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1 **Chronic Intrahippocampal Interleukin-1 β Overexpression in Adolescence Impairs**
2 **Hippocampal Neurogenesis but Not Neurogenesis-Associated Cognition**

3
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26 **Abstract**

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

48

49 **1.0 Introduction**

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

64

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

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

77

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

99 2.0 Methods

100 2.1 Animals and Experimental Design

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

111

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

113 The purified lentiviral particles expressed a full-length Open Reading Frame (ORF) clone on a
114 feline immunodeficiency virus (FIV) backbone, containing a DNA insert encoding for the full
115 length of the IL-1 β gene (from start codon to stop codon without the 5' and 3' end untranslated
116 regions or introns; gene accession NM_008361; LV^{IL-1 β mCherry}) or an empty vector (LV^{mCherry})
117 as control. Expression efficiency was driven by a cytomegalovirus (CMV) promoter with
118 puromycin resistance used as a selection marker. Both plasmids carried an mCherry reporter
119 gene clone driven by IRES promoter. Lentiviral particles were produced and packaged by
120 Genecopoeia (LV^{IL-1 β mCherry}: Cat #LP-Mm03282-Lv80-0205-cs; LV^{mCherry}: Cat# LP-NEG-
121 Lv80-0205-cs) with titers of $> 1 \times 10^7$ transfection units per mL (Genecopoeia, Rockville, MD,
122 USA). It should be noted that some studies have incorporated a signal peptide at the end of the
123 5' end of the mature IL-1 β cDNA (Shaftel et al., 2007). We have not utilized a signal peptide

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

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

149 four hours following acquisition, object O4 was placed in the same position as O1, while object
150 O5 was placed halfway between the acquisition locations of O2 and O3 and rats were allowed
151 to explore for 5 minutes. The objects and order of testing were counterbalanced within and
152 between groups. Time spent with the objects was recorded, and a discrimination index (DI) of
153 object recognition was calculated as $DI = (\text{seconds with O5} - \text{seconds with O4}) / (\text{seconds with}$
154 $O4 + \text{seconds with O5})$. The arena and objects were cleaned with a 70% ethanol solution
155 between exposures of each animal to the arena to remove odour cues.

156

157 2.4 Object Recognition Test

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

168

169 2.5 Spontaneous Alternation Test

170 Spontaneous alternation behavior is used as a measure of hippocampal-dependent working
171 memory (Hughes, 2004). The Y maze consisted of three arms 120° from each other (40 x 10 x
172 20 cm; made in house). Each animal was allowed to explore the maze for five minutes (adapted
173 from Senechal et al., 2007). The number and order of arm entries were recorded. An arm entry

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

178

179 2.6 Confirmation of IL-1 β Overexpression

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

195

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

197 Immunohistochemistry

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

215

216 2.8 Image Acquisition and Analysis

217 DAB staining was visualized at 10x and 20x magnification using the brightfield channel on an
218 Olympus AX70 upright microscope (BioSciences Imaging Centre, Department of Anatomy
219 and Neuroscience, UCC), while fluorescent staining was captured using the green fluorescent
220 channel on the same microscope. Images were acquired across a 1:6 series using Olympus
221 cellSens Entry software and analyzed using the NeuronJ plugin (Meijering et al., 2004) for
222 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

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

240

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

248 length. The extent of neurite branching was determined by counting the number of neurite
249 branch points (nodes) per neuron.

250

251 2.10 Statistical Analysis

252 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

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

262

263 3.2 Lentiviral transduction of the adolescent rat hippocampus resulted in IL-1 β overexpression 264 and microglial activation

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

274

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

277 IL-1 β overexpression in the hippocampus significantly decreased the number of DCX⁺
278 cells/ μm^2 in the GCL of the DG ($[t = 3.637, p = 0.0220]$; Figure 3A-C). IL-1 β overexpression
279 also significantly decreased the number of branch points (nodes) on DCX⁺ cells ($[t = 5.024, p$

280 = 0.0074]; Figure 3D-F), however it did not affect the average total neurite length, nor the
281 length of primary, secondary or tertiary neurites in the hippocampus (all $p > 0.05$; Figure 3G).

282

283 3.4 IL-1 β overexpression in the hippocampus during adolescence had no effect on performance
284 in hippocampus-dependent cognitive tasks

285 Changes in AHN have been reported to affect performance in some cognitive tasks. Thus, we
286 examined the effects of five weeks of hippocampal IL-1 β overexpression in the adolescent
287 brain on three tests of hippocampal-dependent memory (spontaneous alternation in the Y-
288 maze, pattern separation and novel object recognition). Despite the IL-1 β -induced decrease in
289 hippocampal neurogenesis we observed, IL-1 β overexpression during adolescence did not
290 affect hippocampal-dependent memory, as measured by these behavioral tests (Figure 4).

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
293 into the different arms ([$t(18) = 0.1739$, $p = 0.8639$]; Figure 4B). All animals explored the
294 objects equally during acquisition small separation (Figure 4C) and acquisition large separation
295 (Figure 4D), respectively. All animals were able to differentiate the novel from familiar
296 location when tested on small ([$t(18) = 0.9942$, $p = 0.3333$]; Figure 4E) and large pattern
297 separation ([$t(18) = 0.5223$, $p = 0.6078$]; Figure 4E) thus there was no effect of hippocampal
298 IL-1 β overexpression on pattern separation as assessed using the modified spontaneous
299 location recognition test. Performance in the novel object recognition task was also unaffected
300 by hippocampal overexpression of IL-1 β ([$t(18) = 0.9943$, $p = 0.3333$]; Figure 4F).

301

302

303

304 **4.0 Discussion**

305 Given the current paucity of data on the effects of IL-1 β during the adolescent period on
306 hippocampal neurogenesis, we aimed to examine the impact of chronic hippocampal IL-1 β
307 exposure during adolescence on hippocampal-associated cognitive function and the neuronal
308 differentiation and morphology of recently-born neurons. We found that five weeks of
309 hippocampal IL-1 β overexpression induced a significant reduction in neurogenesis and
310 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
313 number of DCX⁺ cells in the hippocampus. This is in agreement with what has been reported
314 when IL-1 β is overexpressed during adulthood, whereby there is a decline in the number of
315 new neurons (Hueston et al., 2018; Koo and Duman, 2008; Ryan et al., 2013). Newly-born
316 neurons are more sensitive to inflammatory insults than mature neurons (Felderhoff-Mueser et
317 al., 2005; Kole et al., 2013), and this has been demonstrated through various methods of chronic
318 hippocampal overexpression of IL-1 β in adulthood including infusion of IL-1 β through a
319 cannula in rats (Koo and Duman, 2008), transgenic murine overexpression in a IL-1 β XAT
320 model (Wu et al., 2013), or treatment of adult rat hippocampal neurosphere cultures with a
321 LV^{IL1 β} virus (Ryan et al., 2013). Further, we found that the complexity of newly-born neurons
322 (as measured by the number of branch points on DCX⁺ cells) was negatively impacted by IL-
323 1 β in the hippocampus, while the length of neurites on these DCX⁺ cells was unaffected.
324 Previous studies have shown that treatment of embryonic rat hippocampal NPCs with IL-1 β
325 reduced neurite length on DCX⁺ cells *in vitro* (Green et al., 2012) and that chronic hippocampal
326 overexpression of IL-1 β in adulthood in rats reduced the neurite length of DCX⁺ cells (Hueston
327 et al., 2018). These results indicate a differential effect of neuroinflammation on neurite length
328 induced by IL-1 β overexpression during the embryonic period, adolescence and adulthood.

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

336

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

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

355

356 We injected IL-1 β into the dorsal hippocampus since lesion studies in rodents have shown that
357 the dorsal hippocampus plays a more predominant role in spatial learning and memory than the
358 ventral region which is predominantly involved in regulating anxiety (Bannerman et al., 2002).

359 For example, dorsal hippocampal lesions in rats hindered spatial memory acquisition on the
360 Morris water maze (Moser et al., 1995), and impaired spatial memory on the radial arm maze
361 (Pothuizen et al., 2004). On the other hand, ventral hippocampal lesions appear to have minimal

362 impact on spatial memory tasks, but instead decrease behaviors linked to anxiety (Bannerman
363 et al., 2014). In parallel, it has been reported that hippocampal neurogenesis might also be

364 functionally segregated along its longitudinal axis whereby neurogenesis in the ventral
365 hippocampus rather than the dorsal hippocampus is preferentially affected by stress and
366 antidepressant drugs (O'Leary and Cryan, 2014; Tanti and Belzung, 2013). As such, we

367 hypothesized that any impact of IL-1 β on neurogenesis in the dorsal region would in turn affect
368 cognitive processes that are dependent on hippocampal neurogenesis (i.e. pattern separation)
369 and that also have a spatial component (i.e. pattern separation, y-maze) and where previous

370 studies have reported that lesions of the dorsal hippocampus disrupt performance in such tests
371 (Hammond et al., 2004; Josey and Brigman, 2015; Lee et al., 2005). While it is somewhat
372 surprising we that found no impact of IL-1 β overexpression in the dorsal hippocampus on these

373 cognitive tasks, we previously found that chronic IL-1 β overexpression in the dorsal
374 hippocampus of adult rats impaired behavioural pattern separation but had no effect on
375 spontaneous alternation and novel object recognition, hippocampus dependent tasks not

376 associated with neurogenesis. Considering the role of inflammation and neurogenesis in stress-
377 related psychiatric disorders (Goshen et al., 2008; Hueston et al., 2017b; Levone et al., 2014;

378 Pereira et al., 2019; Yun et al., 2016) and the role of the ventral hippocampus in the regulation
379 of anxiety and the stress response, it will be of interest for future studies to determine the impact
380 of IL-1 β overexpression in the ventral hippocampus on neurogenesis in this region and anxiety
381 related behaviour. We report no impact of IL-1 β on neurite length of newly-born hippocampal
382 neurons; it is therefore possible that the unaffected neurite length in these animals may have
383 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
386 perturbations such as stress (Hueston et al., 2017), and the long-lasting negative
387 effects of disruptions during this time may leave individuals more susceptible to
388 developing neurological disorders later in life (Mirescu et al., 2004), although further study is
389 needed to validate this. Our current results are important given the clinical relevance of
390 inflammation in disorders with impaired AHN (Ryan and Nolan, 2016). Neurodegenerative
391 disorders such as Alzheimer's and Parkinson's disease, and neuropsychiatric disorders such as
392 major depression, are linked with chronic neuroinflammation (Ben Menachem-Zidon et al.,
393 2008; Dursun et al., 2015; Maes et al., 2012), and have been shown to have modified levels of
394 AHN (Winner and Winkler, 2015). Specifically, IL-1 β has been found to be increased in the
395 cerebrospinal fluid (CSF) of patients with severe depression (Levine et al., 1999) and in women
396 with perinatal depression (Miller et al., 2019). There is some discrepancy in the literature
397 however about whether IL-1 β is increased (Blum-Degen et al., 1995) or unchanged (Martinez
398 et al., 2012) in the CSF of AD patients compared to healthy individuals. However, IL-1 β has
399 been linked to the pathology of AD and has been shown to surround plaques of amyloid-beta
400 in the brain, as well as aiding the deposition of plaques (Griffin et al., 1995; Heneka et al.,
401 2015). It is also plausible that such local increases of IL-1 β in the hippocampus would further
402 stimulate other immune cells, thus inducing cytokine release and resulting in an overall chronic

403 inflammatory state (Netea et al., 2010), including in the CSF. Further, psychiatric disorders
404 such as schizophrenia, which first become symptomatic during adolescence, have also been
405 associated with alterations in AHN (Iannitelli et al., 2017), as well as inflammation (Müller et
406 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

417

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420

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Figures

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430 **Figure 1:** Experimental timeline. Rats were injected with lentivirus overexpressing $LV^{IL-1\beta mCherry}$ or $LV^{mCherry}$ alone as control. All rats underwent behavioral testing at weeks 4 and 5.

432

433 **Figure 2:** Confirmation of viral transduction and microglial activation in response to IL-1 β overexpression. Fluorescence intensity of mCherry (C) and IL-1 β (F) and representative images of mCherry (A-B) and IL-1 β (D-E; scale bar represents 100 μ m) five weeks after lentiviral injection with an IL-1 β -overexpressing $LV^{IL-1\beta mCherry}$ virus. The number of IBA-1 $^{+}$ cells/ μ m 2 (G) and representative images of IBA-1 $^{+}$ cells (H-I, scale bar represents 100 μ m) five weeks after viral injection. The somal size of IBA-1 $^{+}$ cells (J) and representative images (K-L, scale bar represents 5 μ m) are shown. * $p < 0.05$, ** $p < 0.001$ relative to the control group; two-tailed t-test, $n = 3$.

441

442 **Figure 3:** Overexpression of IL-1 β reduced the number of DCX $^{+}$ hippocampal neurons and negatively impacted on their complexity. The number of DCX $^{+}$ cells/ μ m 2 (A) and representative images of the number of DCX $^{+}$ cells in the hippocampus five weeks after lentiviral injection (B-C, scale bar represents 100 μ m). The number of nodes/DCX $^{+}$ cell (D) and tracings of the length of neurites on DCX $^{+}$ cells (E-F, scale bar represents 10 μ m). The neurite length of DCX $^{+}$ cells (G). * $p < 0.05$, ** $p < 0.001$ relative to the control group; two-tailed t-test, $n = 3$.

449

450 **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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