Journal of Neuroimmunology 331 (2019) 87–96

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Absence of the neurogenesis-dependent nuclear receptor TLX induces inflammation in the hippocampus

Danka A. Kozareva[a], Cara M. Hueston[a], Ciarán S. Ó'Léime[a], Suzanne Crotty[a], Peter Dockery[b], John F. Cryan[a,c], Yvonne M. Nolan[a,c,*]

[a] Department of Anatomy and Neuroscience, University College Cork, Ireland
[b] Department of Anatomy, National University of Ireland, Galway, Ireland
[c] APC Microbiome Institute, University College Cork, Ireland

ARTICLE INFO

Keywords:
Microglia
Neurogenesis
Hippocampus
TLX
Interleukin-1β
DCX

ABSTRACT

The orphan nuclear receptor TLX (Nr2e1) is a key regulator of hippocampal neurogenesis. Impaired adult hippocampal neurogenesis has been reported in neurodegenerative and psychiatric conditions including dementia and stress-related depression. Neuroinflammation is also implicated in the neuropathology of these disorders, and has been shown to negatively affect hippocampal neurogenesis. To investigate a role for TLX in hippocampal neuroinflammation, we assessed microglial activation in the hippocampus of mice with a spontaneous deletion of TLX. Results from our study suggest that a lack of TLX is implicated in deregulation of microglial phenotype and that consequently, the survival and function of newborn cells in the hippocampus is impaired. TLX may be an important target in understanding inflammatory-associated impairments in neurogenesis.

1. Introduction

The process of generating functional neurons from stem and progenitor cells in the central nervous system occurs in the adult as well as in the embryonic brain. Neurogenic niches have been identified in the adult mammalian brain including the subgranular zone (SGZ) of the hippocampus. Here, the progeny of SGZ stem cells migrate to the granule cell layer (GCL) of the dentate gyrus (DG) and integrate into hippocampal circuitry as mature excitatory neurons (Gage, 2000). Adult hippocampal neurogenesis has been implicated in learning and memory (Gould et al., 1999; Kempermann, 2008) and has been shown to play a role in mood regulation (Balu and Lucki, 2009; O'Leary and Cryan, 2014). Moreover, decreased hippocampal neurogenesis is recognized as an important mechanism underlying cognitive deficits associated with depression as well as with normal aging (Kuhn et al., 1996; Lazarov and Marr, 2010; Marlatt and Lucassen, 2010; Spalding et al., 2013). The precise mechanisms underlying the impairment of hippocampal neurogenesis and associated cognitive impairment are not yet fully understood, however, a role for a number of intrinsic factors have been proposed (Qu and Shi, 2009; Green and Nolan, 2014; O'Leary et al., 2016b).

One such intrinsic factor is the orphan nuclear receptor subfamily 2 group E member 1 (NR2E1), also known as TLX. The alignment of the TLX gene is highly conserved across species (97% homology between mouse and human; Jackson et al., 1998). Expression of TLX is restricted to the neurogenic niches of the brain as well as the retina (Monaghan et al., 1997; Shi et al., 2004) where it has been shown to be crucial for neural and retinal development (Miyawaki et al., 2004; Li et al., 2008). Specifically, TLX is responsible for the timing of neurogenesis by regulating the proliferation, differentiation and migration of stem cells (Roy et al., 2004) and has been termed a master regulator of neurogenesis (Islam and Zhang, 2015). Moreover, targeted disruption and conditional deletion of TLX in mouse models have implicated TLX as an important factor in the control and maintenance of adult hippocampal neurogenesis (Roy et al., 2004; Niu et al., 2011; Murai et al., 2014), and in hippocampal neurogenesis-associated behavioural tasks in rodents such as Morris Water Maze and contextual fear conditioning (Zhang et al., 2008; Murai et al., 2014). In a spontaneous deletion mouse model, adult TLX knock out mice displayed altered neurogenesis, synaptic plasticity, and an impairment of dendritic structures in the DG.

Abbreviations: ANOVA, one-way analysis of variance; Arg1, Arginase1; BrdU, 5-bromo-2′-deoxyuridine; CD206, C-Type Mannose Receptor 1; DCX, doublecortin; DG, dentate gyrus; GFAP, glial fibrillary acidic protein; GCL, granule cell layer; H & E, haemotoxylin and eosin; Iba-1, ionized calcium binding adaptor molecule 1; IL-1β, interleukin-1 beta; i.p., intraperitoneal; NDS, normal donkey serum; Nr2e1/TLX, tailless homolog orphan nuclear receptor subfamily 2 group E member 1; NSC, neural stem cellPBS, phosphate buffered saline; PFA, paraformaldehyde; SGZ, subgranular zone; SVZ, subventricular zone; TNFα, tumor necrosis factor alpha

* Corresponding author at: Department of Anatomy and Neuroscience, University College Cork, Ireland.
E-mail address: y.nolan@ucc.ie (Y.M. Nolan).

http://dx.doi.org/10.1016/j.jneuroim.2017.08.008
Received 19 May 2017; Received in revised form 18 August 2017; Accepted 18 August 2017

These mice also presented with impairments in motor, cognitive and anxiety-related behaviours (Young et al., 2002; Christie et al., 2006). Mice with constitutive knock out of the TLX gene present with lower body weight, hypoplasia and distortion of the anterior aspects of the brain. In addition, the Nr2e1$^{-/-}$ mice exhibited thin and frail rostral commissure, the cerebrum and olfactory lobes were underdeveloped and the retina suffered diminished vascularization and displacement of retinal ganglion cells and optic nerves (Young et al., 2002).

Microglial cells and pro-inflammatory cytokines in the microenvironment of the subgranular neurogenic niche have also been shown to impact upon neurogenesis (Yirmiya and Goshen, 2011; Green and Nolan, 2014). Microglia are the resident immune cells of the central nervous system and have been shown to regulate adult hippocampal neurogenesis under physiological conditions (Sierra et al., 2014). Quiescent (also known as ramified or resting) microglia display small cell bodies and multiple extending processes, with which they probe the environment for signals of injury, trauma or other disruption of homeostasis. However, under inflammatory conditions, microglia become activated and can suppress neurogenesis (Belarbi and Rosi, 2013). Activated microglia become motile using amoeboid-like movements and display enlarged cell soma and one or less extending process (Kettenmann and Verkhratsky, 2011; Kohman and Rhodes, 2013). Activated microglia have been described as either adopting a pro- (M1) or an alternatively activated-(M2) inflammatory phenotype (Orihuela et al., 2016). Pro-inflammatory or M1 microglia are phagocytic and release inflammatory molecules including cytokines, nitric oxide and other reactive oxygen species which have been shown to have a negative effect on hippocampal neurogenesis (Ekdahl et al., 2003; Fujioka and Akema, 2010; Belarbi et al., 2012). Two such cytokines shown to be released by activated microglia are interleukin − 1 beta (IL-1β) and tumor necrosis factor alpha (TNFα) (Nakamura et al., 1999; Wang et al., 2015). We have previously shown that IL-1β treatment decreases TLX expression in neurosphere cultures prepared from both embryonic and adult rat hippocampus in a time- and dose- dependent manner (Green and Nolan, 2012; Ryan et al., 2013). Alternatively activated (M2) microglia, on the other hand, have been shown to exhibit neuroprotective properties (Orihuela et al., 2016) by releasing growth factors, enzymes and cytokines which facilitate repair and neurite outgrowth (Butovsky et al., 2006; Cherry et al., 2014). For instance, the enzyme Arginase 1 (Arg1) is released in response to wound healing and extracellular matrix deposition (Cherry et al., 2014), while the expression of C-Type Mannose Receptor 1 (CD206) is promoted in response to stimulation by the anti-inflammatory cytokine IL-4 (Roszer, 2015). It should be noted however that it has been suggested that describing activated microglia as M1 or M2 type can be restrictive and can hinder our understanding of the complex pathways *via* which microglia influence the brain parenchyma (Ransohoff, 2016).

The role of TLX in the activation status of microglia in the hippocampal neurogenic niche remains largely unexplored. Thus, the aim of the present study was to investigate microglial phenotypes and the hippocampal architecture in heterozygous and homozygous mice with spontaneous deletion of TLX.

2. Material and methods

2.1. Animals

Two month old male Nr2e1$^{-/-}$ knock-out mice, Nr2e1$^{+/-}$ heterozygous mice, and wildtype controls (129S1/SvImJ background) were group housed under standard housing conditions (temperature 21 °C and relative humidity 55%), with food and water available *ad libitum*. Breeding pairs were kindly provided by Prof. Elizabeth Simpson, University of British Colombia. Nr2e1$^{-/-}$ mice exhibit a spontaneous deletion of the entire TLX allele, including all nine exons. However, the deletion of TLX does not affect the transcription of neighbouring genes (Kumar et al., 2004). All experiments were conducted in accordance with the European Directive 2010/63/EU, and under an authorization issued by the Health Products Regulatory Authority Ireland and approved by the Animal Ethics Committee of University College Cork.

2.2. BrdU administration and tissue preparation

Bromodeoxyuridine (BrdU; Sigma) was administered (4 x intraperitoneal (i.p.) injections over the course of 6 h at 75 μg/10 mL/kg) to one cohort of Nr2e1$^{-/-}$, Nr2e1$^{+/-}$ and wildtype mice at postnatal day (P) 42. Since the effect of silencing and overexpressing TLX on neuronal survival has been thoroughly examined at 3- and 4-week time points (Roy et al., 2004; Niu et al., 2011; Murai et al., 2014), we investigated the effect of TLX knock out on neuronal and cellular survival 2 weeks post BrdU injection. This allowed us to examine whether a lack of TLX interferes at an intermittent point (2 weeks) of the neurogenic process, rather than when adult-born neurons integrate into hippocampal circuitry (4 weeks). At P56 mice were euthanized with an i.p. injection of anaesthetic (1.0 mL/kg) and transcardially perfused using a 0.9% phosphate buffered saline (PBS) solution followed by 4.0% paraformaldehyde (PFA) in PBS. After overnight incubation in PFA, brains were incubated in 30% sucrose until they sank, and subsequently flash frozen using liquid nitrogen. Coronal sections (40 μm) through the hippocampus were collected directly onto slides in a 1:6 series, then stored at − 80 °C.

2.3. Immunohistochemistry

To determine the survival of adult-born hippocampal neurons, sections were double-labelled with BrdU and the neuronal marker NeuN. DNA was denatured in sections by incubation in 2 M HCl for 45 min at 37 °C, renatured in 0.1 M sodium tetraborate (pH 8.5) and then blocked in 3% normal donkey serum (NDS; Sigma D9663). Slides were incubated with rat anti-BrdU antibody (Abcam AB6326; 1:250) followed by AlexaFluor594 donkey anti-rat (Abcam Cat# ab150156; 1:500) and mouse anti-NeuN (Millipore MAB377; 1:100) antibodies. Finally, sections were incubated with AlexaFluor488 donkey anti-mouse (Abcam Cat# ab150105; 1:500) antibody, washed and coverslipped using anti-fade mounting medium (Dako; Cat# S3023).

The number of microglia and newly born neurons in the hippocampus was assessed by staining for ionized calcium binding adaptor molecule 1 (Iba-1) and doublecortin (DCX), respectively. To determine whether TLX was expressed on microglia, double-labelling was performed with TLX and Iba-1. Astrocytic activation was assessed using an antibody raised against the glial fibrillary acidic protein (GFAP). Sections were washed, incubated in 3% NDS and then in anti- Iba-1 (Wako 019-19741; 1:1000) or anti-DCX (Santa Cruz Biotechnology; Z0334; 1:100), the combination of TLX (Abcam ab86276; 1:100) and Iba1 (Abcam ab5076; 1:100), or in GFAP (DAKO z0334; 1:500) antibodies. Sections were incubated in AlexaFluor488 donkey anti-rabbit (GFAP and Iba-1; Abcam ab150073; 1:500) or AlexaFluor488 donkey anti-goat (DCX; Abcam ab150129; 1:500) or the combination of AlexaFluor488 donkey anti-rabbit (TLX; Abcam ab150073; 1:500) and Alexa Fluor594 donkey anti-goat (Iba1; Abcam ab150136; 1:500) antibodies and were subsequently counterstained with DAPI (Sigma D9642; 1:5000), and coverslipped using anti-fade mounting medium (Dako; S3023).

For haemotoxylin and eosin (H & E) staining, slides were washed in distilled water, incubated in haemotoxylin, washed and incubated in eosin. Slides were dehydrated in a series of alcohol concentrations 70%, 90%, 95% and 100%, followed by incubations in histolene.

2.4. Image analysis and cell quantification

Images were obtained using either an Olympus VS120 virtual slide scanning system (courtesy of Prof. Peter Dockery, Department of

Anatomy, NUI Galway, Ireland) or an Olympus FV1000 scanning laser confocal system (BioSciences Imaging Centre, Department of Anatomy and Neuroscience, University College Cork, Ireland). *Z*-stack images with a 1.10 μm or 4.4 μm step size were collected using a 10 × objective (BrdU/NeuN), 20 × objective (DCX/DAPI; Iba1/DAPI) or 40 × objective (GFAP). The DG was imaged bilaterally on all sections. For bright field images an Olympus BX533 upright microscope coupled to an Olympus DP72 camera was used with 10 × and 40 × objectives.

Cell quantification, area and volume measurements were performed using the image processing software packages OlyVIA and ImageJ (National Institute of Health, USA; Schneider et al., 2012). Quantification of BrdU- and BrdU/NeuN- positive cells was performed for the GCL, SGZ, and the hilus of the DG. Quantification of Iba-1-positive cells was performed for the GCL and hilus of the DG. The DCX-expressing cell bodies only emerge in the SGZ and were thus quantified within this area. Systematic random sampling was employed for all cell quantifications, and the unbiased Physical Paired Dissector method (Mayhew, 1992) was performed for unbiased stereological estimation of the number of each immunopositive cell phenotype (Crotty et al., 2008). For area quantification, the area of interest, *i.e.* subareas of the DG or the microglia soma, was outlined manually and its area calculated using the image processing software ImageJ. For analysis of the microglial phenotypes, Iba1 + cells with soma area equal to or below one standard deviation above the mean and more than two visible processes were categorized as ramified or resting, while cells with enlarged soma area (greater than one standard deviation above the mean) and up to one extending process were categorized as activated. The morphology of DCX-positive cells was analysed with NeuronJ, an ImageJ plug-in which facilitates the tracing and quantification of elongated image structures (Meijering et al., 2004; Meijering, 2010). Specifically, the dendritic length, number of dendrites and number of nodes for 10 randomly selected cells through the DG from each of five animals per group were compared across all three groups.

2.5. Quantitative RT-PCR analysis of hippocampal tissue

Fresh brains from wildtype, Nr2e1$^{+/-}$, and Nr2e1$^{-/-}$ were snap frozen, and stored at − 80 °C. The hippocampal region was micropunched according to visual comparison to the mouse brain atlas (Franklin, 2008).

Samples were processed according to the GenElute kit protocol (Sigma; RTN350). Briefly, total cellular RNA was homogenized into lysis solution and homogenized sample was filtered through a binding column to remove non-RNA from the sample. Equal volume of 70% ethanol was added to the filtrate and purified through columns, which were then washed with buffer. Purified mRNA was recovered into 30 μL of elution solution. A further DNAse wipeout step was conducted on the sample using DNase1 (Sigma; AMPD1) to ensure the complete removal of endogenous DNA from the samples. Total RNA yield and purity were determined using the Nanodrop System (Thermo Scientific). Synthesis of cDNA was performed using 0.5 μg of normalized total RNA from each sample using ReadyScript cDNA synthesis mix (Sigma; RDRT-25RXN). Probed cDNA amplification was performed in a 20 μL reaction consisting of 10 μL KiCqStart qPCR Ready Mix with ROX (Sigma; Cat# KCQS02), 0.1 μL of each forward and reverse primer (final concentration 250 nM), 1 μL cDNA template, and 8.8 μL RNase-free water. Real-time RT PCR was performed in duplicate in a 96-well plate (Applied Biosystems) and captured in real time using the StepOne Plus System (Applied Biosystems). Relative gene expression was adjusted to the housekeeper gene *Trfc*, and quantified using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Primer sequences were: 5′-CCCAAGTATTCTCAGATATGATTTCAA-3′ (forward) and 5′-AAAGGTATCCCTCCAACCACTC-3′ (reverse) for *Trfc*; 5′-TGCCACCTTTTGACAGTGATG-3′ (forward) and 5′-TGATGTGCTGCTGCGAGATT-3′ (reverse) for *IL-1β*; 5′-AGGCACTCCCCCAAAAGATG-3′ (forward) and 5′-TTGCTACGACGTGGGCTAC-3′ (reverse) for *TNFα*; 5′-TGGGTGGATGCTCACACTG-3` (forward) and 5′-ACAGGTTGCCCATGCAGATT-3 (reverse) for *Arg1*; and 5′-GTGGGGACCTGGCAAGTATC-3′ (forward) and 5′-CACTGGGGTTCCATCACTCC-3′ (reverse) for *CD206*.

2.6. Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) with an α-level of 0.05. In cases where data were not normally distributed or the assumption of homogeneity of variance was violated, the non-parametric Kruskal-Wallis analysis of variance by ranks was performed. Posthoc analyses were conducted using either Bonferroni's multiple comparisons or the Mann-Whitney multiple comparisons test for parametric and non-parametric data, respectively. All data are presented as mean ± SEM.

3. Results

3.1. Impaired hippocampal architecture and reduced neurogenesis and survival of adult-born cells in Nr2e1$^{-/-}$ but not Nr2e1$^{+/-}$ mice

Histological examination of coronal sections through the hippocampus of Nr2e1$^{-/-}$ mice identified distortion of the hippocampal structure compared with wildtype and Nr2e1$^{+/-}$ littermate controls (Suppl. material; Fig. A.1). The vulnerable nature of the tissue from Nr2e1$^{-/-}$ mice resulted in the appearance of vacuoles in the tissue. Fluorescent labelling with the nuclear stain DAPI illuminated the gross impairments in the structure of the DG of Nr2e1$^{-/-}$ mice that were also apparent in the H & *E*-stained sections. While no differences were evident between the dentate gyri of mice heterozygous for TLX and their wildtype littermates, the animals lacking TLX exhibited a notably smaller dentate structure with a significantly altered shape. Specifically, the upper and lower blades of the DG were both shorter and the hilus appeared wider in the Nr2e1$^{-/-}$ mice (Suppl. material; Fig. A.1). We observed a significant decrease in the number of surviving adult-born cells (BrdU-positive) in the DG of Nr2e1$^{-/-}$ mice compared to either wildtype or Nr2e1$^{+/-}$ mice ($F(2, 10) = 6.354$; $p < 0.001$). This difference was driven by a reduction of BrdU-positive cells in the SGZ of Nr2e1$^{-/-}$ mice ($F(2, 10) = 5.115$; $p < 0.05$) compared to their wildtype and heterozygous littermates; no significant change was observed across the three genotypes in the GCL ($F(2, 10) = 0.159$; $p > 0.05$) or the hilus ($F(2, 10) = 1.285$; $p > 0.05$; Fig. 1A). There was a significant decrease in the mean number of surviving new neurons in the DG of Nr2e1$^{-/-}$ compared to wildtype mice ($H(2) = 6.12$; $p < 0.05$). Additionally, the number of surviving new neurons in the SGZ was significantly lower in Nr2e1$^{-/-}$ compared to wildtype and heterozygous littermates ($H(2) = 7.71$; $p < 0.05$). There was no significant effect of genotype in either the GCL ($H(2) = 1.19$, $p = 0.55$) or the hilus ($H(2) = 0.08$, $p = 0.96$; Fig. 1B; C–E). There was a significant decrease in the number of DCX-positive cells in Nr2e1$^{-/-}$ mice ($F(2, 9) = 22.65$; $p < 0.001$) compared to both wildtype and Nr2e1$^{+/-}$ animals (Fig. 2A). DCX-positive cells which extended processes through the GCL were evident in wildtype and Nr2e1$^{+/-}$, but not in Nr2e1$^{-/-}$ mice (Fig. 2B–D). Morphological analysis of DCX-positive cells revealed that Nr2e1$^{-/-}$ mice exhibited a significant decrease in dendritic length ($F(2, 27) = 8.525$; $p < 0.01$; Fig. 2E), a reduced number of dendrites ($F(2, 27) = 4.219$; $p < 0.05$; Fig. 2F) and a diminished number of branching points of the dendritic tree ($F(2, 27) = 4.643$; $p < 0.05$; Fig. 2G–H) compared to wildtype control mice.

3.2. Microglia quantification and phenotype characterization

There was a significant increase in number of microglia in the DG and GCL of Nr2e1$^{-/-}$ mice compared to either their wildtype or Nr2e1$^{+/-}$ littermates (DG: $F(2, 9) = 16.43$, $p < 0.001$; GCL: $F(2, 9) = 30.51$, $p < 0.001$). There was no difference in microglia density in the hilus ($F(2, 9) = 1.435$; $p = 0.28$) across the three genotypes

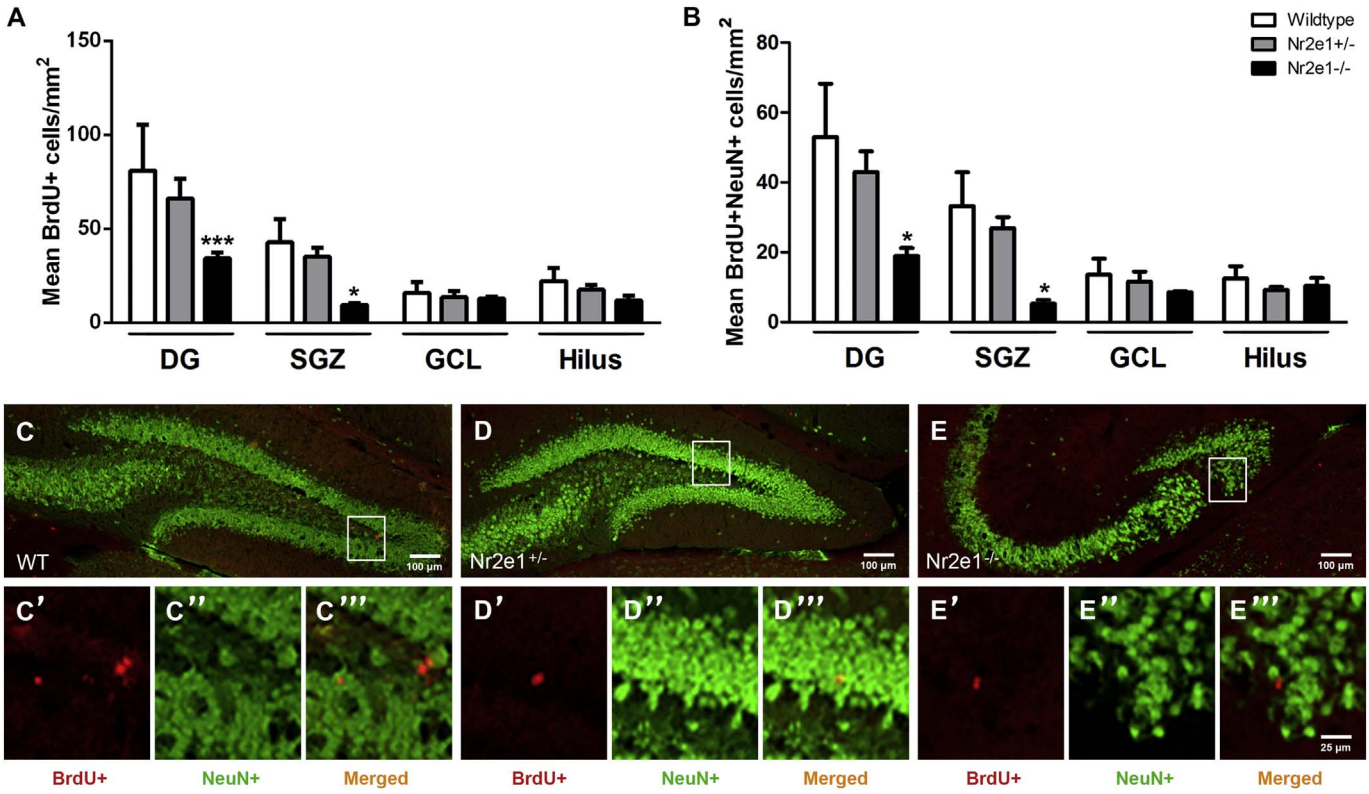

Fig. 1. Lack of TLX causes a reduction in cell survival in the DG of Nr2e1$^{-/-}$ but not Nr2e1$^{+/-}$ mice.
Mean density (cells/mm^2) of adult-born surviving cells (BrdU +; A) and adult-born surviving neurons (BrdU + NeuN +; B) in the DG, SGZ, GCL and hilus of wildtype, Nr2e1$^{+/-}$, and Nr2e1$^{-/-}$ mice. Data are expressed as mean ± SEM. ***$p < 0.001$, *$p < 0.05$ compared to wildtype and Nr2e1$^{+/-}$ mice (ANOVA), $n = 4$–5. Representative confocal images from wildtype (C), Nr2e1$^{+/-}$ (D), and Nr2e1$^{-/-}$ (E) of coronal sections through the DG immunohistochemically stained with BrdU (red), NeuN (green) and BrdU/NeuN (orange) at 10 × magnification. Scale bar = 100 µm. Higher magnification images depict immunopositive cells in the DG of wildtype (C′: BrdU + channel; C″: NeuN channel; C‴: merged channel), Nr2e1$^{+/-}$ (D′: BrdU + channel; D″: NeuN channel; D‴: merged channel) and Nr2e1$^{-/-}$ (E′: BrdU + channel; E″: NeuN channel; E‴: merged channel). Scale bar = 25 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Fig. 3A). Mice lacking TLX displayed microglia with greater cell somal area in the DG and GCL compared to wildtype or Nr2e1$^{+/-}$ mice (DG: $F(2, 9) = 5.387$; $p < 0.05$; GCL: $F(2, 9) = 7.665$; $p = 0.01$; Fig. 3B). Within the hilus we did not observe any differences in microglia size across the three groups ($F(2, 9) = 2.634$; $p = 0.13$; Fig. 3B). The proportion of "resting" or ramified microglia in the DG of mice with complete deletion of the TLX gene was significantly smaller than the proportion of ramified microglia in the wildtype controls ($F(2, 9) = 5.683$; $p < 0.05$). The same pattern was observed in the GCL, but not the hilus (GCL: $F(2, 9) = 5.480$; $p < 0.05$; Hilus: $F(2, 9) = 3.245$; $p = 0.09$; Fig. 3C). Finally, when we compared the proportion of activated microglia (having enlarged soma) across the three genotypes we found a significant increase of activated microglia in Nr2e1$^{-/-}$ mice compared to their wildtype and Nr2e1$^{+/-}$ littermates across all areas examined (DG: $F(2, 9) = 6.531$; $p = 0.01$; GCL: $F(2, 9) = 5.711$; $p < 0.05$; Hilus: $F(2, 9) = 4.119$; $p = 0.05$; Fig. 3D–F). There was a significant increase in the relative mRNA expression of the pro-inflammatory cytokine IL-1β in the hippocampus of mice lacking the TLX gene ($F(2, 15) = 17.79$; $p < 0.001$; Fig. 4A). There was no difference in relative mRNA expression of TNFα across the groups ($F(2, 17) = 0.295$; $p = 0.75$; Fig. 4B). Lastly, when we compared the relative mRNA expression of the alternatively activated markers Arg1 and CD206, we observed no difference across the three genotypes (Arg1: $F(2, 21) = 0.174$; $p > 0.05$; Fig. 4D; CD206: $F(2, 21) = 0.252$; $p > 0.05$; Fig. 4C).

We previously confirmed the expression of TLX in astrocytes derived from cultures of hippocampal neural stem cells (Green and Nolan, 2012). Here we show that immunostaining of hippocampal tissue from wildtype mice revealed no overlap between TLX and Iba-1 expression, (Fig. 5A). Upon examination of GFAP staining, astrocytes appeared to have adopted a reactive or activated phenotype in the hippocampus of Nr2e1$^{-/-}$ mice when compared to Nr2e1$^{+/-}$ and wildtype mice as evidenced by increased fluorescence intensity and hypertrophy of processes (Fig. 5B–D).

4. Discussion

In the present study, we demonstrated a pro-inflammatory phenotype in the DG of Nr2e1$^{-/-}$ mice. Specifically, we observed a significant increase in density of endogenous microglia in the dentate area of Nr2e1$^{-/-}$ mice, while Nr2e1$^{+/-}$ and wildtype mice displayed similar and lower densities of Iba1-positive cells. We found that TLX deficient animals exhibited a higher proportion of microglia that were in an activated state, and reduced percentage of ramified microglia. Furthermore, there was a significant increase in expression of the pro-inflammatory cytokine IL-1β, an indicator of classically activated microglia in the hippocampus of Nr2e1$^{-/-}$ mice, but no difference in the expression levels of TNFα or the alternatively activated markers CD206 and Arg1. These results were coupled with deficits in neuronal morphology and neurogenesis in Nr2e1$^{-/-}$ but not Nr2e1$^{+/-}$ mice as shown by a reduction in the density, dendritic length and nodes of newborn neurons as well as the density of surviving new neuronal and non-neuronal cells in the DG.

The current results support previous work by Monaghan et al. (1997) who showed alteration in the size and architecture of the hippocampus of Nr2e1$^{-/-}$ (in which the gene was functionally knocked down through homologous recombination) compared to wildtype mice (Monaghan et al., 1997). We also showed that the hippocampal architecture of the Nr2e1$^{+/-}$ mice resembles that of wildtype mice. The decrease in the number of adult born neurons in transgenic mice with a

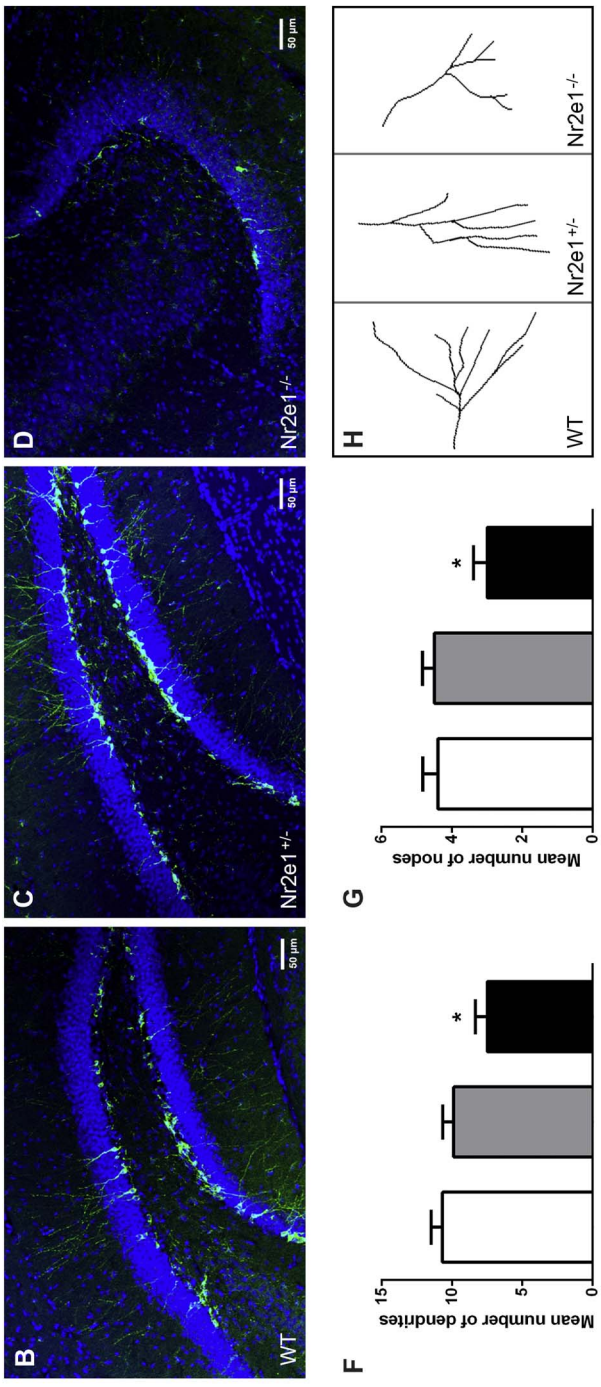

Fig. 2. Lack of TLX is associated with reduction in neurogenesis and impaired morphology of newly born neurons in the DG of Nr2e1$^{-/-}$ but not Nr2e1$^{+/-}$ mice.
Mean number of DCX + cells per mm^3 in wildtype (WT), Nr2e1$^{+/-}$, and Nr2e1$^{-/-}$ animals (A)., Representative confocal images through the DG of DCX + (green) cells in wildtype (B), Nr2e1$^{+/-}$ (C), and Nr2e1$^{-/-}$ (D) mice. Nuclei were counterstained with DAPI (blue). Scale bar = 50 µm. Mean dendritic length (µm) of DCX + cells (E), mean number of dendrites per DCX + cell (F) and mean number of nodes per DCX + cell (G) in wildtype, Nr2e1$^{+/-}$, and Nr2e1$^{-/-}$ mice. Data are expressed as mean ± SEM. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ compared to wildtype and Nr2e1$^{+/-}$ mice (ANOVA), $n = 4$–5. Representative tracings of cells from each group (H). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

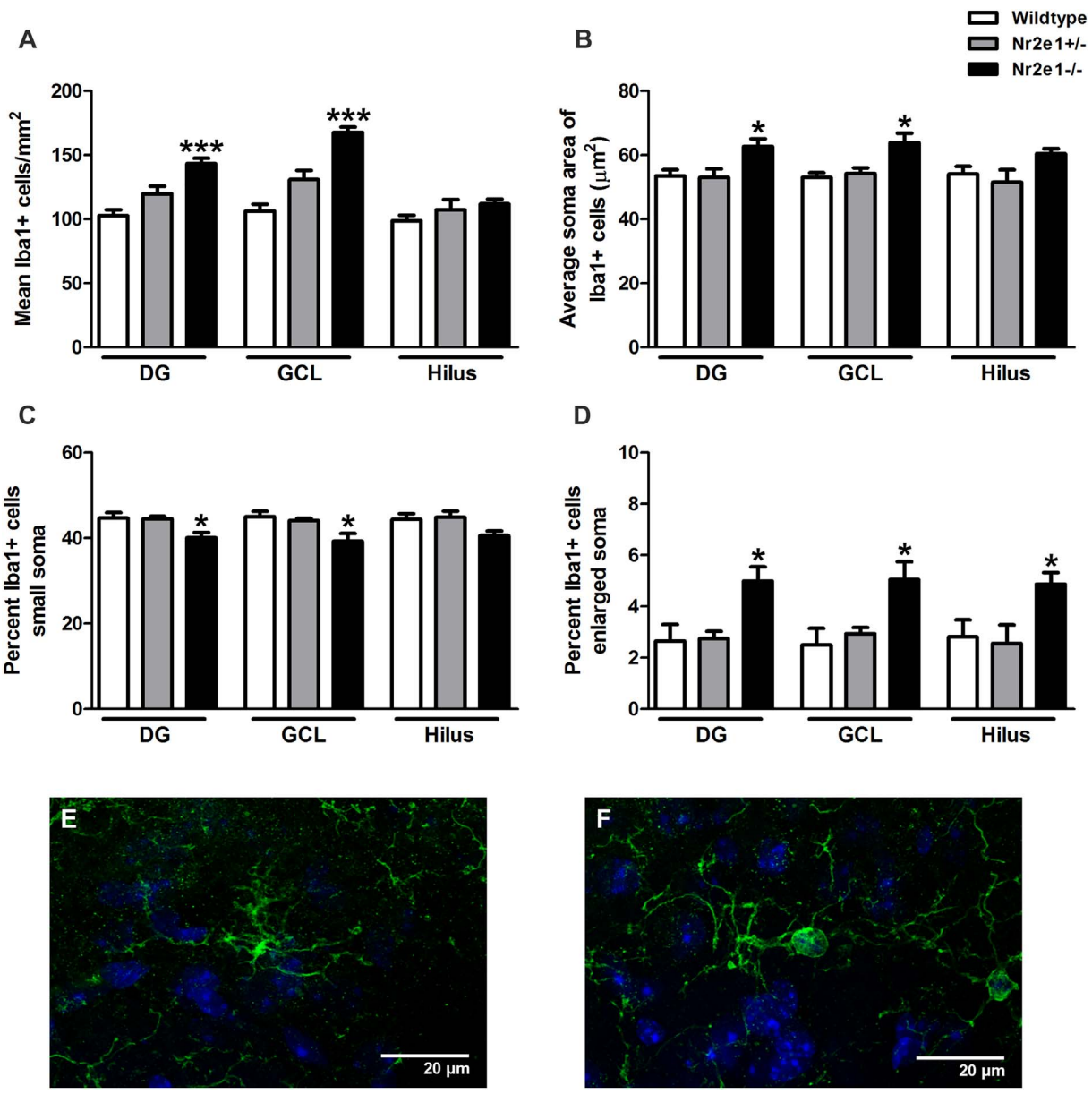

Fig. 3. Lack of TLX is associated with increased microglia density and activation.
The mean density (cells/mm^2) of microglia (Iba1 +) in the DG, GCL and hilus of wildtype (WT), Nr2e1$^{+/-}$, and Nr2e1$^{-/-}$ mice (A). Average microglial soma area (μm^2) in the DG, GCL and hilus of wildtype (WT), Nr2e1$^{+/-}$, and Nr2e1$^{-/-}$ mice (B). Proportion (%) of "resting" (C) and activated (D) microglia in the DG, GCL and hilus of wildtype (WT), Nr2e1$^{+/-}$, and Nr2e1$^{-/-}$ mice. Data are expressed as mean ± SEM. ***$p < 0.001$, *$p < 0.01$ compared to wildtype and Nr2e1$^{+/-}$ mice (ANOVA), $n = 4$. Representative images of a ramified/resting Iba1 + cell (E) and activated Iba1 + cell (F) at 100 × magnification.

targeted deletion of TLX was first described by Shi and colleagues in 2004. Later the Simpson group showed that mice with a spontaneous deletion of TLX not only displayed a decrease in neurogenesis, but also an impairment in synaptic plasticity and in the dendritic structure of pyramidal cells in the DG but not in the CA1 region of the hippocampus (Christie et al., 2006). Here we further demonstrated that mice with a spontaneous deletion of TLX also have reduced survival of newborn neurons as well as newborn non-neuronal cells in the DG as well as impaired morphology of newly born neurons. These findings support previous evidence from studies employing mice with a conditional deletion of TLX that reported that ablation of TLX resulted in a complete loss of transiently amplifying cells and neuroblasts (Roy et al., 2004; Niu et al., 2011). The current results also suggest that a lack of the TLX gene has long term negative consequences for the viability of both neurogenic and gliogenic cells. To the best of our knowledge, we are the first to demonstrate that Nr2e1$^{+/-}$ mice do not exhibit the neurogenic and neuronal survival deficiencies observed in Nr2e1$^{-/-}$ mice. Interestingly, we have previously observed that these Nr2e1$^{+/-}$ heterozygous mice performed similarly to wildtype littermates in contextual fear conditioning, a neurogenesis-associated hippocampal-dependant cognitive task, while Nr2e1$^{-/-}$ mice displayed impaired performance (O'Leary et al., 2016a). Together, these findings suggest that one allele of the TLX gene is sufficient for normal hippocampal neurogenic processes and associated behaviours. This is in line with the proposition that an animal with a heterozygous genotype generally has a higher relative fitness than an animal having a homozygous dominant or homozygous recessive genotype (Charlesworth and Willis, 2009). Indeed, studies have shown, that next to being the master regulator of neural stem cell maintenance (Islam and Zhang, 2015), TLX is also responsible for gliomagenesis in the adult neurogenic niches (Zou et al., 2012). What is more, increased TLX expression in stem cells from gliomas correlated with poor survival of patients (Park et al., 2010),

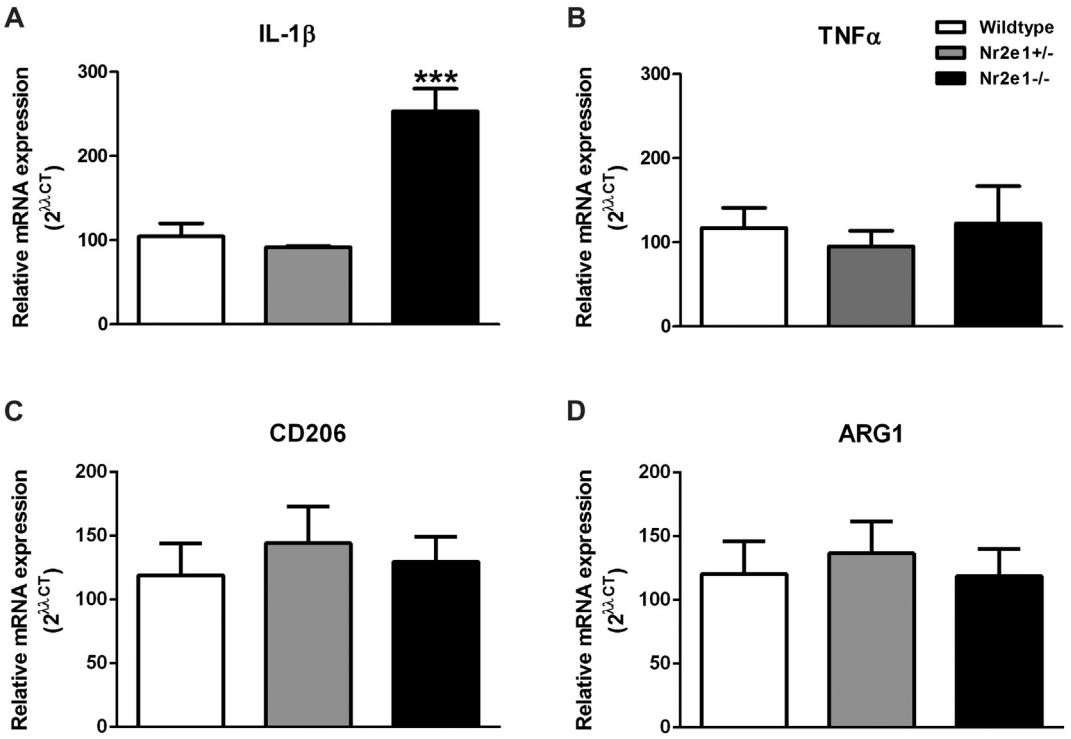

Fig. 4. Lack of TLX is associated with increase in markers of classically but not alternatively activated microglia.
Relative mRNA expression of IL-1β (A) TNFα (B) CD206 (C) and Arg1 (D) in the hippocampus of wildtype, Nr2e1$^{+/-}$ and Nr2e1$^{-/-}$ mice. All values were adjusted to the relative expression of the housekeeping gene *Trfc*. Data are expressed as the mean ± SEM, ***$p < 0.001$ (ANOVA), $n = 7–9$.

while downregulation of TLX was shown to inhibit tumorigenesis (Xie et al., 2014; Cui et al., 2016). Hence, one allele of the TLX gene may not only be sufficient but also beneficial from an evolutionary perspective.

Interestingly, mice with a spontaneous deletion of TLX did not exhibit full ablation of cell survival and neurogenesis as evidenced by a third of BrdU-positive, BrdU/NeuN-positive and DCX-positive cells remaining in the DG. This could be explained by the fact that neurogenesis is regulated by multiple pathways operating in parallel and/or in synergy that could compensate for the lack of TLX. For example, the Wnt/beta-catenin pathway (Qu et al., 2010), Notch signalling (Breunig et al., 2007; Ables et al., 2010) and the sonic hedgehog (Shh) pathway (Machold et al., 2003; Ahn and Joyner, 2005) have been identified as central mediators of neural stem cell (NSC) maintenance. Indeed, TLX has been shown to directly activate both the Wnt/beta-catenin (Qu et al., 2010) as well as the Notch signalling pathway (Shi, 2015). Conversely, Shh signalling appears to take place upstream of TLX (Shimozaki et al., 2012). It is also possible that in the absence of TLX expression, other transcription factors, such as SOX2 (Shimozaki et al., 2012), autonomously maintain, albeit a reduced pool of NSCs.

Despite the reduction in size of the hippocampus and the reduced cell survival observed, we found a pattern towards an increased number of endogenous microglia in the DG and GCL of the hippocampus of Nr2e1$^{-/-}$ mice. In recent years, microglia have been shown to be important effectors of adult hippocampal neurogenesis. In the absence of inflammation, ramified microglia are involved in pruning newborn cells, in providing trophic support for newly forming cells, and in apoptosis of newborn cells that fail to integrate into the existing circuitry (Sierra et al., 2010; Sierra et al., 2014). *In vitro* studies have also shown that in their resting state, microglia release factors which rescue neuroblasts, instruct neuronal cell differentiation and enhance and prolong the neurogenic potential of the cultured cells (Walton et al., 2006). However, microglia that become activated by foreign antigens or by changes in brain homeostasis are predominantly neurotoxic, promote an inflammatory environment and have been shown to suppress hippocampal neurogenesis by reducing the survival of neuroblasts (Ekdahl et al., 2003; Monje et al., 2003). We thus characterized the activation status of the microglia in the DG of Nr2e1$^{-/-}$ mice by firstly assessing the morphology of the cells, and observed that there was a significant increase in the proportion of activated microglia in the whole DG, including the GCL and hilus, of Nr2e1$^{-/-}$ mice. This result was coupled with a significant decrease in the proportion of ramified Iba1-positive cells in the DG of Nr2e1$^{-/-}$ animals. We showed through immunolabelling that TLX is not expressed on microglia. This finding is corroborated by RNA sequencing data (Zhang et al., 2014) and gene expression analysis and histology (Zou et al., 2012). Future studies should examine whether there is causal link between the lack of TLX expression and the increase in microglial activation that we observed. If such causality exists, it would be important to identify the underlying pathway mediating this process as potential therapeutic targets for inflammatory and/or neurodegenerative conditions may be revealed. We speculate that astrocytes may be key mediator of the activation of microglia under reduced or ablated TLX expression. In our transgenic model, we observed increased intensity of GFAP fluorescent staining and extended or hypertrophic GFAP processes in Nr2e1$^{-/-}$ mice. These parameters have been established as markers of astrocyte activation and reactive gliosis (Wilhelmsson et al., 2006; Pekny et al., 2014; Pekny et al., 2016). Furthermore, it has been shown that TLX regulates astrogenesis in the subventricular zone (SVZ) (Qin et al., 2014) as well as astrocyte development in the retina (Miyawaki et al., 2004). Additionally, loss of TLX expression in the SVZ also resulted in increased GFAP fluorescence staining and extended GFAP-positive cell processes (Li et al., 2012). Whether the spontaneous deletion of TLX in our model causes direct activation of astrocytes, which thereby activate microglia, or whether a lack of TLX results in activation of microglia, which cause astrocytes to adopt reactive phenotype remains to be investigated.

In order to determine whether the increased number of activated microglia in Nr2e1$^{-/-}$ mice display a pro-inflammatory or neuroprotective phenotype, we examined the relative mRNA expression of pro-inflammatory cytokines IL-1β and TNFα as well as the expression of alternatively activated markers Arg1 and CD206 in the hippocampus of

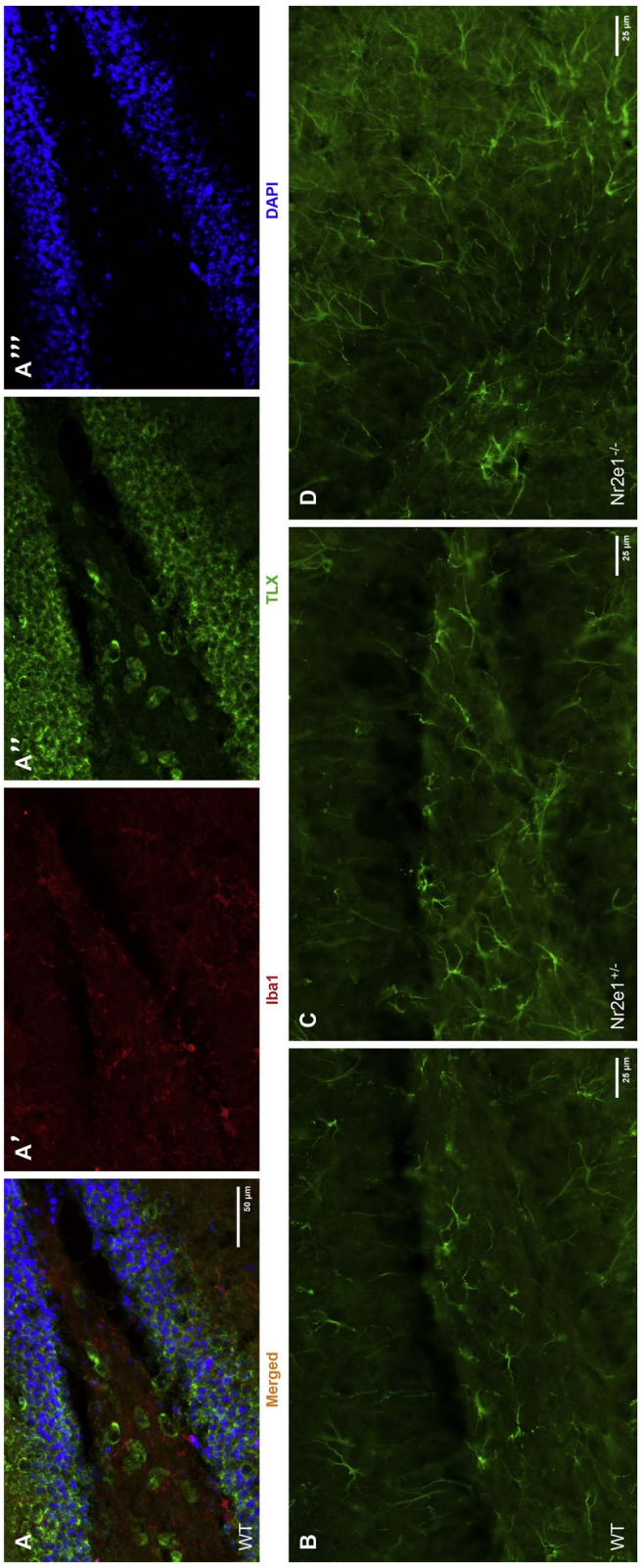

Fig. 5. Lack of TLX expression on microglia and increase in GFAP staining in the absence of TLX.
Representative image of the hippocampus of a wildtype mouse showing lack of colocalizationn between TLX and Iba1 by double immunofluorescence (A). Iba1 + staining appears as red (A'), TLX staining appears as green (A'') and nuclei were counterstained with DAPI (blue; A'''). Scale bar = 50 µm. Fluorescence images of astrocytes stained for GFAP (green) in wildtype (B), $Nr2e1^{+/-}$ (C), and $Nr2e1^{-/-}$ (D) mice. Images were taken at 40 × magnification. Scale bar = 25 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

these mice. We found that there was a significant increase in the mRNA expression of IL-1β but not TNFα in Nr2e1$^{-/-}$ compared to wildtype and Nr2e1$^{+/-}$ mice. Furthermore, no difference was observed in the expression of the alternatively activated markers. The appearance of microglial-derived IL-1β under endotoxic conditions was first investigated several decades ago (van Dam et al., 1992). This and a related classic pioneering study implicated IL-1β as a key player in the signalling pathway between neuronal and microglial cells during inflammatory challenge (van Dam et al., 1992; van Dam et al., 1995). Elevated levels of IL-1β have previously been shown to negatively impact upon neurogenesis. For example, central infusion of IL-1β to rats and overexpression of IL-1β in a conditional transgenic mouse model both significantly reduced neurogenesis in the DG (Goshen et al., 2008; Koo and Duman, 2008; Wu et al., 2012). *In vitro* studies have revealed that IL-1β reduced the number of proliferating cells, proliferating newly-born neurons, reduced neuronal differentiation, and stimulated astroglial differentiation in cultures prepared from adult and embryonic rat hippocampal neural precursor cells (Koo and Duman, 2008; Green and Nolan, 2012; Green et al., 2012; Ryan et al., 2013). We have previously demonstrated the vulnerability of TLX to IL-1β in embryonic rat hippocampal NSCs under both proliferation and differentiation conditions (Green and Nolan, 2012). The IL-1β-induced reduction of the numbers of proliferating cultured neural precursor cells and reduction of TLX expression was rescued by inhibition of GSK-3β signalling, which has been shown to negatively regulate neurogenesis (Green and Nolan, 2012). Additionally, we have previously shown that treatment with IL-1β reduced TLX expression in proliferating NSCs in the adult hippocampus in a dose and time-dependant manner. Further, administration of the IL-1 receptor antagonist or IL-1 receptor silencing prevented this decrease (Ryan et al., 2013). Interestingly, others have shown that astrocyte-mediated secretion of IL-1β occurs under inflammatory conditions in human astrocytes cultures (Didier et al., 2003), and spinal cord injury in rats induces increased expression of IL-1β in astrocytes located around the spinal cord ependyma, a neurogenic region (Paniagua-Torija et al., 2015). Recently it was demonstrated that reactive astrocytes are induced by activated microglia (Liddelow et al., 2017), which points to the question of whether activation of astrocytes or microglia occurs first as a result of lack of TLX expression.

Overall, these studies suggest that a lack of TLX expression may be implicated in microglial and astrocytic activation which is coupled with impaired survival and integration of hippocampal newborn neurons. Moreover, increased levels of endogenous IL-1β in the hippocampus of Nr2e1$^{-/-}$ mice correlate with the observed deficiencies in hippocampal neurogenesis and increased microglia activation. Given our previous studies demonstrating that IL-1β induces a decrease in expression of TLX in hippocampal NSCs (Green and Nolan, 2012), it is also likely that a vicious circle of an IL-1β-induced inflammatory environment and an impaired neurogenic phenotype is perpetuated in the absence of TLX expression. Furthermore, it would be important to establish the temporal relation between a lack of TLX expression, astrocyte activation and microglia activation, which may aid in identifying the primary cause for the observed increase in IL-1β in Nr2e1$^{-/-}$ mice.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jneuroim.2017.08.008.

Acknowledgements

This work was supported by Science Foundation Ireland (SFI) [Grant Number SFI/IA/1537]. The authors declare no conflict of interest. We thank Professor Elisabeth Simpson, University of British Columbia who kindly provided us with the Nr2e1 mice and Patrick Fitzgerald for help with establishing this mouse colony.

References

Ables, J.L., Decarolis, N.A., Johnson, M.A., Rivera, P.D., Gao, Z., Cooper, D.C., Radtke, F., Hsieh, J., Eisch, A.J., 2010. Notch1 is required for maintenance of the reservoir of adult hippocampal stem cells. J. Neurosci. 30, 10484–10492.

Ahn, S., Joyner, A.L., 2005. In vivo analysis of quiescent adult neural stem cells responding to Sonic hedgehog. Nature 437, 894–897.

Balu, D.T., Lucki, I., 2009. Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. Neurosci. Biobehav. Rev. 33, 232–252.

Belarbi, K., Rosi, S., 2013. Modulation of adult-born neurons in the inflamed hippocampus. Front. Cell. Neurosci. 7, 145.

Belarbi, K., Arellano, C., Ferguson, R., Jopson, T., Rosi, S., 2012. Chronic neuroinflammation impacts the recruitment of adult-born neurons into behaviorally relevant hippocampal networks. Brain Behav. Immun. 26, 18–23.

Breunig, J.J., Silbereis, J., Vaccarino, F.M., Sestan, N., Rakic, P., 2007. Notch regulates cell fate and dendrite morphology of newborn neurons in the postnatal dentate gyrus. Proc. Natl. Acad. Sci. U. S. A. 104, 20558–20563.

Butovsky, O., Koronyo-Hamaoui, M., Kunis, G., Ophir, E., Landa, G., Cohen, H., Schwartz, M., 2006. Glatiramer acetate fights against Alzheimer's disease by inducing dendritic-like microglia expressing insulin-like growth factor 1. Proc. Natl. Acad. Sci. U. S. A. 103, 11784–11789.

Charlesworth, D., Willis, J.H., 2009. The genetics of inbreeding depression. Nat. Rev. Genet. 10, 783–796.

Cherry, J.D., Olschowka, J.A., O'Banion, M.K., 2014. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. J. Neuroinflammation 11, 98.

Christie, B.R., Li, A.M., Redila, V.A., Booth, H., Wong, B.K., Eadie, B.D., Ernst, C., Simpson, E.M., 2006. Deletion of the nuclear receptor Nr2e1 impairs synaptic plasticity and dendritic structure in the mouse dentate gyrus. Neuroscience 137, 1031–1037.

Crotty, S., Fitzgerald, P., Tuohy, E., Harris, D.M., Fisher, A., Mandel, A., Bolton, A.E., Sullivan, A.M., Nolan, Y., 2008. Neuroprotective effects of novel phosphatidylglycerol-based phospholipids in the 6-hydroxydopamine model of Parkinson's disease. Eur. J. Neurosci. 27, 294–300.

Cui, Q., Yang, S., Ye, P., Tian, E., Sun, G., Zhou, J., Sun, G., Liu, X., Chen, C., Murai, K., Zhao, C., Azizian, K.T., Yang, L., Warden, C., Wu, X., D'Apuzzo, M., Brown, C., Badie, B., Peng, L., Riqqs, A.D., Rossi, J.J., Shi, Y., 2016. Downregulation of TLX induces TET3 expression and inhibits glioblastoma stem cell self-renewal and tumorigenesis. Nat. Commun. 7, 10637.

van Dam, A.M., Brouns, M., Louisse, S., Berkenbosch, F., 1992. Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? Brain Res. 588, 291–296.

Didier, N., Romero, I.A., Creminon, C., Wijkhuisen, A., Grassi, J., Mabondzo, A., 2003. Secretion of interleukin-1beta by astrocytes mediates endothelin-1 and tumour necrosis factor-alpha effects on human brain microvascular endothelial cell permeability. J. Neurochem. 86, 246–254.

Ekdahl, C.T., Claasen, J.H., Bonde, S., Kokaia, Z., Lindvall, O., 2003. Inflammation is detrimental for neurogenesis in adult brain. Proc. Natl. Acad. Sci. U. S. A. 100, 13632–13637.

Franklin, K.B.J., 2008. In: Paxinos, G. (Ed.), The Mouse Brain in Stereotaxic Coordinates, 3rd ed. Elsevier Academic Press, Amsterdam.

Fujioka, H., Akema, T., 2010. Lipopolysaccharide acutely inhibits proliferation of neural precursor cells in the dentate gyrus in adult rats. Brain Res. 1352, 35–42.

Gage, F.H., 2000. Mammalian neural stem cells. Science 287, 1433–1438.

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.

Gould, E., Beylin, A., Tanapat, P., Reeves, A., Shors, T.J., 1999. Learning enhances adult neurogenesis in the hippocampal formation. Nat. Neurosci. 2, 260–265.

Green, H.F., Nolan, Y.M., 2012. Unlocking mechanisms in interleukin-1beta-induced changes in hippocampal neurogenesis–a role for GSK-3beta and TLX. Transl. Psychiatry 2, e194.

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-1beta in determining the lineage fate of embryonic rat hippocampal neural precursor cells. Mol. Cell. Neurosci. 49, 311–321.

Islam, M.M., Zhang, C.L., 2015. TLX: a master regulator for neural stem cell maintenance and neurogenesis. Biochim. Biophys. Acta 1849, 210–216.

Jackson, A., Panayiotidis, P., Foroni, L., 1998. The human homologue of the Drosophila tailless gene (TLX): characterization and mapping to a region of common deletion in human lymphoid leukemia on chromosome 6q21. Genomics 50, 34–43.

Kempermann, G., 2008. The neurogenic reserve hypothesis: what is adult hippocampal neurogenesis good for? Trends Neurosci. 31, 163–169.

Kettenmann, H., Verkhratsky, A., 2011. Neuroglia–living nerve glue. Fortschr. Neurol. Psychiatr. 79, 588–597.

Kohman, R.A., Rhodes, J.S., 2013. Neurogenesis, inflammation and behavior. Brain Behav. Immun. 27, 22–32.

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.

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

Kumar, R.A., Chan, K.L., Wong, A.H., Little, K.Q., Rajcan-Separovic, E., Abrahams, B.S., Simpson, E.M., 2004. Unexpected embryonic stem (ES) cell mutations represent a concern in gene targeting: lessons from “fierce” mice. Genesis 38, 51–57.

Lazarov, O., Marr, R.A., 2010. Neurogenesis and Alzheimer's disease: at the crossroads.

Exp. Neurol. 223, 267–281.
Li, W., Sun, G., Yang, S., Qu, Q., Nakashima, K., Shi, Y., 2008. Nuclear receptor TLX regulates cell cycle progression in neural stem cells of the developing brain. Mol. Endocrinol. 22, 56–64.
Li, S., Sun, G., Murai, K., Ye, P., Shi, Y., 2012. Characterization of TLX expression in neural stem cells and progenitor cells in adult brains. PLoS One 7, e43324.
Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Munch, A.E., Chung, W.S., Peterson, T.C., Wilton, D.K., Frouin, A., Napier, B.A., Panicker, N., Kumar, M., Buckwalter, M.S., Rowitch, D.H., Dawson, V.L., Dawson, T.M., Stevens, B., Barres, B.A., 2017. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541, 481–487.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(− Delta Delta C(T)) method. Methods 25, 402–408.
Machold, R., Hayashi, S., Rutlin, M., Muzumdar, M.D., Nery, S., Corbin, J.G., Gritli-Linde, A., Dellovade, T., Porter, J.A., Rubin, L.L., Dudek, H., McMahon, A.P., Fishell, G., 2003. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron 39, 937–950.
Marlatt, M.W., Lucassen, P.J., 2010. Neurogenesis and Alzheimer's disease: biology and pathophysiology in mice and men. Curr. Alzheimer Res. 7, 113–125.
Mayhew, T.M., 1992. A review of recent advances in stereology for quantifying neural structure. J. Neurocytol. 21, 313–328.
Meijering, E., 2010. Neuron tracing in perspective. Cytometry A 77, 693–704.
Meijering, E., Jacob, M., Sarria, J.C., Steiner, P., Hirling, H., Unser, M., 2004. Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. Cytometry A 58, 167–176.
Miyawaki, T., Uemura, A., Dezawa, M., Yu, R.T., Ide, C., Nishikawa, S., Honda, Y., Tanabe, Y., Tanabe, T., 2004. Tlx, an orphan nuclear receptor, regulates cell numbers and astrocyte development in the developing retina. J. Neurosci. Off. J. Soc. Neurosci. 24, 8124–8134.
Monaghan, A.P., Bock, D., Gass, P., Schwager, A., Wolfer, D.P., Lipp, H.P., Schurtz, G., 1997. Defective limbic system in mice lacking the tailless gene. Nature 390, 515–517.
Monje, M.L., Toda, H., Palmer, T.D., 2003. Inflammatory blockade restores adult hippocampal neurogenesis. Science 302, 1760–1765.
Murai, K., Qu, Q., Sun, G., Ye, P., Li, W., Asuelime, G., Sun, E., Tsai, G.E., Shi, Y., 2014. Nuclear receptor TLX stimulates hippocampal neurogenesis and enhances learning and memory in a transgenic mouse model. Proc. Natl. Acad. Sci. U. S. A. 111, 9115–9120.
Nakamura, Y., Si, Q.S., Kataoka, K., 1999. Lipopolysaccharide-induced microglial activation in culture: temporal profiles of morphological change and release of cytokines and nitric oxide. Neurosci. Res. 35, 95–100.
Niu, W., Zou, Y., Shen, C., Zhang, C.L., 2011. Activation of postnatal neural stem cells requires nuclear receptor TLX. J. Neurosci. Off. J. Soc. Neurosci. 31, 13816–13828.
O'Leary, O.F., Cryan, J.F., 2014. A ventral view on antidepressant action: roles for adult hippocampal neurogenesis along the dorsoventral axis. Trends Pharmacol. Sci. 35, 675–687.
O'Leary, J.D., Kozareva, D.A., Hueston, C.M., O'Leary, O.F., Cryan, J.F., Nolan, Y.M., 2016a. The nuclear receptor Tlx regulates motor, cognitive and anxiety-related behaviours during adolescence and adulthood. Behav. Brain Res. 306, 36–47.
O'Leary, J.D., O'Leary, O.F., Cryan, J.F., Nolan, Y.M., 2016b. Regulation of behaviour by the nuclear receptor TLX. Genes Brain Behav. 306, 36–47.
Orihuela, R., McPherson, C.A., Harry, G.J., 2016. Microglial M1/M2 polarization and metabolic states. Br. J. Pharmacol. 173, 649–665.
Paniagua-Torija, B., Arevalo-Martin, A., Molina-Holgado, E., Molina-Holgado, F., Garcia-Ovejero, D., 2015. Spinal cord injury induces a long-lasting upregulation of interleukin-1beta in astrocytes around the central canal. Neuroscience 284, 283–289.
Park, H.J., Kim, J.K., Jeon, H.M., Oh, S.Y., Kim, S.H., Nam, D.H., Kim, H., 2010. The neural stem cell fate determinant TLX promotes tumorigenesis and genesis of cells resembling glioma stem cells. Mol. Cells. 30, 403–408.
Pekny, M., Wilhelmsson, U., Pekna, M., 2014. The dual role of astrocyte activation and reactive gliosis. Neurosci. Lett. 565, 30–38.
Pekny, M., Pekna, M., Messing, A., Steinhauser, C., Lee, J.M., Parpura, V., Hol, E.M., Sofroniew, M.V., Verkhratsky, A., 2016. Astrocytes: a central element in neurological diseases. Acta Neuropathol. 131, 323–345.
Qin, S., Niu, W., Iqbal, N., Smith, D.K., Zhang, C.L., 2014. Orphan nuclear receptor TLX regulates astrogenesis by modulating BMP signaling. Front. Neurosci. 8, 74.
Qu, Q., Shi, Y., 2009. Neural stem cells in the developing and adult brains. J. Cell. Physiol. 221, 5–9.
Qu, Q., Sun, G., Li, W., Yang, S., Ye, P., Zhao, C., Yu, R.T., Gage, F.H., Evans, R.M., Shi, Y., 2010. Orphan nuclear receptor TLX activates Wnt/beta-catenin signalling to stimulate neural stem cell proliferation and self-renewal. Nat. Cell biol. 12, 31–40 (sup pp 1-9).
Ransohoff, R.M., 2016. A polarizing question: do M1 and M2 microglia exist? Nat. Neurosci. 19, 987–991.
Roszer, T., 2015. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediat. Inflamm. 2015, 816460.
Roy, K., Kuznicki, K., Wu, Q., Sun, Z., Bock, D., Schutz, G., Vranich, N., Monaghan, A.P., 2004. The Tlx gene regulates the timing of neurogenesis in the cortex. J. Neurosci. Off. J. Soc. Neurosci. 24, 8333–8345.
Ryan, S.M., O'Keeffe, G.W., O'Connor, C., Keeshan, K., Nolan, Y.M., 2013. Negative regulation of TLX by IL-1beta correlates with an inhibition of adult hippocampal neural precursor cell proliferation. Brain Behav. Immun. 33, 7–13.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.
Shi, Y., 2015. Regulation of Adult Neural Stem Cell Activation by Orphan Nuclear Receptor TLX (NR2E1) and Notch Signaling. The Ruperto-Carola University of Heidelberg, Heidelberg, Germany.
Shi, Y., Chichung Lie, D., Taupin, P., Nakashima, K., Ray, J., Yu, R.T., Gage, F.H., Evans, R.M., 2004. Expression and function of orphan nuclear receptor TLX in adult neural stem cells. Nature 427, 78–83.
Shimozaki, K., Zhang, C.L., Suh, H., Denli, A.M., Evans, R.M., Gage, F.H., 2012. SRY-box-containing gene 2 regulation of nuclear receptor tailless (Tlx) transcription in adult neural stem cells. J. Biol. Chem. 287, 5969–5978.
Sierra, A., Encinas, J.M., Deudero, J.J., Chancey, J.H., Enikolopov, G., Overstreet-Wadiche, L.S., Tsirka, S.E., Maletic-Savatic, M., 2010. Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell 7, 483–495.
Sierra, A., Beccari, S., Diaz-Aparicio, I., Encinas, J.M., Comeau, S., Tremblay, M.E., 2014. Surveillance, phagocytosis, and inflammation: how never-resting microglia influence adult hippocampal neurogenesis. Neural Plast. 2014, 610343.
Spalding, K.L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H.B., Bostrom, E., Westerlund, I., Vial, C., Buchholz, B.A., Possnert, G., Mash, D.C., Druid, H., Frisen, J., 2013. Dynamics of hippocampal neurogenesis in adult humans. Cell 153, 1219–1227.
Van Dam, A.M., Bauer, J., Tilders, F.J., Berkenbosch, F., 1995. Endotoxin-induced appearance of immunoreactive interleukin-1 beta in ramified microglia in rat brain: a light and electron microscopic study. Neuroscience 65, 815–826.
Walton, N.M., Sutter, B.M., Laywell, E.D., Levkoff, L.H., Kearns, S.M., Marshall 2nd, G.P., Scheffler, B., Steindler, D.A., 2006. Microglia instruct subventricular zone neurogenesis. Glia 54, 815–825.
Wang, W.Y., Tan, M.S., Yu, J.T., Tan, L., 2015. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann. Transl. Med. 3, 136.
Wilhelmsson, U., Bushong, E.A., Price, D.L., Smarr, B.L., Phung, V., Terada, M., Ellisman, M.H., Pekny, M., 2006. Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. Proc. Natl. Acad. Sci. U. S. A. 103, 17513–17518.
Wu, M.D., Hein, A.M., Moravan, M.J., Shaftel, S.S., Olschowka, J.A., O'Banion, M.K., 2012. Adult murine hippocampal neurogenesis is inhibited by sustained IL-1beta and not rescued by voluntary running. Brain Behav. Immun. 26, 292–300.
Xie, Q., Flavahan, W.A., Bao, S., Rich, J., 2014. The tailless root of glioma: cancer stem cells. Cell Stem Cell 15, 114–116.
Yirmiya, R., Goshen, I., 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav. Immun. 25, 181–213.
Young, K.A., Berry, M.L., Mahaffey, C.L., Saionz, J.R., Hawes, N.L., Chang, B., Zheng, Q.Y., Smith, R.S., Bronson, R.T., Nelson, R.J., Simpson, E.M., 2002. Fierce: a new mouse deletion of Nr2e1; violent behaviour and ocular abnormalities are background-dependent. Behav. Brain Res. 132, 145–158.
Zhang, C.L., Zou, Y., He, W., Gage, F.H., Evans, R.M., 2008. A role for adult TLX-positive neural stem cells in learning and behaviour. Nature 451, 1004–1007.
Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O'Keeffe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N., Deng, S., Liddelow, S.A., Zhang, C., Daneman, R., Maniatis, T., Barres, B.A., Wu, J.Q., 2014. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947.
Zou, Y., Niu, W., Qin, S., Downes, M., Burns, D.K., Zhang, C.L., 2012. The nuclear receptor TLX is required for gliomagenesis within the adult neurogenic niche. Mol. Cell. Biol. 32, 4811–4820.