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Authors	Pawley, Lauren C.;Hueston, Cara M.;O'Leary, James D.;Kozareva, Danka A.;Cryan, John F.;O'Leary, Olivia F.;Nolan, Yvonne M.
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University College Cork, Ireland Coláiste na hOllscoile Corcaigh

1	Chronic Intrahippocampal Interleukin-1ß Overexpression in Adolescence Impairs
2	Hippocampal Neurogenesis but Not Neurogenesis-Associated Cognition
3	
4	Lauren C. Pawley ¹ , Cara M. Hueston ¹ , James D. O'Leary ¹ , Danka A. Kozareva ¹ , John F.
5	Cryan ^{1,2} , Olivia F. O'Leary ^{1,2*} , and Yvonne M. Nolan ^{1, 2*}
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8	¹ Department of Anatomy and Neuroscience, University College Cork, Ireland
9	² APC Microbiome Ireland, University College Cork, Ireland
10	*These authors contributed equally to this work.
11	
12	To whom correspondence should be addressed:
13	Yvonne M. Nolan, Department of Anatomy and Neuroscience, University College Cork,
14	Ireland
15	Tel: (353) 21-420 5476
16	Fax: (353) 21-427 3518
17	Email: y.nolan@ucc.ie
18	
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26 Abstract

Both neuroinflammation and adult hippocampal neurogenesis (AHN) are implicated in many 27 neurodegenerative disorders as well as in neuropsychiatric disorders, which often become 28 29 symptomatic during adolescence. A better knowledge of the impact that chronic neuroinflammation has on the hippocampus during the adolescent period could lead to the 30 discovery of new therapeutics for some of these disorders. The hippocampus is particularly 31 32 vulnerable to altered concentrations of the pro-inflammatory cytokine interleukin-1 β (IL-1 β), with elevated levels implicated in the aetiology of neurodegenerative disorders such as 33 34 Alzheimer's and Parkinson's, and stress-related disorders such as depression. The effect of acutely and chronically elevated concentrations of hippocampal IL-1ß have been shown to 35 reduce AHN in rats and mice. However, the effect of exposure to chronic overexpression of 36 hippocampal IL-1ß during adolescence, a time of increased vulnerability, hasn't been fully 37 38 interrogated. Thus, in this study we utilized a lentiviral approach to induce chronic overexpression of IL-1β in the dorsal hippocampus of adolescent male Sprague Dawley rats 39 for 6 weeks, during which time its impact on cognition and hippocampal neurogenesis were 40 examined. A reduction in hippocampal neurogenesis was observed along with a reduced level 41 of neurite branching on hippocampal neurons. However, there was no effect of IL-1ß 42 overexpression on cognitive performance. Our study has highlighted that chronic IL-1 β 43 44 overexpression in the hippocampus during the adolescent period exerts a negative impact on 45 neurogenesis and neurite branching.

46

47 Key words: adolescence, hippocampus, neurogenesis, behavior, inflammation, IL- 1β

49 **1.0 Introduction**

Neuroinflammation is a key contributing factor to neurodegenerative and neuropsychiatric 50 disorders (Freeman and Ting, 2016; Miller and Raison, 2016; Raison et al., 2006), and has been 51 consistently demonstrated to exert a detrimental effect on hippocampal-dependent processes 52 (Amor et al., 2010; Green and Nolan, 2014; Nolan et al., 2013; Ryan and Nolan, 2016). In 53 particular, chronically elevated concentrations of IL-1 β , which is produced predominantly by 54 55 microglia, has a substantially negative impact on hippocampal-dependent learning and memory processes (Pugh et al., 2001; Yirmiya and Goshen, 2011), and has been implicated in the 56 57 pathophysiology of both Alzheimer's disease (AD) (Griffin and Mrak, 2002) and depression (Koo and Duman, 2009; Maes et al., 2012; Raison et al., 2006). While low levels of IL-1ß are 58 necessary for memory formation (Yirmiya and Goshen, 2011), transgenic overexpression of 59 60 IL-1 β has been shown to induce impairments in both spatial and contextual fear memory (Hein 61 et al., 2010; Moore et al., 2009). Further, increased concentrations of IL-1β have been shown to impair long-term potentiation (LTP; a vital process for memory formation (Morris et al., 62 1986) in the hippocampus (Murray and Lynch, 1998; Vereker et al., 2000)). 63

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As well as influencing the function of mature neurons, it is now established that both acutely 65 and chronically elevated levels of IL-1ß negatively affect adult hippocampal neurogenesis 66 67 (AHN) (Hueston et al., 2018; O'Léime et al., 2017; Ryan et al., 2013), a process in which 68 neurons are generated from neural progenitor cells (NPCs) in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus throughout life (Kempermann et al., 2008). AHN 69 is essential for cognitive functioning such as spatial learning and memory, contextual fear 70 71 conditioning and pattern separation (Clelland et al., 2009; Jessberger et al., 2009; Ryan and Nolan, 2016; Santarelli et al., 2003). AHN has also been implicated in anxiety, stress resilience 72 (Levone et al., 2014; Revest et al., 2009; Snyder et al., 2011) and antidepressant action 73

(Santarelli et al., 2003). Recent evidence demonstrated that chronically elevated levels of hippocampal IL-1 β in the adult rat hippocampus impaired pattern separation, which was coupled with a decrease in AHN (Hueston et al., 2018).

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78 During the adolescent period of life, there is a higher density of granule cells in rodents, and up to four-fold more neurogenesis occurring during this time compared to adulthood (Curlik et 79 al., 2014; Hueston et al., 2017a; Yassa et al., 2011b). It is not yet clear however, if these newly 80 81 born cells serve the same function in the adolescent brain as they do in the adult brain. This is 82 an important area of research because adolescence is a critical period for brain development and maturation, and the brain is especially sensitive to perturbations such as inflammatory 83 84 stressors during this time (Hueston et al., 2017a). For example, it has been demonstrated in vitro that hippocampal NPCs derived from adolescent mice (PND 21) show increased levels of 85 cell proliferation when exposed to IL-1 α , while there was no effect of IL-1 α on NPCs from 86 adult mice (McPherson et al., 2011). Disruptions in hippocampal neurogenesis have been 87 implicated either directly or indirectly to various neuropsychiatric disorders, such as major 88 89 depression and schizophrenia (Kempermann et al., 2008), which also exhibit 90 neuroinflammation (Miller and Raison, 2016; Müller et al., 2015). Interestingly, these disorders tend to first become evident during adolescence, and can have both cognitive and emotional 91 92 elements (Kempermann et al., 2008). Given the role of AHN in the adult brain and the higher rate of hippocampal neurogenesis in the adolescent brain, it is plausible that perturbations of 93 neurogenesis during adolescence might be involved in these neuropsychiatric disorders. 94 However, there is a paucity of data on the effects of IL-1 β on hippocampal neurogenesis during 95 the adolescent period. Thus, the aim of this study was to examine the impact of chronic 96 hippocampal IL-1ß overexpression during adolescence on neuronal differentiation and 97 morphology of recently-born neurons, as well as on cognitive function. 98

99 **2.0 Methods**

100 2.1 Animals and Experimental Design

Adolescent (4 week old) male Sprague-Dawley rats were bred in-house (Biological Services 101 Unit, University College Cork) under veterinary supervision. All rats were pair housed in a 102 colony maintained at $22 \pm 1^{\circ}$ C, with a 12:12 hour light-dark cycle (lights on 0630-1830). All 103 animal procedures were performed under authorizations issued by the Health Products 104 105 Regulatory Authority (HPRA, Ireland), in accordance with the European Communities Council Directive (2010/63/EU) and approved by the Animal Experimentation Ethics Committee of 106 107 University College Cork. The animals were injected with either a lentivirus overexpressing mCherry-tagged IL-1β (LV^{IL-1βmCherry}, n=10) or mCherry alone (LV^{mCherry}, n=10) into the dorsal 108 hippocampus for six weeks (Figure 1). All animals underwent behavioural testing three weeks 109 110 following viral injection as per the timeline in Figure 1.

111

112 2.2 Preparation and Intrahippocampal Administration of Lentivirus Overexpressing IL-1β

The purified lentiviral particles expressed a full-length Open Reading Frame (ORF) clone on a 113 feline immunodeficiency virus (FIV) backbone, containing a DNA insert encoding for the full 114 length of the IL-1 β gene (from start codon to stop codon without the 5` and 3` end untranslated 115 regions or introns; gene accession NM 008361; LV^{IL-1βmCherry}) or an empty vector (LV^{mCherry}) 116 as control. Expression efficiency was driven by a cytomegalovirus (CMV) promoter with 117 118 puromycin resistance used as a selection marker. Both plasmids carried an mCherry reporter gene clone driven by IRES promoter. Lentiviral particles were produced and packaged by 119 Genecopoeia (LV^{IL-1βmCherry}: Cat #LP-Mm03282-Lv80-0205-cs; LV^{mCherry}: Cat# LP-NEG-120 Lv80-0205-cs) with titers of $> 1 \times 10^7$ transfection units per mL (Genecopoeia, Rockville, MD, 121 USA). It should be noted that some studies have incorporated a signal peptide at the end of the 122 5' end of the mature IL-1 β cDNA (Shaftel et al., 2007). We have not utilized a signal peptide 123

in our virus as we have characterized it in our previous studies (Hueston et al., 2018), and in 124 the present study through immunohistochemistry using an IL-1 β antibody raised in mouse, not 125 rat, to ensure that only viral IL-1 β was detected. Rats were anaesthetized with isoflurane, placed 126 into a stereotaxic frame, and 3µL of either the LV^{mCherry} or LV^{IL-1βmCherry} was bilaterally injected 127 into the dorsal hippocampus using the coordinates AP: -3.3, ML: +/- 2.0, DL: 2.7-3.0 128 (dependent on weight) relative to bregma at a rate of 1µL/min followed by a 5 min diffusion 129 130 period (Kozareva et al., 2019). Following lentiviral injection, incisions were sutured, treated with antibacterial ointment (Fucithalmic® 10mg/g), and rats were administered the analgesic 131 132 carprofen (Rimadyl® 5 mg/kg, s.c., Zoetis Ireland Ltd) and a 5% glucose solution.

133

134 2.3 Modified Spontaneous Location Recognition Test

Three weeks following surgery, the rats underwent the modified spontaneous location 135 recognition test that assesses behavioural pattern separation. This was a modified version of 136 the standard novel location recognition task in which animals underwent two consecutive 137 location discrimination tests where the inter-stimulus distances between the novel and familiar 138 locations have been varied to create a state of either high or low contextual overlap. Previous 139 studies have demonstrated that performance during conditions of high contextual overlap 140 require intact hippocampal neurogenesis (Bekinschtein et al., 2014). The task was conducted 141 in an open field arena, covered with bedding under dim light conditions (20 lux) as described 142 previously (Bekinschtein et al., 2013; Hueston et al., 2018). The testing room had three 143 proximal spatial cues and distal standard furniture. Rats were habituated to the arena for 10 144 minutes per day for 5 consecutive days before testing. Rats were exposed to three identical 145 objects for 10 minutes, in either a large separation condition (three objects (O1, O2, and O3) 146 separated by 120° angles) or a small separation condition (two of the objects separated by a 147 50° angle (O2, O3), and the third placed at an equal distance between the two (O1)). Twenty-148

four hours following acquisition, object O4 was placed in the same position as O1, while object O5 was placed halfway between the acquisition locations of O2 and O3 and rats were allowed to explore for 5 minutes. The objects and order of testing were counterbalanced within and between groups. Time spent with the objects was recorded, and a discrimination index (DI) of object recognition was calculated as DI = (seconds with O5 - seconds with O4)/(seconds withO4 + seconds with O5). The arena and objects were cleaned with a 70% ethanol solutionbetween exposures of each animal to the arena to remove odour cues.

156

157 2.4 Object Recognition Test

The object recognition test, a hippocampal-perirhinal cortex-dependent task, was carried out 158 as described previously (Bevins and Besheer, 2006). Rats were habituated to an empty chamber 159 160 (40.5cm L x 36.5cm W x 28.0cm H) under dim light (20 lux) for 10 minutes. Twenty-four hours later, rats were exposed to 2 identical objects (either ceramic mugs or glass bottles) for 161 10 minutes, followed by a 3-hour inter-trial interval. After the delay, recognition memory was 162 tested with a 5-minute exposure to one novel object and one familiar object. All behaviors were 163 recorded, and videos were scored to determine the amount of time the rats spent attending to 164 the novel vs. familiar objects. Objects were counterbalanced between groups. Time spent with 165 the objects was recorded, and a discrimination ratio (DR) of object recognition was calculated 166 as DR = seconds with novel object/(seconds with novel object + seconds with familiar object). 167 168

169 2.5 Sponta

2.5 Spontaneous Alternation Test

Spontaneous alternation behavior is used as a measure of hippocampal-dependent working
memory (Hughes, 2004). The Y maze consisted of three arms 120° from each other (40 x 10 x
20 cm; made in house). Each animal was allowed to explore the maze for five minutes (adapted
from Senechal et al., 2007). The number and order of arm entries were recorded. An arm entry

was defined as all four paws entering the arm (four paw criteria). An alternation was determined
as the number of consecutive entries into the three maze arms. Alternations were divided by
the total number of entries during the five-minute test period. The percentage of alternations
was calculated as % = Alternations/(Entries-2).

178

179 2.6 Confirmation of IL-1β Overexpression

180 Rats were euthanized with an intraperitoneal injection of Sleep-Away (1.0mL/kg) and transcardially perfused using phosphate-buffered saline (PBS) solution, followed by 4.0% 181 182 paraformaldehyde in PBS. Brains were post-fixed in 4% formaldehyde in PBS overnight, before being transferred to a 30% sucrose solution. Coronal sections from the brains were cut 183 at 40µm and mounted onto gelatin-coated slides in a 1:6 series. Virus validation and 184 confirmation of IL-1ß overexpression was carried out as previously described (Hueston et al., 185 2018). Sections were washed in PBS before being blocked in 10% donkey serum blocking 186 solution (G9023 Sigma) in PBS with 0.3% Triton-X (0.3% PBS-T), followed by overnight 187 incubation at 4°C with a primary antibody against mCherry (1:2000 Abcam, rabbit polyclonal 188 ab167453) and IL-1β (goat polyclonal anti-mouse IL-1β 1:500 AF-401-NA R&D Systems) 189 diluted in 0.3% PBS-T with 5% donkey serum. Sections were incubated with secondary 190 antibodies (AlexaFluor 488 donkey anti-rabbit IgG A11055 Abcam and AlexaFluor 594 191 donkey anti-goat IgG A21207 Abcam) in 0.3% PBS-T and coverslipped with Vectashield 192 193 mounting medium. To ensure that only viral-mediated and not endogenous IL-1 β was detected, the primary antibody used was raised in mouse, not rat. 194

195

196 2.7 Ionized calcium binding adapter molecule-1 (IBA-1) and Doublecortin (DCX)197 Immunohistochemistry

Cells that were immunopositive for IBA-1 (microglia) and DCX (immature neurons) were 198 identified in the granule cell layer (GCL) of the DG of the hippocampus. Rehydrated sections 199 200 were treated with 1% hydrogen peroxide (216763 Sigma) in methanol to block endogenous peroxidases, followed by blocking with 10% normal goat serum for IBA-1 staining, or 10% 201 normal rabbit serum (R9133 Sigma) for DCX staining, prepared in 0.3% or 0.1% PBS-T 202 respectively. For IBA-1 staining, sections were incubated overnight at 4°C in rabbit polyclonal 203 204 anti-IBA-1 (1:500 019-19741 WAKO) in 0.1% PBS-T and 5% normal goat serum in PBS. For DCX-staining, sections were incubated overnight at 4°C in goat polyclonal anti-DCX (1:100 205 206 sc-8066 Santa Cruz) in 0.3% PBS-T and 5% normal rabbit serum. The following day, IBA-1 sections were rinsed with PBS and incubated in the secondary antibody solution containing 207 biotinylated goat anti-rabbit (1:200 pk-6101 Vector Laboratories) in 0.1% PBS-T and 1.5% 208 209 normal rabbit serum. DCX-sections were rinsed with PBS, and incubated in biotinylated rabbit 210 anti-goat IgG (1:200 pk-6105 Vector Laboratories), 0.3% PBS-T and 1.5% normal rabbit serum. Detection of the secondary antibodies was enhanced using the Vectastain ABC Elite kit 211 (PK-6105/PK-6101 Vector Laboratories), followed by incubation with 3,3'-Diaminobenzidine 212 (DAB) activated with 0.3% hydrogen peroxide. Slides were cover-slipped using DPX 213 mounting medium. 214

215

216 2.8 Image Acquisition and Analysis

DAB staining was visualized at 10x and 20x magnification using the brightfield channel on an Olympus AX70 upright microscope (BioSciences Imaging Centre, Department of Anatomy and Neuroscience, UCC), while fluorescent staining was captured using the green fluorescent channel on the same microscope. Images were acquired across a 1:6 series using Olympus cellSens Entry software and analyzed using the NeuronJ plugin (Meijering et al., 2004) for Image J software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, 223 Maryland, USA, https://imagej.nih.gov/ij/, 1997-2016). To quantify IL-1 β staining through 224 the DG, mean fluorescence intensity was measured across a randomly selected area of the same 225 dimensions within the DG, and the mean background fluorescence intensity was measured 226 across a randomly selected area of the same size outside of the DG. IL-1 β staining was 227 expressed as the ratio between fluorescence within to fluorescence outside of the DG.

228

229 2.9 Quantification and Morphological Analysis of Cells

A modified stereological approach was performed to estimate the number of IBA-1⁺ and DCX⁺ 230 231 cells in the GCL of the DG. Cells were counted through the whole DG on both hemispheres of each section in 1:6 series (240µm apart). The area of each section of the DG was obtained using 232 the ImageJ programme (Schneider et al., 2012). Measurements were obtained in pixels and 233 234 converted to µm² using a scaled micrometer and ImageJ software (Schneider et al., 2012). Data 235 were expressed as the number of cells per μ m². To assess the degree of microglial activation in response to the IL-1 β treatment, the somal size of IBA-1⁺ microglial cells in the DG was 236 observed at 60x magnification on the Olympus AX70 microscope. Ten randomly selected cells 237 were sampled per animal, thus there were 30 microglia analyzed per experimental group. The 238 area of the soma was measured using ImageJ and expressed as μm^2 . 239

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DCX⁺ neurons and associated neurites were observed at 20x magnification on the Olympus BX40 microscope and were traced to paper using an attached Camera Lucida drawing tube (Wollaston, 1807). Ten randomly selected DCX⁺ neurons were sampled per animal, based on them having minimal overlap with neurites of adjacent neurons, thus there were 30 neurons analyzed per experimental group. The tracings were scanned onto a personal computer and analyzed using the NeuronJ plugin for ImageJ. The length of primary, secondary, tertiary, and quaternary neurites per neuron were measured, with the sum of these being taken as the total length. The extent of neurite branching was determined by counting the number of neuritebranch points (nodes) per neuron.

251	2.10 Statistical Analysis
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- A two-tailed t-test was used for all analyses. An alpha level of 0.05 was used as criterion for
- 253 statistical significance. All data are presented as mean plus/minus standard error of the mean
- 254 (SEM).

255 **3.0 Results**

256 3.1 Confirmation of lentiviral transduction of the hippocampus

Immunopositive staining for mCherry was evident in the GCL of the DG of both the dHi (Figure 2A-C) of all animals at six weeks following surgery, demonstrating successful transduction of cells in the GCL of the DG by the lentivirus. Indeed, a similar level of fluorescence intensity of mCherry was observed in the DG of all animals with no significant difference in expression levels between groups (p > 0.05; Figure 2A-C).

262

3.2 Lentiviral transduction of the adolescent rat hippocampus resulted in IL-1β overexpression
and microglial activation

Representative images of immunopositive staining for non-endogenous IL-1ß in the GCL of 265 the DG of the hippocampus are shown in (Figure 2D-E). There was a significant increase in 266 the fluorescence intensity of IL-1 β in the DG of animals injected with the LV^{IL-1 β mCherry} virus 267 compared to those injected with $LV^{mCherry}$ control virus ([t = 3.435, p = 0.0264]; Figure 2F), 268 thus demonstrating successful transduction of cells by the lentivirus overexpressing mouse IL-269 1β at five weeks following surgery. Five weeks of IL- 1β overexpression in the hippocampus 270 significantly increased the number of microglia (IBA-1⁺ cells)/ μ m² in the GCL of the DG ([t = 271 4.911, p = 0.008]; Figure 2G-I) and increased the somal size of IBA-1⁺ cells in the GCL of the 272 hippocampi in these animals ([t = 5.305, p = 0.0131]; Figure 2J-L). 273

274

275 3.3 IL-1β overexpression in the adolescent rat hippocampus decreased hippocampal
276 neurogenesis

IL-1 β overexpression in the hippocampus significantly decreased the number of DCX⁺ cells/ μ m² in the GCL of the DG ([t = 3.637, p = 0.0220]; Figure 3A-C). IL-1 β overexpression also significantly decreased the number of branch points (nodes) on DCX⁺ cells ([t = 5.024, p = 0.0074]; Figure 3D-F), however it did not affect the average total neurite length, nor the length of primary, secondary or tertiary neurites in the hippocampus (all p > 0.05; Figure 3G).

3.4 IL-1β overexpression in the hippocampus during adolescence had no effect on performance
in hippocampus-dependent cognitive tasks

Changes in AHN have been reported to affect performance in some cognitive tasks. Thus, we 285 286 examined the effects of five weeks of hippocampal IL-1 β overexpression in the adolescent brain on three tests of hippocampal-dependent memory (spontaneous alternation in the Y-287 288 maze, pattern separation and novel object recognition). Despite the IL-1 β -induced decrease in hippocampal neurogenesis we observed, IL-1ß overexpression during adolescence did not 289 affect hippocampal-dependent memory, as measured by these behavioral tests (Figure 4). 290 291 Specifically, hippocampal overexpression of IL-1 β did not affect the percentage of alternations 292 made in the Y-Maze ([t(18) = 0.2102, p = 0.8358]; Figure 4A) nor the number of entries made into the different arms ([t(18) = 0.1739, p = 0.8639]; Figure 4B). All animals explored the 293 objects equally during acquisition small separation (Figure 4C) and acquisition large separation 294 (Figure 4D), respectively. All animals were able to differentiate the novel from familiar 295 location when tested on small ([t(18) = 0.9942, p = 0.3333]; Figure 4E) and large pattern 296 separation ([t(18) = 0.5223, p = 0.6078]; Figure 4E) thus there was no effect of hippocampal 297 IL-1 β overexpression on pattern separation as assessed using the modified spontaneous 298 299 location recognition test. Performance in the novel object recognition task was also unaffected by hippocampal overexpression of IL-1 β ([t(18) = 0.9943, p = 0.3333]; Figure 4F). 300

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304 **4.0 Discussion**

Given the current paucity of data on the effects of IL-1 β during the adolescent period on hippocampal neurogenesis, we aimed to examine the impact of chronic hippocampal IL-1 β exposure during adolescence on hippocampal-associated cognitive function and the neuronal differentiation and morphology of recently-born neurons. We found that five weeks of hippocampal IL-1 β overexpression induced a significant reduction in neurogenesis and neuronal complexity, but had no impact on cognitive performance.

311

312 We report that five weeks of hippocampal IL-1 β during adolescence significantly reduced the number of DCX⁺ cells in the hippocampus. This is in agreement with what has been reported 313 when IL-1 β is overexpressed during adulthood, whereby there is a decline in the number of 314 new neurons (Hueston et al., 2018; Koo and Duman, 2008; Ryan et al., 2013). Newly-born 315 neurons are more sensitive to inflammatory insults than mature neurons (Felderhoff-Mueser et 316 al., 2005; Kole et al., 2013), and this has been demonstrated through various methods of chronic 317 hippocampal overexpression of IL-1\beta in adulthood including infusion of IL-1\beta through a 318 cannula in rats (Koo and Duman, 2008), transgenic murine overexpression in a IL-1βXAT 319 model (Wu et al., 2013), or treatment of adult rat hippocampal neurosphere cultures with a 320 $LV^{IL1\beta}$ virus (Ryan et al., 2013). Further, we found that the complexity of newly-born neurons 321 (as measured by the number of branch points on DCX⁺ cells) was negatively impacted by IL-322 1β in the hippocampus, while the length of neurites on these DCX⁺ cells was unaffected. 323 Previous studies have shown that treatment of embryonic rat hippocampal NPCs with IL-1β 324 reduced neurite length on DCX⁺ cells in vitro (Green et al., 2012) and that chronic hippocampal 325 overexpression of IL-1 β in adulthood in rats reduced the neurite length of DCX⁺ cells (Hueston 326 et al., 2018). These results indicate a differential effect of neuroinflammation on neurite length 327 induced by IL-1 β overexpression during the embryonic period, adolescence and adulthood. 328

While we focused on the use of DCX to identify the differentiation of new neurons as an indicator of neurogenesis, identification of the proliferation or survival of new neurons or glia, could be carried out in future studies by injecting the thymidine analogue BrdU to rats at relevant time points and performing immunohistochemistry with markers for mature neurons and glial cells. Measures could also be taken to identify cells that are undergoing apoptotic death in order to get a complete picture of the effects of neuroinflammation on the neurogenic process.

336

337 We show that five weeks of hippocampal IL-1 β during adolescence had no effect on performance in cognitive tasks. Pattern separation is believed to be dependent on AHN 338 339 (Bekinschtein et al., 2013), and although we observed that hippocampal IL-1ß overexpression during adolescence significantly reduced neurogenesis, pattern separation wasn't impacted by 340 this reduction in new neurons. This may be an age-dependent effect, since it has been well 341 documented that reduced neurogenesis in adulthood impairs cognitive performance on 342 hippocampal-dependent tasks, including pattern separation and spatial and object recognition 343 (Hueston et al., 2018; Jessberger et al., 2009). This is especially likely given the differential 344 effect of neuroinflammation induced by IL-1β overexpression across the lifespan as discussed 345 above. New neurons born in younger stages of life are much the same as those born in later life 346 in terms of their morphological structure (van Praag and Christie, 2015), however it has been 347 reported that the maturation of these new neurons and their successful integration into the 348 existing neuronal circuitry is impaired with age (Trinchero et al., 2017). One possible 349 explanation for this is that age-dependent inflammation is involved (Kuhn et al., 2018), and IL-350 1β is now established as one of the cytokines playing a key role in "inflammaging" (Franceschi 351 et al., 2018). A decline in hippocampal-dependent cognitive functioning with age is well 352

documented (Yassa et al., 2011a), with the level of AHN also found to be related to cognitive
performance in both humans and non-human primates (Aizawa et al., 2009).

355

We injected IL-1ß into the dorsal hippocampus since lesion studies in rodents have shown that 356 the dorsal hippocampus plays a more predominant role in spatial learning and memory than the 357 ventral region which is predominantly involved in regulating anxiety (Bannerman et al., 2002). 358 359 For example, dorsal hippocampal lesions in rats hindered spatial memory acquisition on the Morris water maze (Moser et al., 1995), and impaired spatial memory on the radial arm maze 360 361 (Pothuizen et al., 2004). On the other hand, ventral hippocampal lesions appear to have minimal impact on spatial memory tasks, but instead decrease behaviors linked to anxiety (Bannerman 362 et al., 2014). In parallel, it has been reported that hippocampal neurogenesis might also be 363 functionally segregated along its longitudinal axis whereby neurogenesis in the ventral 364 hippocampus rather than the dorsal hippocampus is preferentially affected by stress and 365 antidepressant drugs (O'Leary and Cryan, 2014; Tanti and Belzung, 2013). As such, we 366 hypothesized that any impact of IL-1 β on neurogenesis in the dorsal region would in turn affect 367 cognitive processes that are dependent on hippocampal neurogenesis (i.e. pattern separation) 368 and that also have a spatial component (i.e. pattern separation, y-maze) and where previous 369 studies have reported that lesions of the dorsal hippocampus disrupt performance in such tests 370 (Hammond et al., 2004; Josey and Brigman, 2015; Lee et al., 2005). While it is somewhat 371 surprising we that found no impact of IL-1ß overexpression in the dorsal hippocampus on these 372 cognitive tasks, we previously found that chronic IL-1ß overexpression in the dorsal 373 hippocampus of adult rats impaired behavioural pattern separation but had no effect on 374 spontaneous alternation and novel object recognition, hippocampus dependent tasks not 375 associated with neurogenesis. Considering the role of inflammation and neurogenesis in stress-376 related psychiatric disorders (Goshen et al., 2008; Hueston et al., 2017b; Levone et al., 2014; 377

Pereira et al., 2019; Yun et al., 2016) and the role of the ventral hippocampus in the regulation of anxiety and the stress response, it will be of interest for future studies to determine the impact of II-1 β overexpression in the ventral hippocampus on neurogenesis in this region and anxiety related behaviour. We report no impact of IL-1 β on neurite length of newly-born hippocampal neurons; it is therefore possible that the unaffected neurite length in these animals may have conferred a degree of resilience to the effects of chronic IL-1 β on cognitive tasks.

384

385 Adolescence is a period during the lifespan when the brain is particularly vulnerable to perturbations such as stress (Hueston et al., 2017), and the long-lasting negative 386 387 effects of disruptions during this time may leave individuals more susceptible to developing neurological disorders later in life (Mirescu et al., 2004), although further study is 388 needed to validate this. Our current results are important given the clinical relevance of 389 inflammation in disorders with impaired AHN (Ryan and Nolan, 2016). Neurodegenerative 390 disorders such as Alzheimer's and Parkinson's disease, and neuropsychiatric disorders such as 391 major depression, are linked with chronic neuroinflammation (Ben Menachem-Zidon et al., 392 2008; Dursun et al., 2015; Maes et al., 2012), and have been shown to have modified levels of 393 AHN (Winner and Winkler, 2015). Specifically, IL-1 β has been found to be increased in the 394 cerebrospinal fluid (CSF) of patients with severe depression (Levine et al., 1999) and in women 395 396 with perinatal depression (Miller et al., 2019). There is some discrepancy in the literature however about whether IL-1 β is increased (Blum-Degen et al., 1995) or unchanged (Martinez 397 et al., 2012) in the CSF of AD patients compared to healthy individuals. However, IL-1 β has 398 399 been linked to the pathology of AD and has been shown to surround plaques of amyloid-beta in the brain, as well as aiding the deposition of plaques (Griffin et al., 1995; Heneka et al., 400 2015). It is also plausible that such local increases of IL-1 β in the hippocampus would further 401 402 stimulate other immune cells, thus inducing cytokine release and resulting in an overall chronic

inflammatory state (Netea et al., 2010), including in the CSF. Further, psychiatric disorders
such as schizophrenia, which first become symptomatic during adolescence, have also been
associated with alterations in AHN (Iannitelli et al., 2017), as well as inflammation (Müller et
al., 2015).

407

408 **5.0 Conclusion**

409 Our data demonstrate that chronic inflammation during adolescence, a critical developmental 410 period during the lifespan, has detrimental effects on hippocampal neurogenesis, but not on 411 associated cognitive functions, nor on the length of neurites on newly-born neurons. We 412 propose that newly-born neurons in the developing hippocampus during adolescence may 413 confer resilience to inflammatory-mediated insults, such that hippocampal-associated 414 cognitive function is not impacted. Harnessing newly-born neurons during adolescence for 415 therapeutic gain is an exciting area for future research.

416

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Figures

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Figure 1: Experimental timeline. Rats were injected with lentivirus overexpressing LV^{IL-} 430 $^{1\beta mCherry}$ or LV^{mCherry} alone as control. All rats underwent behavioral testing at weeks 4 and 5. 431 432 **Figure 2:** Confirmation of viral transduction and microglial activation in response to IL-1 β 433 overexpression. Fluorescence intensity of mCherry (C) and IL-1 β (F) and representative 434 images of mCherry (A-B) and IL-1B (D-E; scale bar represents 100µm) five weeks after 435 lentiviral injection with an IL-1 β -overexpressing LV^{IL-1 β mCherry} virus. The number of IBA-1⁺ 436 cells/µm² (G) and representative images of IBA-1+ cells (H-I, scale bar represents 100µm) five 437 weeks after viral injection. The somal size of IBA-1⁺ cells (J) and representative images (K-L, 438 scale bar represents 5µm) are shown. *p<0.05, **p<0.001 relative to the control group; two-439 440 tailed t-test, n=3.

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Figure 3: Overexpression of IL-1β reduced the number of DCX⁺ hippocampal neurons and negatively impacted on their complexity. The number of DCX⁺ cells/ μ m² (A) and representative images of the number of DCX⁺ cells in the hippocampus five weeks after lentiviral injection (B-C, scale bar represents 100µm). The number of nodes/DCX⁺ cell (D) and tracings of the length of neurites on DCX⁺ cells (E-F, scale bar represents 10µm). The neurite length of DCX⁺ cells (G). *p<0.05, **p<0.001 relative to the control group; twotailed t-test, n=3.

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Figure 4: Five weeks of IL-1 β overexpression in the hippocampus during adolescence had no effect on hippocampal-dependent memory processes. The number of alternations made in the Y-maze (A), the number of entries made into different arms of the Y-maze (B), performance 453 on a small (C) and large (D) acquisition separation, and discrimination in the modified 454 spontaneous location recognition test (E), and novel object recognition (F). P > 0.05; two-tailed 455 Student's t-test; n = 10.

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