 91 or later had lower TNF-α estimates following ELS (p = 0.008 and p < 0.0001, respectively; variance explained = 37.18%). The analyzed tissue was significantly associated with the estimates of heterogeneity of IL-6 and TNF-α meta-analysis. Indicating that analysis performed in the cerebral cortex and hypothalamus had lower IL-6 estimates (p = 0.043 and p < 0.0001, respectively; variance explained = 5.37%) and the striatum had lower TNF-α estimates (p = 0.016; variance explained = 5.37%) following ELS when compared to the hippocampus.

Furthermore, the biological material analyzed was associated to the heterogeneity of IL-6 and TNF-α meta-analysis, indicating that RNA analysis presented lower estimates of both cytokines following ELS when compared to protein analysis estimates (p < 0.0001 for both targets; variance explained = 21.18% and 19.65%, respectively). Finally, secondary manipulations were significantly associated with the estimates of heterogeneity of IL-10 and TNF-α. In detail, animals that experienced behavioral testing had lower IL-10 estimates (p = 0,003; variance explained = 16,41%) and higher TNF-α estimates (p = 0.033; variance explained = 1.34%) following ELS exposure when compared to estimates of animals not exposed to secondary manipulations. Detailed information regarding IL-1β, IL-6, IL-10, and TNF-α heterogeneity sources are respectively displayed in Supplementary Tables 1-4.

Funnel plots were created to evaluate the publication bias and they revealed an asymmetry in all targets (Figure 7). Egger's regression test was used to confirm if the asymmetry was statistically significant. As we predicted, the test evidenced publication bias in IL-1β, IL-6, TNF-α and IL-10 (z = 5.7455, p < 0.0001; z = 7.5297, p < 0.0001; z = 10.1140, p < 0.0001; z = -4.7384, p < 0.0001;). The existence of publication bias may indicate an overestimation of the effect size.

Discussion

In this study we sought to analyze the effects of ELS exposure on the levels of inflammatory cytokines in the brain. To our knowledge, this is the first meta-analytic investigation of such outcomes in rodents. The evidence analyzed in our study indicated that ELS induced a significant increase in the proinflammatory cytokines IL-1β and TNF-α in the brain, especially in the hippocampus. A trend effect (0.052) that indicates an increase in IL-6 levels in animals exposed to ELS was also reported. Our meta-regression analysis showed that extended ELS protocols induce more pronounced alterations in the investigated cytokines, and that ELS effects appear to diminish when the analysis is performed in older animals. Furthermore, publication bias was evidenced for all meta-analysis, which points out negative results might not have been reported in the studies.

Cytokines are key modulators of neuroinflammatory processes, which are directly related with the protection of neural integrity. However, increased pro-inflammatory cytokines expression is harmful when uncontrolled, leading to chronic changes in the patterns of inflammation and aggravating neuronal damage (Kim et al., 2016). Chronic stress exposure may induce changes in the immune system, which could lead to alterations on the HPA axis (Jia et al., 2019; Walker et al., 2019), and trigger a chronic neuroinflammatory state that has been associated with multiple psychiatric conditions (Kim et al., 2016; Na et al., 2014). In our study we observed that ELS increased TNF-α and IL-1β, which is an interesting finding considering that both cytokines have similar pro-inflammatory properties. TNF-α and IL-1β are capable of inducing inflammatory damage by regulating a series of cellular activities in the endothelium trough similar mechanisms (Feghali & Wright, 1997; Marafini et al., 2019; Wojdasiewicz et al., 2014). However, a key function of these cytokines is the ability to stimulate IL-6 synthesis in various cell types during TNF-α and IL-1β activation (Feghali & Wright, 1997), which promotes a cascade of intracellular events that perpetuate the inflammatory response through the release of several cytokines with culminating effects (Feghali & Wright, 1997; Marafini et al., 2019; Warren, 1990). This scenario may favor the development of a chronic inflammatory state that may have a significant impact on sensitive regions of the brain.

Even though a trend was observed regarding IL-6 data, this might be explained by recent studies that reported alterations in IL-6 levels only within a 24-hour period after the end of ELS protocol, which suggests that these changes are more evident during

acute stages (Giridharan et al., 2019; Roque et al., 2016). Cytokine production occurs in large amounts during an acute response, whereas during chronic activation cytokines are produced recurrently but in smaller amounts (Feghali & Wright, 1997). This trend effect leads to the hypothesis that long-term changes in IL-6 levels may be dependent on the chronicity of the stress protocols used. Moreover, we should also consider that if more studies were included in the meta-analysis, we might have reached statistical significance.

No alteration was observed regarding IL-10 levels, which is in agreement with a recent review that reported inconsistencies in studies that investigated IL-10 levels after ELS exposure (Dutcher et al., 2020). Considering that IL-10 has a robust action to counteract an inflammatory response (Feghali & Wright, 1997; Pedersen et al., 2018), it is possible that the stress protocols used by the studies included in our review were not sufficient to induce long-lasting alterations in the levels of this cytokine. In addition, according to Walker et al. (2019), IL-10 expression varies significantly among brain tissues, being more expressed in the hypothalamus and pituitary when compared to the hippocampus. In our review we found that the hippocampus was the predominantly analyzed tissue whereas the hypothalamus was analyzed only in 11,11% of the studies, highlighting that the focus of analysis of IL-10 levels should expand to other brain regions in order to complement this research gap.

Regarding the specific moderators investigated in our meta-analysis, we observed that multiple moderators had an impact on analysis estimates. For instance, we identified that extended ELS protocols induced higher estimates of IL-1β, IL-6, and TNF-α when compared to shorter protocols. This interpretation can be seen in the studies from Burke et al. (2013), in which one day of maternal deprivation resulted an IL-1β increase only in females. Furthermore, Hohmann et al. (2017) used a 6 days MS protocol and no difference was observed in any of the cytokines investigated in our meta-analysis. On the other hand, Wang et al. (2020) exposed rats to 19 days of MS and reported increased levels of IL-1β, IL-6, and TNF-α in both the PFC and hippocampus. Additionally, we identified an overexpression of TNF-α on female animals compared to males, which can support the hypothesis that females are more prone to a pro-inflammatory state following stressful events, and might be related to the development of later in life psychiatric disorders (Bekhbat & Neigh, 2018; Engler et al., 2016).

Even though an overexpression of pro-inflammatory cytokines was observed during adolescence and early adulthood in animals previously exposed to ELS (Fagundes & Way, 2014; Majcher-Maślanka et al., 2019), we identified a desensitization effect of ELS in older animals. When analyzing animals past PND 90, ELS-induced cytokine alterations were hardly even present. For example, Hoeijmakers et al. (2017) reported no effect of ELS on animals with 10 months of age. Moreover, Banquieri et al. (2019) utilized a robust MS protocol of 21 days and 4 hours per day of separation and only reported a single increase in IL-6 levels in the hippocampus at PND 100. For this reason, it is possible to hypothesize that ELS indeed primes a neuroinflammatory response, but it cannot ensure long-lasting alterations in cytokine levels. Previous evidence has shown that a secondary stress exposure, especially during adolescence could be the key factor to trigger an irreversible neuroinflammatory malfunction (Dutcher et al., 2020; Kiank et al., 2009; Wohleb et al., 2012).

Considering that RNA transcripts do not necessarily correlate with protein levels, our meta-analysis sought to deepen the knowledge about the differences between cytokines RNA and protein levels (Koussounadis et al., 2015). We observed that protein analysis presented higher estimates when compared to studies that investigated only RNA expression. In fact, we suggest that future studies investigate both protein and RNA levels of cytokines, since only RNA might not give a proper estimative of how these alterations may influence the immune state of the brain (Vogel & Marcotte, 2012). For example, Lajud et al. (2021), performed MS protocol on rats and investigated RNA expression, but no difference was observed in the cytokines analyzed. On the other hand, Reus et al. (2017) , performed a similar MS protocol, but analyzed protein levels of cytokines and saw multiple alterations on the targets. Considering the brain regions analyzed in the included studies, we observed that the hippocampus was the region with more significant effects. The high number of studies in our meta-analysis that investigated this region, and also the significant relationship of the hippocampus with neuroinflammatory function could be in part associated with those results (Calcia et al., 2016; Frank et al., 2014; González-Pardo et al., 2020; Wohleb et al., 2012; Çalışkan et al., 2020).

We must consider some limitations for the present study. First, we identified publication bias in all the analyzed targets included in this study, which might suggest that some of the effect sizes of these cytokines could be overestimated. In addition,

pro-inflammatory cytokines analysis gathered 46 effect sizes or even more, while few studies analyzed anti-inflammatory cytokines, hence only IL-10 could be included in the meta-analysis, but still not being present in many studies and having only 20 effect sizes, which is less than half when compared to other cytokines. We are aware that the included studies have different methodological approaches and aim for a range of brain regions, to overcome this issue we applied meta-regression analysis with different moderators. Unfortunately, we could not investigate specific differences between ELS protocols due to the predominance of MS, so model to model singularities could not be identified.

In conclusion, our analysis revealed that ELS exposure can alter the expression of pro-inflammatory cytokines, especially regarding protein levels of IL-1β and TNF-α. The analysis also suggests that these alterations were more apparent in the hippocampus of adult animals that were exposed to at least 8 days of ELS protocol. Moreover, the meta-regression results indicate that these inflammatory changes might not be long-lasting, and we hypothesize that a further stressful challenge could trigger them also later in life. Finally, it would be important for future studies to focus on the effects of ELS exposure on anti-inflammatory cytokines because the existent evidence is still too little to fully understand the relationship between these targets and ELS.

Figure Legends

Figure 1. Flowchart of the systematic review.

Figure 2. Methodological quality assessment. Percentage of studies that reported each item of the checklist.

Figure 3. Forest plot showing the effect size of IL-1β. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Figure 4. Forest plot showing the effect size of TNF-α. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Figure 5. Forest plot showing the effect size of IL-10. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Figure 6. Forest plot showing the effect size of IL-6. SMD = Standardized Mean Difference; RE Model = Random Effects Model; 95% CI.

Figure 7. Funnel plots indicating publication bias of included studies. A) IL-1β; B) TNF-α; C) IL-10; D) IL-6.

References

Agorastos, A., Pervanidou, P., Chrousos, G. P., & Baker, D. G. (2019). Developmental Trajectories of Early Life Stress and Trauma: A Narrative Review on Neurobiological Aspects Beyond Stress System Dysregulation. *Front Psychiatry*, *10*, 118. https://doi.org/10.3389/fpsyt.2019.00118

Aleksić, D., Aksić, M., Radonjić, N. V., Jovanović, A., Marković, B., Petronijević, N., . . . Filipović, B. (2016). Long-term Effects of Maternal Deprivation on the Volume, Number and Size of Neurons in the Amygdala and Nucleus Accumbens of Rats. *Psychiatr Danub*, *28*(3), 211-219.

Amini-Khoei, H., Mohammadi-Asl, A., Amiri, S., Hosseini, M. J., Momeny, M., Hassanipour, M., . . . Dehpour, A. R. (2017). Oxytocin mitigated the depressive-like behaviors of maternal separation stress through modulating mitochondrial function and neuroinflammation. *Prog Neuropsychopharmacol Biol Psychiatry*, *76*, 169-178. https://doi.org/10.1016/j.pnpbp.2017.02.022

Arabi, M., Nasab, S. H., Lorigooini, Z., Boroujeni, S. N., Mortazavi, S. M., Anjomshoa, M., & Amini-Khoei, H. (2021). Auraptene exerts protective effects on maternal separation stress-induced changes in behavior, hippocampus, heart and serum of mice. *Int Immunopharmacol*, *93*, 107436. https://doi.org/10.1016/j.intimp.2021.107436

Banqueri, M., Méndez, M., Gómez-Lázaro, E., & Arias, J. L. (2019). Early life stress by repeated maternal separation induces long-term neuroinflammatory response in glial cells of male rats. *Stress*, *22*(5), 563-570. https://doi.org/10.1080/10253890.2019.1604666

Bauer, M. E., & Teixeira, A. L. (2019). Inflammation in psychiatric disorders: what comes first? *Ann N Y Acad Sci*, *1437*(1), 57-67. https://doi.org/10.1111/nyas.13712

Bekhbat, M., & Neigh, G. N. (2018). Sex differences in the neuro-immune consequences of stress: Focus on depression and anxiety. *Brain Behav Immun*, *67*, 1-12. https://doi.org/10.1016/j.bbi.2017.02.006

Brenhouse, H. C., Danese, A., & Grassi-Oliveira, R. (2019). Neuroimmune Impacts of Early-Life Stress on Development and Psychopathology. *Curr Top Behav Neurosci*, *43*, 423-447. https://doi.org/10.1007/7854_2018_53

Burke, N. N., Llorente, R., Marco, E. M., Tong, K., Finn, D. P., Viveros, M. P., & Roche, M. (2013). Maternal deprivation is associated with sex-dependent alterations in nociceptive behavior and neuroinflammatory mediators in the rat following peripheral nerve injury. *J Pain*, *14*(10), 1173-1184. https://doi.org/10.1016/j.jpain.2013.05.003

Calcia, M. A., Bonsall, D. R., Bloomfield, P. S., Selvaraj, S., Barichello, T., & Howes, O. D. (2016). Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology (Berl)*, *233*(9), 1637-1650. https://doi.org/10.1007/s00213-016-4218-9

Cochrane Infectious Diseases Group. (2007). How to develop a search strategy for a Cochrane Review. In: The Cochrane Library.

Danese, A., & J Lewis, S. (2017). Psychoneuroimmunology of Early-Life Stress: The Hidden Wounds of Childhood Trauma? *Neuropsychopharmacology*, *42*(1), 99-114. https://doi.org/10.1038/npp.2016.198

de Azeredo, L. A., Wearick-Silva, L. E., Viola, T. W., Tractenberg, S. G., Centeno-Silva, A., Orso, R., . . . Grassi-Oliveira, R. (2017). Maternal separation induces hippocampal changes in cadherin-1 (CDH-1) mRNA and recognition memory impairment in

adolescent mice. *Neurobiol Learn Mem*, *141*, 157-167. https://doi.org/10.1016/j.nlm.2017.04.006

Diaz-Chávez, A., Lajud, N., Roque, A., Cheng, J. P., Meléndez-Herrera, E., Valdéz-Alarcón, J. J., . . . Kline, A. E. (2020). Early life stress increases vulnerability to the sequelae of pediatric mild traumatic brain injury. *Exp Neurol*, *329*, 113318. https://doi.org/10.1016/j.expneurol.2020.113318

Druzhkova, T., Pochigaeva, K., Yakovlev, A., Kazimirova, E., Grishkina, M., Chepelev, A., . . . Gulyaeva, N. (2019). Acute stress response to a cognitive task in patients with major depressive disorder: potential metabolic and proinflammatory biomarkers. *Metab Brain Dis*, *34*(2), 621-629. https://doi.org/10.1007/s11011-018-0367-3

Dugue, R., Nath, M., Dugue, A., & Barone, F. C. (2017). Roles of pro-and anti-inflammatory cytokines in traumatic brain injury and acute ischemic stroke. *Mech. Neuroinflamm*, *211*.

Dutcher, E. G., Pama, E. A. C., Lynall, M. E., Khan, S., Clatworthy, M. R., Robbins, T. W., . . . Dalley, J. W. (2020). Early-life stress and inflammation: A systematic review of a key experimental approach in rodents. *Brain Neurosci Adv*, *4*, 2398212820978049. https://doi.org/10.1177/2398212820978049

Engler, H., Benson, S., Wegner, A., Spreitzer, I., Schedlowski, M., & Elsenbruch, S. (2016). Men and women differ in inflammatory and neuroendocrine responses to endotoxin but not in the severity of sickness symptoms. *Brain Behav Immun*, *52*, 18-26. https://doi.org/10.1016/j.bbi.2015.08.013

Fabbri, C., Hosak, L., Mössner, R., Giegling, I., Mandelli, L., Bellivier, F., . . . Serretti, A. (2017). Consensus paper of the WFSBP Task Force on Genetics: Genetics, epigenetics and gene expression markers of major depressive disorder and antidepressant response. *World J Biol Psychiatry*, *18*(1), 5-28. https://doi.org/10.1080/15622975.2016.1208843

Fagundes, C. P., & Way, B. (2014). Early-life stress and adult inflammation. *Current Directions in Psychological Science*, *23*(4), 277-283.

Feghali, C. A., & Wright, T. M. (1997). Cytokines in acute and chronic inflammation. *Front Biosci*, *2*(1), d12-d26.

Frank, M. G., Hershman, S. A., Weber, M. D., Watkins, L. R., & Maier, S. F. (2014). Chronic exposure to exogenous glucocorticoids primes microglia to pro-inflammatory stimuli and induces NLRP3 mRNA in the hippocampus. *Psychoneuroendocrinology*, *40*, 191-200. https://doi.org/10.1016/j.psyneuen.2013.11.006

Ganguly, P., Honeycutt, J. A., Rowe, J. R., Demaestri, C., & Brenhouse, H. C. (2019). Effects of early life stress on cocaine conditioning and AMPA receptor composition are sex-specific and driven by TNF. *Brain Behav Immun*, *78*, 41-51. https://doi.org/10.1016/j.bbi.2019.01.006

Giridharan, V. V., Réus, G. Z., Selvaraj, S., Scaini, G., Barichello, T., & Quevedo, J. (2019). Maternal deprivation increases microglial activation and neuroinflammatory markers in the prefrontal cortex and hippocampus of infant rats. *J Psychiatr Res*, *115*, 13-20. https://doi.org/10.1016/j.jpsychires.2019.05.001

González-Pardo, H., Arias, J. L., Gómez-Lázaro, E., López Taboada, I., & Conejo, N. M. (2020). Sex-Specific Effects of Early Life Stress on Brain Mitochondrial Function, Monoamine Levels and Neuroinflammation. *Brain Sci*, *10*(7). https://doi.org/10.3390/brainsci10070447

Grassi-Oliveira, R., Honeycutt, J. A., Holland, F. H., Ganguly, P., & Brenhouse, H. C. (2016). Cognitive impairment effects of early life stress in adolescents can be predicted with early biomarkers: Impacts of sex, experience, and cytokines. *Psychoneuroendocrinology*, *71*, 19-30. https://doi.org/10.1016/j.psyneuen.2016.04.016

Hanson, J. L., Nacewicz, B. M., Sutterer, M. J., Cayo, A. A., Schaefer, S. M., Rudolph, K. D., . . . Davidson, R. J. (2015). Behavioral problems after early life stress: contributions of the hippocampus and amygdala. *Biol Psychiatry*, *77*(4), 314-323. https://doi.org/10.1016/j.biopsych.2014.04.020

Hoeijmakers, L., Ruigrok, S. R., Amelianchik, A., Ivan, D., van Dam, A. M., Lucassen, P. J., & Korosi, A. (2017). Early-life stress lastingly alters the neuroinflammatory response to amyloid pathology in an Alzheimer's disease mouse model. *Brain Behav Immun*, *63*, 160-175. https://doi.org/10.1016/j.bbi.2016.12.023

Hohmann, C. F., Odebode, G., Naidu, L., & Koban, M. (2017). Early Life Stress Alters Adult Inflammatory Responses in a Mouse Model for Depression. *Ann Psychiatry Ment Health*, *5*(2).

Hooijmans, C. R., de Vries, R., Leenaars, M., Curfs, J., & Ritskes-Hoitinga, M. (2011). Improving planning, design, reporting and scientific quality of animal experiments by using the Gold Standard Publication Checklist, in addition to the ARRIVE guidelines. *Br J Pharmacol*, *162*(6), 1259-1260. https://doi.org/10.1111/j.1476-5381.2010.01128.x

Jia, Y., Liu, L., Sheng, C., Cheng, Z., Cui, L., Li, M., . . . Chen, L. (2019). Increased Serum Levels of Cortisol and Inflammatory Cytokines in People With Depression. *J Nerv Ment Dis*, *207*(4), 271-276. https://doi.org/10.1097/NMD.0000000000000957

Kiank, C., Mundt, A., & Schuett, C. (2009). Mild postnatal separation stress reduces repeated stress-induced immunosuppression in adult BALB/c mice. *Neuro Endocrinol Lett*, *30*(6), 761-768.

Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*, *8*(6), e1000412. https://doi.org/10.1371/journal.pbio.1000412

Kim, Y. K., Na, K. S., Myint, A. M., & Leonard, B. E. (2016). The role of pro-inflammatory cytokines in neuroinflammation, neurogenesis and the neuroendocrine system in major depression. *Prog Neuropsychopharmacol Biol Psychiatry*, *64*, 277-284. https://doi.org/10.1016/j.pnpbp.2015.06.008

Koussounadis, A., Langdon, S. P., Um, I. H., Harrison, D. J., & Smith, V. A. (2015). Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Sci Rep*, *5*, 10775. https://doi.org/10.1038/srep10775

Kuhlman, K. R., Robles, T. F., Haydon, M. D., Dooley, L., Boyle, C. C., & Bower, J. E. (2020). Early life stress sensitizes individuals to the psychological correlates of mild fluctuations in inflammation. *Dev Psychobiol*, *62*(3), 400-408. https://doi.org/10.1002/dev.21908

Lajud, N., Roque, A., Cheng, J. P., Bondi, C. O., & Kline, A. E. (2021). Early Life Stress Preceding Mild Pediatric Traumatic Brain Injury Increases Neuroinflammation but Does Not Exacerbate Impairment of Cognitive Flexibility during Adolescence. *J Neurotrauma*, *38*(4), 411-421. https://doi.org/10.1089/neu.2020.7354

Lo Iacono, L., Catale, C., Martini, A., Valzania, A., Viscomi, M. T., Chiurchiù, V., . . . Carola, V. (2018). From Traumatic Childhood to Cocaine Abuse: The Critical Function of the

Immune System. *Biol Psychiatry*, *84*(12), 905-916. https://doi.org/10.1016/j.biopsych.2018.05.022

Lorigooini, Z., Boroujeni, S. N., Sayyadi-Shahraki, M., Rahimi-Madiseh, M., Bijad, E., & Amini-Khoei, H. (2021). Limonene through Attenuation of Neuroinflammation and Nitrite Level Exerts Antidepressant-Like Effect on Mouse Model of Maternal Separation Stress. *Behav Neurol*, *2021*, 8817309. https://doi.org/10.1155/2021/8817309

Majcher-Maślanka, I., Solarz, A., & Chocyk, A. (2019). Maternal separation disturbs postnatal development of the medial prefrontal cortex and affects the number of neurons and glial cells in adolescent rats. *Neuroscience*, *423*, 131-147. https://doi.org/10.1016/j.neuroscience.2019.10.033

Marafini, I., Sedda, S., Dinallo, V., & Monteleone, G. (2019). Inflammatory cytokines: from discoveries to therapies in IBD. *Expert Opin Biol Ther*, *19*(11), 1207-1217. https://doi.org/10.1080/14712598.2019.1652267

Massart, R., Nemoda, Z., Suderman, M. J., Sutti, S., Ruggiero, A. M., Dettmer, A. M., . . . Szyf, M. (2016). Early life adversity alters normal sex-dependent developmental dynamics of DNA methylation. *Dev Psychopathol*, *28*(4pt2), 1259-1272. https://doi.org/10.1017/S0954579416000833

McLaughlin, K. A., Sheridan, M. A., & Lambert, H. K. (2014). Childhood adversity and neural development: deprivation and threat as distinct dimensions of early experience. *Neurosci Biobehav Rev*, *47*, 578-591. https://doi.org/10.1016/j.neubiorev.2014.10.012

Na, K. S., Jung, H. Y., & Kim, Y. K. (2014). The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*, *48*, 277-286. https://doi.org/10.1016/j.pnpbp.2012.10.022

Nouri, A., Hashemzadeh, F., Soltani, A., Saghaei, E., & Amini-Khoei, H. (2020). Progesterone exerts antidepressant-like effect in a mouse model of maternal separation stress through mitigation of neuroinflammatory response and oxidative stress. *Pharm Biol*, *58*(1), 64-71. https://doi.org/10.1080/13880209.2019.1702704

Oliveira, C., Toledo, R. S., Scarabelot, V. L., Vercelino, R., da Silva, L. S., Regner, G. G., . . . Torres, I. L. S. (2020). Neonatal morphine exposure and maternal deprivation alter nociceptive response and central biomarkers' levels throughout the life of rats. *Neurosci Lett*, *738*, 135350. https://doi.org/10.1016/j.neulet.2020.135350

Orso, R., Creutzberg, K. C., Kestering-Ferreira, E., Wearick-Silva, L. E., Tractenberg, S. G., & Grassi-Oliveira, R. (2020). Maternal Separation Combined With Limited Bedding Increases Anxiety-Like Behavior and Alters Hypothalamic-Pituitary-Adrenal Axis Function of Male BALB/cJ Mice. *Front Behav Neurosci*, *14*, 600766. https://doi.org/10.3389/fnbeh.2020.600766

Orso, R., Creutzberg, K. C., Wearick-Silva, L. E., Wendt Viola, T., Tractenberg, S. G., Benetti, F., & Grassi-Oliveira, R. (2019). How Early Life Stress Impact Maternal Care: A Systematic Review of Rodent Studies. *Front Behav Neurosci*, *13*, 197. https://doi.org/10.3389/fnbeh.2019.00197

Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan-a web and mobile app for systematic reviews. *Syst Rev*, *5*(1), 210. https://doi.org/10.1186/s13643-016-0384-4

Pace, T. W., Mletzko, T. C., Alagbe, O., Musselman, D. L., Nemeroff, C. B., Miller, A. H., & Heim, C. M. (2006). Increased stress-induced inflammatory responses in male

patients with major depression and increased early life stress. *American Journal of Psychiatry*, *163*(9), 1630-1633.
Park, H. J., Kim, S. A., Kang, W. S., & Kim, J. W. (2021). Early-Life Stress Modulates Gut Microbiota and Peripheral and Central Inflammation in a Sex-Dependent Manner. *Int J Mol Sci*, *22*(4). https://doi.org/10.3390/ijms22041899
Park, H. J., Kim, S. K., Kang, W. S., Woo, J. M., & Kim, J. W. (2014). Effects of essential oil from Chamaecyparis obtusa on cytokine genes in the hippocampus of maternal separation rats. *Can J Physiol Pharmacol*, *92*(2), 95-101. https://doi.org/10.1139/cjpp-2013-0224
Pedersen, J. M., Mortensen, E. L., Christensen, D. S., Rozing, M., Brunsgaard, H., Meincke, R. H., . . . Lund, R. (2018). Prenatal and early postnatal stress and later life inflammation. *Psychoneuroendocrinology*, *88*, 158-166. https://doi.org/10.1016/j.psyneuen.2017.12.014
Pei, X., Jiang, H., Liu, X., Li, L., Li, C., Xiao, X., . . . Tang, S. (2021). Targeting HMGB1 inhibits T-2 toxin-induced neurotoxicity via regulation of oxidative stress, neuroinflammation and neuronal apoptosis. *Food Chem Toxicol*, *151*, 112134. https://doi.org/10.1016/j.fct.2021.112134
Peña, C. J., Kronman, H. G., Walker, D. M., Cates, H. M., Bagot, R. C., Purushothaman, I., . . . Nestler, E. J. (2017). Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. *Science*, *356*(6343), 1185-1188. https://doi.org/10.1126/science.aan4491
Pfau, M. L., & Russo, S. J. (2015). Peripheral and Central Mechanisms of Stress Resilience. *Neurobiol Stress*, *1*, 66-79. https://doi.org/10.1016/j.ynstr.2014.09.004
Pinheiro, R. M., de Lima, M. N., Portal, B. C., Busato, S. B., Falavigna, L., Ferreira, R. D., . . . Schröder, N. (2015). Long-lasting recognition memory impairment and alterations in brain levels of cytokines and BDNF induced by maternal deprivation: effects of valproic acid and topiramate. *J Neural Transm (Vienna)*, *122*(5), 709-719. https://doi.org/10.1007/s00702-014-1303-2
Provençal, N., & Binder, E. B. (2015). The effects of early life stress on the epigenome: From the womb to adulthood and even before. *Exp Neurol*, *268*, 10-20. https://doi.org/10.1016/j.expneurol.2014.09.001
Reed, R. G., & Raison, C. L. (2016). Stress and the immune system. In *Environmental influences on the immune system* (pp. 97-126). Springer.
Rice, C. J., Sandman, C. A., Lenjavi, M. R., & Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology*, *149*(10), 4892-4900. https://doi.org/10.1210/en.2008-0633
Romeo, R. D., Fossella, J. A., Bateup, H. S., Sisti, H. M., Brake, W. G., & McEwen, B. S. (2004). Maternal separation suppresses TGF alpha mRNA expression in the prefrontal cortex of male and female neonatal C57BL/6 mice. *Brain Res Dev Brain Res*, *152*(1), 73-77. https://doi.org/10.1016/j.devbrainres.2004.05.007
Roque, A., Ochoa-Zarzosa, A., & Torner, L. (2016). Maternal separation activates microglial cells and induces an inflammatory response in the hippocampus of male rat pups, independently of hypothalamic and peripheral cytokine levels. *Brain Behav Immun*, *55*, 39-48. https://doi.org/10.1016/j.bbi.2015.09.017
Réus, G. Z., Dos Santos, M. A., Abelaira, H. M., Ribeiro, K. F., Petronilho, F., Vuolo, F., . . . Quevedo, J. (2013). Imipramine reverses alterations in cytokines and BDNF levels

induced by maternal deprivation in adult rats. *Behav Brain Res*, *242*, 40-46. https://doi.org/10.1016/j.bbr.2012.11.044

Réus, G. Z., Fernandes, G. C., de Moura, A. B., Silva, R. H., Darabas, A. C., de Souza, T. G., . . . Quevedo, J. (2017). Early life experience contributes to the developmental programming of depressive-like behaviour, neuroinflammation and oxidative stress. *J Psychiatr Res*, *95*, 196-207. https://doi.org/10.1016/j.jpsychires.2017.08.020

Réus, G. Z., Nacif, M. P., Abelaira, H. M., Tomaz, D. B., dos Santos, M. A., Carlessi, A. S., . . . Quevedo, J. (2015). Ketamine ameliorates depressive-like behaviors and immune alterations in adult rats following maternal deprivation. *Neurosci Lett*, *584*, 83-87. https://doi.org/10.1016/j.neulet.2014.10.022

Saavedra, L. M., Fenton Navarro, B., & Torner, L. (2017). Early Life Stress Activates Glial Cells in the Hippocampus but Attenuates Cytokine Secretion in Response to an Immune Challenge in Rat Pups. *Neuroimmunomodulation*, *24*(4-5), 242-255. https://doi.org/10.1159/000485383

Ströher, R., de Oliveira, C., Stein, D. J., de Macedo, I. C., Goularte, J. F., da Silva, L. S., . . . Torres, I. L. S. (2020). Maternal Deprivation and Sex Alter Central Levels of Neurotrophins and Inflammatory Cytokines in Rats Exposed to Palatable Food in Adolescence. *Neuroscience*, *428*, 122-131. https://doi.org/10.1016/j.neuroscience.2019.12.027

Tang, H. L., Zhang, G., Ji, N. N., Du, L., Chen, B. B., Hua, R., & Zhang, Y. M. (2017). Toll-Like Receptor 4 in Paraventricular Nucleus Mediates Visceral Hypersensitivity Induced by Maternal Separation. *Front Pharmacol*, *8*, 309. https://doi.org/10.3389/fphar.2017.00309

Tannous, J., Godlewska, B. R., Tirumalaraju, V., Soares, J. C., Cowen, P. J., & Selvaraj, S. (2020). Stress, inflammation and hippocampal subfields in depression: A 7 Tesla MRI Study. *Transl Psychiatry*, *10*(1), 78. https://doi.org/10.1038/s41398-020-0759-0

Teissier, A., Le Magueresse, C., Olusakin, J., Andrade da Costa, B. L. S., De Stasi, A. M., Bacci, A., . . . Gaspar, P. (2020). Early-life stress impairs postnatal oligodendrogenesis and adult emotional behaviour through activity-dependent mechanisms. *Mol Psychiatry*, *25*(6), 1159-1174. https://doi.org/10.1038/s41380-019-0493-2

Tractenberg, S. G., Levandowski, M. L., de Azeredo, L. A., Orso, R., Roithmann, L. G., Hoffmann, E. S., . . . Grassi-Oliveira, R. (2016). An overview of maternal separation effects on behavioural outcomes in mice: Evidence from a four-stage methodological systematic review. *Neurosci Biobehav Rev*, *68*, 489-503. https://doi.org/10.1016/j.neubiorev.2016.06.021

van Bodegom, M., Homberg, J. R., & Henckens, M. J. A. G. (2017). Modulation of the Hypothalamic-Pituitary-Adrenal Axis by Early Life Stress Exposure. *Front Cell Neurosci*, *11*, 87. https://doi.org/10.3389/fncel.2017.00087

Viola, T. W., Creutzberg, K. C., Zaparte, A., Kestering-Ferreira, É., Tractenberg, S. G., Centeno-Silva, A., . . . Grassi-Oliveira, R. (2019). Acute neuroinflammation elicited by TLR-3 systemic activation combined with early life stress induces working memory impairments in male adolescent mice. *Behav Brain Res*, *376*, 112221. https://doi.org/10.1016/j.bbr.2019.112221

Viviani, B., Boraso, M., Valero, M., Gardoni, F., Marco, E. M., Llorente, R., . . . Viveros, M. P. (2014). Early maternal deprivation immunologically primes hippocampal synapses by redistributing interleukin-1 receptor type I in a sex dependent manner. *Brain Behav Immun*, *35*, 135-143. https://doi.org/10.1016/j.bbi.2013.09.008

Vogel, C., & Marcotte, E. M. (2012). Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat Rev Genet*, *13*(4), 227-232. https://doi.org/10.1038/nrg3185

Walker, D. J., Zimmer, C., Larriva, M., Healy, S. D., & Spencer, K. A. (2019). Early-life adversity programs long-term cytokine and microglia expression within the HPA axis in female Japanese quail. *J Exp Biol*, *222*(Pt 6). https://doi.org/10.1242/jeb.187039

Wang, R., Wang, W., Xu, J., Liu, D., Wu, H., Qin, X., . . . Pan, F. (2020). Jmjd3 is involved in the susceptibility to depression induced by maternal separation via enhancing the neuroinflammation in the prefrontal cortex and hippocampus of male rats. *Exp Neurol*, *328*, 113254. https://doi.org/10.1016/j.expneurol.2020.113254

Warren, J. S. (1990). Interleukins and tumor necrosis factor in inflammation. *Crit Rev Clin Lab Sci*, *28*(1), 37-59. https://doi.org/10.3109/10408369009105897

Weber, M. D., Frank, M. G., Tracey, K. J., Watkins, L. R., & Maier, S. F. (2015). Stress induces the danger-associated molecular pattern HMGB-1 in the hippocampus of male Sprague Dawley rats: a priming stimulus of microglia and the NLRP3 inflammasome. *J Neurosci*, *35*(1), 316-324. https://doi.org/10.1523/JNEUROSCI.3561-14.2015

White, J. D., & Kaffman, A. (2019). The Moderating Effects of Sex on Consequences of Childhood Maltreatment: From Clinical Studies to Animal Models. *Front Neurosci*, *13*, 1082. https://doi.org/10.3389/fnins.2019.01082

Wohleb, E. S., Fenn, A. M., Pacenta, A. M., Powell, N. D., Sheridan, J. F., & Godbout, J. P. (2012). Peripheral innate immune challenge exaggerated microglia activation, increased the number of inflammatory CNS macrophages, and prolonged social withdrawal in socially defeated mice. *Psychoneuroendocrinology*, *37*(9), 1491-1505. https://doi.org/10.1016/j.psyneuen.2012.02.003

Wojdasiewicz, P., Poniatowski, Ł., & Szukiewicz, D. (2014). The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm*, *2014*, 561459. https://doi.org/10.1155/2014/561459

Ye, Y., Yao, S., Wang, R., Fang, Z., Zhong, K., Nie, L., & Zhang, Q. (2019). PI3K/Akt/NF-κB signaling pathway regulates behaviors in adolescent female rats following with neonatal maternal deprivation and chronic mild stress. *Behav Brain Res*, *362*, 199-207. https://doi.org/10.1016/j.bbr.2019.01.008

Zhu, Y., Wang, Y., Yao, R., Hao, T., Cao, J., Huang, H., . . . Wu, Y. (2017). Enhanced neuroinflammation mediated by DNA methylation of the glucocorticoid receptor triggers cognitive dysfunction after sevoflurane anesthesia in adult rats subjected to maternal separation during the neonatal period. *J Neuroinflammation*, *14*(1), 6. https://doi.org/10.1186/s12974-016-0782-5

Çalışkan, G., Müller, A., & Albrecht, A. (2020). Long-Term Impact of Early-Life Stress on Hippocampal Plasticity: Spotlight on Astrocytes. *Int J Mol Sci*, *21*(14). https://doi.org/10.3390/ijms21144999