bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Early and Late Effects of Maternal Experience on Hippocampal Neurogenesis, Microglia, and the Circulating Cytokine Milieu

Rand Mahmoud[1], Jessica A Chaiton[2], Stephanie E Lieblich[2], Tamara S Bodnar[3], Joanne Weinberg[1,3], and Liisa AM Galea[1,2,4]

[1] Graduate Program in Neuroscience, University of British Columbia, Vancouver, BC, Canada
[2] Department of Psychology, University of British Columbia, Vancouver, BC, Canada
[3] Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada
[4] Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada

Corresponding author:

Dr. Liisa Galea
Djavad Mowafaghian Centre for Brain Health
University of British Columbia
2215 Wesbrook Mall
Vancouver, BC
Canada, V6T 1Z3
Tel: +1 (604) 822 6536
Email: liisa.galea@ubc.ca

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 **Abstract**

2 The maternal brain displays considerable plasticity, and motherhood is associated with changes in
3 affective and cognitive function. Motherhood can alter the trajectory of brain ageing, including
4 modifications to neuroplasticity and cognition. Here, we investigated the short- and long-term effects of
5 motherhood on hippocampal neurogenesis, microglial density and morphology, and circulating
6 cytokines, domains known to be altered with age and implicated in cognition and mood. Female rats
7 were bred then euthanized during gestation or at various postpartum timepoints, culminating in middle
8 age, and nulliparous rats served as age-matched controls. Hippocampal neurogenesis was significantly
9 suppressed during gestation and the postpartum period. Interestingly, neurogenesis declined significantly
10 in middle-aged nulliparous rats, but increased in primiparous rats across the same period. Transient
11 postpartum adaptations to the neuroimmune environment of the hippocampus were evidenced, as Iba-1-
12 immunoreactive microglia assumed a de-ramified morphology followed by increased density.
13 Intriguingly, ageing-related changes in circulating cytokines were dependent on parity. These
14 adaptations in neurogenic and immune processes may have ramifications for maternal mood and
15 cognition across the peripartum period and beyond.

16 **Keywords:** pregnancy, postpartum, motherhood, doublecortin, cell proliferation, Iba-1,
17 proinflammatory cytokines, anti-inflammatory cytokines

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

18 **1. Introduction**

19 Dramatic physiological adaptations occur during pregnancy and the postpartum period to ensure
20 offspring development and survival (Dulac et al., 2014; Hall et al., 2011; Lain and Catalano, 2007;
21 PrabhuDas et al., 2015; Rossant and Cross, 2001). The maternal brain exhibits substantial plasticity,
22 including large-scale volumetric changes (Galea et al., 2000; Hoekzema et al., 2016; Oatridge et al.,
23 2002), alterations in cellular architecture (Leuner and Gould, 2010; Pawluski and Galea, 2006), and
24 hippocampal neurogenesis (Darnaudéry et al., 2007; Leuner et al., 2007; Pawluski and Galea, 2007).
25 While this capacity for plasticity is likely essential for the onset of a repertoire of maternal behaviours
26 (Bridges, 2015), motherhood is also associated with changes in affective function (Bennett et al., 2004;
27 Darcy et al., 2011; O'Hara, 2009), hypothalamic-pituitary-adrenal axis (HPA) regulation (Slattery and
28 Neumann, 2008), and cognition (Cuttler et al., 2011; De Groot et al., 2006; Galea et al., 2000; Kinsley et
29 al., 1999; Pawluski et al., 2006). Interestingly, motherhood may improve the ageing trajectory in terms
30 of cognition (Colucci et al., 2006; Cui et al., 2014; Gatewood et al., 2005), neuroplasticity (Barha et al.,
31 2015; Barha and Galea, 2011; Galea et al., 2018), and cellular aging (Barha et al., 2016), suggesting that
32 the effects of the motherhood on the brain may be long lasting. However, the mechanisms underlying
33 alterations in the ageing maternal brain are not known, but may include modifications in neurogenic or
34 immune processes, both of which were examined in the current study.

35 The hippocampus produces new neurons across the lifespan (Altman and Das, 1965; Boldrini et
36 al., 2018; Eriksson et al., 1998) and these neurons play a role in certain aspects of learning and memory
37 (Yau et al., 2015), mood regulation (Sahay and Hen, 2007), and the stress response (Snyder et al., 2011).
38 Importantly, several studies found postpartum reductions in hippocampal cell proliferation (Darnaudéry
39 et al., 2007; Leuner et al., 2007; Pawluski and Galea, 2007), cell survival (Pawluski and Galea, 2007)
40 and the density of immature neurons (Workman et al., 2015). Interestingly, motherhood may have
41 contrasting effects on hippocampal neurogenesis with age, as studies have found increased neurogenesis
42 in middle-aged primiparous and multiparous rats relative to age-matched nulliparous controls (Barha et
43 al., 2015; Galea et al., 2018). This finding signifies that parity can have delayed pro-neurogenic effects,
44 thus the current study aimed to determine the timeline by which these changes may emerge.

45 Adaptations to the maternal immune system are well documented, and necessary for the
46 establishment and maintenance of pregnancy (Mor and Cardenas, 2010). In contrast, and despite the
47 growing recognition of plasticity in the maternal brain, there is a paucity of research on potential
48 neuroimmune adaptations with maternal experience. Few studies to date have examined microglia, the

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

49 innate immune cells of the brain, in pregnant and postpartum rats (Haim et al., 2017; Posillico and
50 Schwarz, 2016). Microglia alterations were found in several regions of the maternal brain, and
51 normalized by postpartum day 21 in all regions except the hippocampus (Haim et al., 2017). This
52 indicates that changes in the neuroimmune environment of the maternal hippocampus may be longer
53 lasting. The hippocampus undergoes considerable plasticity in the peripartum period (Galea et al., 2014),
54 perhaps not surprisingly given its role in cognitive function (Sweatt, 2004) and mood regulation
55 (Campbell and MacQueen, 2004). Neuroimmune processes are implicated in cognition (Lee et al., 2008;
56 Parkhurst et al., 2013; vom Berg et al., 2012), stress (Hodes et al., 2014; Kreisel et al., 2014), and mood
57 (Setiawan et al., 2015), raising the possibility that changes in the neuroimmune environment of the
58 hippocampus may represent a substrate for motherhood-related changes in hippocampal function.

59 In the non-maternal brain, immune processes have been implicated in the regulation of adult
60 hippocampal neurogenesis, under basal and inflammatory conditions (reviewed in Sierra et al., 2014).
61 For example, inflammation was first demonstrated to suppress neurogenesis by studies utilizing systemic
62 or intrahippocampal administration of the bacterial endotoxin lipopolysaccharide (Ekdahl et al., 2009;
63 Monje, 2003). Microglia also maintain homeostasis in the healthy adult neurogenic niche via
64 phagocytosis of apoptotic new cells (Sierra et al., 2010). In the maternal brain, one study found that
65 alterations in T-cell activity accounted for at least some of the postpartum-associated reductions in
66 neurogenesis (Rolls et al., 2008). To date, however, no studies have concurrently examined adaptations
67 in microglia and neurogenesis in the maternal hippocampus, and therefore the experiments reported here
68 aimed to fill this gap.

69 Given the extensive cross-talk between the central nervous system and the immune system
70 (Louveau et al., 2015), motherhood-related adaptations in the immune system can potentially drive
71 plasticity in the brain. Reproductive immunology research has been primarily focused on aspects of
72 immune function that affect fetal development and the success of pregnancy (PrabhuDas et al., 2015).
73 Although many of the maternal immune adaptations normalize in the postpartum period (Groer et al.,
74 2015), there is evidence indicating that maternal experience can leave a lasting footprint on the immune
75 system (Barrat et al., 1997a, 1997b, 1999; Helle et al., 2004). For example, the risk of dying of
76 infectious disease after the age of 65 was increased in mothers of twins, compared to mothers of
77 singletons (Helle et al., 2004). This effect may be related to reproductive effort, and is perhaps indicative
78 of accelerated immunosenescence (Helle et al., 2004). Although the long-term effects of parity on the
79 immune system have received little attention in animal models, delayed senescence in certain aspects of
80 immune function is evidenced in parous relative to non-parous mice (Barrat et al., 1997b, 1997a, 1999).

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

81 In tandem with neurogenic and neuroimmune markers, our current study aimed to assess whether
82 maternal experience can alter the circulating cytokine profile at various intervals following parturition,
83 ending well after the reproductive event itself. The circulating cytokine profile is not only informative to
84 the general inflammatory state, but may have ramifications for brain and behaviour as peripheral
85 cytokine signals propagate to the brain (Miller et al., 2014; Quan and Banks, 2007). Preclinical cytokine
86 data may also be valuable for comparative purposes, as circulating cytokines levels are accessible
87 biomarkers in clinical populations (Guerreiro et al., 2007).

88 In this study, we examined the short- and long-term effects of parity on microglia density and
89 morphology, and on neurogenesis in the hippocampus. These measures were examined across age and
90 time since parturition, extending into middle age. To gain information about the peripheral inflammatory
91 milieu, we also quantified concentrations of various serum cytokines across the same time points. We
92 expected parity to suppress hippocampal neurogenesis in the short term, and to increase neurogenesis in
93 middle age. At least in the short term, we expected parity to modify microglial density and morphology
94 in the dentate gyrus. Finally, we expected alterations in the circulating cytokine profile during pregnancy
95 and the early postpartum period, and hypothesized that parity would modulate the age-related changes in
96 the circulating cytokine milieu.

97 **2. Materials and Methods**

98 ***2.1. Animals***

99 Young adult female and male Sprague-Dawley rats were purchased from Charles River Laboratories
100 (Montreal, Canada), weighing at 200–250g. All rats arrived at our facility at the same time. Rats were
101 maintained on a 12-hour light/dark cycle (lights on at 07:00 h), in standard laboratory conditions
102 (21 ± 1°C; 50 ± 10% humidity) and given *ad libitum* access to water and food (Purina Rat Chow).
103 Female rats were initially pair-housed, and except for the breeding period, all rats were housed in
104 female-only colony rooms. Males were used for breeding purposes only. Nulliparous rats were never
105 housed in the same colony room as primiparous rats when they were breeding or had active litters. To
106 minimize potential environmental exposure differences between nulliparous and primiparous groups,
107 primiparous rats were transferred to the nulliparous colony room on the day that their litters were
108 weaned (postpartum day 21). All procedures were performed in accordance with ethical guidelines set
109 by the Canadian Council on Animal Care and approved by the Animal Care Committee at the University
110 of British Columbia.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

111 ***2.2. Breeding procedure and experimental groups***

112 Female rats were bred at approximately 7 months of age. At 18:00 h daily, each pair of female cage-
113 mates was placed with one male. Vaginal lavage samples were obtained the following morning, between
114 08:00 and 09:00 h, and examined for the presence of sperm cells. The detection of sperm cells indicated
115 Gestation Day 1 (GD1), at which point the pregnant female was weighed and single-housed. Primigravid
116 or primiparous rats (i.e. pregnant or mothering for the first time; n=30) were randomly assigned to one
117 of six groups (n=5 each) according to the timeline of euthanasia relative to gestation. This included one
118 gestational group at Gestation Day 13 (GD13), and four postpartum groups: Postpartum Day 8 (PPD8),
119 Postpartum Day 30 (PPD30), Postpartum Day 90 (PPD90), and Postpartum Day 180 (PPD180).
120 Nulliparous rats (i.e. never pregnant; n=30) were randomly assigned to control groups (n=5 each) that
121 were age-matched to each of the primiparous groups. Specifically, nulliparous rats at approximately 7,
122 7.5, 8, 10, and 13 months of age served as control groups for primiparous rats at GD13, PPD8, PPD30,
123 PPD90, and PPD180, respectively (experimental groups are detailed in **Figure 1**). These timepoints
124 were chosen to capture: mid-gestation (GD13), as a previous study found reductions in the survival of
125 hippocampal neurons produced at this time (Rolls et al., 2008); an early postpartum timepoint (PPD8)
126 that avoids the acute inflammatory state surrounding parturition (Catalano et al., 2010) and is associated
127 with declines in cell proliferation (Leuner et al., 2007); a post-weaning, late postpartum timepoint
128 (PPD30) shown to be associated with reduced neurogenesis (Workman et al., 2015); and finally, for a
129 time-course analysis of the effects of parity on the ageing trajectory, two further timepoints were
130 selected leading to middle age (PPD180), as previous studies reported increased neurogenesis in middle-
131 aged rats with previous maternal experience (Barha et al., 2015; Galea et al., 2018). To account for
132 potential effects of social housing, nulliparous controls were single-housed at GD1 of their primiparous-
133 counterparts. For all postpartum groups, litters were culled to include between 8-10 pups, with
134 approximately 50% males and females. When the original sex ratio or litter size was not sufficient to
135 achieve this, pups were cross-fostered between dams that had given birth the same day. Pups were
136 weaned at PPD21 for all postpartum groups, except for the PPD8 group in which the dams remained
137 with their litter until just prior to euthanasia. One rat from the nulliparous 10-month-old group was
138 eliminated from the study due to a mammary gland tumor.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

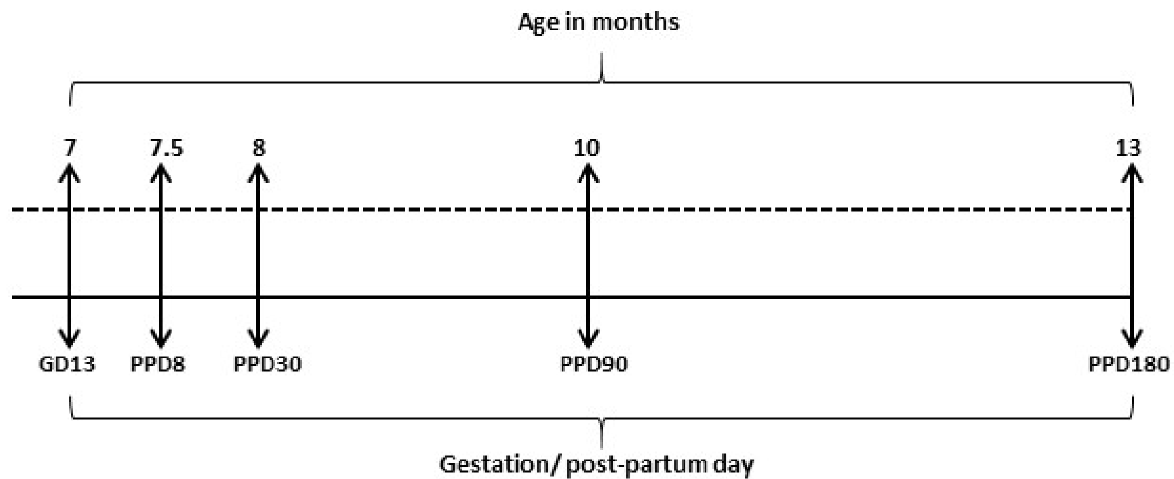

Figure 1. Experimental groups. Primigravid or primiparous rats (n=5/group) were euthanized at gestation day 13 (GD13), postpartum day 8 (PPD8), postpartum day 30 (PPD30), postpartum day 90 (PPD90), or postpartum day 180 (PPD180). Nulliparous rats (n=5/group) were age-matched to their primiparous counterparts, and euthanized along the same timeline, depicted as approximate age in months. Arrows indicate euthanasia day.

139 ***2.3. Perfusion and tissue collection***

140 All perfusions were completed between 9:00 and 11:00 am. The rats were deeply anesthetized with an
141 overdose of sodium pentobarbital (i.p.), and blood was collected via cardiac puncture. Brains were
142 collected immediately after transcardial perfusion with 60ml of cold 0.9% saline, followed by 120ml of
143 cold 4% paraformaldehyde (PFA). Brains were stored at 4°C in 4% PFA for 24 hours, then transferred
144 into a 30% sucrose solution (in 0.1 M Phosphate Buffer) until sectioning. In the group euthanized during
145 gestation, the uterus was dissected to confirm pregnancy. To obtain serum, blood samples were allowed
146 to clot overnight at 4°C, then centrifuged at 10g for 15 minutes and serum aliquots were stored at -20°C
147 until processing.

148 ***2.4. Brain Tissue Processing and Immunohistochemistry***

149 Brains were sliced into 40 µm coronal sections using a Leica SM2000R Microtome (Richmond Hill,
150 Ontario, Canada). Sections were collected in series of 10 along the rostral-caudal axis of the
151 hippocampus, then stored at -20 °C in a cryoprotectant consisting of 30% ethylene glycol (Sigma-
152 Aldrich, St. Louis, MO, USA) and 20% glycerol (Sigma-Aldrich) in 0.1 M phosphate-buffer (PB, pH
153 7.4). Sections were thoroughly rinsed (5 x 10 mins) in PBS prior to staining to remove the
154 cryoprotective medium. All immunohistochemical procedures were conducted on free-floating brain
155 sections, and on a rotator at room temperature unless otherwise noted.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

156 ***2.4.1. Doublecortin (DCX):*** DCX is a microtubule-associated protein expressed in immature neurons for
157 21 days after production in adult rats (Brown et al., 2003), and thus was used as a marker of adult
158 hippocampal neurogenesis. Tissue was rinsed in 0.1M PBS (pH 7.4; 5 x 10 minutes) between each of the
159 following procedures. Tissue was treated with 0.3% hydrogen peroxide (H_2O_2, in dH_2O) for 30 minutes,
160 then incubated for 24 hours at 4°C in a primary antibody solution containing 1:1000 goat anti-
161 doublecortin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 3% normal rabbit serum (NRS) and
162 0.4% Triton-X in 0.1M PBS. Next, tissue was transferred to a secondary antibody solution consisting of
163 1:500 rabbit anti-goat (Vector Laboratories, Burlington, ON, Canada) in 0.1 M PBS for 24 hours at 4°C.
164 Finally, tissue was transferred to an avidin-biotin complex (ABC; Elite kit; 1:1000, Vector Laboratories)
165 in PBS for 4 hours, then immunoreactants were visualized with a Nickel-enhanced DAB reaction
166 (Vector Laboratories). Sections were mounted onto glass slides and allowed to dry, then dehydrated in
167 increasing graded ethanol, defatted with xylenes, and cover-slipped with Permount (Fisher Scientific).

168 ***2.4.2. Ionized calcium binding adaptor molecule-1 (Iba-1):*** Iba-1 is a calcium-binding protein widely
169 used as a microglial marker (Korzhevskii and Kirik, 2016). Tissue was rinsed in 0.1 M PBS (pH 7.4; 3 x
170 10 minutes) between each of the following procedures. Tissue was incubated in 0.3% hydrogen peroxide
171 (H_2O_2, in dH_2O) for 25 minutes, then blocked with 10% normal goat serum (NGS) in 0.5% Triton-X in
172 0.1M PBS. Tissue was then transferred to a primary antibody solution for 18 hours at 4°C, consisting of
173 1:1000 anti-Iba-1 (Wako, Osaka, Japan) in 10% NGS and 0.4% Triton-X in 0.1M PBS. Next, tissue was
174 incubated in a secondary antibody solution for 1 hour, containing 1:500 biotinylated anti-rabbit (Vector
175 Laboratories) in 2.5% NGS and 0.4% Triton X in PBS. Finally, tissue was transferred to an avidin-biotin
176 complex (ABC; Elite kit; 1:50, Vector Laboratories) in 0.4% Triton-X in PBS for 1 hour, and
177 immunoreactivity was visualized with a Nickel-enhanced DAB reaction (Vector Laboratories). Sections
178 were mounted onto glass slides and allowed to dry, then counterstained with cresyl violet, dehydrated in
179 a series of ethanol solutions of increasing concentrations, defatted with xylenes, and cover-slipped with
180 Permount (Fisher Scientific).

181 ***2.4.3. Ki67:*** Ki67 is expressed during all active phases of the cell cycle, but not during G_0 phase
182 (Scholzen and Gerdes, 2000), and therefore was used as a marker of cell proliferation in the dentate
183 gyrus. Tissue was rinsed in 0.1 M PBS (pH 7.4; 3 x 10 minutes) between each of the following
184 procedures. Tissue was incubated in a primary antibody solution for 48 hours at 4°C, consisting of 1:200
185 mouse anti-Ki67 (NCL-L-Ki67-MM1; Leica Biosystems, Newcastle, UK) in 3% normal donkey serum
186 (NDS), and 0.3% Triton-X in 0.1M PBS. Next, tissue was incubated for 18 hours at 4°C in a secondary
187 antibody solution consisting of 1:200 donkey anti-mouse IgG, Alexa Fluor 555 (Molecular Probes,

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

188 Eugene, Oregon, USA) in 3% NDS in 0.1M PBS. Sections were counterstained with DAPI (2.5 minutes;
189 300nM; ThermoFisher, Waltham, WA, USA), mounted onto glass slides, and cover-slipped with an anti-
190 fade medium (2.5% Polyvinyl alcohol-Dabco).

191 ***2.5. Microscopy, Cell Quantification, and Morphological analyses***

192 An investigator blinded to experimental conditions quantified DCX- and Iba-1-, and Ki67-
193 immunoreactive cells and analyzed cell morphology. See **Fig. 3B and 5** for representative
194 photomicrographs.

195 ***2.5.1. Iba-1*****:** Under a 400x objective on a Nikon E600 microscope, an exhaustive quantification of Iba-
196 1-IR cells was completed in four hippocampal slices from each animal, as we have done previously
197 (Mahmoud et al., 2016). This included two dorsal and two ventral sections, with approximate Bregma
198 coordinates of -3.12, -3.48, 6.00, and -6.36. Iba-1-IR cells were quantified in the dentate gyrus,
199 specifically in the granule cell layer (GCL), the subgranular zone (SGZ), and within an approximately
200 50μm band of the molecular layer (ML).

201 To obtain a proxy-measure of microglial activation, Iba-1-IR cell morphology was analyzed utilizing
202 NIS Elements Basic Research software (Nikon) under a Nikon E600 microscope. Using the measure
203 feature, soma size, in addition to cell process length and number were measured live at 400x for every
204 cell within a 23672.24 μm^2 region of interest (ROI), with 3 ROIs each for the dorsal and ventral
205 hippocampus. Further, no more than one ROI was taken from an individual tissue slice, and ROIs were
206 defined in 3 consistent locations in the GCL for both the dorsal and ventral hippocampus within each
207 animal. Cells were defined by the presence of an Iba-1-IR cell body within the ROI, and this definition
208 did not necessitate the presence of cell processes, by that ensuring the inclusion of any cells with
209 amoeboid morphology. The average process length per cell was calculated using the total length and
210 number of processes for each cell, and subsequently an average process length was calculated for each
211 animal. Both primary (extending directly from the cell body) and secondary processes were taken into
212 account in the analyses. There were no significant differences between groups in the number of Iba-1-
213 IR cells that fell within the ROIs and were used for morphological analyses (see Table 1).

Table 1. Mean Iba-1-IR cells used for morphological analyses ± standard error of the mean. There were no significant differences between groups.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Group	Number of Iba-1-IR cells analyzed
Nulliparous – 7 mo.	23.40 ± 1.03
Nulliparous – 7.5 mo.	21.00 ± 2.04
Nulliparous – 8 mo.	20.80 ± 2.13
Nulliparous – 10 mo.	21.20 ± 1.16
Nulliparous – 13 mo.	22.25 ± 2.32
Primiparous – GD13	23.67 ± 1.67
Primiparous – PPD8	22.00 ± 0.77
Primiparous – PPD30	23.60 ± 1.47
Primiparous – PPD90	20.60 ± 1.03
Primiparous – PPD180	23.20 ± 0.37

214 ***2.5.2. DCX*:** Under a 1000x objective on an Olympus CX22LED brightfield microscope, DCX-IR cells
215 in the granule cell layer (GCL) were exhaustively counted in every 10th section of the hippocampus
216 along the rostral-caudal axis. Thus, raw counts were multiplied by a factor of 10 to obtain an estimate of
217 the total number of DCX-IR cells in the hippocampus.

218 Using the 1000× objective on an Olympus CX22LED brightfield microscope, 50 DCX-IR cells (25
219 dorsal GCL and 25 ventral GCL; each taken from 3 slices) were randomly selected for each animal.
220 Cells were categorized into one of three maturational stages, based on previously established criteria
221 (Plümpe et al., 2006): proliferative (no process or short process), intermediate (medium process with no
222 branching), or post-mitotic (long processes with branching in the GCL and ML).

223 ***2.5.3. Ki67:*** Under a 1000x objective on an Olympus CX22LED microscope equipped with
224 epifluorescence, Ki67-IR cells in the GCL and SGZ of the DG were exhaustively counted in every 10th
225 section of the hippocampus along the rostral-caudal axis. Raw counts were multiplied by a factor of 10
226 to obtain an estimate of the total number of Ki67-IR cells in the DG.

227 ***2.6. Serum cytokine quantification***

228 A multiplex immunoassay kit (V-PLEX Proinflammatory Panel 2, Rat) was purchased from Meso-Scale
229 Discovery (Rockville, MD) and used according to manufacturer instructions to measure serum cytokine
230 levels. The antibody pre-coated plates allowed for the simultaneous quantification of the following
231 cytokines: Interferon-gamma (IFN-γ), Interleukin-1beta (IL-1β), Interleukin-4 (IL-4), Interleukin-5 (IL-
232 5), Interleukin-6 (IL-6), chemokine (C-X-C motif) ligand 1 (CXCL1), Interleukin-10 (IL-10),
233 Interleukin-13 (IL-13), and tumor necrosis factor alpha (TNF-α). Samples were run in duplicates, and
234 plates were read with a Sector Imager 2400 (Meso Scale Discovery), and data was analyzed using the

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

235 Discovery Workbench 4.0 software (Meso Scale Discovery). The assays' lower limits of detection
236 (LLOD), which varied between analytes and plates (2 plates total), were as follows (pg/mL): IFN-ɣ:
237 0.163-0.266; IL-10: 0.233-0.313; IL-13: 0.78-2.7; IL-1β:1.48-1.62; IL-4:0.179-0.298; IL-5: 7.64-9.8;
238 IL-6: 2.48-2.49; CXCL1: 0.085-0.164; and TNF-α: 0.156–0.186. Any values below the LLOD were
239 assigned 0 pg/mL, as we have done previously (Bodnar et al., 2017). All samples were within the
240 detection range for TNF-α, CXCL1, and IL-10. One sample fell below the LLOD for each of IFN-γ, IL-
241 4, and IL-13. For three cytokines, a number of samples fell below the LLOD (n=12 for IL-6, n=17 for
242 IL-1β, and n=36 for IL-5). This panel was chosen as it includes a broad range of cytokines, some
243 traditionally considered proinflammatory (IL-1β, IFN-ɣ, TNF-α), anti-inflammatory (IL-4, IL-10), and
244 pleiotropic (IL-6), in addition to the chemokine CXCL1 which is important for neurotrophil recruitment.
245 Therefore, combined, these markers provide a comprehensive view of the inflammatory milieu.

246 ***2.7. Statistical analyses***

247 Statistical analyses were performed using Statistica software (Tulsa, OK). Neural measures (DCX- and
248 Ki67-IR cell number, Iba-1-IR density, length and number of processes) and serum cytokine levels
249 (IFN-γ, IL-1β, IL-4, IL-5, IL-6, CXCL1, IL-10, IL-13, and TNF-α) were each analyzed using factorial
250 analysis of variance (ANOVA), with time (GD13/7 mo., PPD8/7.5 mo., PPD30/8 mo., PPD90/10 mo.,
251 PPD180/13 mo.) and reproductive status (primi-gravid/parous, nulliparous) as the between-subject
252 factors. Post-hoc analyses utilized Fisher's LSD. A priori we expected parity to modulate the age-related
253 changes in cytokine levels, and density/morphology of Iba-1-IR cells. Any a priori comparisons were
254 subjected to a Bonferroni correction. Pearson's correlations were performed on dependent variables of
255 interest. Finally, as an exploratory approach, and to complement our findings from ANOVA analyses, we
256 ran a principal component analysis (PCA) on the cytokine data, with the purpose of deriving information
257 about the amount of variance accounted for by potential cytokine networks within the dataset.

258 **3. Results**

259 **3.1. Parity and age had no significant effect on granule cell layer volume**

260 Granule cell layer volume was not significantly affected by age, parity, or age by parity interaction (all
261 p's >0.48), thus all further analyses were performed on the number, rather than density, of Ki67- and
262 DCX-IR cells.

263 **3.2. The number of doublecortin-IR cells was significantly reduced during pregnancy and the**

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

264 **postpartum period, and declined in middle-age in nulliparous rats only**

265 The number of DCX-IR cells was significantly reduced in primi-gravid and -parous rats relative to age-
266 matched nulliparous controls at GD13 (p=0.000056), PPD8 (p=0.00029), and PPD30 (p=0.000015;
267 significant time by reproductive status interaction; F(4, 38)=6.7213, p=.00034; **Fig. 2**). There were also
268 significant main effects of time and reproductive status (all p's < 0.0000031).

269 There was a significant age-related decline in the number of DCX-IR cells in nulliparous rats,
270 such that each nulliparous group had a significantly lower number of DCX-IR cells than all previous
271 nulliparous age groups (all p's<0.014), with the exception of a non-significant decline from 10 to 13
272 months (p=0.11; significant time by reproductive status interaction; F(4, 38)=6.7213, p=0.00034; **Fig.**
273 **2**). There was also a significant decline in DCX-IR cell number in primiparous rats at PPD30 relative to
274 GD13 and PPD8 (p's<0.017; **Fig. 2**). However, the trajectory of age-related change in DCX-IR cell
275 number was significantly altered by parity in middle-age; unlike nulliparous rats, primiparous groups
276 had no significant difference in the number of DCX-IR cells from 8 to 10 months of age (p=0.10). Based
277 on previous findings (Barha et al., 2015; Galea et al., 2018), we expected parity to increase neurogenesis
278 in middle age. Indeed, we found a significant increase in DCX-IR cells from 8 to 13 months of age in
279 primiparous rats (p=0.044; one-tailed).

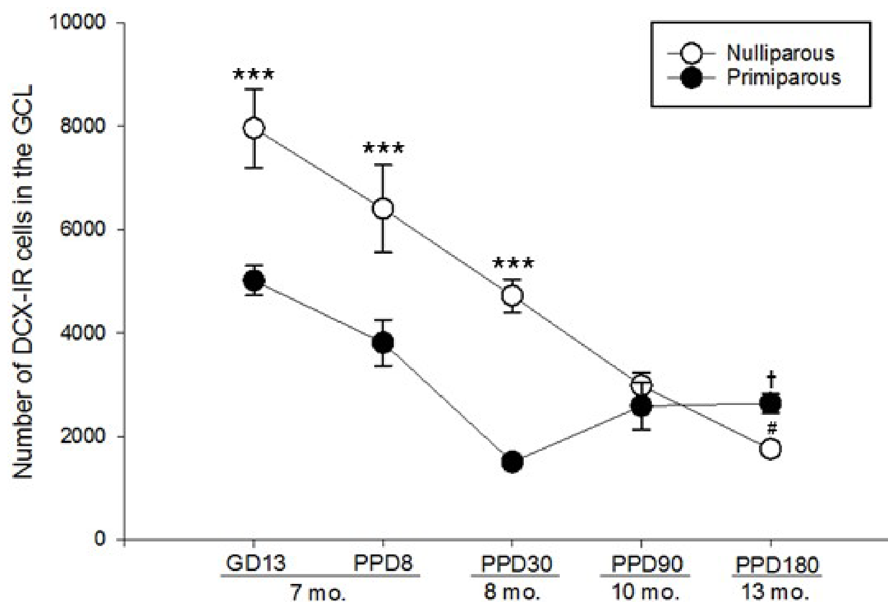

Figure 2. Estimated total number of doublecortin (DCX)-immunoreactive (IR) cells in the granule cell layer of primiparous and nulliparous rats across 7-13 months of age. The x-axis represents time relative to gestation and parturition in primiparous groups, and approximate age in months. DCX-IR cell number was significantly reduced in primiparous rats in mid-gestation and until postpartum day 30. Between 8 and 13 months of age, DCX-IR cell number significantly declined in nulliparous rats, but significantly increased in primiparous rats. Data are represented in mean values ± standard error of the mean (SEM). GCL= granule cell layer, DCX-IR= doublecortin-immunoreactive, GD= Gestations Day, PPD= Postpartum Day, mo.= approximate age in months. *** indicates

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

p<0.0003, significantly different from age-matched primiparous group. # denotes p=0.0012, significantly different from 8-month-old nulliparous group. † indicates p=0.044, significantly different from primiparous rats at PPD30.

280 **3.3. Ki67-IR cells declined in mid-gestion and the early postpartum period in primiparous rats,**
281 **and with age regardless of reproductive status**

282 Parity significantly reduced the number of Ki67-IR cells in the GCL ($F(1, 38)=9.2681$, $p=0.0042$;
283 significant main effect of reproductive status; **Fig. 3A**). Regardless of reproductive status, Ki67-IR cells
284 declined significantly with age ($F(4, 38)=16.505$, $p<0.00001$; main effect of time; **Fig. 3A**), in which
285 there was a significant difference between all age groups (p's < 0.005), with the exception of non-
286 significant differences from 7 to 7.5 months, 8 to 10 months, and 10 to 13 months (p's > 0.05). Although
287 there was no significant age by parity interaction (p=0.13), a priori we expected a decline in cell
288 proliferation in the early postpartum period based on previous work (Leuner et al., 2007). Planned
289 comparisons revealed a significant decline in Ki67-IR cells on PPD8 (p=0.013; one-tailed), in addition
290 to a significant decline on GD13 (p= 0.0042), relative to age-matched nulliparous controls in both
291 instances (Figure3A).

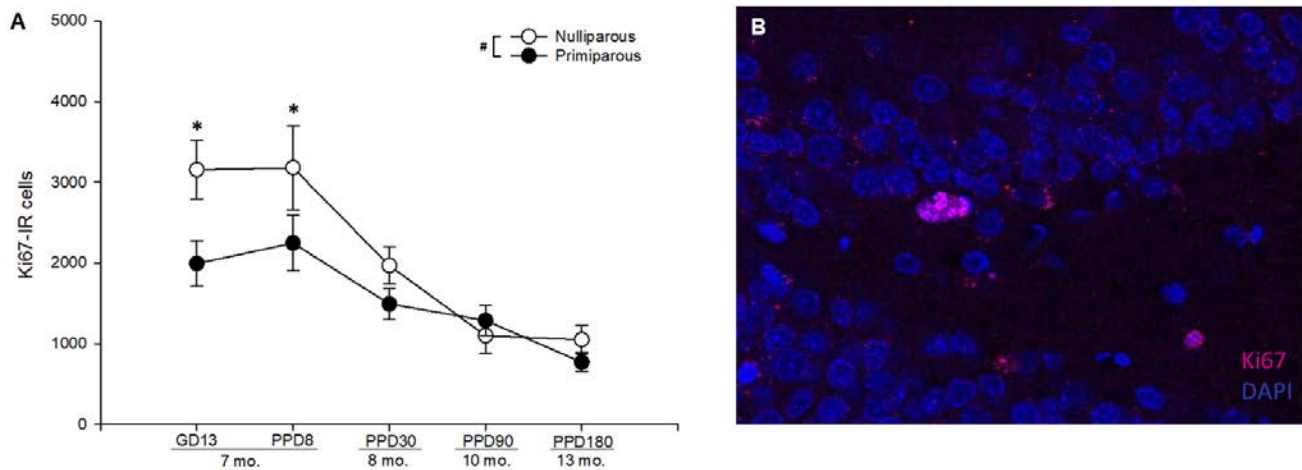

Figure 3. (**A**) Estimated total number of Ki67-immunoreactive (IR) cells in the granule cell layer and subgranular zone of primiparous and nulliparous rats across 7-13 months of age. The x-axis represents time relative to gestation and parturition in primiparous groups, and approximate age in months. Ki67-IR cell number was significantly reduced in primiparous rats in mid-gestation and the early postpartum period, and declined with age regardless of reproductive status. Data are represented in mean values ± standard error of the mean (SEM). Ki67-IR = Ki67-immunoreactive, GD= Gestations Day, PPD= Postpartum Day, mo.= approximate age in months. * indicates p<0.014, significantly different from age-matched primiparous group. # denotes p=0.0042, significantly main effect of reproductive status. **(B)** Representative photomicrograph of the dentate gyrus, showing Ki67-IR cells (pink), counterstained with DAPI (blue).

292 **3.4. Parity and age had no significant effect on the maturational stage of DCX-IR cells**

293 Regardless of age and reproductive status, the percentage of proliferative DCX-IR cells was

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

294 significantly higher than that of intermediate DCX-IR cells (p=0.000007), and the percentage of post-
295 mitotic DCX-IR cells was significantly higher than that of intermediate and proliferative DCX-IR cells
296 (p's<0.000001; significant main effect of DCX maturational stage, F(2, 76)=107.17, p<0.000001; **Table**
297 **2**). There were no other significant main effects, and no significant interactions (all p's >0.14).

Table 2. Mean percentage of proliferative, intermediate, and post-mitotic doublecortin (DCX)-immunoreactive (IR) cells in the granule cell layer ± standard error of the mean. Parity and age did not significantly affect the maturational stage of DCX-IR cells.

Group	% Proliferative	% Intermediate	% Post-mitotic
Nulliparous – 7 mo.	37.60 ± 1.60	17.20 ± 2.15	45.20 ± 2.65
Nulliparous – 7.5 mo.	26.40 ± 2.86	18.80 ± 1.85	54.80 ± 2.87
Nulliparous – 8 mo.	35.60 ± 4.71	20.80 ± 1.02	46.40 ± 4.45
Nulliparous – 10 mo.	29.60 ± 6.52	21.60 ± 2.64	48.80 ± 4.84
Nulliparous – 13 mo.	25.33 ± 8.74	20.00 ± 1.15	54.67 ± 8.97
Primiparous – GD13	32.00 ± 6.23	18.40 ± 2.93	49.60 ± 5.84
Primiparous – PPD8	28.40 ± 5.31	16.00 ± 1.10	56.40 ± 6.71
Primiparous – PPD30	27.60 ± 4.26	17.20 ± 0.80	55.20 ± 4.88
Primiparous – PPD90	23.60 ± 3.19	16.80 ± 3.01	59.60 ± 4.53
Primiparous – PPD180	28.40 ± 5.71	16.00 ± 2.45	56.00 ± 6.23

298 **3.5. Density of Iba-1-IR cells increased in the late postpartum period and fluctuated significantly**
299 **with age in primiparous but not in nulliparous rats**

300 Because we quantified Iba-1-IR cells in 4 sections per animal, the density of IR cells was analyzed, as
301 we have done previously (Mahmoud et al., 2016). Age significantly affected the density of Iba-1-IR
302 cells in the dentate gyrus, with higher density at 8 and 13 months relative to 7.5 and 10 months of age
303 (p's<0.02; significant main effect of time, F(4, 39)=3.43, p=0.017). We expected alterations in the
304 density of Iba-1-IR cells in the postpartum period due to previous findings (Haim et al., 2017). A priori
305 comparisons show that the density of Iba-1-IR cells fluctuated significantly across time in primiparous
306 rats; density was increased in the late postpartum period at PPD30 relative to PPD8 (p=0.0020), and in
307 middle-aged rats at PPD180 relative to PPD8 (p=0.0027; **Fig. 4A**). On the other hand, density did not
308 significantly change across age in nulliparous rats (all p's >0.17; **Fig. 4A**). There were no significant
309 differences between nulliparous and primiparous groups across age (all p's >0.3), except for a trend for
310 higher density at PPD30 relative to age-matched nulliparous controls (p=0.09; **Fig. 4A**). There was no
311 significant main effect of reproductive status and no significant interaction (p's>0.28).

312 **3.6. The average length of Iba-1-IR cell processes was transiently reduced in the early postpartum**
313 **period, and declined with age regardless of reproductive status.**

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

314 Iba-1-IR cells displayed significantly shorter processes in the early postpartum period at PPD8 relative
315 to nulliparous controls (p=0.0081; **Fig. 4B**), and to primiparous rats at GD13 (p=0.0025;). There was a
316 decline in average length of Iba-1-IR cell processes at 10 months of age, relative to 7 months in
317 primiparous rats, and to 7, 7.5, and 8 months in nulliparous rats (all p's <0.037; significant time by
318 reproductive status interaction F(4, 38)=2.75, p=0.04; **Fig. 4B**). However, there was no further
319 significant change in average process length between 10 and 13 months of age, regardless of
320 reproductive status (p's > 0.23). There was a significant main effect of time (p=0.032), but not of
321 reproductive status (p=0.86).

322 **3.7. The average number of Iba-1-IR cell processes increased with age in nulliparous but not**
323 **primiparous rats**

324 The average number of Iba-1-IR cell processes increased significantly with age in nulliparous rats,
325 where significantly more processes were found in 13- compared to 8-month-old rats (0.0016), but
326 missed significance compared to 7.5-month-old rats (p=0.027; planned comparisons; **Fig. 4C**). On the
327 other hand, there were no significant differences in the average number of cell processes between
328 primiparous rats across age (all p's >0.06). There were trends towards significant main effects of time
329 (p=0.054) and reproductive status (p=0.056) but no significant time by reproductive status interaction
330 (p=0.11).

331 **3.8. Soma size of Iba-1-IR cells was reduced by parity**

332 Interestingly, primiparity significantly decreased the soma size of Iba-1-IR cells relative to nulliparity
333 (F(1, 38)=5.2646, p=0.027; main effect of reproductive status; **Fig. 4D**). While there was no significant
334 age by parity interaction (F(4, 38)=.81004, p=0.53), the effects of primiparity to reduce soma size
335 appears to be driven by the PPD30, 90, and 180 groups. In addition, there was a significant main effect
336 of time (F(4, 38)=6.8580, p=0.00029).

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

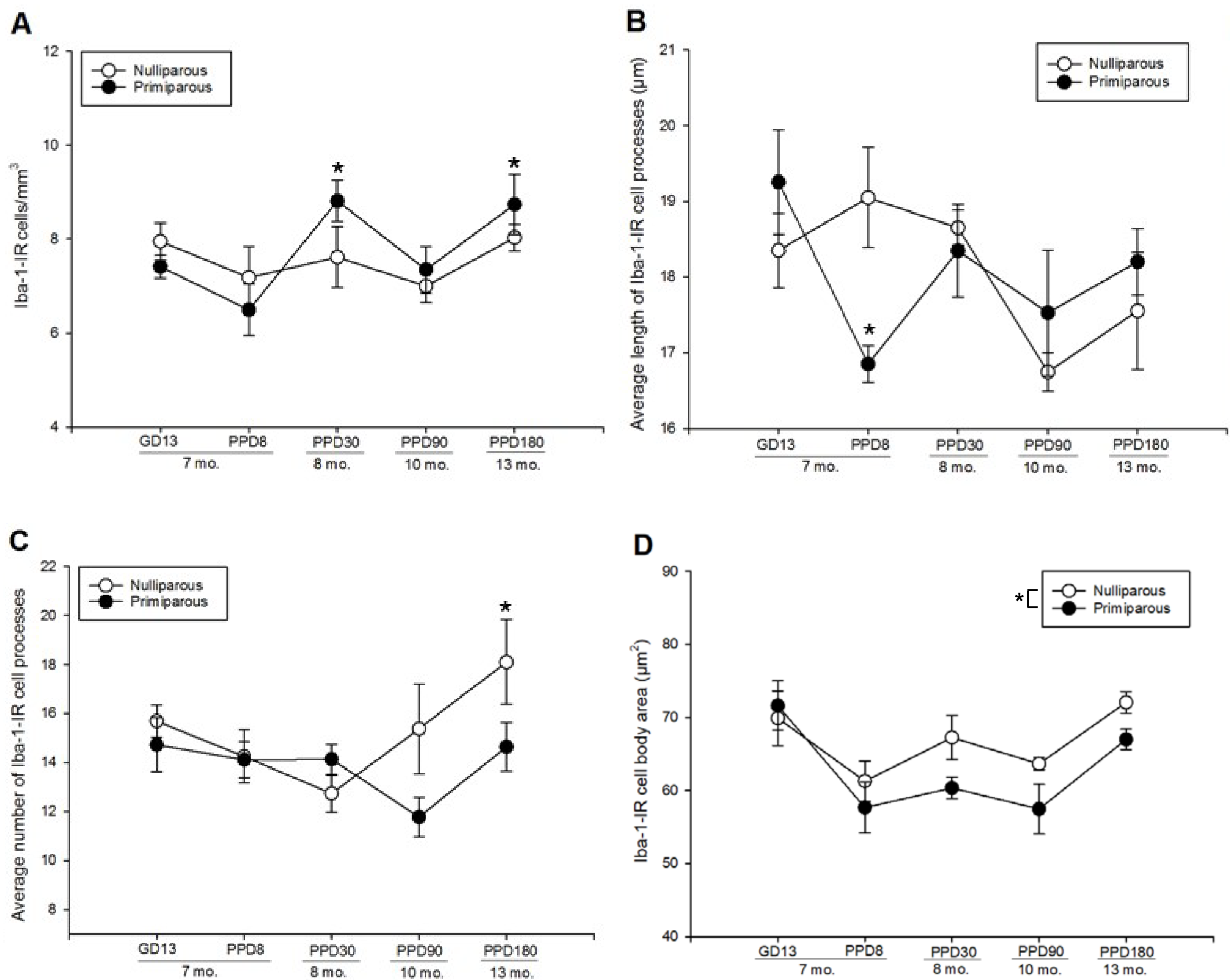

Figure 4. Density and morphology of Iba-1-immunoreactive cells in the dentate gyrus of primiparous and nulliparous rats. The x-axis represents time relative to gestation and parturition in primiparous rats, and approximate age in months. **(A)** Iba-1-IR cell density was stable across age in nulliparous rats, but fluctuated significantly in primiparous rats, with increased density at PPD30 and PPD180, relative to PPD8. * indicates $p<0.003$, significantly different from primiparous group at PPD8. **(B)** Average length of Iba-1-immunoreactive cell processes was reduced at PPD8, and decreased with age regardless of reproductive status. * denotes $p<0.0082$, significantly different from age-matched nulliparous controls, and from primiparous group at GD13 **(C)** Average number of Iba-1-immunoreactive cell processes increased significantly with age in nulliparous but not primiparous rats. * indicates $p=0.0016$, significantly different from 8-month-old nulliparous group. **(D)** Soma area of Iba-1-IR cells was reduced by parity. * indicates $p=0.027$, main effect of reproductive status. Data are represented in mean values ± SEM. GD= gestation day, PPD= postpartum day, mo.= age in months, Iba-1-IR= Iba-1-immunoreactive.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

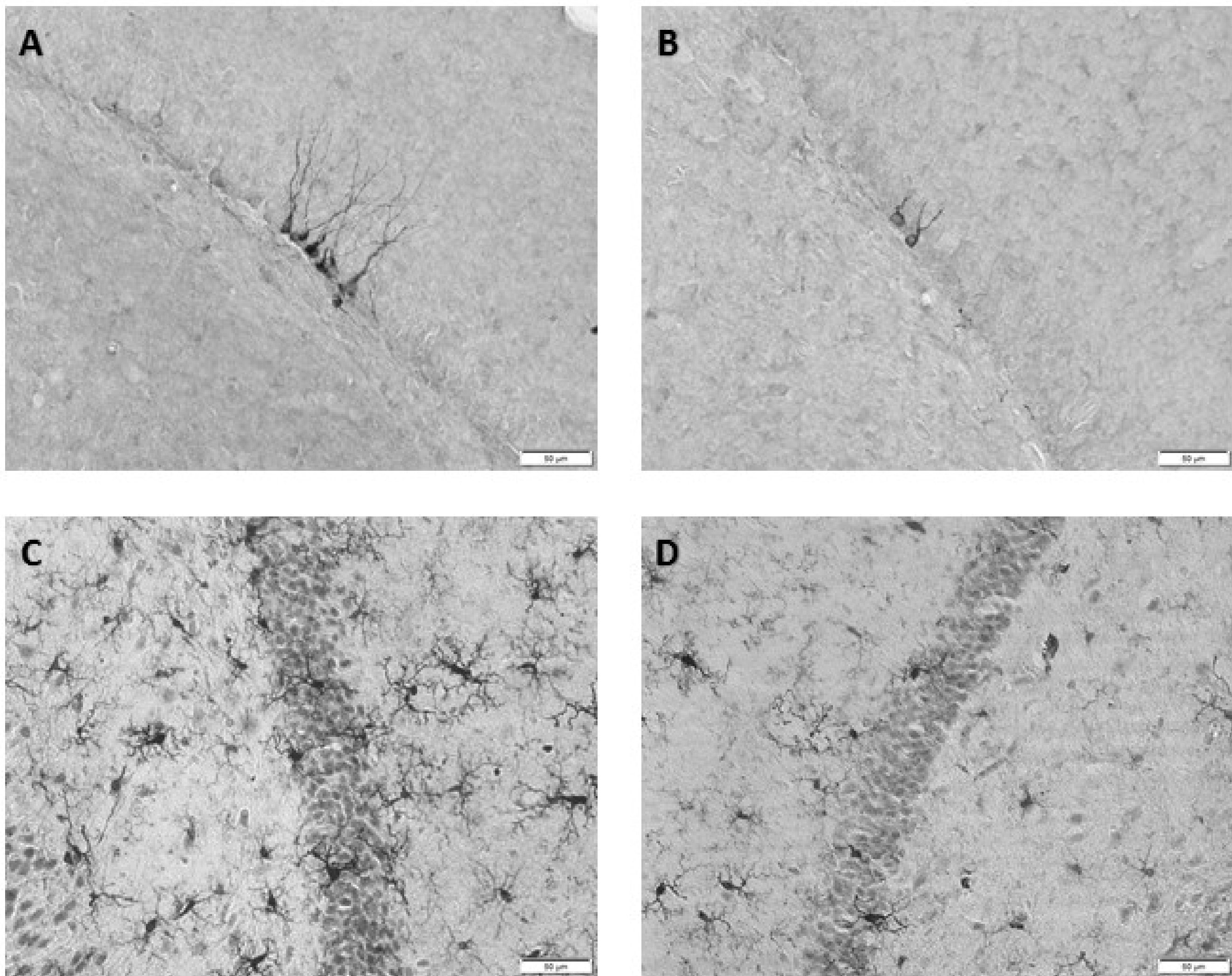

Figure 5. Representative photomicrographs of the granule cell layer in the dentate gyrus. **(A)** Doublecortin-immunoreactive cells in an 8-month-old nulliparous rat. **(B)** Doublecortin-immunoreactive cells in an 8-month-old primiparous rat on postpartum day 30. **(C)** Iba-1-immunoreactive cells in a 7.5-month-old nulliparous rat. **(D)** Iba-1-immunoreactive cells in a 7.5-month-old primiparous rat on postpartum day 8.

337 **3.9. Serum IFN-γ and IL-10 showed an age-related increase in nulliparous but not primiparous**
338 **rats.**

339 There was a significant ageing-related increase in IFN-γ in nulliparous rats, in which 13-month-old
340 nulliparous rats had significantly higher IFN-γ levels than all other nulliparous groups (all p's <0.0044;
341 planned comparisons; **Fig. 6A**). No significant differences were found in IFN-γ levels between any
342 primiparous groups (all p's >0.04, non-significant due to Bonferroni correction). Further, at 13 months,
343 IFN-γ levels were higher in nulliparous relative to primiparous rats ($p = 0.023$; **Fig. 6A**). There was also
344 a significant main effect of time ($p=0.00042$), but not reproductive status ($p=0.14$) nor an interaction
345 ($p=0.325$).

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

346 Similarly, serum IL-10 increased significantly with age in nulliparous but not primiparous rats;
347 significantly higher levels of IL-10 were detected in 13-month old nulliparous rats relative to all other
348 nulliparous groups (P<0.0015; **Fig. 6B**), but no significant differences were found between any of the
349 primiparous groups (all p's>0.05; planned comparisons; **Fig. 6B**). There was also a significant main
350 effect of time (p=0.0024), but not reproductive status (p=0.11) nor an interaction (p=0.45).

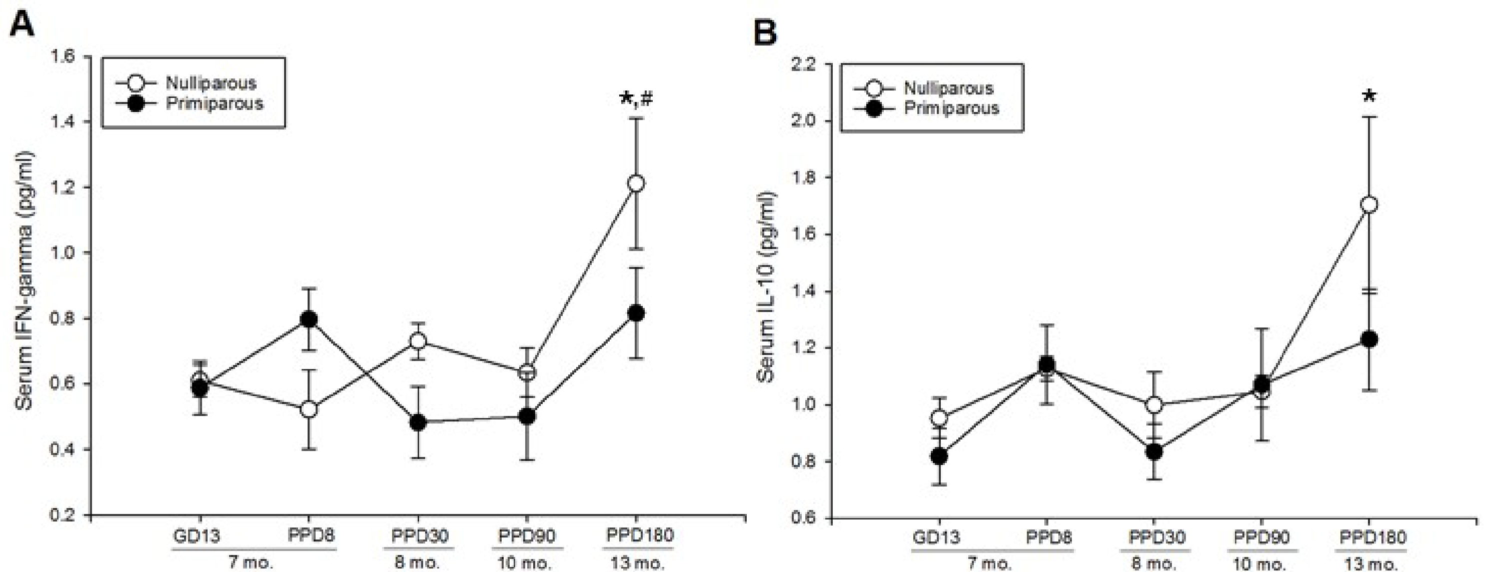

Figure 6. Serum levels of interferon-γ **(A)** and interleukin-10 **(B)** in primiparous and nulliparous rats. The x-axis represents time relative to gestation and parturition in primiparous groups, and approximate age in months. There was a significant ageing-related increase in IFN-γ **(A)** and IL-10 **(B)** in nulliparous but not primiparous rats. * indicates p <0. 005, significantly different from all other nulliparous groups. # indicates p = 0.023, significantly different from13-month-old primiparous rats. Data are represented in mean values ± SEM. IFN-γ = interferon gamma, IL-10= interleukin 10, GD= gestation day, PPD= postpartum day.

351 **3.10. Serum IL-4 was transiently increased in the early postpartum then persistently reduced by**
352 **parity**

353 Nulliparous rats had higher levels of IL-4 than primiparous rats, regardless of time point (main effect of
354 reproductive status: F(1, 38)=7.63, p=0.0088, **Fig. 7A**). Regardless of reproductive status, there was an
355 age-related increase in serum IL-4, with significantly elevated levels at 13 months compared to all
356 groups at 8 months of age and younger (all p's< 0.04; main effect of time: F(4, 38)=4.29, p=0.0058).
357 There was no significant reproductive status by time interaction (F(4, 38)=1.73, p=0.16), but a priori we
358 expected cytokine levels to be altered in the early postpartum period in primiparous rats. Indeed,
359 primiparous rats showed a trend for a transient increase in serum IL-4 in the early postpartum period,
360 with higher levels at PPD8 relative to GD13 (p=0.029) and PPD30 (0.027; a priori comparisons, missing

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

361 significance with correction; **Fig. 7A**). In age-matched nulliparous control groups, serum IL-4 was not
362 significantly different in 7.5- relative to 7-or 8-month-old rats (p's >0.45).

363 **3.11. Serum IL-5 was transiently reduced during gestation and the postpartum period, and**
364 **showed an age-related decline in nulliparous but not primiparous rats.**

365 Serum IL-5 levels were reduced during pregnancy (GD13) and the early postpartum period (PPD8) in
366 primiparous rats relative to age matched nulliparous controls (all p's <0.006; time by reproductive status
367 interaction, F(4, 37)=2.762, p=0.042; **Fig. 7B**). Further, IL-5 levels declined with age in nulliparous
368 animals, as higher levels were detected at 7 months relative to 8 (p=0.017), 10 (p=0.0095), and 13
369 months (p=0.015; **Fig. 7B**). Although non-significant, IL-5 levels increased in primiparous animals with
370 age, suggesting a reversed pattern of age-related changes in IL-5 compared to nulliparous rats. There
371 were no significant main effects of time or reproductive status (all p's >0.28).

372 **3.12. Serum IL-13 increased with age regardless of reproductive status**

373 Regardless of reproductive status, IL-13 levels were elevated at 13 months compared to all other time
374 points (all p's<0.025; main effect of time: F(4, 37)=4.390, p=0.0053; **Fig. 7C**). There was no significant
375 main effect of reproductive status and no interaction (all p's >0.3).

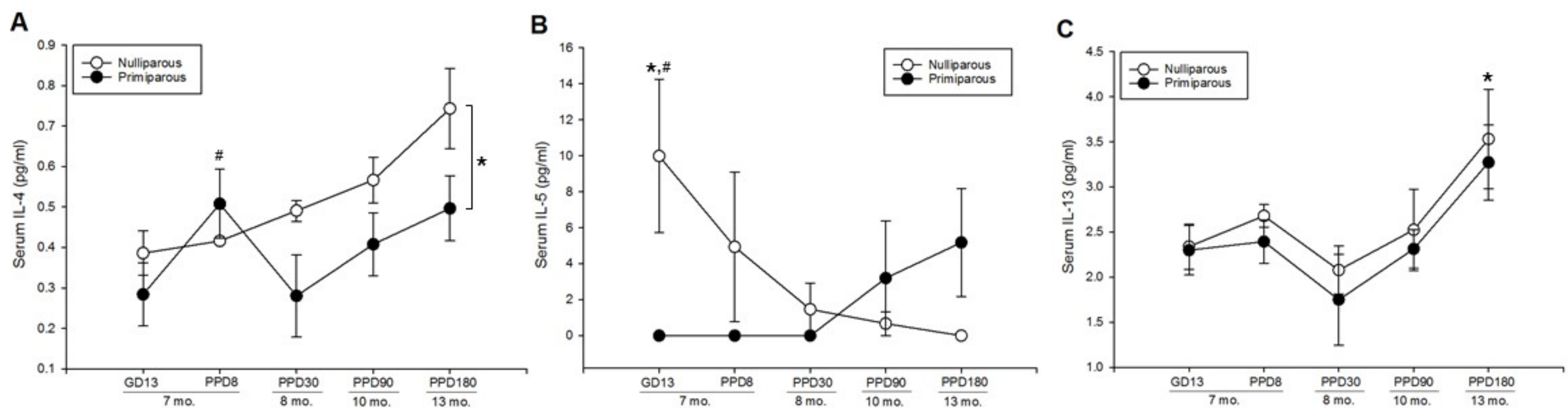

Figure 7. Serum levels of interleukin-4 **(A)**, interleukin-5 **(B)**, and interleukin-13 **(C)** in primiparous and nulliparous rats. The x-axis represents time relative to gestation and parturition in primiparous groups, and approximate age in months. **(A)** Serum IL-4 levels were transiently increased in primiparous rats at PPD8, but persistently suppressed by parity thereafter. # indicates p<0.03, trend towards significance relative to GD13 and PPD30. * indicates p=0.0088, main effect of primiparity to reduce IL-4 levels **(B)** Serum IL-5 was transiently blunted during gestation and the early postpartum period. A significant age-related decline in serum IL-5 was found only in nulliparous rats. * indicates p<0.006, significantly higher than primiparous groups at GD13 and PPD8. # indicates p<0.02, significantly higher than all nulliparous groups between 8 and 13 months of age. **(C)** Serum IL-13 increased significantly with age, regardless of reproductive status. * indicates p<0.02, significantly different from all other time points. Data are represented in mean values ± SEM. IL-4= interleukin-4; IL-5 = interleukin-5; IL-13 = interleukin-13; GD= gestation day; PPD = postpartum day; mo.= age in months.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

376 **3.13. Serum IL6, CXCL1, TNF-α, and IL-1β were not significantly altered by parity or age**

377 There were no significant main effects of reproductive status or age, nor an interaction for serum levels
378 of IL-6 (all p's >0.46, **Fig. 8A**), CXCL1 (all p's >0.40 **Fig. 8B**), or TNF-α (all p's >0.33 **Fig. 8C**). There
379 was a weak trend towards a significant main effect for parity to increase serum IL-1β levels (p=0.093,
380 **Fig. 8D**), but no significant main effect of time or an interaction (all p's>0.4).

381 **3.14. Principal Component Analysis of serum cytokines**

382 The model generated 4 principal components, which accounted for 82.6% of the variance within the
383 dataset, with the first principal component explaining 40.4% of the variance, the second 18.2%, the third
384 12.6%, and the fourth 11.4%. Interestingly, IFN-γ, IL-10, IL-13, and IL-4 loaded heavily onto Principal
385 Component 1 (PC1; see **Table 3**). These same cytokines also showed the most robust alterations with
386 parity and age with ANOVA, therefore the PCA ultimately verified our individual ANOVA analyses.
387 Subsequently, we analyzed PC1 scores using ANOVA, which revealed a main effect of time (F(4,
388 37)=3.78, p=.011; **Fig. 8E**), with higher scores at 13 months of age relative to all other age groups (all
389 p's <0.033). Planned comparisons reveal a more robust age-related increase in PC1 scores in nulliparous
390 rats, with higher scores in 13- relative to 7- and 8-month-old rats (p's<0.0072). In contrast, there were no
391 significant differences in PC1 scores between any primiparous groups (p's>0.038; non-significant due to
392 Bonferroni correction). There was no significant main effect of reproductive status, nor an interaction
393 (p's >0.11). Interestingly, the pattern observed here is akin to the age-related increase in IL-10, IFN- γ,
394 and IL-4 in nulliparous rats, obtained with individual ANOVA analyses. Further, IL-6 and TNF-α loaded
395 heavily onto PC2, and IL-1β and IL-5 loaded heavily onto PC3 and PC4, respectively (Table 1).

Table 3. Principal Component Analysis loading table.

	PC1	PC2	PC3	PC4
IFN-γ	**0.87**	-0.28	0.01	-0.04
IL-10	**0.91**	-0.15	-0.12	0.027
IL-13	**0.87**	-0.18	0.27	0.12
IL-1ß	-0.06	0.25	**0.78**	-0.46
IL-4	**0.89**	-0.17	-0.05	0.03
IL-5	-0.24	0.10	0.30	**0.87**
IL-6	0.46	**0.77**	-0.11	0.091
CXCL1	0.41	0.46	0.42	0.11
TNF-α	0.25	**0.77**	-0.39	-0.096

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

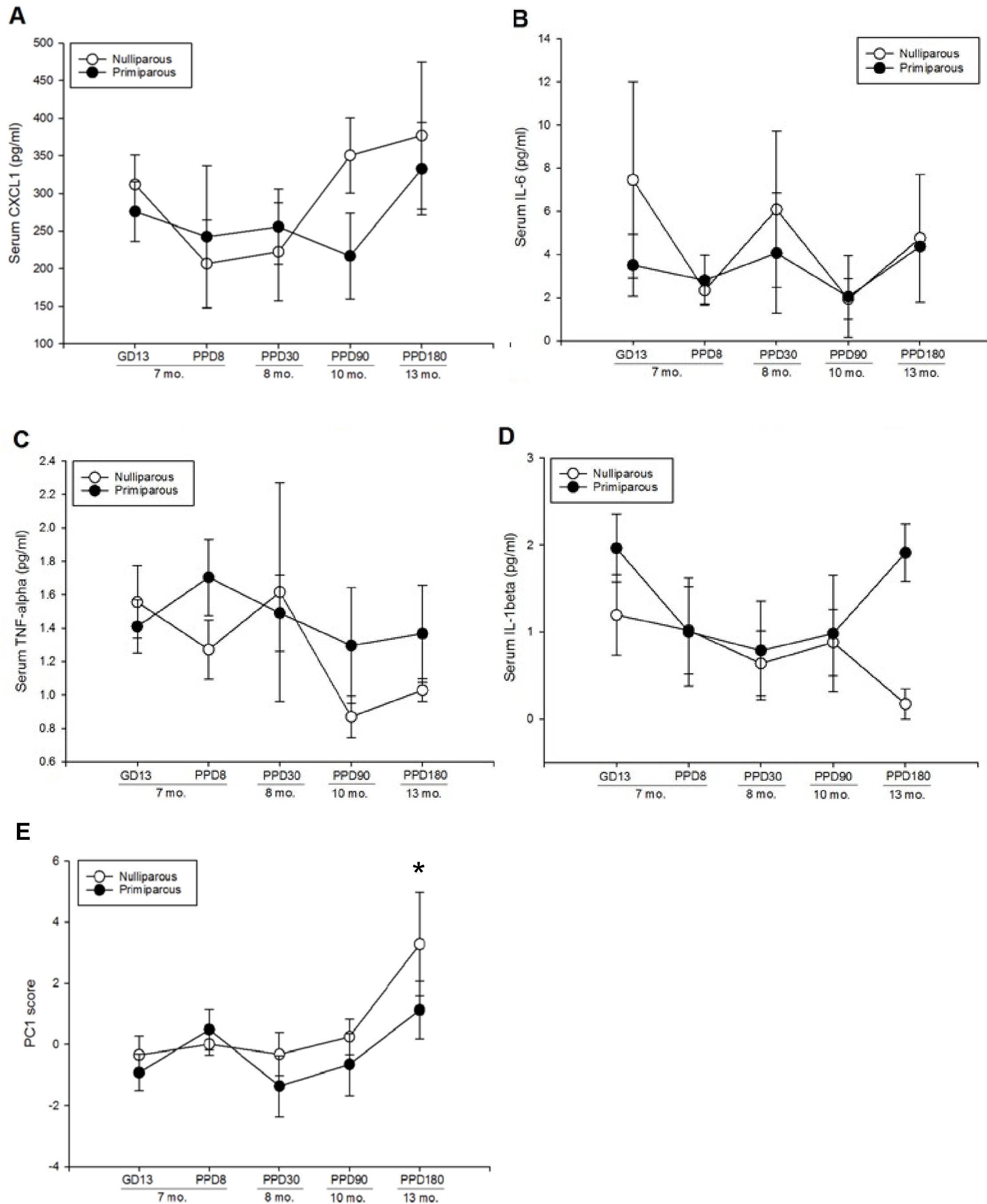

Figure 8. Serum concentrations of CXCL1 **(A)**, interleukin-6 **(B)**, TNF-α **(C)**, and interleukin-β **(D)** in primiparous and nulliparous rats. The x-axis represents time relative to gestation and parturition in primiparous rats, and approximate age in months. Reproductive status and age had no significant effects on serum levels of CXCL1 **(A)**, interleukin-6 **(B)**, TNF-α **(C)**, and interleukin- β **(D)**. Principal Component 1 scores in primiparous and nulliparous rats **(E)**, * indicates p's<0.0072, significantly higher PC1 scores in 13- relative to 7- and 8-month-old nulliparous rats. Data are represented in mean values ± SEM. GD= gestation day, PPD= postpartum day.

396 **3.15. Increased average length of Iba-1-IR cell processes was associated with more Ki67- and DCX-**
397 **IR cells in nulliparous but not primiparous rats**

398 Increased average length of processes was significantly correlated with a higher number of Ki67-IR cells

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

399 in nulliparous ($r = 0.58$, $p = 0.005$; **Fig. 9A**), but not primiparous rats ($r = 0.089$, $p = 0.67$). Similarly,
400 increased average length of processes was significantly correlated with a higher number of DCX-IR cells
401 in nulliparous ($r = 0.62$, $p = 0.002$; **Fig. 9B**), but not primiparous rats ($r = 0.11$, $p = 0.60$; **Fig. 9B**).

402 **3.16. Higher Iba-1-IR cell density was associated with fewer Ki-67- and DCX-IR cells in primiparous**
403 **but not nulliparous rats**

404 Increased Iba-1-IR cell density was significantly correlated with fewer Ki67-IR cells in primiparous rats
405 ($r = -0.45$, $p = 0.025$; **Fig. 9C**) but not nulliparous rats ($r = -0.018$, $p = 0.94$; **Fig. 9C**). Similarly, increased
406 Iba-1-IR cell density was significantly correlated with fewer DCX-IR cells in primiparous rats ($r = -0.43$,
407 $p = 0.032$; **Fig. 9D**) but not nulliparous rats ($r = -$ 0. 19, $p = 0.19$; **Fig. 9D**).

408 **3.17. Serum IL-10, IL-4, and IFN-γ correlated negatively with average Iba-1-IR cell process length**
409 **in primiparous but not nulliparous rats.**

410 Interestingly, in primiparous rats, the average length of Iba-1-IR cell processes was negatively correlated
411 with serum IL-10 ($r = -0.56$, $p = 0.005$; **Fig. 9E**), IL-4 ($r = -0.6$, $p = 0.002$; **Fig. 9F**), and IFN-γ ($r = -$
412 0.47, $p = 0.022$, **Fig. 9G**). On the other hand, length of cell processes was not significantly correlated
413 with IL-10, IL-4, or IFN-γ in nulliparous rats (all p's > 0.36). Regardless of reproductive status, no other
414 significant correlations were found between length of Iba-1-IR cell processes and all other measured
415 cytokines (all p's>0.056; all trends towards significance appear in primiparous groups only). Further,
416 there were no significant correlations between any of the cytokines and Iba-1-IR cell density, regardless
417 of reproductive status (all p's > 0.23).

418 **3.17. Higher concentrations of serum IL-1β were associated with larger Iba-1-IR soma size in**
419 **primiparous but not nulliparous rats**

420 In primiparous rats, serum IL-1β was positively correlated with Iba-1-IR soma size ($r = 0.44$, $p = 0.048$;
421 **Fig. 9H**), but this relationship did not exist in nulliparous rats ($p=0.62$). Regardless of reproductive
422 status, no other significant correlations were found between Iba-1-IR soma size and all other measured
423 cytokines (all p's>0.1).

424 **3.18. Higher Serum IL-4 concentrations were associated with fewer Ki67- and DCX-IR cell in**
425 **nulliparous but not primiparous rats**

426 In nulliparous rats, increased serum concentrations of IL-4 were associated with fewer DCX-IR and Ki67-
427 IR cells (Ki67: -0.57, $p=0.006$; **Fig. 9I**; and DCX: $r = -$ 0.70, $p<0.001$; **Fig. 9J**). There were no significant

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

428 correlations between any cytokines and Ki67- or DCX-IR cells in primiparous rats (all p's > 0.19).

429 **3.19. DCX-IR and Ki67-IR cell numbers were positively correlated in primiparous but not**
430 **nulliparous rats**

431 Increased Ki-67-IR cell number was significantly associated with more DCX-IR cells in nulliparous rats
432 (r = 0.86, p<0.0001; **Fig. 9K**), but not in primiparous rats (r = 0.30, p = 0.15; **Fig. 9K**)

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Figure 9. Correlations between dependent variables of interest. Increased average length of Iba-1-immunoreactive (IR) cell processes was associated with higher Ki67 **(A)** and doublecortin (DCX; **B**) expression in nulliparous rats. Higher Iba-1 density was associated with increased Ki67 **(C)** and DCX expression **(D)** in primiparous rats Elevated serum concentrations of IL-10 **(E)**, IL-4 **(F)**, and IFN-γ **(G)** were correlated with shorter Iba-1-IR cell processes in primiparous rats. Higher concentrations of serum IL-1β were associated with larger Iba-1-IR soma size in primiparous but not nulliparous rats (**H**). In nulliparous rats only, increased Ki67-IR **(I)** and DCX-IR **(J)** cell number was significantly associated with lower serum IL-4. KI67-IR cell number was positively correlated with DCX-IR cell number in nulliparous rats only **(K)**.

433 **4. Discussion**

434 Here, we report short- and long-term effects of maternal experience on hippocampal neurogenesis,
435 microglial density and morphology in the dentate gyrus, and circulating cytokine levels, culminating six
436 months after parturition. We found that adult hippocampal neurogenesis was suppressed in mid-
437 gestation and up to one month postpartum. Interestingly, the ageing trajectory of neurogenesis was
438 modulated by reproductive experience, as neurogenesis levels declined from 7 to 13 months of age in
439 nulliparous rats, but showed a slight increase in primiparous rats across the same period. Hippocampal
440 cell proliferation was suppressed in mid-gestation and the early postpartum period in primiparous rats,
441 but normalized thereafter, as an age-related decline in cell proliferation was observed regardless of
442 previous parity. Further, microglia in the dentate gyrus displayed a more activated morphology in the
443 early postpartum period, followed by a transient increase in microglial density in the later postpartum
444 period and overall smaller microglia soma size in primiparous compared to nulliparous rats. We found
445 alterations in circulating cytokine levels during pregnancy and the early postpartum period, and more
446 intriguingly, we show that the age-related changes in circulating cytokine levels were dependent on
447 parity. Finally, we observed that reproductive status shifted the associations between microglia and
448 neurogenesis, with Iba-1-IR density being negatively associated with neurogenesis in primiparous but
449 not nulliparous rats, and length of Iba-1-IR cell processes being positively associated with neurogenesis
450 in nulliparous but not primiparous. Further, parity modulated the correlations between serum cytokines
451 and microglial morphology, and between serum cytokines and neurogenesis levels. These correlations
452 suggest that the relationships between immune processes and neurogenesis may be modified with parity.
453 Collectively our data suggest that maternal experience has transient and delayed effects on hippocampal
454 neurogenesis, microglia, and the peripheral inflammatory milieu.

455 **4.1. Adult hippocampal neurogenesis was suppressed during gestation and the postpartum period**

456 We report that adult hippocampal neurogenesis, measured via the expression of doublecortin, was
457 suppressed beginning in mid-gestation in primigravid rats. Few studies to date have examined the
458 survival of new cells in the maternal hippocampus during gestation (Pawluski et al., 2010; Rolls et al.,

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

459 2008). Our findings are, however, consistent with a study that found suppressed neurogenesis in
460 pregnant mice during mid- and late-gestation (Rolls et al., 2008). In contrast, the expression of PSA-
461 NCAM was increased in the dentate gyrus of pregnant rats at GD18 (Banasr et al., 2001), indicating a
462 potential increase in neurogenesis, as PSA-NCAM is expressed on newly generated and migrating
463 neurons (Rutishauser, 2008). However, because PSA-NCAM is also expressed on neurons undergoing
464 other forms of plasticity (Rutishauser, 2008), its expression provides limited and non-specific
465 information regarding neurogenesis levels. Another study in rats found that the survival of cells
466 produced on gestation day 1 was not significantly altered when examined across gestation (Pawluski et
467 al., 2010), partially contrasting with our current findings. Importantly, however, DCX is expressed in
468 immature neurons between 2 hours and 21 days after production (Brown et al., 2003). Therefore, our
469 current data provide information on the population of cells produced as early as 8 days prior to
470 impregnation, and as late as the day of euthanasia (GD13). Therefore, inconsistencies between the two
471 studies are not surprising, considering that the cell populations examined were produced under different
472 conditions. We also observe a concurrent reduction in cell proliferation on GD13 indicating that this
473 may underlie the decline in immature neurons at this time. Although no prior studies have examined
474 cell proliferation in mid-gestation, cell proliferation was not altered on GD1 (Pawluski et al., 2010),
475 GD7 (Shingo et al., 2003), or GD21 (Furuta and Bridges, 2005). Therefore, a more detailed time-course
476 analysis of hippocampal plasticity during pregnancy is warranted.

477 Consistent with a prior study (Workman et al., 2015), we also found reduced DCX expression in
478 the postpartum period, evident until PPD30. This finding is also in keeping with past work showing
479 reduced survival in new cells labelled on PPD2 and examined 21 days later (Pawluski and Galea, 2007),
480 and in new cells labelled in mid-gestation (GD11-12) and examined 14 days later, in the early postpartum
481 period (Rolls et al., 2008). We found reductions in hippocampal cell proliferation in the early postpartum
482 period, which normalized by PPD30, in line with previous data (Darnaudéry et al., 2007; Leuner et al.,
483 2007; Pawluski and Galea, 2007; Rolls et al., 2008). Thus, the suppression in immature neurons found at
484 PPD8 likely resulted from a reduction in both cell proliferation and survival, whereas the suppression at
485 PPD30 is likely due to decreased cell survival rather than proliferation. Although the functional
486 significance is not known, suppressed neurogenesis in the maternal brain may be mechanistically
487 associated with the enhanced susceptibility to mood disorders in the peripartum period (Hendrick et al.,
488 1998). Further, separate lines of evidence indicate that adult neurogenesis is involved in hippocampal
489 regulation of the HPA axis at least in males (Schloesser et al., 2009; Snyder et al., 2011), and that the HPA
490 axis undergoes substantial adaptations during pregnancy and the postpartum (De Weerth and Buitelaar,

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

491 2005; Lightman et al., 2001; Slattery and Neumann, 2008). Therefore, reductions in neurogenesis in the
492 maternal hippocampus could influence HPA axis function. Suppressed neurogenesis may also be linked
493 to deficits in hippocampus-dependent learning and memory reported in late pregnancy and the early
494 postpartum period (reviewed in (Workman et al., 2012)).

495 **4.2. Maternal experience altered the trajectory of age-related changes in hippocampal neurogenesis**

496 Between 8 and 13 months of age, immature neurons in the dentate gyrus declined significantly in
497 nulliparous rats but showed a slight increase in primiparous rats. We observe an age-related decline in
498 cell proliferation (Ki67-IR cells) regardless of reproductive status, suggesting that the differential effects
499 in immature neurons (DCX-IR cells) are driven by differences in cell survival. Previous work indicates
500 that hippocampal neurogenesis steadily declines with age, with the most substantial decline occurring
501 between adulthood and middle age in female rats (Driscoll et al., 2006; Kuhn et al., 1996; Nacher et al.,
502 2003), consistent with our current data from nulliparous rats. Thus, the increase in neurogenesis levels in
503 middle-aged primiparous rats suggests that reproductive experience can modify the trajectory of age-
504 related alterations in neurogenesis. Alternatively, it may also be reasonable to interpret this finding as
505 merely a normalization of neurogenesis to nulliparous levels. However, two previous reports indicate
506 higher neurogenesis levels in primiparous and multiparous relative to nulliparous middle-aged rats
507 (Barha et al., 2015; Galea et al., 2018), and as such an altered aging trajectory is conceivable. It is
508 possible that a more robust difference in neurogenesis levels would arise only after multiple
509 reproductive experiences or later into middle age. There is emerging evidence from human and rodent
510 studies suggesting that motherhood can alter the course of age-related cognitive decline (Beeri et al.,
511 2009; Colucci et al., 2006; Cui et al., 2014; Gatewood et al., 2005). For example, reproductive
512 experience mitigated the age-related decline in spatial memory in rats (Gatewood et al., 2005) and mice
513 (Cui et al., 2014). Other studies indicate that parity is associated with cognitive impairment in the ageing
514 female (Beeri et al., 2009; Colucci et al., 2006). These inconsistencies may be reconciled by more
515 complex interactions with genetic factors that have been associated with pathological cognitive ageing
516 (Corbo et al., 2007; Cui et al., 2014). While speculative, the modest increase in hippocampal
517 neurogenesis in middle-aged primiparous rats may be associated with enhanced hippocampus-dependent
518 cognition that is seen at that time.

519 Interestingly, we observed differences in the relationship between levels of cell proliferation and
520 immature neurons depending on reproductive status, such that a significant positive correlation between
521 the two measures was only seen in nulliparous rats. In addition, increased IL-4 concentrations were

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

522 associated with reduced proliferation and immature neurons in the hippocampus of nulliparous but not
523 primiparous rats. Previous work points to a role of IL-4 in the regulation of cell proliferation under
524 conditions of neurodegeneration (Bhattarai et al., 2016), therefore our findings suggest that this role may
525 be altered by parity. These relationships should be further investigated, as they appear when
526 reproductive status groups are collapsed across age, but nonetheless indicate that parity may modulate
527 the effects of immune signaling on hippocampal neurogenesis.

528 **4.3. Microglia assumed a de-ramified morphology in the early postpartum period**

529 Microglia display a predominantly ramified morphology under basal conditions, and de-ramification is
530 thought to be indicative of increased classical activation under inflammatory conditions (Luo and Chen,
531 2012). Here, we show that microglia in the dentate gyrus exhibited significantly shortened processes at 8
532 days postpartum, suggesting an increase in microglial activation in the early postpartum period. This
533 was a transient morphological modification as the average length of processes was not significantly
534 different from nulliparous controls by PPD30. Only two studies to date have examined microglia in the
535 maternal brain (Haim et al., 2017; Posillico and Schwarz, 2016), with findings partially consistent with
536 our current data. Haim et al. (2017) reported a decrease in the number of microglia with a ramified
537 morphology on postpartum day 8, in several regions including the dorsal hippocampus, in line with the
538 microglial de-ramification that we observed on the same postpartum day. In the current study, we found
539 an increase in the density of microglia in the dorsal and ventral dentate gyrus at PPD30, but no alteration
540 in density during gestation or the early postpartum period. While no other studies have examined
541 microglia in the maternal brain as late as 30 days postpartum, our finding that microglial density in the
542 hippocampus remains unchanged during gestation and the early postpartum period contrasts previous
543 reports (Haim et al., 2017; Posillico and Schwarz, 2016). These previous studies found reduced
544 microglial density in several brain regions from late gestation to the early postpartum (GD20, and PPD1,
545 8, and 21: Haim et al., 2017; PPD0: Posillico and Schwarz, 2016). Further, Haim and colleagues (2017)
546 found that microglial density normalized to nulliparous control levels by PPD21 in all regions examined
547 except the dorsal hippocampus. It appears, however, that the inconsistencies in findings may be
548 accounted for by differences in microglial densities within sub-regions of the dentate gyrus, or by
549 methodological differences related to density measurement. For example, Haim et al. (2017) examined
550 Iba-1 density within the dorsal dentate gyrus only, whereas we included samples from both the dorsal
551 and ventral dentate gyrus. In addition, as we were primarily interested in the neurogenic niche, we
552 quantified Iba-1-IR cells within the GCL, the SGZ, and a thin band of the ML, whereas Haim and
553 colleagues did not specify sub-regions within the dentate gyrus. Finally, Haim et al (2017) utilized

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

554 optical density, whereas density here was defined as the number of cells per volume of dentate gyrus.
555 Interestingly, the increase in microglial density that we find at PPD30 coincides with a return to
556 normalized microglial morphology. We speculate that this may represent a resolution from the pro-
557 inflammatory state at PPD8. Overall, our novel data provide an important addition to the literature
558 indicating that pregnancy-related immune adaptations are not limited to the periphery, but also exist in
559 the brain. More specifically, our data indicate the existence of a pro-inflammatory hippocampal
560 environment in the early postpartum period. Importantly, increased microglial activation may be central
561 to the pathophysiology of depression (Kreisel et al., 2014; Miller and Raison, 2015; Setiawan et al.,
562 2015), thus it is conceivable that similar processes are implicated in postpartum depression. Our current
563 finding suggesting increased microglial activation in the early postpartum may represent a neural
564 mechanism of enhanced susceptibility to mood disorders in the postpartum period.

565 The effects of parity on microglial morphology were not limited to the early postpartum period.
566 Specifically, although the number of microglial cell processes increased significantly with age in
567 nulliparous rats, this effect was prevented by parity. Further, parity reduced microglial soma size, an
568 effect which appears to emerge at PPD30 onwards. The functional significance of these alterations
569 cannot be determined from the current study, but as increased microglial soma size is indicative of
570 classical activation, it is possible that parity may dampen microglial activation in the ageing brain. To
571 gain better insight into the functional significance of these changes, future studies should investigate
572 how previous parity may impact microglial structure and function in the ageing brain in response to an
573 immune challenge.

574 Interestingly, we found that reproductive status affected the associations between microglia and
575 neurogenesis in the hippocampus. Specifically, increased cell proliferation and immature neurons were
576 significantly associated with more ramified microglial morphology in nulliparous rats only. On the other
577 hand, increased cell proliferation and immature neurons were associated with higher microglial density
578 in primiparous rats only. These observations suggest that the neuroimmune regulation of adult
579 hippocampal neurogenesis is affected by reproductive status. Importantly, in addition phagocytic activity
580 during development and disease, microglia are important players in the regulation of adult hippocampal
581 neurogenesis, where they phagocytose apoptotic new cells, while maintaining ramified morphology and
582 a non-inflammatory environment (Sierra et al., 2010). Thus, future research should directly examine the
583 possibilities of microglial phagocytosis in the regulation of neurogenesis during pregnancy and the
584 postpartum period.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

585 **4.5. Maternal experience modifies the age-related changes in circulating cytokine levels**

586 In addition to expected cytokine alterations during pregnancy and the early postpartum period (Holtan et
587 al., 2015; Shimaoka et al., 2000), we observed both persistent and delayed effects of reproductive
588 experience on the circulating cytokine profile. Specifically, after an initial increase at PPD8, IL-4 levels
589 were persistently blunted in primiparous rats relative to nulliparous controls. Further, in nulliparous but
590 not primiparous rats, IFN-γ and IL-10 increased and IL-5 declined significantly with age. Adaptations to
591 the immune systems during pregnancy and the early postpartum period are well established (PrabhuDas
592 et al., 2015). On the other hand, little attention has been paid to potential long-term effects of
593 motherhood on the immune system. To our knowledge, only a few studies have examined the effect of
594 parity on immune systems in aged female mice and one in aged rats. Together these studies suggested
595 that parity may delay certain indicators of immune senescence, as they relate to alterations in cytokine
596 production *in vitro* from activated spleen cells (Barrat et al., 1997a), and the distribution of immune cell
597 populations in the spleen (Barrat et al., 1997b), and bone marrow (Barrat et al., 1999). Another study
598 from our laboratory found a trend for increased serum levels of IL-6 in primiparous compared to
599 nulliparous rats at 15 months of age (Galea et al., 2018). Here, we report that a single reproductive
600 experience alters serum cytokine levels when examined in middle age, up to six months after the
601 reproductive event.

602 Importantly, peripheral cytokines can affect brain function, as they can access the central nervous
603 system through various mechanisms, including active and passive transport, and the activation of
604 cytokine receptors on afferent nerve fibers (reviewed in Miller et al., 2014; Quan and Banks, 2007).
605 Thus, the effects of parity to modify age-related changes in peripheral cytokines may have ramifications
606 for the ageing brain in general, and more specifically the hippocampus, as it contains one of the highest
607 densities of proinflammatory cytokine receptors in the brain (reviewed in Loftis et al., 2010). For
608 instance, the effect of reproductive experience in moderating the age-related increase in IFN-γ may be
609 associated with the amelioration of age-related cognitive decline reported in primiparous animals (Cui et
610 al., 2014; Gatewood et al., 2005). This is a possibility given that IFN-γ has been shown to impair
611 cognitive function; for example, mice lacking IFN-γ exhibit enhanced performance on hippocampus-
612 dependent cognitive tasks and increased hippocampal neurogenesis (Monteiro et al., 2016).
613 Inflammation is a core characteristic of the ageing processes, and the pro-inflammatory cytokine IFN-γ
614 increases with age (Oxenkrug, 2011; Rodríguez et al., 2007). Thus, our current data suggest that parity
615 may prevent or delay at least certain aspects of ageing-related inflammation. Interestingly, from PPD30
616 onwards, we observed a sustained suppression in serum IL-4 in primiparous rats relative to nulliparous

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

617 controls. Traditionally considered an anti-inflammatory cytokine (Hart et al., 1989), elevated IL-4 in
618 nulliparous rats may be suggestive of a compensatory response to attenuate a pro-inflammatory state that
619 is indicated by elevated IFN- γ. However, IL-4 is pleiotropic (Milner et al., 2010), and as such also can
620 have pro-inflammatory properties. For example, sustained exposure to elevated levels of IL-4 was
621 associated with increased inflammation (Milner et al., 2010). The same study found prolonged IL-4
622 exposure to be associated specifically with elevated levels of IL-10 and IFN- γ, but not IL-6 and TNF-α.
623 This is in line with our current data in which IL-4, IL-10, and IFN- γ were concurrently elevated in
624 middle-aged nulliparous rats. Thus, the cytokine profile in nulliparous groups may be alternatively
625 driven by an increase in IL-4. Importantly, IL-4 and IL-10 have also been implicated in cognitive
626 function. For example, IL-4 deficient mice show impaired cognition (Derecki et al., 2010), and IL-10
627 mitigated cognitive deficits under inflammatory conditions (Richwine et al., 2009). Because the
628 cytokines that we observe to be elevated in nulliparous middle-aged rats have been shown to have
629 contradictory effects of cognition, it is difficult to form hypotheses regarding the potential net effects on
630 cognitive function. Further, parity affected the age-related changes in IL-5, with declining levels in
631 nulliparous but not primiparous rats. IL-5 has anti-inflammatory properties, however its effects on the
632 brain are less studied. We investigated correlations between peripheral cytokines and microglial
633 morphology as a plethora of work suggests that systemic inflammation can profoundly affect microglial
634 activation (Reviewed in Hoogland et al., 2015). Interestingly, we found that higher concentrations of IL-
635 10, IL-4, and IFN-γ were associated with more de-ramified microglial morphology in primiparous but
636 not nulliparous rats. On the other hand, higher concentrations of IL-1β were associated with increase
637 microglial soma size in primiparous but not nulliparous rats. The mechanisms and consequences of these
638 altered relationships between peripheral cytokines and microglial morphology are not known, and these
639 findings should be interpreted with caution, as the correlations were detected when age was not included
640 as a factor. In the future, it is also important to examine whether parity may have similar effects on age-
641 related changes in brain cytokines, particularly in the hippocampus. While the functional consequences
642 of the observed differences in cytokine profiles are only speculative, our current findings clearly
643 demonstrate that the trajectory of immune senescence is altered by parity.

644 **5. Conclusions**

645 In summary, we report that maternal experience suppressed hippocampal neurogenesis (proliferation and
646 immature neurons) during gestation and the postpartum period and mitigated the decline in neurogenesis
647 in middle age (immature neurons). Maternal experience also resulted in transient microglial de-
648 ramification in the dentate gyrus, suggesting the existence of a pro-inflammatory hippocampal

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

649 environment in the early postpartum period. In addition to short-term cytokine alterations, maternal
650 experience modified the trajectory of age-related changes in circulating cytokine levels. These findings
651 should encourage future work aimed at delineating the functional consequences for behaviour and
652 immune function across the peripartum period and beyond, especially in relation to maternal mood and
653 cognition. Importantly, our data provide support for the notion that female reproductive history should
654 be regarded as an important determinant of ageing-related changes in physiology.

655 **Acknowledgments**

656 The Authors thank Dr. Timothy Kieffer and Travis Webber for generously providing access to their
657 Sector Imager, and Arianne Albert for her assistance with principal component analyses.

658 **Funding**

659 This work was supported by a grant from the Canadian Institute of Health Research to LAMG
660 (PJT148662), and a Four-Year Doctoral Fellowship from the University of British Columbia to RM.

661 **Conflicts of Interest**

662 The authors declare no conflicts of interest.

663 **References**

664 Altman, J., Das, G.D., 1965. Autoradiographic and histological evidence of postnatal hippocampal
665 neurogenesis in rats. J. Comp. Neurol. 124, 319–335. doi:10.1002/cne.901240303

666 Banasr, M., Hery, M., Brezun, J.M., Daszuta, A., 2001. Serotonin mediates oestrogen stimulation of cell
667 proliferation in the adult dentate gyrus. Eur. J. Neurosci. 14, 1417–1424. doi:10.1046/j.0953-
668 816X.2001.01763.x

669 Barha, C.K., Galea, L.A.M., 2011. Motherhood alters the cellular response to estrogens in the
670 hippocampus later in life. Neurobiol. Aging 32, 2091–2095.
671 doi:10.1016/j.neurobiolaging.2009.12.004

672 Barha, C.K., Hanna, C.W., Salvante, K.G., Wilson, S.L., Robinson, W.P., Altman, R.M., Nepomnaschy,
673 P.A., 2016. Number of children and telomere length in women: A prospective, longitudinal

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

674 evaluation. PLoS One 11. doi:10.1371/journal.pone.0146424

675 Barha, C.K., Lieblich, S.E., Chow, C., Galea, L.A.M., 2015. Multiparity-induced enhancement of
676 hippocampal neurogenesis and spatial memory depends on ovarian hormone status in middle age.
677 Neurobiol. Aging 36, 2391–2405. doi:10.1016/j.neurobiolaging.2015.04.007

678 Barrat, F., Lesourd, B., Boulouis, H.J., Thibault, D., Vincent-Naulleau, S., Gjata, B., Louise, A., Neway,
679 T., Pilet, C., 1997a. Sex and parity modulate cytokine production during murine ageing. Clin. Exp.
680 Immunol. 109, 562–568. doi:10.1046/j.1365-2249.1997.4851387.x

681 Barrat, F., Lesourd, B.M., Louise, A., Boulouis, H.J., Vincent-Naulleau, S., Thibault, D., Sanaa, M.,
682 Neway, T., Pilet, C.H., 1997b. Surface antigen expression in spleen cells of C57B1/6 mice during
683 ageing: influence of sex and parity. Clin. Exp. Immunol. 107, 593–600. doi:10.1046/j.1365-
684 2249.1997.3021199.x

685 Barrat, F.S., Lesourd, B.M., Louise, A.S., Boulouis, H.J., Thibault, D.J., Neway, T., Pilet, C.A., 1999.
686 Pregnancies modulate B lymphopoiesis and myelopoiesis during murine ageing. Immunology 98,
687 604–611. doi:10.1046/j.1365-2567.1999.00918.x

688 Beeri, M.S., Rapp, M., Schmeidler, J., Reichenberg, A., Purohit, D.P., Perl, D.P., Grossman, H.T.,
689 Prohovnik, I., Haroutunian, V., Silverman, J.M., 2009. Number of children is associated with
690 neuropathology of Alzheimer’s disease in women. Neurobiol. Aging 30, 1184–1191.
691 doi:10.1016/j.neurobiolaging.2007.11.011

692 Bennett, H.A., Einarson, A., Taddio, A., Koren, G., Einarson, T.R., 2004. Prevalence of depression
693 during pregnancy: systematic review. Obs. Gynecol 103, 698–709.
694 doi:10.1097/01.AOG.0000116689.75396.5f

695 Bhattarai, P., Thomas, A.K., Cosacak, M.I., Papadimitriou, C., Mashkaryan, V., Froc, C., Reinhardt, S.,
696 Kurth, T., Dahl, A., Zhang, Y., Kizil, C., 2016. IL4/STAT6 Signaling Activates Neural Stem Cell
697 Proliferation and Neurogenesis upon Amyloid-β42 Aggregation in Adult Zebrafish Brain. Cell Rep.
698 17, 941–948. doi:10.1016/j.celrep.2016.09.075

699 Bodnar, T.S., Taves, M.D., Lavigne, K.M., Woodward, T.S., Soma, K.K., Weinberg, J., 2017.
700 Differential activation of endocrine-immune networks by arthritis challenge: Insights from colony-
701 specific responses. Sci. Rep. 7, 1–14. doi:10.1038/s41598-017-00652-4

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

702 Boldrini, M., Fulmore, C.A., Tartt, A.N., Simeon, L.R., Pavlova, I., Poposka, V., Rosoklija, G.B.,
703 Stankov, A., Arango, V., Dwork, A.J., Hen, R., Mann, J.J., 2018. Human Hippocampal
704 Neurogenesis Persists throughout Aging. Cell Stem Cell 589–599. doi:10.1016/j.stem.2018.03.015

705 Bridges, R.S., 2015. Neuroendocrine regulation of maternal behavior. Front. Neuroendocrinol.
706 doi:10.1016/j.yfrne.2014.11.007

707 Brown, J.P., Couillard-Després, S., Cooper-Kuhn, C.M., Winkler, J., Aigner, L., Kuhn, H.G., 2003.
708 Transient Expression of Doublecortin during Adult Neurogenesis. J. Comp. Neurol. 467, 1–10.
709 doi:10.1002/cne.10874

710 Campbell, S., MacQueen, G., 2004. The role of the hippocampus in the pathophysiology of major
711 depression. [Review] [121 refs]. J. Psychiatry & Neurosci. 29, 417–426.

712 Catalano, R.D., Lannagan, T.R.M., Gorowiec, M., Denison, F.C., Norman, J.E., Jabbour, H.N., 2010.
713 Prokineticins: Novel mediators of inflammatory and contractile pathways at parturition? Mol. Hum.
714 Reprod. doi:10.1093/molehr/gaq014

715 Colucci, M., Cammarata, S., Assini, A., Croce, R., Clerici, F., Novello, C., Mazzella, L., Dagnino, N.,
716 Mariani, C., Tanganelli, P., 2006. The number of pregnancies is a risk factor for Alzheimer's
717 disease. Eur. J. Neurol. 13, 1374–1377. doi:10.1111/j.1468-1331.2006.01520.x

718 Corbo, R.M., Gambina, G., Ulizzi, L., Monini, P., Broggio, E., Rosano, A., Scacchi, R., 2007.
719 Combined effect of apolipoprotein e genotype and past fertility on age at onset of Alzheimer's
720 disease in women. Dement. Geriatr. Cogn. Disord. 24, 82–85. doi:10.1159/000103866

721 Cui, J., Jothishankar, B., He, P., Staufenbiel, M., Shen, Y., Li, R., 2014. Amyloid precursor protein
722 mutation disrupts reproductive experience-enhanced normal cognitive development in a mouse
723 model of alzheimer's disease. Mol. Neurobiol. 49, 103–112. doi:10.1007/s12035-013-8503-x

724 Cuttler, C., Graf, P., Pawluski, J.L., Galea, L. a M., 2011. Everyday life memory deficits in pregnant
725 women. Can. J. Exp. Psychol. 65, 27–37. doi:10.1037/a0022844

726 Darcy, J.M., Grzywacz, J.G., Stephens, R.L., Leng, I., Clinch, C.R., Arcury, T.A., 2011. Maternal
727 Depressive Symptomatology: 16-Month Follow-up of Infant and Maternal Health-Related Quality
728 of Life. J. Am. Board Fam. Med. 24, 249–257. doi:10.3122/jabfm.2011.03.100201

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

729 Darnaudéry, M., Perez-Martin, M., Del Favero, F., Gomez-Roldan, C., Garcia-Segura, L.M., Maccari,
730 S., 2007. Early motherhood in rats is associated with a modification of hippocampal function.
731 Psychoneuroendocrinology 32, 803–812. doi:10.1016/j.psyneuen.2007.05.012

732 De Groot, R.H., Vuurman, E.F., Hornstra, G., Jolles, J., 2006. Differences in cognitive performance
733 during pregnancy and early motherhood. Psychol. Med. 36, 1023–1032.
734 doi:10.1017/S0033291706007380

735 De Weerth, C., Buitelaar, J.K., 2005. Physiological stress reactivity in human pregnancy - A review, in:
736 Neuroscience and Biobehavioral Reviews. pp. 295–312. doi:10.1016/j.neubiorev.2004.10.005

737 Derecki, N.C., Cardani, A.N., Yang, C.H., Quinnies, K.M., Crihfield, A., Lynch, K.R., Kipnis, J., 2010.
738 Regulation of learning and memory by meningeal immunity: a key role for IL-4. J. Exp. Med. 207,
739 1067–1080. doi:10.1084/jem.20091419

740 Driscoll, I., Howard, S.R., Stone, J.C., Monfils, M.H., Tomanek, B., Brooks, W.M., Sutherland, R.J.,
741 2006. The aging hippocampus: A multi-level analysis in the rat. Neuroscience 139, 1173–1185.
742 doi:10.1016/j.neuroscience.2006.01.040

743 Dulac, C., O'Connell, L.A., Wu, Z., 2014. Neural control of maternal and paternal behaviors. Science
744 (80-.). 345, 765–770. doi:10.1126/science.1253291

745 Ekdahl, C.T., Kokaia, Z., Lindvall, O., 2009. Brain inflammation and adult neurogenesis: The dual role
746 of microglia. Neuroscience 158, 1021–1029. doi:10.1016/j.neuroscience.2008.06.052

747 Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., Gage,
748 F.H., 1998. Neurogenesis in the adult human hippocampus. Nat. Med. 4, 1313–7. doi:10.1038/3305

749 Furuta, M., Bridges, R.S., 2005. Gestation-induced cell proliferation in the rat brain. Brain Res Dev
750 Brain Res 156, 61–66.

751 Galea, L.A.M., Leuner, B., Slattery, D.A., 2014. Hippocampal plasticity during the peripartum period:
752 Influence of sex steroids, stress and ageing. J. Neuroendocrinol. doi:10.1111/jne.12177

753 Galea, L.A.M., Ormerod, B.K., Sampath, S., Kostaras, X., Wilkie, D.M., Phelps, M.T., 2000. Spatial
754 Working Memory and Hippocampal Size across Pregnancy in Rats. Horm. Behav. 37, 86–95.
755 doi:10.1006/hbeh.1999.1560

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

756 Galea, L.A.M., Roes, M.M., Dimech, C.J., Chow, C., Mahmoud, R., Lieblich, S.E., Duarte-Guterman,
757 P., 2018. Premarin has opposing effects on spatial learning, neural activation, and serum cytokine
758 levels in middle-aged female rats depending on reproductive history. Neurobiol. Aging 70, 291–
759 307. doi:10.1016/j.neurobiolaging.2018.06.030

760 Gatewood, J.D., Morgan, M.D., Eaton, M., McNamara, I.M., Stevens, L.F., MacBeth, A.H., Meyer,
761 E.A.A., Lomas, L.M., Kozub, F.J., Lambert, K.G., Kinsley, C.H., 2005. Motherhood mitigates
762 aging-related decrements in learning and memory and positively affects brain aging in the rat. Brain
763 Res. Bull. 66, 91–98. doi:10.1016/j.brainresbull.2005.03.016

764 Groer, M.E., Jevitt, C., Ji, M., 2015. Immune Changes and Dysphoric Moods Across the Postpartum.
765 Am. J. Reprod. Immunol. 73, 193–198. doi:10.1111/aji.12322

766 Guerreiro, R.J., Santana, I., Brás, J.M., Santiago, B., Paiva, A., Oliveira, C., 2007. Peripheral
767 inflammatory cytokines as biomarkers in Alzheimer's disease and mild cognitive impairment.
768 Neurodegener. Dis. 4, 406–412. doi:10.1159/000107700

769 Haim, A., Julian, D., Albin-Brooks, C., Brothers, H.M., Lenz, K.M., Leuner, B., 2017. A survey of
770 neuroimmune changes in pregnant and postpartum female rats. Brain. Behav. Immun. 59, 67–78.
771 doi:10.1016/j.bbi.2016.09.026

772 Hall, M.E., George, E.M., Granger, J.P., 2011. The Heart During Pregnancy. Rev. Española Cardiol.
773 (English Ed. 64, 1045–1050. doi:10.1016/j.rec.2011.07.008

774 Hart, P.H., Vitti, G.F., Burgess, D.R., Whitty, G.A., Piccoli, D.S., Hamilton, J.A., 1989. Potential
775 antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor
776 alpha, interleukin 1, and prostaglandin E2. Proc. Natl. Acad. Sci. 86, 3803–3807.
777 doi:10.1073/pnas.86.10.3803

778 Helle, S., Lummaa, V., Jokela, J., 2004. Accelerated immunosenescence in preindustrial twin mothers.
779 Proc. Natl. Acad. Sci. U. S. A. 101, 12391–12396. doi:10.1073/pnas.0402215101

780 Hendrick, V., Altshuler, L.L., Suri, R., 1998. Hormonal Changes in the Postpartum and Implications for
781 Postpartum Depression. Psychosomatics 39, 93–101. doi:10.1016/S0033-3182(98)71355-6

782 Hodes, G.E., Pfau, M.L., Leboeuf, M., Golden, S.A., Christoffel, D.J., Bregman, D., Rebusi, N.,
783 Heshmati, M., Aleyasin, H., Warren, B.L., Labonté, B., Horn, S., Lapidus, K.A., Stelzhammer, V.,

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

784 Wong, E.H.F., Bahn, S., Krishnan, V., Bolaños-Guzman, C.A., Murrough, J.W., Merad, M., Russo,
785 S.J., 2014. Individual differences in the peripheral immune system promote resilience versus
786 susceptibility to social stress. Proc. Natl. Acad. Sci. 111, 16136–16141.
787 doi:10.1073/pnas.1415191111

788 Hoekzema, E., Barba-Müller, E., Pozzobon, C., Picado, M., Lucco, F., García-García, D., Soliva, J.C.,
789 Tobeña, A., Desco, M., Crone, E.A., Ballesteros, A., Carmona, S., Vilarroya, O., 2016. Pregnancy
790 leads to long-lasting changes in human brain structure. Nat. Neurosci. doi:10.1038/nn.4458

791 Holtan, S.G., Chen, Y., Kaimal, R., Creedon, D.J., Enninga, E.A.L., Nevala, W.K., Markovic, S.N.,
792 2015. Growth modeling of the maternal cytokine milieu throughout normal pregnancy:
793 Macrophage-derived chemokine decreases as inflammation/counterregulation increases. J.
794 Immunol. Res. 2015. doi:10.1155/2015/952571

795 Hoogland, I.C.M., Houbolt, C., van Westerloo, D.J., van Gool, W.A., van de Beek, D., 2015. Systemic
796 inflammation and microglial activation: systematic review of animal experiments. J.
797 Neuroinflammation 12, 114. doi:10.1186/s12974-015-0332-6

798 Kinsley, C.H., Madonia, L., Gifford, G.W., Tureski, K., Griffin, G.R., Lowry, C., Williams, J., Collins,
799 J., McLearie, H., Lambert, K.G., 1999. Motherhood improves learning and memory. Nature 402,
800 137–138. doi:10.1038/45957

801 Korzhevskii, D.E., Kirik, O. V., 2016. Brain Microglia and Microglial Markers. Neurosci. Behav.
802 Physiol. 46, 284–290. doi:10.1007/s11055-016-0231-z

803 Kreisel, T., Frank, M.G., Licht, T., Reshef, R., Ben-Menachem-Zidon, O., Baratta, M. V, Maier, S.F.,
804 Yirmiya, R., 2014. Dynamic microglial alterations underlie stress-induced depressive-like behavior
805 and suppressed neurogenesis. Mol. Psychiatry 19, 699–709. doi:10.1038/mp.2013.155

806 Kuhn, H.G., Dickinson-Anson, H., Gage, F.H., 1996. Neurogenesis in the dentate gyrus of the adult rat:
807 age-related decrease of neuronal progenitor proliferation. J Neurosci 16, 2027–2033. doi:0270-6474

808 Lain, K.Y., Catalano, P.M., 2007. Metabolic changes in pregnancy. Clin. Obstet. Gynecol. 50, 938–948.
809 doi:10.1097/GRF.0b013e31815a5494

810 Lee, J., Lee, Y., Yuk, D., Choi, D., Ban, S., Oh, K., Hong, J., 2008. Neuro-inflammation induced by
811 lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

812 J. Neuroinflammation 5, 37. doi:10.1186/1742-2094-5-37

813 Leuner, B., Christian, M., Liron, N., Elizabeth, G., 2007. Maternal Experience Inhibits the Production of
814 Immature Neurons in the Hippocampus During the Postpartum Period Through. Hippocampus 17,
815 434–442. doi:10.1002/hipo

816 Leuner, B., Gould, E., 2010. Dendritic growth in medial prefrontal cortex and cognitive flexibility are
817 enhanced during the postpartum period. J. Neurosci. 30, 13499–503.
818 doi:10.1523/JNEUROSCI.3388-10.2010

819 Lightman, S.L., Windle, R.J., Wood, S.A., Kershaw, Y.M., Shanks, N., Ingram, C.D., 2001. Peripartum
820 plasticity within the hypothalamo-pituitary-adrenal axis, in: Progress in Brain Research. pp. 111–
821 129. doi:10.1016/S0079-6123(01)33009-1

822 Loftis, J.M., Huckans, M., Morasco, B.J., 2010. Neuroimmune mechanisms of cytokine-induced
823 depression: Current theories and novel treatment strategies. Neurobiol. Dis.
824 doi:10.1016/j.nbd.2009.11.015

825 Louveau, A., Harris, T.H., Kipnis, J., 2015. Revisiting the Mechanisms of CNS Immune Privilege.
826 Trends Immunol. doi:10.1016/j.it.2015.08.006

827 Luo, X.-G., Chen, S.-D., 2012. The changing phenotype of microglia from homeostasis to disease.
828 Transl. Neurodegener. 1, 9. doi:10.1186/2047-9158-1-9

829 Mahmoud, R., Wainwright, S.R., Chaiton, J.A., Lieblich, S.E., Galea, L.A.M., 2016. Ovarian hormones,
830 but not fluoxetine, impart resilience within a chronic unpredictable stress model in middle-aged
831 female rats. Neuropharmacology 107, 278–293. doi:10.1016/j.neuropharm.2016.01.033

832 Miller, A.H., Haroon, E., Raison, C.L., Felger, J.C., 2014. Cytokine Targets in the Brain: Impact on
833 Neurotransmitters and Neurocircuits Andrew. Depress Anxiety 30, 297–306. doi:10.1146/annurev-
834 cellbio-092910-154240.Sensory

835 Miller, A.H., Raison, C.L., 2015. The role of inflammation in depression: from evolutionary imperative
836 to modern treatment target. Nat. Rev. Immunol. 16, 22–34. doi:10.1038/nri.2015.5

837 Milner, J.D., Orekov, T., Ward, J.M., Cheng, L., Torres-Velez, F., Junttila, I., Sun, G., Buller, M.,
838 Morris, S.C., Finkelman, F.D., Paul, W.E., 2010. Sustained IL-4 exposure leads to a novel pathway

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

839 for hemophagocytosis, inflammation, and tissue macrophage accumulation. Blood 116, 2476–2483.
840 doi:10.1182/blood-2009-11-255174

841 Monje, M.L., 2003. Inflammatory Blockade Restores Adult Hippocampal Neurogenesis. Science (80-.).
842 302, 1760–1765. doi:10.1126/science.1088417

843 Monteiro, S., Ferreira, F.M., Pinto, V., Roque, S., Morais, M., de Sá-Calçada, D., Mota, C., Correia-
844 Neves, M., Cerqueira, J.J., 2016. Absence of IFNγ promotes hippocampal plasticity and enhances
845 cognitive performance. Transl. Psychiatry 6, e707. doi:10.1038/tp.2015.194

846 Mor, G., Cardenas, I., 2010. The Immune System in Pregnancy: A Unique Complexity. Am. J. Reprod.
847 Immunol. doi:10.1111/j.1600-0897.2010.00836.x

848 Nacher, J., Alonso-Llosa, G., Rosell, D.R., McEwen, B.S., 2003. NMDA receptor antagonist treatment
849 increases the production of new neurons in the aged rat hippocampus. Neurobiol Aging 24, 273–
850 284. doi:S0197458002000969 [pii]

851 O'Hara, M.W., 2009. Postpartum depression: What we know. J. Clin. Psychol. doi:10.1002/jclp.20644

852 Oatridge, A., Holdcroft, A., Saeed, N., Hajnal, J. V., Puri, B.K., Fusi, L., Bydder, G.M., 2002. Change in
853 brain size during and after pregnancy: Study in healthy women and women with preeclampsia. Am.
854 J. Neuroradiol. 23, 19–26.

855 Oxenkrug, G.F., 2011. Interferon-gamma-inducible kynurenines/pteridines inflammation cascade:
856 Implications for aging and aging-associated psychiatric and medical disorders. J. Neural Transm.
857 118, 75–85. doi:10.1007/s00702-010-0475-7

858 Parkhurst, C.N., Yang, G., Ninan, I., Savas, J.N., Yates, J.R., Lafaille, J.J., Hempstead, B.L., Littman,
859 D.R., Gan, W.B., 2013. Microglia promote learning-dependent synapse formation through brain-
860 derived neurotrophic factor. Cell 155, 1596–1609. doi:10.1016/j.cell.2013.11.030

861 Pawluski, J.L., Barakauskas, V.E., Galea, L.A.M., 2010. Pregnancy decreases oestrogen receptor alpha
862 expression and pyknosis, but not cell proliferation or survival, in the hippocampus. J.
863 Neuroendocrinol. 22, 248–257. doi:10.1111/j.1365-2826.2010.01960.x

864 Pawluski, J.L., Galea, L.A.M., 2007. Reproductive experience alters hippocampal neurogenesis during
865 the postpartum period in the dam. Neuroscience 149, 53–67.

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

866 doi:10.1016/j.neuroscience.2007.07.031

867 Pawluski, J.L., Galea, L.A.M., 2006. Hippocampal morphology is differentially affected by reproductive
868 experience in the mother. J. Neurobiol. 66, 71–81. doi:10.1002/neu.20194

869 Pawluski, J.L., Walker, S.K., Galea, L.A.M., 2006. Reproductive experience differentially affects spatial
870 reference and working memory performance in the mother. Horm. Behav. 49, 143–149.
871 doi:10.1016/j.yhbeh.2005.05.016

872 Plümpe, T., Ehninger, D., Steiner, B., Klempin, F., Jessberger, S., Brandt, M., Römer, B., Rodriguez,
873 G.R., Kronenberg, G., Kempermann, G., 2006. Variability of doublecortin-associated dendrite
874 maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell
875 proliferation. BMC Neurosci. 7, 77. doi:10.1186/1471-2202-7-77

876 Posillico, C.K., Schwarz, J.M., 2016. An investigation into the effects of antenatal stressors on the
877 postpartum neuroimmune profile and depressive-like behaviors. Behav. Brain Res. 298, 218–228.
878 doi:10.1016/j.bbr.2015.11.011

879 PrabhuDas, M., Bonney, E., Caron, K., Dey, S., Erlebacher, A., Fazleabas, A., Fisher, S., Golos, T.,
880 Matzuk, M., McCune, J.M., Mor, G., Schulz, L., Soares, M., Spencer, T., Strominger, J., Way, S.S.,
881 Yoshinaga, K., Loreau, M., Naeem, S., Inchausti, P., 2015. Immune mechanisms at the maternal-
882 fetal interface: perspectives and challenges. Nat. Immunol. 16, 237–243.
883 doi:10.1038/ni.3131.Immune

884 Quan, N., Banks, W.A., 2007. Brain-immune communication pathways. Brain. Behav. Immun.
885 doi:10.1016/j.bbi.2007.05.005

886 Richwine, A.F., Sparkman, N.L., Dilger, R.N., Buchanan, J.B., Johnson, R.W., 2009. Cognitive deficits
887 in interleukin-10-deficient mice after peripheral injection of lipopolysaccharide. Brain. Behav.
888 Immun. 23, 794–802. doi:10.1016/j.bbi.2009.02.020

889 Rodríguez, M.I., Escames, G., López, L.C., López, A., García, J.A., Ortiz, F., Acuña-Castroviejo, D.,
890 2007. Chronic melatonin treatment reduces the age-dependent inflammatory process in senescence-
891 accelerated mice. J. Pineal Res. 42, 272–279. doi:10.1111/j.1600-079X.2006.00416.x

892 Rolls, A., Schori, H., London, A., Schwartz, M., 2008. Decrease in hippocampal neurogenesis during
893 pregnancy: a link to immunity. Mol. Psychiatry 13, 468–469. doi:10.1038/sj.mp.4002126

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

894 Rossant, J., Cross, J.C., 2001. PLACENTAL DEVELOPMENT: LESSONS FROM MOUSE
895 MUTANTS. Nat. Rev. Genet. 2, 538–548. doi:10.1038/35080570

896 Rutishauser, U., 2008. Polysialic acid in the plasticity of the developing and adult vertebrate nervous
897 system. Nat. Rev. Neurosci. 9, 26–35. doi:10.1038/nrn2285

898 Sahay, A., Hen, R., 2007. Adult hippocampal neurogenesis in depression. Nat. Neurosci. 10, 1110–5.
899 doi:10.1038/nn1969

900 Schloesser, R.J., Manji, H.K., Martinowich, K., 2009. Suppression of adult neurogenesis leads to an
901 increased hypothalamo-pituitary-adrenal axis response. Neuroreport 20, 553–557.
902 doi:10.1097/WNR.0b013e3283293e59

903 Scholzen, T., Gerdes, J., 2000. The Ki-67 protein: From the known and the unknown. J. Cell. Physiol.
904 doi:10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9

905 Setiawan, E., Wilson, A.A., Mizrahi, R., Rusjan, P.M., Miler, L., Rajkowska, G., Suridjan, I., Kennedy,
906 J.L., Rekkas, P.V., Houle, S., Meyer, J.H., 2015. Role of Translocator Protein Density, a Marker of
907 Neuroinflammation, in the Brain During Major Depressive Episodes. JAMA Psychiatry 72, 268.
908 doi:10.1001/jamapsychiatry.2014.2427

909 Shimaoka, Y., Hidaka, Y., Tada, H., Amino, N., Nakamura, T., Murata, Y., Mitsuda, N., Morimoto, Y.,
910 2000. Changes in cytokine production during and after normal pregnancy. Am J Reprod Immunol
911 44, 143–147.

912 Shingo, T., Gregg, C., Enwere, E., Fujikawa, H., Hassam, R., Geary, C., Cross, J.C., Weiss, S., 2003.
913 Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. Science
914 299, 117–20. doi:10.1126/science.1076647

915 Sierra, A., Beccari, S., Diaz-Aparicio, I., Encinas, J.M., Comeau, S., Tremblay, M.È., 2014.
916 Surveillance, phagocytosis, and inflammation: How never-resting microglia influence adult
917 hippocampal neurogenesis. Neural Plast. doi:10.1155/2014/610343

918 Sierra, A., Encinas, J.M., Deudero, J.J.P., Chancey, J.H., Enikolopov, G., Overstreet-Wadiche, L.S.,
919 Tsirka, S.E., Maletic-Savatic, M., 2010. Microglia shape adult hippocampal neurogenesis through
920 apoptosis-coupled phagocytosis. Cell Stem Cell 7, 483–495. doi:10.1016/j.stem.2010.08.014

bioRxiv preprint doi: https://doi.org/10.1101/388009; this version posted August 8, 2018. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

921 Slattery, D.A., Neumann, I.D., 2008. No stress please! Mechanisms of stress hyporesponsiveness of the
922 maternal brain. J. Physiol. 586, 377–385. doi:10.1113/jphysiol.2007.145896

923 Snyder, J.S., Soumier, A., Brewer, M., Pickel, J., Cameron, H.A., 2011. Adult hippocampal
924 neurogenesis buffers stress responses and depressive behaviour. Nature 476, 458–461.
925 doi:10.1038/nature10287

926 Sweatt, J.D., 2004. Hippocampal function in cognition. Psychopharmacology (Berl). 174.
927 doi:10.1007/s00213-004-1795-9

928 vom Berg, J., Prokop, S., Miller, K.R., Obst, J., Kälin, R.E., Lopategui-Cabezas, I., Wegner, A., Mair,
929 F., Schipke, C.G., Peters, O., Winter, Y., Becher, B., Heppner, F.L., 2012. Inhibition of IL-12/IL-
930 23 signaling reduces Alzheimer's disease–like pathology and cognitive decline. Nat. Med. 18,
931 1812–1819. doi:10.1038/nm.2965

932 Workman, J.L., Barha, C.K., Galea, L.A.M., 2012. Endocrine substrates of cognitive and affective
933 changes during pregnancy and postpartum. Behav. Neurosci. 126, 54–72. doi:10.1037/a0025538

934 Workman, J.L., Raineki, C., Weinberg, J., Galea, L.A.M., 2015. Alcohol and pregnancy: Effects on
935 maternal care, HPA axis function, and hippocampal neurogenesis in adult females.
936 Psychoneuroendocrinology 57, 37–50. doi:10.1016/j.psyneuen.2015.03.001

937 Yau, S., Li, A., So, K., 2015. Involvement of Adult Hippocampal Neurogenesis in 2015.