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

Lauren C. Pawley[1], Cara M. Hueston[1], James D. O'Leary[1], Danka A. Kozareva[1], John F. Cryan[1,2], Olivia F. O'Leary[1,2*], and Yvonne M. Nolan[1, 2*]

[1] Department of Anatomy and Neuroscience, University College Cork, Ireland

[2] APC Microbiome Ireland, University College Cork, Ireland

*These authors contributed equally to this work.

To whom correspondence should be addressed:

Yvonne M. Nolan, Department of Anatomy and Neuroscience, University College Cork, Ireland

Tel: (353) 21-420 5476

Fax: (353) 21-427 3518

Email: y.nolan@ucc.ie

**Word count:** 7002

**Declaration of interest:** None

**Abstract**

Both neuroinflammation and adult hippocampal neurogenesis (AHN) are implicated in many neurodegenerative disorders as well as in neuropsychiatric disorders, which often become symptomatic during adolescence. A better knowledge of the impact that chronic neuroinflammation has on the hippocampus during the adolescent period could lead to the discovery of new therapeutics for some of these disorders. The hippocampus is particularly vulnerable to altered concentrations of the pro-inflammatory cytokine interleukin-1β (IL-1β), with elevated levels implicated in the aetiology of neurodegenerative disorders such as Alzheimer's and Parkinson's, and stress-related disorders such as depression. The effect of acutely and chronically elevated concentrations of hippocampal IL-1β have been shown to reduce AHN in rats and mice. However, the effect of exposure to chronic overexpression of hippocampal IL-1β during adolescence, a time of increased vulnerability, hasn't been fully 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 for 6 weeks, during which time its impact on cognition and hippocampal neurogenesis were examined. A reduction in hippocampal neurogenesis was observed along with a reduced level of neurite branching on hippocampal neurons. However, there was no effect of IL-1β overexpression on cognitive performance. Our study has highlighted that chronic IL-1β overexpression in the hippocampus during the adolescent period exerts a negative impact on neurogenesis and neurite branching.

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

## 1.0 Introduction

Neuroinflammation is a key contributing factor to neurodegenerative and neuropsychiatric disorders (Freeman and Ting, 2016; Miller and Raison, 2016; Raison et al., 2006), and has been consistently demonstrated to exert a detrimental effect on hippocampal-dependent processes (Amor et al., 2010; Green and Nolan, 2014; Nolan et al., 2013; Ryan and Nolan, 2016). In particular, chronically elevated concentrations of IL-1β, which is produced predominantly by 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 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-1β are necessary for memory formation (Yirmiya and Goshen, 2011), transgenic overexpression of IL-1β has been shown to induce impairments in both spatial and contextual fear memory (Hein 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., 1986) in the hippocampus (Murray and Lynch, 1998; Vereker et al., 2000)).

As well as influencing the function of mature neurons, it is now established that both acutely and chronically elevated levels of IL-1β negatively affect adult hippocampal neurogenesis (AHN) (Hueston et al., 2018; O'Léime et al., 2017; Ryan et al., 2013), a process in which 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 is essential for cognitive functioning such as spatial learning and memory, contextual fear 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 (Levone et al., 2014; Revest et al., 2009; Snyder et al., 2011) and antidepressant action

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

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 al., 2014; Hueston et al., 2017a; Yassa et al., 2011b). It is not yet clear however, if these newly born cells serve the same function in the adolescent brain as they do in the adult brain. This is 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 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 cell proliferation when exposed to IL-1α, while there was no effect of IL-1α on NPCs from adult mice (McPherson et al., 2011). Disruptions in hippocampal neurogenesis have been implicated either directly or indirectly to various neuropsychiatric disorders, such as major depression and schizophrenia (Kempermann et al., 2008), which also exhibit 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 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 neurogenesis during adolescence might be involved in these neuropsychiatric disorders. However, there is a paucity of data on the effects of IL-1β on hippocampal neurogenesis during the adolescent period. Thus, the aim of this study was to examine the impact of chronic hippocampal IL-1β overexpression during adolescence on neuronal differentiation and morphology of recently-born neurons, as well as on cognitive function.

## 2.0 Methods

### 2.1 Animals and Experimental Design

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

### 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 feline immunodeficiency virus (FIV) backbone, containing a DNA insert encoding for the full length of the IL-1β gene (from start codon to stop codon without the 5` and 3` end untranslated regions or introns; gene accession NM_008361; $LV^{IL\text{-}1\beta mCherry}$) or an empty vector ($LV^{mCherry}$) as control. Expression efficiency was driven by a cytomegalovirus (CMV) promoter with 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 Genecopoeia ($LV^{IL\text{-}1\beta mCherry}$: Cat #LP-Mm03282-Lv80-0205-cs; $LV^{mCherry}$: Cat# LP-NEG-Lv80-0205-cs) with titers of > $1x10^7$ transfection units per mL (Genecopoeia, Rockville, MD, USA). It should be noted that some studies have incorporated a signal peptide at the end of the 5' end of the mature IL-1β cDNA (Shaftel et al., 2007). We have not utilized a signal peptide

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

### 2.3 Modified Spontaneous Location Recognition Test

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

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 with O4 + seconds with O5). The arena and objects were cleaned with a 70% ethanol solution between exposures of each animal to the arena to remove odour cues.

### 2.4 Object Recognition Test

The object recognition test, a hippocampal-perirhinal cortex-dependent task, was carried out as described previously (Bevins and Besheer, 2006). Rats were habituated to an empty chamber (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 10 minutes, followed by a 3-hour inter-trial interval. After the delay, recognition memory was tested with a 5-minute exposure to one novel object and one familiar object. All behaviors were recorded, and videos were scored to determine the amount of time the rats spent attending to the novel vs. familiar objects. Objects were counterbalanced between groups. Time spent with the objects was recorded, and a discrimination ratio (DR) of object recognition was calculated as DR = seconds with novel object/(seconds with novel object + seconds with familiar object).

### 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).

2.6 Confirmation of IL-1β Overexpression

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% 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 at 40μm and mounted onto gelatin-coated slides in a 1:6 series. Virus validation and confirmation of IL-1β overexpression was carried out as previously described (Hueston et al., 2018). Sections were washed in PBS before being blocked in 10% donkey serum blocking solution (G9023 Sigma) in PBS with 0.3% Triton-X (0.3% PBS-T), followed by overnight incubation at 4˚C with a primary antibody against mCherry (1:2000 Abcam, rabbit polyclonal ab167453) and IL-1β (goat polyclonal anti-mouse IL-1β 1:500 AF-401-NA R&D Systems) diluted in 0.3% PBS-T with 5% donkey serum. Sections were incubated with secondary antibodies (AlexaFluor 488 donkey anti-rabbit IgG A11055 Abcam and AlexaFluor 594 donkey anti-goat IgG A21207 Abcam) in 0.3% PBS-T and coverslipped with Vectashield mounting medium. To ensure that only viral-mediated and not endogenous IL-1β was detected, the primary antibody used was raised in mouse, not rat.

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

Cells that were immunopositive for IBA-1 (microglia) and DCX (immature neurons) were identified in the granule cell layer (GCL) of the DG of the hippocampus. Rehydrated sections 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% normal rabbit serum (R9133 Sigma) for DCX staining, prepared in 0.3% or 0.1% PBS-T respectively. For IBA-1 staining, sections were incubated overnight at 4°C in rabbit polyclonal 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 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 biotinylated goat anti-rabbit (1:200 pk-6101 Vector Laboratories) in 0.1% PBS-T and 1.5% normal rabbit serum. DCX-sections were rinsed with PBS, and incubated in biotinylated rabbit 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 (PK-6105/PK-6101 Vector Laboratories), followed by incubation with 3,3′-Diaminobenzidine (DAB) activated with 0.3% hydrogen peroxide. Slides were cover-slipped using DPX mounting medium.

## 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,

Maryland, USA, https://imagej.nih.gov/ij/, 1997-2016). To quantify IL-1β staining through the DG, mean fluorescence intensity was measured across a randomly selected area of the same dimensions within the DG, and the mean background fluorescence intensity was measured across a randomly selected area of the same size outside of the DG. IL-1β staining was expressed as the ratio between fluorescence within to fluorescence outside of the DG.

2.9 Quantification and Morphological Analysis of Cells

A modified stereological approach was performed to estimate the number of IBA-1$^+$ and DCX$^+$ 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 the ImageJ programme (Schneider et al., 2012). Measurements were obtained in pixels and converted to μm² using a scaled micrometer and ImageJ software (Schneider et al., 2012). Data were expressed as the number of cells per μm². To assess the degree of microglial activation in response to the IL-1β treatment, the somal size of IBA-1$^+$ microglial cells in the DG was observed at 60x magnification on the Olympus AX70 microscope. Ten randomly selected cells were sampled per animal, thus there were 30 microglia analyzed per experimental group. The area of the soma was measured using ImageJ and expressed as $\mu m^2$.

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 neurite branch points (nodes) per neuron.

## 2.10 Statistical Analysis

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

## 3.0 Results

### 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).

### 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-1β in the GCL of the DG of the hippocampus are shown in (Figure 2D-E). There was a significant increase in the fluorescence intensity of IL-1β in the DG of animals injected with the $LV^{IL\text{-}1\beta mCherry}$ virus compared to those injected with $LV^{mCherry}$ control virus ([$t = 3.435$, $p = 0.0264$]; Figure 2F), thus demonstrating successful transduction of cells by the lentivirus overexpressing mouse IL-1β at five weeks following surgery. Five weeks of IL-1β overexpression in the hippocampus significantly increased the number of microglia (IBA-$1^+$ cells)/μm² in the GCL of the DG ([$t = 4.911$, $p = 0.008$]; Figure 2G-I) and increased the somal size of IBA-$1^+$ cells in the GCL of the hippocampi in these animals ([$t = 5.305$, $p = 0.0131$]; Figure 2J-L).

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

IL-1β overexpression in the hippocampus significantly decreased the number of $DCX^+$ cells/μm² in the GCL of the DG ([$t = 3.637$, $p = 0.0220$]; Figure 3A-C). IL-1β 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 examined the effects of five weeks of hippocampal IL-1β overexpression in the adolescent brain on three tests of hippocampal-dependent memory (spontaneous alternation in the Y-maze, pattern separation and novel object recognition). Despite the IL-1β -induced decrease in hippocampal neurogenesis we observed, IL-1β overexpression during adolescence did not affect hippocampal-dependent memory, as measured by these behavioral tests (Figure 4). Specifically, hippocampal overexpression of IL-1β did not affect the percentage of alternations 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 objects equally during acquisition small separation (Figure 4C) and acquisition large separation (Figure 4D), respectively. All animals were able to differentiate the novel from familiar location when tested on small ([$t(18) = 0.9942$, $p = 0.3333$]; Figure 4E) and large pattern separation ([$t(18) = 0.5223$, $p = 0.6078$]; Figure 4E) thus there was no effect of hippocampal IL-1β overexpression on pattern separation as assessed using the modified spontaneous location recognition test. Performance in the novel object recognition task was also unaffected by hippocampal overexpression of IL-1β ([$t(18) = 0.9943$, $p = 0.3333$]; Figure 4F).

## 4.0 Discussion

Given the current paucity of data on the effects of IL-1β during the adolescent period on hippocampal neurogenesis, we aimed to examine the impact of chronic hippocampal IL-1β 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-1β overexpression induced a significant reduction in neurogenesis and neuronal complexity, but had no impact on cognitive performance.

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

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.

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

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).

We injected IL-1β into the dorsal hippocampus since lesion studies in rodents have shown that the dorsal hippocampus plays a more predominant role in spatial learning and memory than the ventral region which is predominantly involved in regulating anxiety (Bannerman et al., 2002). 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 (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 et al., 2014). In parallel, it has been reported that hippocampal neurogenesis might also be functionally segregated along its longitudinal axis whereby neurogenesis in the ventral hippocampus rather than the dorsal hippocampus is preferentially affected by stress and antidepressant drugs (O'Leary and Cryan, 2014; Tanti and Belzung, 2013). As such, we hypothesized that any impact of IL-1β on neurogenesis in the dorsal region would in turn affect cognitive processes that are dependent on hippocampal neurogenesis (i.e. pattern separation) and that also have a spatial component (i.e. pattern separation, y-maze) and where previous studies have reported that lesions of the dorsal hippocampus disrupt performance in such tests (Hammond et al., 2004; Josey and Brigman, 2015; Lee et al., 2005). While it is somewhat surprising we that found no impact of IL-1β overexpression in the dorsal hippocampus on these cognitive tasks, we previously found that chronic IL-1β overexpression in the dorsal hippocampus of adult rats impaired behavioural pattern separation but had no effect on spontaneous alternation and novel object recognition, hippocampus dependent tasks not associated with neurogenesis. Considering the role of inflammation and neurogenesis in stress-related psychiatric disorders (Goshen et al., 2008; Hueston et al., 2017b; Levone et al., 2014;

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 Il-1β overexpression in the ventral hippocampus on neurogenesis in this region and anxiety related behaviour. We report no impact of IL-1β 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-1β on cognitive tasks.

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 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 needed to validate this. Our current results are important given the clinical relevance of inflammation in disorders with impaired AHN (Ryan and Nolan, 2016). Neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and neuropsychiatric disorders such as major depression, are linked with chronic neuroinflammation (Ben Menachem-Zidon et al., 2008; Dursun et al., 2015; Maes et al., 2012), and have been shown to have modified levels of AHN (Winner and Winkler, 2015). Specifically, IL-1β has been found to be increased in the cerebrospinal fluid (CSF) of patients with severe depression (Levine et al., 1999) and in women with perinatal depression (Miller et al., 2019). There is some discrepancy in the literature however about whether IL-1β is increased (Blum-Degen et al., 1995) or unchanged (Martinez et al., 2012) in the CSF of AD patients compared to healthy individuals. However, IL-1β has 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., 2015). It is also plausible that such local increases of IL-1β in the hippocampus would further 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).

## 5.0 Conclusion

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

## Funding Sources

This work was funded by Science Foundation Ireland (SFI) under Grant Number SFI/IA/1537.

## Acknowledgements

We wish to thank Suzanne Crotty, Gerard Moloney and Tara Foley for technical assistance.

## Figures

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

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

**Figure 3:** Overexpression of IL-1β reduced the number of $DCX^+$ hippocampal neurons and negatively impacted on their complexity. The number of $DCX^+$ cells/$\mu m^2$ (A) and representative images of the number of $DCX^+$ cells in the hippocampus five weeks after lentiviral injection (B-C, scale bar represents 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; two-tailed t-test, n=3.

**Figure 4:** Five weeks of IL-1β 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; $n = 10$.
456
457

## References


Aizawa, K., Ageyama, N., Yokoyama, C., Hisatsune, T., 2009. Age-dependent alteration in hippocampal neurogenesis correlates with learning performance of macaque monkeys. Exp Anim 58, 403-407.

Amor, S., Puentes, F., Baker, D., van der Valk, P., 2010. Inflammation in neurodegenerative diseases. Immunology 129, 154-169.

Bannerman, D.M., Deacon, R.M., Offen, S., Friswell, J., Grubb, M., Rawlins, J.N., 2002. Double dissociation of function within the hippocampus: spatial memory and hyponeophagia. Behav Neurosci 116, 884-901.

Bannerman, D.M., Sprengel, R., Sanderson, D.J., McHugh, S.B., Rawlins, J.N., Monyer, H., Seeburg, P.H., 2014. Hippocampal synaptic plasticity, spatial memory and anxiety. Nat Rev Neurosci 15, 181-192.

Bekinschtein, P., Kent, B.A., Oomen, C.A., Clemenson, G.D., Gage, F.H., Saksida, L.M., Bussey, T.J., 2013. BDNF in the dentate gyrus is required for consolidation of "pattern-separated" memories. Cell Rep 5, 759-768.

Bekinschtein, P., Kent, B.A., Oomen, C.A., Clemenson, G.D., Gage, F.H., Saksida, L.M., Bussey, T.J., 2014. Brain-derived neurotrophic factor interacts with adult-born immature cells in the dentate gyrus during consolidation of overlapping memories. Hippocampus 24, 905-911.

Ben Menachem-Zidon, O., Goshen, I., Kreisel, T., Ben Menahem, Y., Reinhartz, E., Ben Hur, T., Yirmiya, R., 2008. Intrahippocampal transplantation of transgenic neural precursor cells overexpressing interleukin-1 receptor antagonist blocks chronic isolation-induced impairment in memory and neurogenesis. Neuropsychopharmacology 33, 2251-2262.

Bevins, R.A., Besheer, J., 2006. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat Protoc 1, 1306-1311.

Clelland, C.D., Choi, M., Romberg, C., Clemenson, G.D., Fragniere, A., Tyers, P., Jessberger, S., Saksida, L.M., Barker, R.A., Gage, F.H., Bussey, T.J., 2009. A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325, 210-213.

Curlik, D.M., Difeo, G., Shors, T.J., 2014. Preparing for adulthood: thousands upon thousands of new cells are born in the hippocampus during puberty, and most survive with effortful learning. Front Neurosci 8, 70.

Dursun, E., Gezen-Ak, D., Hanağası, H., Bilgiç, B., Lohmann, E., Ertan, S., Atasoy, İ., Alaylıoğlu, M., Araz, Ö., Önal, B., Gündüz, A., Apaydın, H., Kızıltan, G., Ulutin, T., Gürvit, H., Yılmazer, S., 2015. The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. J Neuroimmunol 283, 50-57.

Felderhoff-Mueser, U., Sifringer, M., Polley, O., Dzietko, M., Leineweber, B., Mahler, L., Baier, M., Bittigau, P., Obladen, M., Ikonomidou, C., Bührer, C., 2005. Caspase-1-processed interleukins in hyperoxia-induced cell death in the developing brain. Ann Neurol 57, 50-59.

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., Santoro, A., 2018. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 14, 576-590.

Freeman, L.C., Ting, J.P., 2016. The pathogenic role of the inflammasome in neurodegenerative diseases. J Neurochem 136 Suppl 1, 29-38.

Goshen, I., Kreisel, T., Ben-Menachem-Zidon, O., Licht, T., Weidenfeld, J., Ben-Hur, T., Yirmiya, R., 2008. Brain interleukin-1 mediates chronic stress-induced depression in mice

via adrenocortical activation and hippocampal neurogenesis suppression. Mol Psychiatry 13, 717-728.
Green, H.F., Nolan, Y.M., 2014. Inflammation and the developing brain: consequences for hippocampal neurogenesis and behavior. Neurosci Biobehav Rev 40, 20-34.
Green, H.F., Treacy, E., Keohane, A.K., Sullivan, A.M., O'Keeffe, G.W., Nolan, Y.M., 2012. A role for interleukin-1β in determining the lineage fate of embryonic rat hippocampal neural precursor cells. Mol Cell Neurosci 49, 311-321.
Griffin, W.S., Mrak, R.E., 2002. Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. J Leukoc Biol 72, 233-238.
Hammond RS, Tull LE, Stackman RW., 2004. On the delay-dependent involvement of the hippocampus in object recognition memory. Neurobiol Learn Mem. 82, 26-34.
Hein, A.M., Stasko, M.R., Matousek, S.B., Scott-McKean, J.J., Maier, S.F., Olschowka, J.A., Costa, A.C., O'Banion, M.K., 2010. Sustained hippocampal IL-1beta overexpression impairs contextual and spatial memory in transgenic mice. Brain Behav Immun 24, 243-253.
Hueston, C.M., Cryan, J.F., Nolan, Y.M., 2017a. Stress and adolescent hippocampal neurogenesis: diet and exercise as cognitive modulators. Transl Psychiatry 7, e1081.
Hueston CM, Cryan JF, Nolan YM. 2017b Adolescent social isolation stress unmasks the combined effects of adolescent exercise and adult inflammation on hippocampal neurogenesis and behavior. Neuroscience. 365:226-236.
Hueston, C.M., O'Leary, J.D., Hoban, A.E., Kozareva, D.A., Pawley, L.C., O'Leary, O.F., Cryan, J.F., Nolan, Y.M., 2018. Chronic interleukin-1β in the dorsal hippocampus impairs behavioural pattern separation. Brain Behav Immun 74, 252-264.
Hughes, R.N., 2004. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neurosci Biobehav Rev 28, 497-505.
Iannitelli, A., Quartini, A., Tirassa, P., Bersani, G., 2017. Schizophrenia and neurogenesis: A stem cell approach. Neurosci Biobehav Rev 80, 414-442.
Jessberger, S., Clark, R.E., Broadbent, N.J., Clemenson, G.D., Consiglio, A., Lie, D.C., Squire, L.R., Gage, F.H., 2009. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. Learn Mem 16, 147-154.
Josey M, Brigman JL., 2015. Loss of hippocampal function impairs pattern separation on a mouse touch-screen operant paradigm. Neurobiol Learn Mem. 125, 85-92.
Kempermann, G., Krebs, J., Fabel, K., 2008. The contribution of failing adult hippocampal neurogenesis to psychiatric disorders. Curr Opin Psychiatry 21, 290-295.
Kole, A.J., Annis, R.P., Deshmukh, M., 2013. Mature neurons: equipped for survival. Cell Death Dis 4, e689.
Koo, J.W., Duman, R.S., 2008. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc Natl Acad Sci U S A 105, 751-756.
Koo, J.W., Duman, R.S., 2009. Evidence for IL-1 receptor blockade as a therapeutic strategy for the treatment of depression. Curr Opin Investig Drugs 10, 664-671.
Kozareva, D.A., Foley, T., Moloney, G.M., Cryan, J.F., Nolan, Y.M., 2019. TLX knockdown in the dorsal dentate gyrus of juvenile rats differentially affects adolescent and adult behaviour. Behav Brain Res 360, 36-50.
Kuhn, H.G., Toda, T., Gage, F.H., 2018. Adult Hippocampal Neurogenesis: A Coming-of-Age Story. J Neurosci 38, 10401-10410.
Lee I, Jerman TS, Kesner RP. 2005. Disruption of delayed memory for a sequence of spatial locations following CA1- or CA3-lesions of the dorsal hippocampus. Neurobiol Learn Mem. 84, 138-47.

556 Levone, B.R., Cryan, J.F., O'Leary, O.F., 2014. Role of adult hippocampal neurogenesis in
557 stress resilience. Neurobiol Stress 1, 147-155.
558 Maes, M., Song, C., Yirmiya, R., 2012. Targeting IL-1 in depression. Expert Opin Ther
559 Targets 16, 1097-1112.
560 McPherson, C.A., Aoyama, M., Harry, G.J., 2011. Interleukin (IL)-1 and IL-6 regulation of
561 neural progenitor cell proliferation with hippocampal injury: differential regulatory
562 pathways in the subgranular zone (SGZ) of the adolescent and mature mouse brain. Brain
563 Behav Immun 25, 850-862.
564 Meijering, E., Jacob, M., Sarria, J.C., Steiner, P., Hirling, H., Unser, M., 2004. Design and
565 validation of a tool for neurite tracing and analysis in fluorescence microscopy images.
566 Cytometry A 58, 167-176.
567 Miller, A.H., Raison, C.L., 2016. The role of inflammation in depression: from evolutionary
568 imperative to modern treatment target. Nat Rev Immunol 16, 22-34.
569 Mirescu, C., Peters, J.D., Gould, E., 2004. Early life experience alters response of adult
570 neurogenesis to stress. Nat Neurosci 7, 841-846.
571 Moore, A.H., Wu, M., Shaftel, S.S., Graham, K.A., O'Banion, M.K., 2009. Sustained
572 expression of interleukin-1beta in mouse hippocampus impairs spatial memory.
573 Neuroscience 164, 1484-1495.
574 Morris, R.G., Anderson, E., Lynch, G.S., Baudry, M., 1986. Selective impairment of learning
575 and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist,
576 AP5. Nature 319, 774-776.
577 Moser, M.B., Moser, E.I., Forrest, E., Andersen, P., Morris, R.G., 1995. Spatial learning with
578 a minislab in the dorsal hippocampus. Proc Natl Acad Sci U S A 92, 9697-9701.
579 Murray, C.A., Lynch, M.A., 1998. Evidence that increased hippocampal expression of the
580 cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in
581 long-term potentiation. J Neurosci 18, 2974-2981.
582 Müller, N., Weidinger, E., Leitner, B., Schwarz, M.J., 2015. The role of inflammation in
583 schizophrenia. Front Neurosci 9, 372.
584 Nolan, Y.M., Sullivan, A.M., Toulouse, A., 2013. Parkinson's disease in the nuclear age of
585 neuroinflammation. Trends Mol Med 19, 187-196.
586 Pereira JDC, Rea K, Nolan YM, O'Leary OF, Dinan TG, Cryan JF. 2019. Depression's
587 Unholy Trinity: Dysregulated Stress, Immunity, and the Microbiome. Annu Rev Psychol.
588 doi: 10.1146/annurev-psych-122216-011613.
589 O'Leary OF, Cryan JF. 2014. A ventral view on antidepressant action: roles for adult
590 hippocampal neurogenesis along the dorsoventral axis. Trends Pharmacol Sci. 35, 675-87.
591 O'Léime, C.S., Cryan, J.F., Nolan, Y.M., 2017. Nuclear deterrents: Intrinsic regulators of IL-
592 1β-induced effects on hippocampal neurogenesis. Brain Behav Immun 66, 394-412.
593 Pothuizen, H.H., Zhang, W.N., Jongen-Rêlo, A.L., Feldon, J., Yee, B.K., 2004. Dissociation
594 of function between the dorsal and the ventral hippocampus in spatial learning abilities of
595 the rat: a within-subject, within-task comparison of reference and working spatial memory.
596 Eur J Neurosci 19, 705-712.
597 Pugh, C., Fleshner, M., Watkins, L.R., Maier, S.F., Rudy, J.W., 2001. The immune system
598 and memory consolidation: a role for the cytokine IL-1beta. Neurosci Biobehav Rev 25,
599 29-41.
600 Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and
601 the pathogenesis of depression. Trends Immunol 27, 24-31.
602 Revest, J.M., Dupret, D., Koehl, M., Funk-Reiter, C., Grosjean, N., Piazza, P.V., Abrous,
603 D.N., 2009. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol
604 Psychiatry 14, 959-967.

Ryan, S.M., Nolan, Y.M., 2016. Neuroinflammation negatively affects adult hippocampal neurogenesis and cognition: can exercise compensate? Neurosci Biobehav Rev 61, 121-131.
Ryan, S.M., O'Keeffe, G.W., O'Connor, C., Keeshan, K., Nolan, Y.M., 2013. Negative regulation of TLX by IL-1β correlates with an inhibition of adult hippocampal neural precursor cell proliferation. Brain Behav Immun 33, 7-13.
Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., Hen, R., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805-809.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9, 671-675.
Senechal, Y., Kelly, P.H., Cryan, J.F., Natt, F., Dev, K.K., 2007. Amyloid precursor protein knockdown by siRNA impairs spontaneous alternation in adult mice. J Neurochem 102, 1928-1940.
Shaftel, S.S., Kyrkanides, S., Olschowka, J.A., Miller, J.N., Johnson, R.E., O'Banion, M.K., 2007. Sustained hippocampal IL-1 beta overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology. J Clin Invest 117, 1595-1604.
Snyder, J.S., Soumier, A., Brewer, M., Pickel, J., Cameron, H.A., 2011. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 476, 458-461.
Tanti A, Belzung C. 2013. Neurogenesis along the septo-temporal axis of the hippocampus: are depression and the action of antidepressants region-specific? Neuroscience. 252, 234-52.
Trinchero, M.F., Buttner, K.A., Sulkes Cuevas, J.N., Temprana, S.G., Fontanet, P.A., Monzón-Salinas, M.C., Ledda, F., Paratcha, G., Schinder, A.F., 2017. High Plasticity of New Granule Cells in the Aging Hippocampus. Cell Rep 21, 1129-1139.
van Praag, H., Christie, B., 2015. Tracking Effects of Exercise on Neuronal Plasticity. Brain Plast 1, 3-4.
Winner, B., Winkler, J., 2015. Adult neurogenesis in neurodegenerative diseases. Cold Spring Harb Perspect Biol 7, a021287.
Wollaston, W.H., 1807. Description of the camera lucida. The Philosophical Magazine 27, 343-347.
Wu, M.D., Montgomery, S.L., Rivera-Escalera, F., Olschowka, J.A., O'Banion, M.K., 2013. Sustained IL-1β expression impairs adult hippocampal neurogenesis independent of IL-1 signaling in nestin+ neural precursor cells. Brain Behav Immun 32, 9-18.
Yassa, M.A., Lacy, J.W., Stark, S.M., Albert, M.S., Gallagher, M., Stark, C.E., 2011a. Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. Hippocampus 21, 968-979.
Yassa, M.A., Mattfeld, A.T., Stark, S.M., Stark, C.E., 2011b. Age-related memory deficits linked to circuit-specific disruptions in the hippocampus. Proc Natl Acad Sci U S A 108, 8873-8878.
Yirmiya, R., Goshen, I., 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun 25, 181-213.
Yun S, Reynolds RP, Masiulis I, Eisch AJ. 2016. Re-evaluating the link between neuropsychiatric disorders and dysregulated adult neurogenesis. Nat Med. 22, 1239-1247.