CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Prior maternal separation stress alters the dendritic complexity of new hippocampal neurons and neuroinflammation in response to an inflammatory stressor in juvenile female rats
Authors	Nicolas, Sarah;McGovern, Andrew J.;Hueston, Cara M.;O'Mahony, Siobhain M.;Cryan, John F.;O'Leary, Olivia F.;Nolan, Yvonne M.
Publication date	2021-10-31
Original Citation	Nicolas, S., McGovern, A. J., Hueston, C. M., O'Mahony, S. M., Cryan, J. F., O'Leary, O. F. and Nolan, Y. M. (2021) 'Prior maternal separation stress alters the dendritic complexity of new hippocampal neurons and neuroinflammation in response to an inflammatory stressor in juvenile female rats', Brain, Behavior, and Immunity, 99, pp.327-338. https://doi.org/10.1016/j.bbi.2021.10.016
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.bbi.2021.10.016
Rights	© 2021, Elsevier Inc. All rights reserved. This manuscript version is made available under the CC BY-NC-ND 4.0 license. - https://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2026-10-30 08:14:33
Item downloaded from	https://hdl.handle.net/10468/12150

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Journal Pre-proofs



Prior maternal separation stress alters the dendritic complexity of new hippocampal neurons and neuroinflammation in response to an inflammatory stressor in juvenile female rats

Sarah Nicolas, Andrew J. McGovern, Cara M. Hueston, Siobhain M. O'Mahony, John F. Cryan, Olivia F. O'Leary, Yvonne M. Nolan

PII: S0889-1591(21)00592-4
DOI: https://doi.org/10.1016/j.bbi.2021.10.016
Reference: YBRBI 4698

To appear in: *Brain, Behavior, and Immunity*

Received Date: 30 March 2021
Revised Date: 19 October 2021
Accepted Date: 23 October 2021

Please cite this article as: Nicolas, S., McGovern, A.J., Hueston, C.M., O'Mahony, S.M., Cryan, J.F., O'Leary, O.F., Nolan, Y.M., Prior maternal separation stress alters the dendritic complexity of new hippocampal neurons and neuroinflammation in response to an inflammatory stressor in juvenile female rats, *Brain, Behavior, and Immunity* (2021), doi: https://doi.org/10.1016/j.bbi.2021.10.016

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Inc.

Journal Pre-proofs

Prior maternal separation stress alters the dendritic complexity of new hippocampal neurons and neuroinflammation in response to an inflammatory stressor in juvenile female rats

Sarah Nicolas[1], Andrew J. McGovern[1], Cara M. Hueston[1], Siobhain M. O'Mahony[1,2], John F. Cryan[1,2], Olivia F. O'Leary[1,2+], Yvonne M. Nolan[1,2+]

1 Department of Anatomy and Neuroscience, University College Cork, Ireland.

2 APC Microbiome Ireland, University College Cork, Ireland.

Corresponding author: Y. M. Nolan, Department of Anatomy and Neuroscience, University College Cork, Ireland, Email: y.nolan@ucc.ie

+ equal contribution

Journal Pre-proofs

Abstract

Stress during critical periods of neurodevelopment is associated with an increased risk of developing stress-related psychiatric disorders which are more common in women than men. Hippocampal neurogenesis (the birth of new neurons) is vulnerable to maternal separation and inflammatory stressors, and emerging evidence suggests that hippocampal neurogenesis is more sensitive to stress in the ventral hippocampus (vHi) than in the dorsal hippocampus (dHi). Although research into the effects of maternal separation stress on hippocampal neurogenesis is well documented in male rodents, the effect in females remains underexplored. Similarly, reports on the impact of inflammatory stressors on hippocampal neurogenesis in females are limited, especially when female bias the in prevalence of stress-related psychiatric disorders begins to emerge. Thus, in this study we investigated the effects of maternal separation (MS) followed by an inflammatory stressor (lipopolysaccharide, LPS) in early adolescence on peripheral and hippocampal inflammatory responses and hippocampal neurogenesis in juvenile female rats. We show that MS enhanced an LPS-induced increase in the pro-inflammatory cytokine IL-1β in the vHi but not in the dHi. However, microglial activation was similar following LPS alone or MS alone in both hippocampal regions, while MS prior to LPS reduced microglial activation in both dHi and vHi. The production of new neurons was unaffected by MS and LPS. MS and LPS independently reduced the dendritic complexity of new neurons, and MS exacerbated LPS-induced reductions in complexity of distal dendrites of new neurons in the vHi but not dHi. These data highlight that MS differentially primes the physiological response to LPS in the juvenile female rat hippocampus.

Journal Pre-proofs

Keywords

Maternal separation, stress, juvenile, female, hippocampal neurogenesis, ventral hippocampus, microglia, immune challenge, IL-1β

Highlights

- Maternal Separation altered the LPS-induced inflammatory response in plasma of juvenile female rats
- Maternal separation enhanced an LPS-induced increase in IL-1β in the ventral but not dorsal hippocampus.
- Maternal separation attenuated an LPS-induced increase in microglial activation in dorsal and ventral hippocampus.
- Maternal separation and LPS, and the combination of both, reduced dendritic complexity of newly born neurons in the dentate gyrus.

Journal Pre-proofs

1.Introduction

Postnatal and juvenile periods are considered important periods of neurodevelopment due to high rates of ontogenetic change [1,2]. Stress during these critical periods can interrupt normal development of neurological and immunological systems, which may have functional consequences later in life[3]. One brain structure which is highly vulnerable to early life stress is the hippocampus[4]. Neurogenesis, a form of brain plasticity in which new neurons are generated throughout life in the dentate gyrus (DG) of the hippocampus, occurs at higher rates during early life and adolescence compared to adulthood[5,6]. Hippocampal neurogenesis plays important roles in learning and spatial memory[7–9] and is implicated in anxiety, forgetting, the stress response and in antidepressant effects[10,11]. Accumulating evidence suggests that the hippocampus is functionally segregated along its longitudinal axis into dorsal and ventral regions whereby the dorsal region plays a more predominant role in spatial learning and memory while the ventral region plays a more predominant role in anxiety regulation and the stress response[12,13]. There is also emerging evidence that neurogenesis in the ventral hippocampus (vHi) is more sensitive to stress regulation than that in the dorsal hippocampus (dHi)[14–16]. Whether the vHi and dHi of the juvenile brain exhibit similar differences in stress sensitivity has not yet been fully explored.

The impact of early life stress on behaviour and physiology has been widely studied in rodents using the rat maternal separation (MS) paradigm[17]. Several studies have demonstrated that MS increased anxiety-like behaviour and decreased hippocampal neurogenesis in adult male rats[18,19]. Similarly, MS decreased neurogenesis in the hippocampus of adolescent male rats[20,21]. Although research into the effects of MS on hippocampal neurogenesis is well documented in males, the immediate or long-term effect of MS on

hippocampal neurogenesis in females and in particular adolescent females remains underexplored. Given that women are twice more likely than men to develop the stress-related psychiatric disorders depression and post-traumatic stress disorder[22], and that 75% of mental illnesses emerge during adolescence (World Health Organisation), it is important that the impact of stress on the female brain is interrogated.

Throughout adolescence, and in particular during the juvenile period, the hippocampus is vulnerable to environmental stressors[19,23] including inflammation, which is a negative regulator of hippocampal neurogenesis[24,25]. It is now well-established that pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) or tumour necrosis factor-α (TNF-α) released following an inflammatory stress negatively affects hippocampal neurogenesis[26–31]. Peripheral injection of lipopolysaccharide (LPS) which mimics a bacterial infection by inducing pro-inflammatory cytokine expression and the activation of microglia in the hippocampus of rodents, also impairs hippocampal neurogenesis[32–34]. However, similar to studies on the effect of MS on hippocampal neurogenesis, the effects of inflammation on hippocampal neurogenesis have been primarily reported in adult males. Given that microglia undergo sex-dependent maturation processes during adolescence[35,36], males and females might also differentially respond to inflammatory stressors during adolescence that would impact upon microglial function and hippocampal neurogenesis. In this regard, little is known about the temporal effect of MS on immune responses in females during the juvenile period. While MS generally leads to increased peripheral and brain inflammation in adult and adolescent male rodents[37–39], studies examining the effects of MS on the inflammatory status of the brain in females are limited to assessment in adulthood. Moreover, the impact of MS on the inflammatory status in the hippocampus of juvenile females and the response of juvenile females to an inflammatory stress after prior MS stress has not yet been investigated.

We hypothesized that adverse events that occur in early life would prime physiological responses in the juvenile female rat to an exogenous inflammatory stress. Thus, in this study we investigated the effects of either MS alone or juvenile acute LPS administration on peripheral and hippocampal inflammatory stress responses and on neurogenesis along the longitudinal axis of the hippocampus of juvenile female rats. We further investigated whether MS primed these physiological responses to juvenile acute LPS administration.

2. Methods

The experimental design is summarised in figure 1a.

2.1. Animals

Adult male and female Sprague-Dawley rats (Biological Services Unit, University College Cork) were used as breeding partners to generate the 63 female offspring used in this study. Females were pair-housed until gestation day 19, after which they were singly housed to give birth. All animals were housed in a colony maintained at 21 ± 2°C, with a 12:12 hour light-dark cycle (lights on 0730-1930). All animal procedures were performed under authorizations issued by the Irish Medicine's Board (IMB), in accordance with the European Communities Council Directive (2010/63/EU) and approved by the Animal Experimentation Ethics Committee of University College Cork.

2.2. Maternal separation

Pups were randomly assigned to the non-separated (NS) or maternal separation (MS) groups. The MS procedure consisted of the separation of the pups from the dams between post-natal days (PND) 2-12 (inclusive) starting at 9 am for 180 minutes[17]. For the MS procedure the pups were separated as whole litters (approximately 8-10 pups per litters) in a different room to

the dams to prevent olfactory or vocal communication. They were placed into clean cages on each separation day and the cages were placed on heating pads to maintain a temperature of 30-33°C. The dams were returned to the holding room in their original cages each day. NS rat pups and dams were removed them from their original cage, moved into a new cage, and then returned to the original cage. Following the separation procedure, animals were left undisturbed except for routine cage cleaning. The complete litters (females and males) were kept during the MS protocol (PND2-12) and sexed at PND 21, according to previous protocols[17,40,41]. The male pups were used in a separate experiment and thus could not be used in this study. Two cohorts of animals were used in the current study. The first cohort was used for immunohistochemical (IHC) analysis of the hippocampus and a second cohort was used for cytokine measures in plasma and hippocampus. For each cohort, three litters comprised the NS group and three litters underwent the MS procedure. On PND21, rats were randomly assigned to control or LPS groups. To avoid a litter effect, rats were assigned to experimental groups such that the minimal number of pups from the same litter were in each group [17,42].

2.3. Lipopolysaccharide administration

On PND 21, rats received an intraperitoneal (IP) LPS injection (250 µg/kg; Sigma UK) or a volume equivalent IP injection of saline. PND21 is the time at which the peak in synaptic density[43,44] and myelination[45] occurs, as well as changes in neurotransmitter and receptor levels[46]. Thus, PND21 is a sensitive period at which an inflammatory challenge could impact brain function. PND21 also occurs during the prepubertal or juvenile period in the female rat. Injecting LPS at a later timepoint (on/after PND30) would necessitate taking into account ovarian maturation and the role of the oestrous cycle in our findings[47]. The dose of LPS used

(250 µg/) is based on evidence that it induces neuroinflammation in the hippocampus[48], which is associated with sickness behaviour[49] in adult female rats. In males, this dose induced neuroinflammation in adult[50–52]; PND13[53] and PND21[54] rats.

2.4. Tissue collection

Twenty-four hours following LPS or saline injections, rats from cohort 1 were euthanized with an IP injection of pentobarbital (1.0 mL/kg) and then transcardially perfused with saline followed by 4% (v/v) paraformaldehyde in a 0.1 M phosphate buffer of pH 7.2. The brains were removed and post-fixed in 4% (v/v) paraformaldehyde at 4°C overnight and then placed into a sucrose solution (30% (w/v)) for 24 hours, after which brains were frozen at -80°C. Rats from cohort 2 were euthanised by cervical dislocation 24 hours after LPS/saline challenge and the dorsal and ventral hippocampus was dissected out and stored in RNAlater (Sigma, UK) for 48 h at 4˚C, after which the RNAlater was removed and the tissue was frozen at −80˚C until subsequent use. Tail blood samples were taken immediately before LPS/saline injection at PND21 (T0) and at 2 hours (T2) after LPS/saline injection. Blood samples were collected into EDTA tubes, centrifuged (15 min, 5000 rpm) and the plasma was collected and stored at −80°C until corticosterone and cytokine analysis.

2.5. Immunohistochemistry

Brains were coronally sectioned (40µm) and mounted onto gelatin-coated slides in a 1:6 series through the hippocampus and frozen at -80°C.

2.5.1 Ionized calcium Binding Adapter 1

To analyse the presence and activation of microglia[55–58], hippocampal sections were stained for ionized calcium-binding adapter molecule 1 (Iba1), a microglia/macrophage-specific

calcium binding protein. Rehydrated sections were treated with 0.1% hydrogen peroxide (Sigma, UK) in methanol to block endogenous peroxidases, followed by 3% normal rabbit serum (Sigma, UK) prepared in PBS with 0.3% Triton-X for 1 hour at room temperature. Sections were then incubated for 24h at 4°C with rabbit Iba1 primary antibody (Wako) solution (1:500 dilution in 1% rabbit serum and 0.3% PBS-T), rinsed in PBS, and incubated for two hours in biotinylated goat anti-rabbit antibody (Vector laboratories; 1:200 dilution) made up in PBS, 0.3% Triton-X and 1.5% normal rabbit serum.

2.5.2 Doublecortin

For analysis of newly born neurons, doublecortin (DCX) immunohistochemistry was carried out. Sections were treated with 0.1% hydrogen peroxide (Sigma, UK) in PBS for 30 min to block endogenous peroxidases, followed by 10% normal rabbit serum (Sigma, UK) prepared in PBS with 0.3% Triton-X for 2 hours at room temperature. Sections were then incubated for 48h at 4°C with goat polyclonal DCX primary antibody (Santa Cruz) solution (1:100 dilution in 5% rabbit serum and 0.3% PBS-T), rinsed in PBS, and incubated for two hours at room temperature in biotinylated rabbit anti-goat antibody (Vector laboratories, 1:200 dilution) diluted in PBS, 0.3% Triton-X and 1.5% normal rabbit serum. Immunopositive cells were detected using the Vectastain ABC Elite kit, followed by incubation with 3,3'-diaminobenzidine activated with 0.3% hydrogen peroxide. Sections were rinsed, dehydrated, cleared in histolene and cover-slipped using DPX mounting medium.

2.6 Imaging, cell quantification and cell morphology analysis

2.6.1 Imaging and Counting Frames

The hippocampus was visualized at 10x (DCX+ counts), 20x (dendritic crossings and Iba1$^+$ counts), and 40x (Iba1$^+$ area) magnification using an Olympus BX53 Upright Microscope (Bioscience Imaging Centre, Department of Anatomy and Neuroscience, UCC). The Olympus CellSens software was used to change the focus in order to differentiate between overlapping cell bodies and dendritic crossings. The dHi was defined as AP -1.8 to -5.2mm and the vHi as AP -5.2 to -6.7mm as previously described[59,60]. As shown in supplemental figure 1A, frames of a height of 50μm were overlaid on the GCL to capture neurons in the subgranular zone and the GCL (proximal frame). Then, frames of a height of 50μm were placed directly on top of these GCL frames to capture cells/dendrites in the molecular layer (ML) (distal frame). Together, the total height of a proximal plus a distal frame is 100μm. The length of each frame is 300μm. Cell quantification in the dentate gyrus using counting frames (supplemental figure 1A) was adapted from Nishijima et al.[61] others[62,63]. A cumulative mean analysis was first conducted to determine the optimal number of counting frames to be used on the upper and lower blades of the DG. This resulted in two distal and two proximal frames on the upper blade, and one distal and one proximal frame on the lower blade of each hemisphere (Supp. Fig. 1b). Every 6th section of both the dorsal and ventral hippocampus was immunostained. Cells were analysed in 3 proximal and 3 distal frames in each hemisphere. For DCX analysis, the number of sections analysed per experimental group was 4 - *9* (~39 frames per animal) for the dHi and 4 - 7 sections (~33 frames per animal) for the vHi. For Iba1 analysis, the number of sections analysed per experimental group was 5 - 9 (~42 frames per animal) for the dHi and 3 - 5 sections (24~ frames per animal) for the vHi. For each animal, the average number of Iba1$^+$ or DCX$^+$ cells per section, the number of proximal dendritic crossings in the GCL and the number of distal dendritic crossings in the ML was determined.

2.6.2 Iba1 cell analysis

Microglial activation was determined by counting the number of Iba1$^+$ cells and measuring the cell soma size, based on previously published approaches[55–58]. The cell soma size of six randomly chosen Iba1$^+$ cells in both hemispheres of one dHi section and six randomly chosen Iba1$^+$ cells in both hemispheres of one vHi section per animal were analysed using ImageJ and expressed as μm^2.

2.6.3 DCX cell analysis

The number of DCX-positive cells and their dendritic development were measured in the DG of the hippocampus (adapted from[61]). The number of cell bodies present in the proximal frames (which were positioned in the GCL) was counted (Supp. Fig. 1d). The number of dendrites that crossed from the proximal frame into the distal frame (line 1; proximal dendritic crossings) and the number that emerged out of the distal frame were counted (line 2; distal dendritic crossing) (Supp. Fig. 1d).

2.7 Blood measurements

2.7.1 Corticosterone

Plasma corticosterone levels were measured using a corticosterone immunoassay kit according to the manufacturers' instructions (Enzo Life Sciences, UK) at T0 and T2.

2.7.2 Cytokines

The concentration of cytokines (interleukin-1β (IL-1β), IL-4, IL-6, IL-10, tumour necrosis factor-α (TNF-α) and interferon-γ (IFNγ)) was measured in plasma collected 2 hours (T2) after

Journal Pre-proofs

LPS/saline injection using an electrochemoluminescence (ECL)-based assay (V-plex, MesoScale Discovery, USA) following the manufacturer's instructions.

2.8 Gene expression analysis

Total RNA was extracted from dHi and the vHi using GenElute kit and treated using a Turbo DNA-free kit (Ambion/life technologies, UK) as per the manufacturer's instructions. Total RNA yield and purity were determined using the Nanodrop System (Thermo Scientific, UK). Synthesis of cDNA was performed using the high capacity cDNA reverse transcription kit (Applied Biosystems, UK) using the SureCycler® 8800 (Agilent Technologies, UK) and diluted to a final concentration of 10ng/µl. All qPCR measurements were performed in 2 technical replicates for each biological sample on a LightCycler® 480 Instrument II (Roche). Each reaction consisted of 1µl of sample (5ng/µl), 5µl of Sybr MasterMix (KiCqStart® SYBR® Green qPCR ReadyMix™ with ROX™ for ABI instruments, Sigma-Aldrich, UK), 0.1µl of both forward (Fw) and reverse (Rv) primers for IL-1β (Fw-AAAGAAGAAGATGGAAAAGCGGTT; Rv-GGGAACTGTGCAGACTCAAACTC), IL-6 (Fw-TCCTACCCCAACTTCCAATGCTC; Rv-TTGGATGGTCTTGGTCCTTAGCC) and TNF-α (Fw-AGGCACTCCCCCAAAAGATG; Rv-TTGCTACGACGTGGGCTAC) and 3.8µl of RNase free H_2O. Relative gene expression was adjusted to β-Actin (Fw-GCGAGTACAACCTTCTTGCAGCTC; Rv–TGGCATGAGGGAGCGCGTAA) and quantified using the 2−ΔΔCT method.

2.9 Statistical analysis

All data were analyzed using SPSS statistical software (SPSS, Chicago, IL). All datasets were assessed for outliers and normal distribution using Shapiro-Wilk test. No outliers were removed from the datasets, and they were normally distributed. Thus, the assumption of

homogeneity of variance was not violated and all the datasets (corticosterone and cytokines levels, microglia phenotype and new neurons number/morphology) were each analyzed using two-factorial analysis of variance (ANOVA), with early-life stress (non separated/maternal separation) and inflammatory challenge (Saline/LPS) as independent factors. Effect sizes (eta squared (η^2)) are reported. *Post hoc* analyses used Fisher's least significant difference. *A priori*, we expected either maternal separation alone and/or LPS alone to affect corticosterone[64,65] , cytokine[66,67] levels or morphology/density of Iba1[+68,69] or DCX cells [18,70]. Any *a priori* comparisons were subjected to a Bonferroni correction. A p-value of less than 0.05 was considered significant. All data are expressed as the mean + SEM.

3. Results

3.1 Maternal separation stress modestly altered juvenile LPS-induced corticosterone and inflammatory response in plasma of juvenile females.

To investigate the effects of prior maternal separation stress on a juvenile response to physiological stress, rats were subjected to an inflammatory challenge with LPS injection at PND21 and plasma was collected 2 hours later. Since an inflammatory challenge can drive HPA-axis activation, we also measured plasma corticosterone levels after the LPS challenge. At baseline (T0) (Fig. 1b) and two hours (T2h) after LPS/saline injection (Fig. 1c), plasma corticosterone levels did not differ between NS and MS groups (Fig. 1b). However, two-way ANOVA revealed a significant effect of LPS on plasma corticosterone concentrations at T2h ($F_{(1, 29)} = 30.61$, $p<0.0001$, $\eta^2=0.4684$), while no effect of MS ($F_{(1, 29)} = 4.050$, $p=0.0535$, $\eta^2=0.0619$) was observed. Although ANOVA did not reveal a significant MS X LPS interaction effect ($F_{(1, 29)} = 0.8132$, $p=0.3746$, $\eta^2=0.0125$) we investigated the effect of LPS in both NS and MS groups by

performing planned comparison. Planned comparison revealed that LPS increased corticosterone concentrations in both NS rats (p=0.0092) and MS rats (p=0.0004) (Fig. 1c).

We assessed circulating (plasma) cytokine levels two hours after systemic administration of LPS to validate that the LPS exposure induced an inflammatory response. Indeed, reported increases in pro-inflammatory cytokines IL-1β, TNF-α and IL-6 in the plasma in response to LPS is well-documented in this timeframe and is standard practice to validate LPS as an immune system stimulator[71]. In addition, a systemic inflammatory response determined at this time point allowed us to investigate if the prior exposure to maternal separation primed the body for an exaggerated physiological stress response to a subsequent stressor (LPS)(Figs. 1d-f). LPS increased plasma levels of IL-1β ($F_{(1, 24)}$ = 11.56, p=0.0024, η^2=0.313) but there was no significant effect of MS ($F_{(1, 24)}$ = 0.8262, p=0.3724, η^2=0.0225). ANOVA did not reveal a significant MS x LPS interaction ($F_{(1, 24)}$ = 0.7541, p=0.3938, η^2=0.0203). We investigated the effect of LPS alone on IL-1β with planned comparisons. LPS increased plasma IL-1β in MS animals (p=0.0203) but not in NS animals (p=0.344) (Fig. 1f). LPS also increased plasma levels of IL-6 ($F_{(1, 24)}$ = 18.38, p=0.0003, η^2=0.4335), while MS ($F_{(1, 24)}$ = 0.00947, p=0.9233, η^2=0.0002) and the interaction between MS X LPS ($F_{(1, 24)}$ = 0.0134, p=0.9086, η^2=0.0003) had no effect (Fig. 1e). Similarly, as for IL-1β, planned comparisons showed that LPS alone increased plasma levels of IL-6 to the same extent in both NS (p=0.028) and MS (p=0.0188) animals. Finally, TNF-α levels were increased by LPS ($F_{(1, 24)}$ = 15.03, p=0.0007, η^2=0.3402), but there was no effect of MS ($F_{(1, 24)}$ = 2.628, p=0.1180, η^2=0.0595). ANOVA analysis showed no interaction effect ($F_{(1, 24)}$ = 2.519, p=0.1255, η^2=0.05703). We investigated the effect of LPS with planned comparisons and found that LPS induced an increase in systemic TNF-α in NS animals (p=0.0028) but not in MS rats (p=0.474) (Fig. 1d). The

plasma concentrations of the pro-inflammatory cytokine IFNγ and anti-inflammatory cytokines IL-4 and IL-10 were below the detection threshold in all groups of animals when measured at these time points (data not shown).

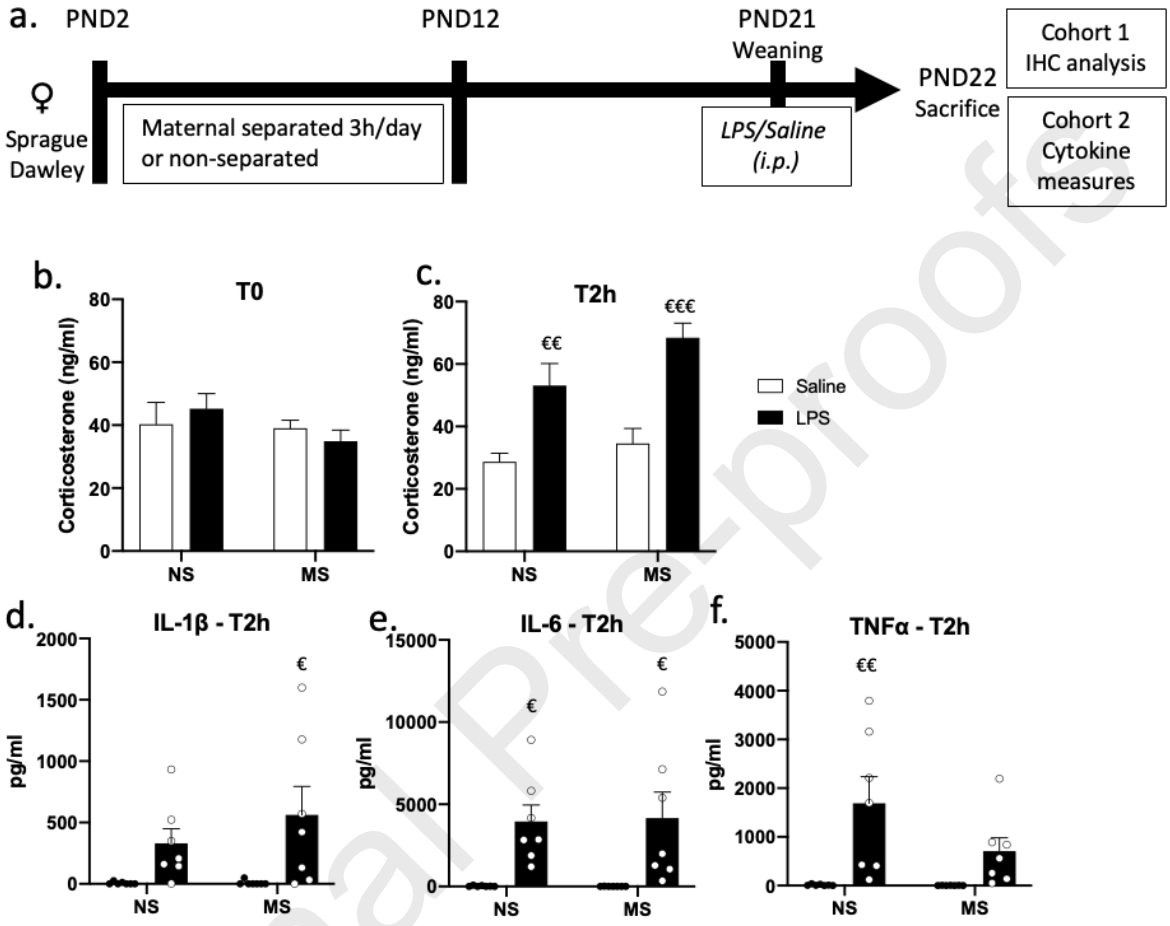


Figure 1. a. Experimental design; Plasma corticosterone (ng/ml) at **b.** baseline (T0) and **c.** 2h after LPS challenge; Plasma concentrations of **d.** IL-1β; **e.** IL-6 and **f.** TNF-α, (pg/ml) at 2h after LPS; Data are expressed as mean + sem. n=7-9. €p<0.05, €€p<0.01, €€€p<0.001 compared to corresponding saline groups following planned comparison focusing on the effect of LPS.

3.2 Maternal separation with juvenile LPS differentially affected cytokine expression in the dorsal and ventral hippocampus.

Because peripheral inflammation induced by an LPS challenge can induce a neuroinflammatory response[67], we measured these same cytokines in the hippocampus to

determine if the trend in the periphery was mirrored in the brain. Thus, expression levels of IL-1β, IL-6 and TNF-α were assessed in the dorsal and ventral hippocampus at 24 hours to align with the time at which microglial activation was assessed (section 3.3). Relative mRNA expression was assessed to determine the coincidence of gene transcripts of pro-inflammatory cytokines with activated microglia, which produce pro-inflammatory cytokines. There was a significant effect of LPS on IL-1β gene expression in both the dorsal and ventral hippocampus (dHi: ($F_{(1,\ 24)}$ = 18.71,p=0.0002, η^2=0.436); vHi ($F_{(1,\ 24)}$ = 20.42,p=0.0001, η^2=0.419)) while no effect of MS (dHi: ($F_{(1,\ 24)}$ = 0.1964,p=0.6616, η^2=0.00457); vHi ($F_{(1,\ 24)}$ = 2.181,p=0. 1527, η^2=0.0447)) was reported. Two-way ANOVA analysis showed no interaction between LPS and MS (dHi: ($F_{(1,\ 24)}$ = 0.006877,p=0. 9346, η^2=0.0001); vHi: ($F_{(1,\ 24)}$ = 2.143,p=0.1562, η^2=0.0439)). Planned comparisons to investigate the effect of LPS in both NS and MS conditions revealed that in MS rats, LPS increased IL-1β expression in both the dorsal (p=0.0248) and ventral (p=0.0012) hippocampus while in NS rats, LPS increased IL-1β expression in the dorsal (p=0.0188) but not the ventral (p=0.1636) hippocampus suggesting that prior exposure to MS exacerbated the LPS-induced increase in IL-1β in the vHi (Fig. 2a). Two-way ANOVA showed that there was a significant effect of LPS on IL-6 expression in the dHi ($F_{(1,\ 24)}$ = 4.489, p=0.0447, η^2=0.1501) but not in the vHi ($F_{(1,\ 24)}$ = 0.02508,p=0.8755, η^2=0.000759) and on MS in the ventral ($F_{(1,\ 24)}$ = 8.869,p=0.0065, η^2=0.2685) but not in the dorsal ($F_{(1,\ 24)}$ = 1.122,p=0.300, η^2=0.0375) hippocampus (Fig. 2b). The interaction between MS and LPS was not significant in either region ((dHi: ($F_{(1,\ 24)}$ = 0.2996, p=0.5892, η^2=0.01002); vHi: ($F_{(1,\ 24)}$ = 0.1362,p=0.7153, η^2=0.00413)). There was a significant effect of LPS on TNF-α expression in the dorsal ($F_{(1,\ 24)}$ = 7.116, p=0.0135, η^2=0.1884) but not the ventral ($F_{(1,\ 24)}$ = 1.543,p=0.2262, η^2=0.0446) hippocampus, and of MS on TNF-α expression in both regions (dHi: ($F_{(1,\ 24)}$ = 6.648, p=0.0165, η^2=0.1760); vHi: ($F_{(1,\ 24)}$ = 9.042,p=0.0061, η^2=0.2614)).

However, ANOVA showed that the interaction between LPS and MS was not significant in either region (dHi: ($F_{(1,\ 24)}$ = 0.0009506, p=0.9757, η^2=0.00025); vHi: ($F_{(1,\ 24)}$ = 0.001218,p=0.9725, η^2=0.000035)) (Fig 2c). Because the plasma concentrations of the pro-inflammatory cytokine IFN-γ and anti-inflammatory cytokines IL-4 and IL-10 were below the detection threshold they were not analysed in hippocampal tissue.

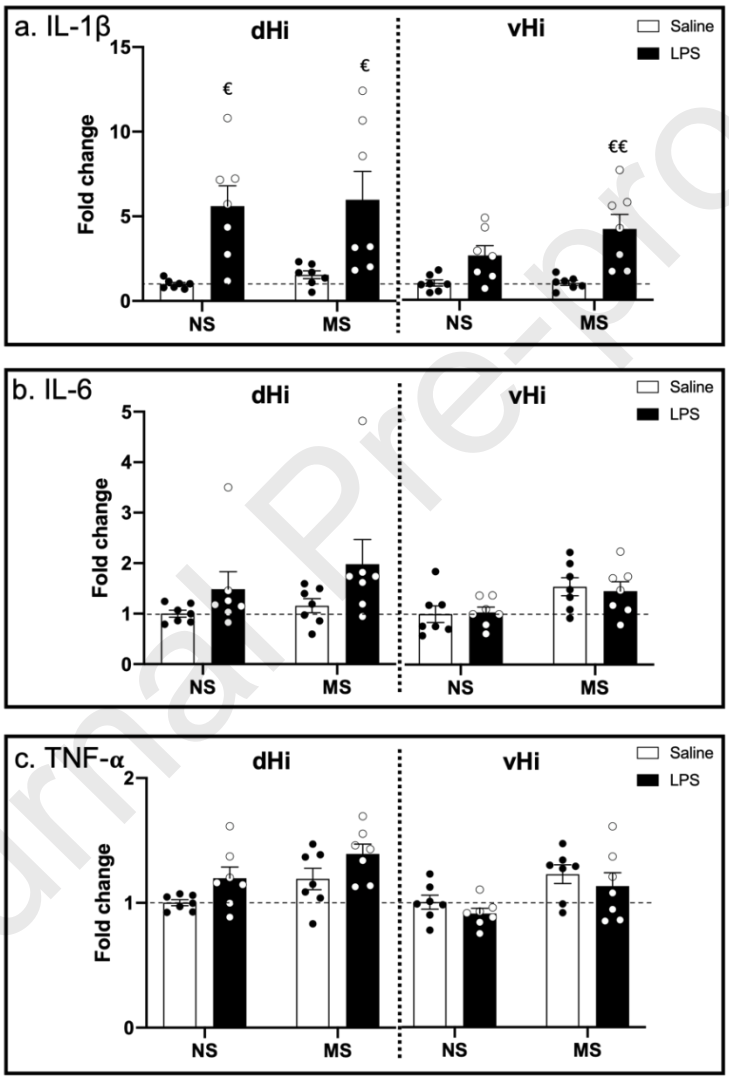


Figure 2. Relative mRNA expression of **a.** IL-1β; **b.** IL-6 and **c.** TNFα in the dorsal hippocampus (dHi) and in the ventral hippocampus (vHi). Data are expressed as mean + sem. n=7 €p<0.05, €€p<0.01 compared to corresponding saline groups following planned comparison focusing on the effect of LPS.

3.3 Juvenile LPS or MS alone increased microglial soma size without altering microglial density, while MS attenuated LPS-induced increase in microglia soma size.

To further assess the impact of juvenile LPS and MS on neuroinflammation, we measured the cell soma size of microglia in the DG of the hippocampus as increased soma size has been associated with an activated status (Fig. 3a). LPS increased microglia soma size in non-separated animals (p=0.0001) but not in MS animals (p=0.116). MS increased the microglia soma size in saline treated animals compared to saline treated NS rats (p=0.0352). The effects of LPS on soma size were attenuated in MS animals compared with NS animals (p=0.0223), with a significant MS X LPS interaction ($F_{(1, 12)}$ = 12.48, P=0.0041, η^2=0.2092). Two-way ANOVA showed that there was a significant effect of LPS ($F_{(1, 12)}$ = 35.15, p<0.0001, η^2=0.5892) but no main effect of MS alone ($F_{(1, 12)}$ = 0.03092, p=0.8633, η^2=0.00052) on the cell soma size of microglia across the whole hippocampus.

This same pattern of effects of LPS, MS and LPS x MS on microglial cell soma size in the whole hippocampus were also observed in both the dorsal and ventral hippocampus thus there were no subregional differences. LPS alone increased microglia soma size in non-separated animals only (dHi: p=0.0002, vHi: p=0.0001) but not in MS animals. MS increased the microglia soma size in saline treated animals compared to saline treated NS rats (dHi: p=0.0216; vHi: p=0.0142). The effects of LPS on soma size were attenuated in MS animals compared with NS animals (dHi p=0.0048; vHi p=0.0296), with a significant interaction MS x LPS (dHi: $F_{(1, 12)}$ = 18.58, P=0.0010, η^2=0.3191; vHi: $F_{(1, 12)}$ = 14.23, P=0.0027, η^2=0.2592). Two-way ANOVA analysis revealed a significant effect of LPS (dHi: $F_{(1, 12)}$ = 27.32, p=0.0002, η^2=0.4691; vHi: $F_{(1, 12)}$ = 28.59, p=0.0002, η^2=0.5208) but no main effect of MS alone (dHi: $F_{(1,}$

$_{12)}$ = 0.3322, p=0.5750, η^2=0.05706; vHi: $F_{(1, 12)}$ = 0.079, p=0.7834, η^2=0.01439) on microglia soma size.

We also investigated the effects of MS and juvenile LPS on the number of microglia in the hippocampus of juvenile female rats (Fig. 3b). Two-way ANOVA analysis revealed that there was a significant effect of MS on the number of microglia in the DG of the whole hippocampus ($F_{(1, 12)}$ = 6.647, p=0.0242, η^2=0.2827) but no effect of LPS ($F_{(1, 12)}$ = 0.3856, p=0.5462, η^2=0.01641). Despite a large size effect, ANOVA did not reveal a significant MS x LPS interaction effect ($F_{(1, 12)}$ = 4.477, p=0.0569, η^2=0.1904). We performed planned comparisons to investigate the effect of MS alone on the number of microglia and we found that MS decreased the number of microglia only in LPS treated animals (p=0.0244).

Upon segregation into dorsal and ventral regions we found that the effects observed in the whole hippocampus were due to changes in the dHi and not the vHi. In the dHi, MS ($F_{(1, 12)}$ = 9.011, p=0.0110, η^2=0.3819) but not LPS ($F_{(1, 12)}$ = 1.107, p=0.3134, η^2=0.04693) significantly decreased the number of microglia. There was no significant interaction between MS and LPS ($F_{(1, 12)}$ = 1.478, p=0.2475, η^2=0.06264). Planned comparisons revealed MS decreased the number microglia in LPS treated animals compared to the corresponding group of NS animals (p=0.0456).

There were no significant effects in the vHi [MS ($F_{(1, 12)}$ = 0.2955, p=0.5966, η^2=0.01572); LPS ($F_{(1, 12)}$ = 0.02195, p=0.8847, η^2=0.001168); MS X LPS ($F_{(1, 12)}$ = 4.478, p=0.0874, η^2=0.1006)].

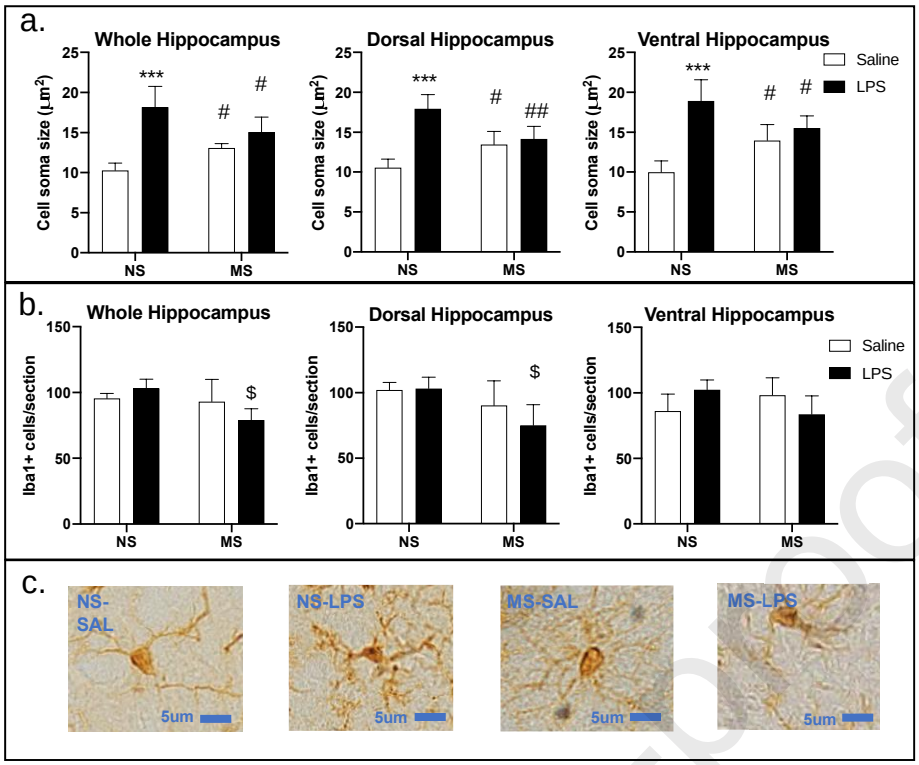


Figure 3. a. Microglia soma size (μm^2) and **b.** Number of Iba1$^+$ cells per section in the dentate gyrus of the whole hippocampus and dorsal and ventral regions **c.** Representative images of microglia used for the cell soma size analysis from the hippocampus of non-separated control/saline-treated, non-separated/LPS-treated, maternally-separated/saline-treated and maternally-separated/LPS-treated female rats. Data are expressed as mean + sem. n=4 ***p<0.001 compared to corresponding saline groups and #p<0.05 compared to corresponding non-separated groups; $p<0.05 compared to corresponding non-separated group after planned comparison.

3.4. MS prevented LPS-induced reductions in proximal (but not distal) dendritic complexity of newly born hippocampal neurons in the vHi but not dHi.

We next investigated the effects of maternal separation and LPS on neurogenesis and dendritic development of newly born neurons in the dorsal and ventral hippocampus. To assess the effects of MS and LPS on neurogenesis in juvenile female rats we counted the number of positively stained DCX cells. Two-way ANOVA revealed that there was no effect of LPS ($F_{(1, 12)} = 1.101$, p=0.3148, η^2=0.07673), MS ($F_{(1, 12)} = 1.201$, p=0.2947, η^2=0.0837), nor a MS x LPS interaction ($F_{(1, 12)} = 0.04403$, p=0.8373, η^2=0.003069) on the number of DCX-positive

cells across the whole hippocampus, the dHi (LPS ($F_{(1, 12)}$ = 1.856, p=0.1981, η^2=0.1247), MS ($F_{(1, 12)}$ = 1.024, p=0.3316, η^2=0.06878), LPS x MS ($F_{(1, 12)}$ = 0.01020, p=0.9212, η^2=0.00024)) or vHi (LPS ($F_{(1, 12)}$ = 0.05781, p=0.8141, η^2=0.03558), MS ($F_{(1, 12)}$ = 3.416, p=0.0893, η^2=0.2103), MS x LPS interaction ($F_{(1, 12)}$ = 0.7741, p=0.3962, η^2=0.4764)) (Fig. 4a).

To assess the effects of MS and LPS on dendritic development of newly born neurons, the number of dendrites of DCX-positive cells that crossed 50µm into the GCL (proximal dendritic crossing) were counted (Fig. 4b). Two-way ANOVA revealed that there was no effect of LPS ($F_{(1, 12)}$ = 0.00717, p=0.9339, η^2=0.00019) or MS ($F_{(1, 12)}$ = 1.203, p=0.2943, η^2=0.0319) but a significant MS x LPS interaction ($F_{(1, 12)}$= 24.39, p=0.0003, η^2=0.64871) on the number of proximal dendritic crossings in the whole hippocampus. Segregation of the hippocampus into dorsal and ventral regions revealed the same pattern of effects. In both the dHi and the vHi there was no effect of LPS (dHi: $F_{(1, 12)}$ = 0.03413, p=0.8565, η^2=0.00166; vHi ($F_{(1, 12)}$ = 0.06198, p=0.8076, η^2=0.001504) or MS (dHi $F_{(1, 12)}$ = 0.04995, p=0.8269, η^2=0.00243; vHi:$F_{(1, 12)}$ = 1.048, p=0.3261, η^2=0.02544). The interaction MS x LPS was significant in both dHi and vHi (dHi: $F_{(1, 12)}$= 8.449, p=0.0132, η^2=0.4115; vHi ($F_{(1, 12)}$= 28.10, p=0.0002, η^2=0.6818) on the number of proximal dendritic crossings.

MS alone and LPS alone significantly decreased proximal dendritic crossings in comparison to their respective controls (p=0.0011 and p=0.005, respectively). MS combined with LPS reversed the MS-induced and LPS-induced decreases in proximal dendritic crossings (p=0.0187). The effect was specific to the ventral region as MS alone and LPS alone significantly decreased proximal dendritic crossings in the dHi (p=0.0494 and p=0.0470, and vHi (p=0.0046 and p=0.0230, respectively) but MS combined with LPS reversed the MS-

induced and LPS-induced decreases in proximal dendritic crossings in the vHi (p=0.0106) but not in the dHi (p=0.0821) when compared to the MS-SAL group.

Finally, the number of dendrites which crossed 50 µm into the ML (distal dendritic crossings) were counted (Fig. 4c). In the whole hippocampus, two-way ANOVA showed that there was a significant effect of LPS ($F_{(1, 12)}$= 56.23, p<0.0001, η^2=0.6767) and MS ($F_{(1, 12)}$= 10.28, p=0.0076, η^2=0.1237) on the number of distal dendritic crossings of new born neurons. ANOVA analysis showed that MS X LPS interaction was not significant ($F_{(1, 12)}$= 4.583, p=0.0535, η^2=0.05516). Planned comparisons showed that both LPS alone (p=0.0001 in NS group and p=0.0104 in MS group) and MS alone (p=0.0104) decreased the number of distal dendritic crossings in the whole hippocampus. In the dHi, MS alone and LPS alone (all p<0.0001) decreased the number of distal dendritic crossings with a statistically significant effect of LPS alone ($F_{(1, 12)}$= 52.80, P<0.0001, η^2=0.4667), MS alone ($F_{(1, 12)}$= 21.32, p=0.0006, η^2=0.1884) and a MS x LPS interaction ($F_{(1, 12)}$= 27.02, p=0.0002, η^2=0.2388). In the vHi, both LPS alone and MS alone (all p=0.0001) decreased the number of distal dendritic crossings with a statistically significant effect of LPS ($F_{(1, 12)}$= 39.45, p<0.0001, η^2=0.3777), MS ($F_{(1, 12)}$= 42.80, p<0.0001, η^2=0.4098) and MS x LPS interaction ($F_{(1, 12)}$= 10.20, p=0.0077, η^2=0.0976).

Taken together, MS alone and LPS alone decreased distal dendritic crossings in both the dHi and vHi and in the whole, dorsal and ventral hippocampus, while MS only exacerbated LPS-induced reductions in distal dendritic crossings in the whole hippocampus, an effect that was not specific to either the dorsal or ventral regions.

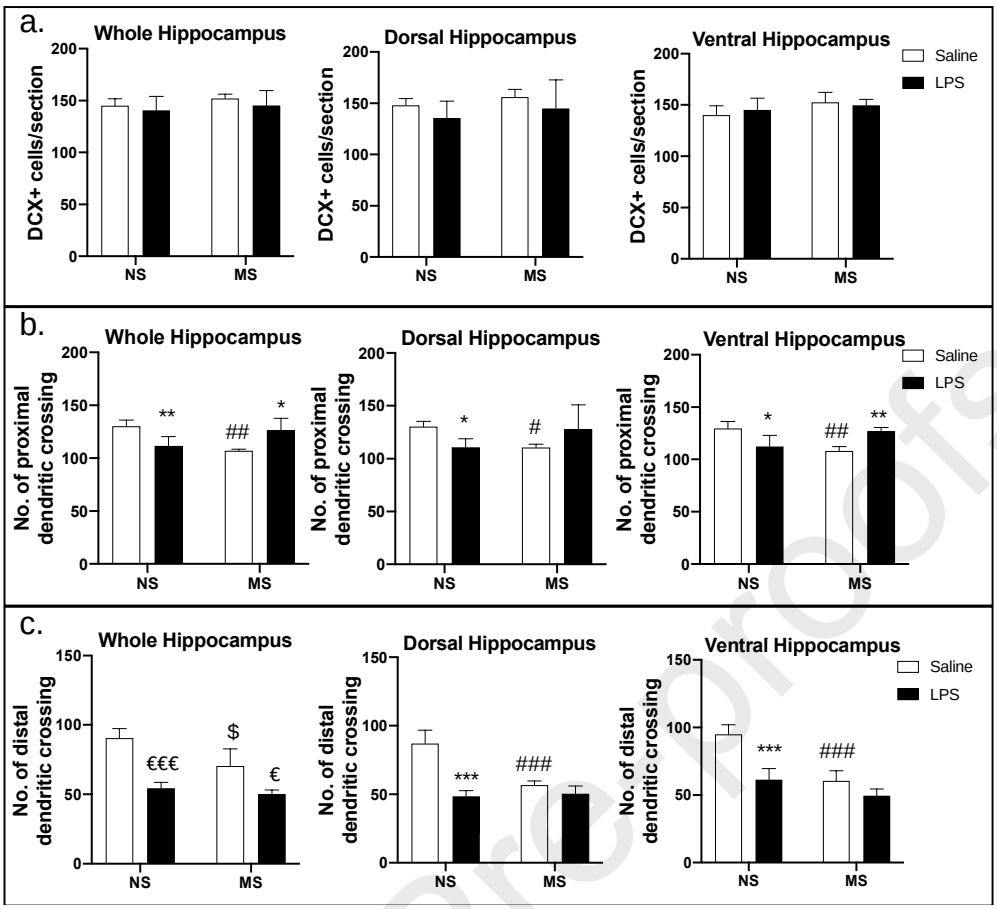


Figure 4. a. Number of DCX-positive cells per section in the dentate gyrus in the whole, ventral and dorsal hippocampus; **b.** Number of proximal dendritic crossings in the GCL and **c.** Number of distal dendritic crossings in the ML in the whole hippocampus and dorsal and ventral regions. Data are expressed as mean + sem. n=4 *p<0.05, **p<0.01, ***p<0.001 compared to corresponding saline groups and #p<0.05, ##p<0.01, ###p<0.001 compared to corresponding non-separated groups. €p<0.05, €€€p<0.001 compared to corresponding saline group after planned comparison; $p<0.05 compared to corresponding non-separated group after planned comparison.

4. Discussion

Understanding the influence of stressors at different times during early life in females is critical to identifying risk factors for the development of stress-related psychiatric disorders[72], such as depression which are twice as prevalent in females than males[22]. We investigated whether the early life stressor, maternal separation (MS) would prime peripheral and brain responses to an acute inflammatory stressor, LPS, in juvenile female rats. We found that MS potentiated the HPA-axis response to LPS and altered the peripheral immune

response to LPS. In the dHi, we found that LPS increased IL-1β in both NS and MS groups while in the vHi, only LPS increased IL-1β expression in the MS group. We also found that LPS alone or MS alone induced microglial activation (as indicated by increased soma size) in the whole hippocampus, but that MS attenuated this effect of LPS. Finally, we found that LPS and MS independently reduced the dendritic complexity of newly born hippocampal neurons, and that MS prevented LPS-induced reductions in proximal dendritic complexity. These effects were not accompanied by alterations in the production of new neurons. Taken together, our results suggest that MS differentially primes the physiological response (HPA and immune) to a juvenile inflammatory stressor in female rats.

In the present study, LPS increased plasma corticosterone concentrations in juvenile female rats. This finding is in agreement with a previous study that reported that LPS increased plasma corticosterone levels 2 hours post-LPS injection in male rats (PND6, PND12 and PND18)[65]. We also found that MS potentiated the LPS-induced increases in corticosterone, which is in agreement with studies done at early life stages in male rodents. Indeed, it was reported that MS increased the corticosterone response to LPS in PND9 male mice[73] and in PND14 male rats[39]. As expected, we found that LPS increased plasma levels of the pro-inflammatory cytokines TNF-α and IL-6 in NS rats at two hours post-injection, which is in line with what has previously been reported in male rodents[74]. While MS had no impact on LPS-induced secretion of IL-6, plasma levels of IL-1β were increased in LPS-injected rats subjected to MS, while plasma level of TNF-α were reduced, suggesting that the systemic inflammatory response may be affected by this early life stressor. In agreement, Saavedra and colleagues reported that maternally separated PND14 male rats had lower plasma levels of TNF-α 1h30 post-LPS injection compared to the non-separated controls[39]. In contrast to our study,

Saavedra and colleagues also reported that maternally separated PND14 male rats had lower plasma levels of IL-6 and IL-1β. However, the biological age and sex were different in the current study suggesting the importance of investigating LPS response kinetics in the periphery of both juvenile female and male rats. In addition, it is important to note that the reduction of TNF-α was concomitant with the increase of the corticosterone levels at 2h. This observation is not surprising as it has already been described that TNF-α levels are repressed by glucocorticoids[76].

We found that LPS and/or MS impacted pro-inflammatory cytokine expression along the longitudinal axis of the hippocampus. Specifically, LPS increased IL-1β in the dHi in both NS and MS rats, and in the vHi LPS increased IL-1β only in MS rats compared to NS rats. Similarly with IL-6, we found a main effect of LPS only in the dHi but not the vHi. Our findings in females are similar to a previous report showing that pro-inflammatory cytokine expression of IL-1β is greater in the dorsal than in the vHi in adult male rats following a peripheral LPS injection[77]. However, the data presented here are the first showing a difference between the dHi and vHi response to both LPS and MS stressors during the juvenile period of life. With regard to MS, we found a main effect of MS on IL-6 expression in the vHi but not the dHi. Regarding TNFα expression, we found a main effect of MS in both the vHi and dHi. Previous studies have shown that MS increased TNF-α but not IL-6 in the hippocampus of PND14 male rats[37] and IL-6 in the hippocampus of adult male rats[38]. These observations in males and the current observations in females suggest that the neuroinflammatory response in the hippocampus following MS is sex-dependent, yet further research is needed to determine the changes along the longitudinal axis of the hippocampus in males. In line with this idea, Saavedra and colleagues showed that MS did not affect LPS-induced IL-1β expression in the

hippocampus of PND14 male rats 90min post injection[39] while we showed that MS exacerbated an LPS-induced increase in IL-1β, in the vHi in females.

Changes in the concentration of some but not all cytokines in the periphery were reflected in the hippocampus in response to the stressors applied in this study. It should be noted that the responsive and transient nature of pro-inflammatory cytokine expression in both the periphery and hippocampus is due to the concentration of the inflammatory stimulus (or the degree of stress)[78], the timing of the inflammatory/stress insult[79,80], and the age[81,82] at which exposure occurs. The complexity of the inflammatory response is further highlighted in the current study due to the priming effect of MS in early life, followed by the inflammatory insult LPS during the prepubertal period, on hippocampal neuron integrity. For example, we found that MS followed by LPS increased peripheral IL-1β two hours after LPS, and increased IL-1β expression in the vHi 24 hours after LPS, while other cytokines (IL-6 and TNF-α) were less sensitive to these stimuli. These data highlight the need for further research to tease out the relationship between the peripheral and central expression of inflammatory factors such as IL-1β in response to stressors especially as IL-1β has been associated with the onset of neuropsychiatric disorders such as depression[83,84] and Alzheimer's disease[85].

We found that juvenile LPS alone or MS alone increased microglial soma size without altering microglial density, while MS attenuated the LPS-induced increase in microglia soma size in both the dorsal and ventral hippocampus. In agreement with our findings, it has been reported that LPS increased the microglia cell soma size in both male and female adult mice[69], while in contrast with our findings, others have reported that LPS alone increased the number of microglia in the hippocampus of male mice[80] and male rats[86]. One contributing factor to this difference could be that microglial phenotypical heterogeneity across brain regions[80,87]

occurs in a sex-dependent manner. We found that MS alone did not affect the number of microglia in the hippocampus of juvenile female rats. In contrast, previous studies in male mice found that MS (PND1-14) increased the number of microglia in the DG at PND14 but not 2 weeks after at PND28[68]. In male rats, the effect of MS (PND1-10) on microglia number was detected between PND10-30 but not from PND40 or PND60[88], while at PND100 the number of microglia was still increased but only in the CA3 region of the hippocampus[38]. The differences between our results and those that are already published suggest that the effect of MS on microglia number varies depending upon the duration of the MS paradigm, the subregion of the hippocampus analysed, and the sex and age of the rodent. We found that MS increased microglia cell soma size which is in agreement with others that have reported that MS induces microglial activation in the hippocampus of male rats[37] and mice[68]. We also found that LPS administration to MS rats reduced the number of microglia in the whole hippocampus and this reduction was specific to the dorsal region. In agreement with our findings, previous studies in juvenile male rats found that MS (PND1-14) immediately followed by LPS, reduced the number of microglia in the hilus and CA3 regions of the hippocampus[39]. Intriguingly, in addition to a decrease in the number of microglia, we observed that MS attenuated LPS-induced increased in microglial soma size. This finding is surprising given our observation that MS alone increased microglial soma size and that it has previously been reported that MS alone increased microglial soma size in PND15 male rats[37], with a greater MS-induced increase in cell soma size in adult female than in male mice[66]. It is plausible that there may also be biological sex differences in microglial activation in response to LPS and MS during early life. Indeed, there is evidence that inflammation during early life stress and during adolescence may induce sex-dependent neuroinflammatory responses[89,90].

Since both stress and inflammation are regulators of the production and development of newly born hippocampal neurons[91,92], we investigated the impact of MS, LPS and their interaction on the production and development of newly born hippocampal neurons. We found that the number of newly born hippocampal neurons was unaffected, but that juvenile LPS and maternal separation independently reduced the dendritic complexity of newly born hippocampal neurons in both the dHi and vHi, and that MS prevented LPS-induced reductions in distal dendritic complexity in the whole and ventral hippocampus in juvenile female rats. It has been widely reported that inflammation decreases hippocampal neurogenesis from early developmental stages to adulthood in males[24,91,93], and previous findings from our group described that chronic hippocampal IL-1β overexpression in adolescence decreased neurogenesis and dendritic complexity of newly born hippocampal neurons in male rats[25]. As oestrogen has neurogenic effects on neurons in the hippocampus[94,95], it could be hypothesized that in the current study, the hippocampal neurogenesis in juvenile female rats may have been protected from the negative impact of peripheral inflammation by an interaction with the female hypothalamic pituitary gonadal axis[96]. In fact, there is evidence of a sexual dimorphism in hippocampal neurogenesis in response to inflammatory insults[93,97]. However, we cannot exclude that in our experimental condition the short time (24 hours) between the LPS challenge and the analysis of DCX cells number may be such that DCX-positive cells could still be viable but apoptotic, as the reduced dendritic complexity might suggest. We also found that MS did not affect the number of newly born hippocampal neurons but decreased their dendritic complexity, while previous work has reported that MS either decreased the number of mature neurons[98] or DCX neurons[18], or increased apoptosis in the dentate gyrus[20] in juvenile male mice or rats. To the best of our knowledge, this study is the first to describe the effect of MS on hippocampal neurogenesis in juvenile female rats. In

agreement with our finding that MS decreased dendritic complexity of newly born hippocampal neurons in juvenile females, another study using the same MS protocol and analytical approach to assess dendritic complexity reported that MS decreased the dendritic complexity, independently of biological sex, in adult mice[99]. Intriguingly, we found that the LPS-induced decrease in the dendritic complexity of new neurons in the proximal region was attenuated by MS in the whole hippocampus, which was reflected in the vHi. However, the LPS-induced reductions in dendritic complexity in the distal region were prevented by MS in the whole hippocampus without specifically affecting the dHi or vHi. The different response between the proximal and distal regions observed in our study is in line with a previous finding showing that MS alone reduced the number of distal crossing much more than the number of proximal crossing in juvenile female rats[99] suggesting a higher sensitivity of the distal region to MS which is potentiated by LPS in our study.

To date there is no study investigating the correlation between changes in dendrite length and behavioural changes in the context of MS in females. One study has shown that MS decreased dendritic complexity of hippocampal new neurons in adult male rats, and this was associated with a decrease in spatial memory[100]. Another study has shown that MS reduced the number of primary dendrites concomitantly with a decrease in spatial learning in adult male rats[101]. The effect of the reduction of dendritic length in response to MS or physiological stress in females is less documented. However, one study reported that chronic juvenile stress decreased the apical dendritic length of pyramidal cells in the hippocampus and this decrease was correlated with anhedonia, anxiety and behavioural despair in female rats[102].

The response to MS or other forms of stress has previously been shown to have a greater negative impact on neurogenesis in the vHi than the dHi[15,77,92], while the impact of inflammatory insults on hippocampal neurogenesis has not been shown to be specific to either the dorsal or ventral hippocampus[24]. LPS has previously been reported to induce higher levels of corticosterone in the vHi than the dHi of adult male rats[77]. We found a modest potentiation of LPS-induced corticosterone by MS, which may have contributed to the effects we observed on proximal dendritic complexity in the vHi. Therefore, it is plausible that the vHi may be more sensitive to the effects of LPS on the dendritic complexity of new neurons via the potential mediating effect of corticosterone[9,15,92]. It should be noted however, that we cannot directly confirm differences in neuroinflammation and hippocampal neurogenesis in response to maternal separation and/or LPS in males and females due to methodological differences in previous studies.

This study is one of the first investigating the neurogenic response of juvenile female rats to an early life physiological stressor followed by an immune challenge in early adolescence. The results suggest an interaction between MS and LPS that induces aberrant microglial activity that could impact the dendritic complexity of new neurons in the juvenile period and potentially also in later in life. Although we did not include subregion as a factor in statistical analysis, the results of the current study still show that MS potentiated LPS-induced increase in IL-1β in the vHi. However, there was no difference in sensitivity between the dHi and vHi to MS or LPS-induced changes in microglial activation and the number of DCX positive cells. On the other hand, it should be noted that MS exacerbated LPS-induced reductions in complexity of distal dendrites of new neurons in whole hippocampus without a distinction between the dHi and vHi. Finally, further research is needed to determine the effects of early life (postnatal and juvenile) stress in females, on behavioural consequences particularly in

adolescence which is when a female bias in prevalence of stress-related psychiatric disorders begins to emerge.

Funding details

This work was funded by Science Foundation Ireland (SFI) under Grant Number SFI/FFP/6820.

Acknowledgments:

We thank Dr. Erin Harris, Dr. Gerard Moloney, Suzanne Crotty and Tara Foley for technical assistance.

Declaration of interest statement:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References list:

1. van den Berg GJ, Lundborg P, Nystedt P, Rooth D-O. Critical Periods During Childhood and Adolescence. J Eur Econ Assoc [Internet]. 2014 Dec 1;12(6):1521–1557. Available from: https://doi.org/10.1111/jeea.12112
2. Selemon LD, Zecevic N. Schizophrenia: a tale of two critical periods for prefrontal cortical development. Transl Psychiatry. 2015 Aug;5(8):e623. PMID: 26285133
3. VanRyzin JW, Pickett LA, McCarthy MM. Microglia: Driving critical periods and sexual differentiation of the brain. Dev Neurobiol. 2018 Jun;78(6):580–592. PMID: 29243403
4. Hoeijmakers L, Ruigrok SR, Amelianchik A, Ivan D, van Dam A-M, Lucassen PJ, Korosi A. Early-life stress lastingly alters the neuroinflammatory response to amyloid pathology in an Alzheimer's disease mouse model. Brain Behav Immun. Netherlands; 2017 Jul;63:160–175. PMID: 28027926
5. Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, Ávila J, Llorens-Martín M. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat Med. 2019; PMID: 30911133

6. Kozareva DA, Cryan JF, Nolan YM. Born this way: Hippocampal neurogenesis across the lifespan. Aging Cell. 2019; PMID: 31298475

7. Terranova JI, Ogawa SK, Kitamura T. Adult hippocampal neurogenesis for systems consolidation of memory. Behavioural Brain Research. 2019. PMID: 31201874

8. Alam MJ, Kitamura T, Saitoh Y, Ohkawa N, Kondo T, Inokuchi K. Adult neurogenesis conserves hippocampal memory capacity. J Neurosci. 2018; PMID: 29986876

9. Anacker C, Hen R. Adult hippocampal neurogenesis and cognitive flexibility-linking memory and mood. Nature Reviews Neuroscience. 2017. PMID: 28469276

10. David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED, Hen R. Neurogenesis-Dependent and -Independent Effects of Fluoxetine in an Animal Model of Anxiety/Depression. Neuron. 2009; PMID: 19477151

11. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science (80-). 2003; PMID: 12907793

12. Fanselow MS, Dong HW. Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? Neuron. 2010. PMID: 20152109

13. Bannerman DM, Matthews P, Deacon RMJ, Rawlins JNP. Medial septal lesions mimic effects of both selective dorsal and ventral hippocampal lesions. Behav Neurosci. 2004; PMID: 15506885

14. O'Leary OF, Cryan JF. A ventral view on antidepressant action: Roles for adult hippocampal neurogenesis along the dorsoventral axis. Trends in Pharmacological Sciences. 2014. PMID: 25455365

15. Levone BR, Codagnone MG, Moloney GM, Nolan YM, Cryan JF, O' Leary OF. Adult-born neurons from the dorsal, intermediate, and ventral regions of the longitudinal axis of the hippocampus exhibit differential sensitivity to glucocorticoids. Mol Psychiatry. England; 2020 Jul; PMID: 32709996

16. Tanti A, Belzung C. Neurogenesis along the septo-temporal axis of the hippocampus: Are depression and the action of antidepressants region-specific? Neuroscience. 2013. PMID: 23973415

17. O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho A-M, Quigley EMM, Cryan JF, Dinan TG. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol Psychiatry. United States; 2009 Feb;65(3):263–267. PMID: 18723164

18. Lajud N, Roque A, Cajero M, Gutiérrez-Ospina G, Torner L. Periodic maternal separation decreases hippocampal neurogenesis without affecting basal corticosterone during the stress hyporesponsive period, but alters HPA axis and coping behavior in adulthood. Psychoneuroendocrinology. 2012; PMID: 21862224

19. Mirescu C, Peters JD, Gould E. Early life experience alters response of adult neurogenesis to stress. Nat Neurosci. 2004; PMID: 15273691

20. Baek S-B, Bahn G, Moon S-J, Lee J, Kim K-H, Ko I-G, Kim S-E, Sung Y-H, Kim B-K, Kim T-S, Kim C-J, Shin M-S. The phosphodiesterase type-5 inhibitor, tadalafil, improves depressive symptoms, ameliorates memory impairment, as well as suppresses apoptosis and enhances cell proliferation in the hippocampus of maternal-separated rat pups. Neurosci Lett [Internet]. 2011 Jan;488(1):26–30. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304394010014370 PMID: 21056623

21. Baek S-S, Jun T-W, Kim K-J, Shin M-S, Kang S-Y, Kim C-J. Effects of postnatal treadmill exercise on apoptotic neuronal cell death and cell proliferation of maternal-separated rat pups. Brain Dev. Netherlands; 2012 Jan;34(1):45–56. PMID: 21353411

22. Bangasser DA, Valentino RJ. Sex differences in stress-related psychiatric disorders: Neurobiological perspectives. Frontiers in Neuroendocrinology. 2014. PMID: 24726661

23. Nicolas S, Léime CSÓ, Hoban AE, Hueston CM, Cryan JF, Nolan YM. Enduring effects of an unhealthy diet during adolescence on systemic but not neurobehavioural measures in adult rats. Nutr Neurosci. Taylor and Francis Ltd.; 2020; PMID: 32723167

24. Green HF, Nolan YM. Inflammation and the developing brain: consequences for hippocampal neurogenesis and behavior. Neurosci Biobehav Rev. United States; 2014 Mar;40:20–34. PMID: 24462889

25. Pawley LC, Hueston CM, O'Leary JD, Kozareva DA, Cryan JF, O'Leary OF, Nolan YM. Chronic intrahippocampal interleukin-1β overexpression in adolescence impairs hippocampal neurogenesis but not neurogenesis-associated cognition. Brain Behav Immun. 2020; PMID: 31604142

26. Iosif RE, Ekdahl CT, Ahlenius H, Pronk CJH, Bonde S, Kokaia Z, Jacobsen S-EW, Lindvall O. Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. J Neurosci. 2006 Sep;26(38):9703–9712. PMID: 16988041

27. Kohman RA, Rhodes JS. Neurogenesis, inflammation and behavior. Brain Behav Immun. 2013 Jan;27(1):22–32. PMID: 22985767

28. Borsini A, Pariante CM, Zunszain PA, Hepgul N, Russell A, Zajkowska Z, Mondelli V, Thuret S. The role of circulatory systemic environment in predicting interferon-alpha–induced depression: The neurogenic process as a potential mechanism. Brain Behav Immun. 2019; PMID: 31207337

29. Keohane A, Ryan S, Maloney E, Sullivan AM, Nolan YM. Tumour necrosis factor-α impairs neuronal differentiation but not proliferation of hippocampal neural precursor cells: Role of Hes1. Mol Cell Neurosci. 2010; PMID: 19840854

30. Green HF, Treacy E, Keohane AK, Sullivan AM, O'Keeffe GW, Nolan YM. A role for interleukin-1β in determining the lineage fate of embryonic rat hippocampal neural precursor cells. Mol Cell Neurosci. 2012; PMID: 22270046

31. Hueston CM, O'Leary JD, Hoban AE, Kozareva DA, Pawley LC, O'Leary OF, Cryan JF, Nolan YM. Chronic interleukin-1β in the dorsal hippocampus impairs behavioural pattern separation. Brain Behav Immun. 2018; PMID: 30217534

32. Valero J, Mastrella G, Neiva I, Sánchez S, Malva JO. Long-term effects of an acute and systemic administration of LPS on adult neurogenesis and spatial memory. Front Neurosci. 2014; PMID: 24795557

33. Fujioka H, Akema T. Lipopolysaccharide acutely inhibits proliferation of neural precursor cells in the dentate gyrus in adult rats. Brain Res. 2010; PMID: 20647006

34. Monje ML, Toda H, Palmer TD. Inflammatory Blockade Restores Adult Hippocampal Neurogenesis. Science (80-). 2003;

35. Weinhard L, Neniskyte U, Vadisiute A, di Bartolomei G, Aygün N, Riviere L, Zonfrillo F, Dymecki S, Gross C. Sexual dimorphism of microglia and synapses during mouse postnatal development. Dev Neurobiol. 2018; PMID: 29239126

36. Premachandran H, Zhao M, Arruda-Carvalho M. Sex Differences in the Development of the Rodent Corticolimbic System. Frontiers in Neuroscience. 2020.

37. Roque A, Ochoa-Zarzosa A, Torner L. 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 [Internet]. 2016 Jul;55:39–48. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0889159115300246 PMID: 26431692

38. Banqueri M, Méndez M, Gómez-Lázaro E, Arias JL. Early life stress by repeated maternal separation induces long-term neuroinflammatory response in glial cells of male rats. Stress [Internet]. 2019 Sep 3;22(5):563–570. Available from: https://www.tandfonline.com/doi/full/10.1080/10253890.2019.1604666 PMID: 31007117

39. Saavedra LM, Navarro BF, Torner L. Early Life Stress Activates Glial Cells in the Hippocampus but Attenuates Cytokine Secretion in Response to an Immune Challenge in Rat Pups. Neuroimmunomodulation. 2018; PMID: 29332092

40. Moloney RD, Sajjad J, Foley T, Felice VD, Dinan TG, Cryan JF, O'Mahony SM. Estrous cycle influences excitatory amino acid transport and visceral pain sensitivity in the rat: effects of early-life stress. Biol Sex Differ. 2016;7:33. PMID: 27429736

41. Pusceddu MM, Kelly P, Ariffin N, Cryan JF, Clarke G, Dinan TG. n-3 PUFAs have beneficial effects on anxiety and cognition in female rats: Effects of early life stress. Psychoneuroendocrinology. England; 2015 Aug;58:79–90. PMID: 25965872

42. McVey Neufeld K-A, O'Mahony SM, Hoban AE, Waworuntu R V, Berg BM, Dinan TG, Cryan JF. Neurobehavioural effects of Lactobacillus rhamnosus GG alone and in combination with prebiotics polydextrose and galactooligosaccharide in male rats exposed to early-life stress. Nutr Neurosci. England; 2019 Jun;22(6):425–434. PMID: 29173065

43. Huttenlocher PR. Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res. Netherlands; 1979 Mar;163(2):195–205. PMID: 427544

44. Micheva KD, Beaulieu C. Quantitative aspects of synaptogenesis in the rat barrel field cortex with special reference to GABA circuitry. J Comp Neurol. United States; 1996 Sep;373(3):340–354. PMID: 8889932

45. Keshavan MS, Diwadkar VA, DeBellis M, Dick E, Kotwal R, Rosenberg DR, Sweeney JA, Minshew N, Pettegrew JW. Development of the corpus callosum in childhood, adolescence and early adulthood. Life Sci. Netherlands; 2002 Mar;70(16):1909–1922. PMID: 12005176

46. Romijn HJ, Hofman MA, Gramsbergen A. At what age is the developing cerebral cortex of the rat comparable to that of the full-term newborn human baby? Early Hum Dev. Ireland; 1991 Jul;26(1):61–67. PMID: 1914989

47. Westwood FR. The female rat reproductive cycle: a practical histological guide to staging. Toxicol Pathol. United States; 2008 Apr;36(3):375–384. PMID: 18441260

48. Pyter LM, El Mouatassim Bih S, Sattar H, Prendergast BJ. Peripheral tumors alter neuroinflammatory responses to lipopolysaccharide in female rats. Brain Res. 2014 Mar;1552:55–63. PMID: 24457042

49. Azizi-Malekabadi H, Hosseini M, Pourganji M, Zabihi H, Saeedjalali M, Anaeigoudari A. Deletion of Ovarian Hormones Induces a Sickness Behavior in Rats Comparable to the Effect of Lipopolysaccharide. Geula C, editor. Neurol Res Int [Internet]. Hindawi Publishing Corporation; 2015;2015:627642. Available from: https://doi.org/10.1155/2015/627642

50. Lee JW, Lee YK, Yuk DY, Choi DY, Ban SB, Oh KW, Hong JT. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. J Neuroinflammation. 2008 Aug;5:37. PMID: 18759972

51. Dornelles GL, de Oliveira JS, de Almeida EJR, Mello CBE, E Rodrigues BR, da Silva CB, Petry LDS, Pillat MM, Palma TV, de Andrade CM. Ellagic Acid Inhibits Neuroinflammation and Cognitive Impairment Induced by Lipopolysaccharides. Neurochem Res. United States; 2020 Oct;45(10):2456–2473. PMID: 32779097

52. Yassa NW, Khalil S, Saleh SR, Ghareeb DA, El Demellawy MA, El-Sayed MM. Ipriflavone and Ipriflavone loaded albumin nanoparticles reverse lipopolysaccharide induced neuroinflammation in rats. PLoS One. 2020;15(8):e0237929. PMID: 32822403

53. Berkiks I, Boulbaroud S, Garcia-Segura LM, Mesfioui A, Ouichou A, Mouden S, Benmhammed H, El hasnaoui A, Nakache R, Bahbiti Y, El hessni A. Thymelaea lythroides extract attenuates microglial activation and depressive-like behavior in LPS-induced inflammation in adult male rats. Biomed Pharmacother. 2018;99(October 2017):655–663. PMID: 29710462

54. Ortega A, Jadeja V, Zhou H. Postnatal development of lipopolysaccharide-induced inflammatory response in the brain. Inflamm Res. 2011;60(2):175–185. PMID: 20865294

55. Bowyer JF, Sarkar S, Burks SM, Hess JN, Tolani S, O'Callaghan JP, Hanig JP. Microglial activation and responses to vasculature that result from an acute LPS exposure. Neurotoxicology. 2020 Mar;77:181–192. PMID: 32014511

56. Hadar R, Dong L, Del-Valle-Anton L, Guneykaya D, Voget M, Edemann-Callesen H, Schweibold R, Djodari-Irani A, Goetz T, Ewing S, Kettenmann H, Wolf SA, Winter C. Deep brain stimulation during early adolescence prevents microglial alterations in a model of maternal immune activation. Brain Behav Immun. Netherlands; 2017 Jul;63:71–80. PMID: 27939248

57. Wadhwa M, Prabhakar A, Ray K, Roy K, Kumari P, Jha PK, Kishore K, Kumar S, Panjwani U. Inhibiting the microglia activation improves the spatial memory and adult neurogenesis in rat hippocampus during 48 h of sleep deprivation. J Neuroinflammation. 2017 Nov;14(1):222. PMID: 29141671

58. Romana Rizzo F, Guadalupi L, Sanna K, Vanni V, Fresegna D, De Vito F, Musella A, Caioli S, Balletta S, Bullitta S, Bruno A, Dolcetti E, Stampanoni Bassi M, Buttari F, Gilio L, Mandolesi G, Centonze D, Gentile A. Exercise protects from hippocampal inflammation and neurodegeneration in experimental autoimmune encephalomyelitis. Brain Behav Immun [Internet]. Elsevier Inc.; 2021;98(August):13–27. Available from: https://doi.org/10.1016/j.bbi.2021.08.212

59. Brummelte S, Galea LAM. Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. Neuroscience. United States; 2010 Jul;168(3):680–690. PMID: 20406669

60. Ramos Costa AP, Levone BR, Gururajan A, Moloney G, Hoeller AA, Lino-de-Oliveira C, Dinan TG, O'Leary OF, Monteiro de Lima TC, Cryan JF. Enduring effects of muscarinic receptor activation on adult hippocampal neurogenesis, microRNA expression and behaviour. Behav Brain Res. Netherlands; 2019 Apr;362:188–198. PMID: 30650342

61. Nishijima T, Kawakami M, Kita I. Long-term exercise is a potent trigger for ΔFosB induction in the hippocampus along the dorso-ventral axis. PLoS One. 2013;8(11):e81245. PMID: 24282574

62. Nishijima T, Kamidozono Y, Ishiizumi A, Amemiya S, Kita I. Negative rebound in hippocampal neurogenesis following exercise cessation. Am J Physiol - Regul Integr Comp Physiol. 2017;312(3):R347–R357. PMID: 28052868

63. Crouzier L, Gilabert D, Rossel M, Trousse F, Maurice T. Topographical memory analyzed in mice using the Hamlet test, a novel complex maze. Neurobiol Learn Mem [Internet]. Elsevier; 2018;149(December 2017):118–134. Available from: https://doi.org/10.1016/j.nlm.2018.02.014 PMID: 29458097

64. Pinheiro ML, Ferraz-de-Paula V, Ribeiro A, Sakai M, Bernardi MM, Palermo-Neto J. Long-Term Maternal Separation Differentially Alters Serum Corticosterone Levels and Blood Neutrophil Activity in A/J and C57BL/6 Mouse Offspring. Neuroimmunomodulation [Internet]. 2011;18(3):184–190. Available from: https://www.karger.com/DOI/10.1159/000323516

65. Dent GW, Smith MA, Levine S. The ontogeny of the neuroendocrine response to endotoxin. Dev Brain Res [Internet]. 1999 Oct;117(1):21–29. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165380699000917 PMID: 10536228

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

67. Cazareth J, Guyon A, Heurteaux C, Chabry J, Petit-Paitel A. Molecular and cellular neuroinflammatory status of mouse brain after systemic lipopolysaccharide challenge: Importance of CCR2/CCL2 signaling. J Neuroinflammation. 2014; PMID: 25065370

68. Delpech J-C, Wei L, Hao J, Yu X, Madore C, Butovsky O, Kaffman A. Early life stress perturbs the maturation of microglia in the developing hippocampus. Brain Behav Immun [Internet]. 2016 Oct;57:79–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0889159116301556 PMID: 27301858

69. Savage JC, St-Pierre MK, Hui CW, Tremblay ME. Microglial Ultrastructure in the Hippocampus of a Lipopolysaccharide-Induced Sickness Mouse Model. Front Neurosci. 2019;

70. Zonis S, Ljubimov VA, Mahgerefteh M, Pechnick RN, Wawrowsky K, Chesnokova V. p21Cip restrains hippocampal neurogenesis and protects neuronal progenitors from apoptosis during acute systemic inflammation. Hippocampus. 2013;23(12):1383–1394. PMID: 23966332

71. Givalois L, Dornand J, Mekaouche M, Solier MD, Bristow AF, Ixart G, Siaud P, Assenmacher I, Barbanel G. Temporal cascade of plasma level surges in ACTH, corticosterone, and cytokines in endotoxin-challenged rats. Am J Physiol. United States; 1994 Jul;267(1 Pt 2):R164-70. PMID: 8048620

72. Davis BM, Salinas-Navarro M, Cordeiro MF, Moons L, Groef L De. Characterizing microglia activation: A spatial statistics approach to maximize information extraction. Sci Rep. 2017; PMID: 28484229

73. Zajdel J, Zager A, Blomqvist A, Engblom D, Shionoya K. Acute maternal separation potentiates the gene expression and corticosterone response induced by inflammation. Brain Behav Immun. 2019; PMID: 30590109

74. Dinel AL, Joffre C, Trifilieff P, Aubert A, Foury A, Le Ruyet P, Layé S. Inflammation early in life is a vulnerability factor for emotional behavior at adolescence and for lipopolysaccharide-induced spatial memory and neurogenesis alteration at adulthood. J Neuroinflammation. 2014; PMID: 25224537

75. Dutcher EG, Pama EAC, Lynall M-E, Khan S, Clatworthy MR, Robbins TW, Bullmore ET, Dalley JW. Early-life stress and inflammation: A systematic review of a key experimental approach in rodents. Brain Neurosci Adv. 2020;

76. Silverman MN, Macdougall MG, Hu F, Pace TWW, Raison CL, Miller AH. Endogenous glucocorticoids protect against TNF-alpha-induced increases in anxiety-like behavior in virally infected mice. Mol Psychiatry. 2007; PMID: 17389906

77. Onufriev M V., Uzakov SS, Freiman S V., Stepanichev MY, Moiseeva Y V., Lazareva NA, Markevich VA, Gulyaeva N V. The Dorsal and Ventral Hippocampus Have Different Reactivities to Proinflammatory Stress: Corticosterone Levels, Cytokine Expression, and Synaptic Plasticity. Neurosci Behav Physiol [Internet]. 2018 Oct 10;48(8):1024–1031. Available from: http://link.springer.com/10.1007/s11055-018-0665-6

78. Matelski L, Keil Stietz KP, Sethi S, Taylor SL, Van de Water J, Lein PJ. The influence of sex, genotype, and dose on serum and hippocampal cytokine levels in juvenile mice developmentally exposed to a human-relevant mixture of polychlorinated biphenyls. Curr Res Toxicol [Internet]. The Author(s); 2020;1:85–103. Available from: https://doi.org/10.1016/j.crtox.2020.09.001

79. Erickson MA, Banks WA. Cytokine and chemokine responses in serum and brain after single and repeated injections of lipopolysaccharide: Multiplex quantification with path analysis. Brain Behav Immun. 2011;25(8):1637–1648. PMID: 21704698

80. Furube E, Kawai S, Inagaki H, Takagi S, Miyata S. Brain Region-dependent Heterogeneity and Dose-dependent Difference in Transient Microglia Population Increase during Lipopolysaccharide-induced Inflammation. Sci Rep. 2018 Feb;8(1):2203. PMID: 29396567

81. Mouton PR, Kelley-Bell B, Tweedie D, Spangler EL, Perez E, Carlson OD, Short RG, deCabo R, Chang J, Ingram DK, Li Y, Greig NH. The effects of age and lipopolysaccharide (LPS)-mediated peripheral inflammation on numbers of central catecholaminergic neurons. Neurobiol Aging [Internet]. 2010/11/19. 2012 Feb;33(2):423.e27-423.e4.23E36. Available from: https://pubmed.ncbi.nlm.nih.gov/21093964

82. Nikodemova M, Small AL, Kimyon RS, Watters JJ. Age-dependent differences in microglial responses to systemic inflammation are evident as early as middle age. Physiol Genomics. 2016;48(5):336–344. PMID: 26884461

83. Koo JW, Duman RS. Evidence for IL-1 receptor blockade as a therapeutic strategy for the treatment of depression. Curr Opin Investig Drugs. 2009 Jul;10(7):664–671. PMID: 19579172

84. Maes M, Song C, Yirmiya R. Targeting IL-1 in depression. Expert Opin Ther Targets. England; 2012 Nov;16(11):1097–1112. PMID: 22925041

85. Griffin WST, Mrak RE. Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. J Leukoc Biol. 2002 Aug;72(2):233–238. PMID: 12149413

86. Lana D, Ugolini F, Nosi D, Wenk GL, Giovannini MG. Alterations in the interplay between neurons, astrocytes and microglia in the rat dentate gyrus in experimental models of neurodegeneration. Front Aging Neurosci. 2017;

87. Tan Y-L, Yuan Y, Tian L. Microglial regional heterogeneity and its role in the brain. Mol Psychiatry. 2020 Feb;25(2):351–367. PMID: 31772305

88. Réus GZ, Silva RH, de Moura AB, Presa JF, Abelaira HM, Abatti M, Vieira A, Pescador B, Michels M, Ignácio ZM, Dal-Pizzol F, Quevedo J. Early Maternal Deprivation Induces

Microglial Activation, Alters Glial Fibrillary Acidic Protein Immunoreactivity and Indoleamine 2,3-Dioxygenase during the Development of Offspring Rats. Mol Neurobiol. United States; 2019 Feb;56(2):1096–1108. PMID: 29873040

89. Kentner AC, McLeod SA, Field EF, Pittman QJ. Sex-dependent effects of neonatal inflammation on adult inflammatory markers and behavior. Endocrinology. 2010; PMID: 20392837

90. Pyter LM, Kelly SD, Harrell CS, Neigh GN. Sex differences in the effects of adolescent stress on adult brain inflammatory markers in rats. Brain Behav Immun. 2013; PMID: 23348027

91. Ryan SM, Nolan YM. Neuroinflammation negatively affects adult hippocampal neurogenesis and cognition: can exercise compensate? Neurosci Biobehav Rev [Internet]. Department of Anatomy and Neuroscience, University College Cork, Western Gate Building, Cork, Ireland. Department of Anatomy and Neuroscience, University College Cork, Western Gate Building, Cork, Ireland. Electronic address: y.nolan@ucc.ie.; 2016;61:121–131. Available from: internal-pdf://177.130.6.28/1-s2.0-S014976341530049X-main.pdf

92. Levone BR, Cryan JF, O'Leary OF. Role of adult hippocampal neurogenesis in stress resilience. Neurobiology of Stress. 2015.

93. Perez-Dominguez M, Ávila-Muñoz E, Domínguez-Rivas E, Zepeda A. The detrimental effects of lipopolysaccharide-induced neuroinflammation on adult hippocampal neurogenesis depend on the duration of the pro-inflammatory response. Neural Regen Res. 2019 May;14(5):817–825. PMID: 30688267

94. Rummel J, Epp JR, Galea LAM. Estradiol does not influence strategy choice but place strategy choice is associated with increased cell proliferation in the hippocampus of female rats. Horm Behav. United States; 2010 Sep;58(4):582–590. PMID: 20688068

95. Tzeng W-Y, Chen L-H, Cherng CG, Tsai Y-N, Yu L. Sex differences and the modulating effects of gonadal hormones on basal and the stressor-decreased newly proliferative cells and neuroblasts in dentate gyrus. Psychoneuroendocrinology. England; 2014 Apr;42:24–37. PMID: 24636498

96. Green MR, McCormick CM. Sex and stress steroids in adolescence: Gonadal regulation of the hypothalamic-pituitary-adrenal axis in the rat. Gen Comp Endocrinol. United States; 2016 Aug;234:110–116. PMID: 26851306

97. Järlestedt K, Naylor AS, Dean J, Hagberg H, Mallard C. Decreased survival of newborn neurons in the dorsal hippocampus after neonatal LPS exposure in mice. Neuroscience. 2013 Dec;253:21–28. PMID: 23994184

98. Oreland S, Nylander I, Pickering C. Prolonged maternal separation decreases granule cell number in the dentate gyrus of 3-week-old male rats. Int J Dev Neurosci. 2010; PMID: 20079421

99. Leslie AT, Akers KG, Krakowski AD, Stone SSD, Sakaguchi M, Arruda-Carvalho M, Frankland PW. Impact of early adverse experience on complexity of adult-generated

neurons. Transl Psychiatry. 2011 Aug;1(8):e35. PMID: 22832609

100. Eiland L, McEwen BS. Early life stress followed by subsequent adult chronic stress potentiates anxiety and blunts hippocampal structural remodeling. Hippocampus. 2012;22(1):82–91. PMID: 20848608

101. Oomen CA, Soeters H, Audureau N, Vermunt L, Van Hasselt FN, Manders EMM, Joëls M, Lucassen PJ, Krugers H. Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. J Neurosci. 2010;30(19):6635–6645. PMID: 20463226

102. Eiland L, Ramroop J, Hill MN, Manley J, McEwen BS. Chronic Juvenile Stress Produces Corticolimbic Dendritic Architectural Remodeling and Modulates Emotional Behavior in Male and Female Rats. Psychoneuroendocrinology. 2013;23(1):1–7. PMID: 1000000221

Highlights

- Maternal Separation altered the LPS-induced inflammatory response in plasma of juvenile female rats
- Maternal separation enhanced an LPS-induced increase in IL-1β in the ventral but not dorsal hippocampus.
- Maternal separation attenuated an LPS-induced increase in microglial activation in dorsal and ventral hippocampus.
- Maternal separation and LPS, and the combination of both, reduced dendritic complexity of newly born neurons in the dentate gyrus.

Journal Pre-proofs