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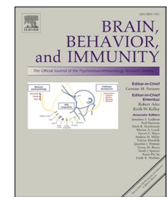

Cell line-specific modulation of inflammation by oestradiol in an *in vitro* model of antenatal depression☆

Madeline Kirkpatrick [a,*], Silvia Alboni [b], Federico Imbeni [b], Nicole Mariani [a], Nuriza Tukiran [a], Erin Mason-White [a], Carmine M. Pariante [a], Alessandra Borsini [a,**]

[a] *Stress, Psychiatry and Immunology Laboratory, Institute of Psychiatry, Psychology and Neuroscience, Department of Psychological Medicine, King's College London, UK*
[b] *Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy*

A R T I C L E I N F O

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A B S T R A C T

Antenatal depression is linked to adverse neurodevelopmental outcomes in offspring, likely mediated by a multitude of biological mechanisms, including elevated maternal cytokines. However, factors modulating fetal vulnerability or resilience to inflammatory exposure remain unclear. This study examines whether the steroid hormone, oestradiol, can modulate inflammatory responses in an *in vitro* model of hippocampal neurogenesis, using two fetal hippocampal progenitor cell lines: female-derived HPC0A07/03C and male-derived HIP-009. Cells were pre-treated with oestradiol for 24- and 48-hours during proliferation, followed by interleukin-1beta (IL-1β) exposure prior to the initiation of differentiation. Markers of proliferation and neurogenesis, as well as inflammatory cytokines and kynurenine pathway metabolites, were assessed. In female HPC0A07/03C cells, oestradiol pre-treatment prevented IL-1β-induced proliferation and apoptosis, and reduced cytokine production. Conversely, in male HIP-009 cells, oestradiol pre-treatment did not prevent IL-1β-induced reduction of proliferation and apoptosis and indeed enhanced inflammatory responses after 48 h. In terms of differentiation, IL-1β produced opposite effects on neurogenesis across cell lines, increasing neuronal maturation in female HPC0A07/03C cells, but decreasing neurite complexity in male HIP-009 cells. Notably, oestradiol pre-treatment in both lines reduced neuronal differentiation and increased kynurenine levels, suggesting potentially detrimental long-term effects. These results highlight complex, potentially cell-line-dependent, sex-specific hormone-immune interactions shaping fetal neurodevelopment and underscore the need to investigate these interactions when assessing risks and developing therapeutic interventions for inflammation-related neurodevelopmental disorders.

1. Introduction

A growing body of evidence indicates that antenatal depression, or depression experienced during pregnancy, is linked to a range of adverse outcomes in offspring (Srinivasan et al., 2020; Rogers et al., 2020; Davis et al., 2007; Stein et al., 2014). Studies estimate that 40–50 % of children exposed to antenatal depression develop some form of psychopathology later in life (Biaggi et al., 2025). Additionally, maternal depression during pregnancy is associated with negative birth outcomes, including preterm birth and low birth weight (Dadi et al., 2020). Notably, the increased risk of poor outcomes persists even after accounting for other contributing factors such as genetic vulnerability, postnatal depression, and maternal health (Pearson et al., 2013; Rice et al., 2010; O'Connor et al., 2002; Pina-Camacho et al., 2015). This suggests the existence of a specific antenatal effect, whereby biological changes within the *in-utero* environment during key developmental windows of prenatal programming directly influence fetal neurodevelopment. As such, uncovering these mechanisms is crucial to identify therapeutic targets and biomarkers for risk progression.

Among other mechanisms, such as increased foetal exposure to cortisol, growing evidence points to activation of the maternal immune system due to depression in pregnancy as a mediator of adverse offspring

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* Corresponding author at: Stress, Psychiatry and Immunology Lab & Perinatal Psychiatry, Institute of Psychiatry, Psychology and Neuroscience, King's College London, G.33.65, The Maurice Wohl Clinical Neuroscience Institute, 5 Cutcombe Road, London SE5 9RT, UK.
** Corresponding author at: Institute of Psychiatry, Psychology & Neuroscience, King's College London, The Maurice Wohl Clinical Neuroscience Institute K0.35, 5 Cutcombe Road, London SE5 9RT, UK.
E-mail addresses: madeline.kirkpatrick@kcl.ac.uk (M. Kirkpatrick), alessandra.borsini@kcl.ac.uk (A. Borsini).

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outcomes (Graham et al., 2022; Osborne et al., 2018; Hantsoo et al., 2019; Gustafsson et al., 2018). Indeed, clinical studies link depression in pregnancy with an increase in pro-inflammatory cytokines, including tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-1β and IL-17 (Osborne et al., 2018; Sha et al., 2022; Christian et al., 2009; Naudé et al., 2022). Additionally, in the Psychiatry Research and Motherhood – Depression (PRAM-D) study, conducted by our group, it was revealed that increases in maternal inflammatory cytokines were associated with suboptimal neurobehavioral outcomes (Osborne et al., 2018; Osborne et al., 2022). One potential mechanism underlying these disrupted neurobehavioral outcomes is the alteration of neural progenitor cell (NPC) fate by antenatal inflammatory challenges. Indeed, animal models of maternal immune activation (MIA) show that inflammatory exposure during the antenatal period disrupts NPC differentiation (Couch et al., 2021; Kiriyama et al., 2025). Additionally, in *in vitro* studies, exposure of NPCs to inflammatory cytokines leads to changes in neurogenesis, apoptosis, and an epigenetic priming of inflammatory pathways (for review, see (Kirkpatrick et al., 2025).

One important pathway in this context is the kynurenine pathway, an enzymatic pathway which degrades tryptophan. Through activation of the enzyme, indolamine-2,3-dioxygenase (IDO), inflammatory signalling can promote tryptophan degradation down the kynurenine pathway rather than towards serotonin synthesis. Importantly, this pathway is implicated in several psychiatric disorders, including depression both within and outside of pregnancy (Achtyes et al., 2020; Kadriu et al., 2021; Sha et al., 2024; van Zundert et al., 2022). This is thought to be underscored by increased production of neurotoxic metabolites, such as quinolinic acid, over neuroprotective metabolites, such as kynurenic acid. Indeed, depressive symptoms during pregnancy are associated with neurotoxic kynurenine pathway metabolites in the maternal serum (Sha et al., 2022) as well as in the placenta (Sha et al., 2024). Importantly, maternal stress during pregnancy can enhance hippocampal kynurenine and quinolinic acid concentrations in male mice (Moura et al., 2022), suggesting the kynurenine pathway may play a role in the mechanisms underlying intergenerational transmission. In our *in vitro* work, we have demonstrated that inflammatory signaling can reduce neurogenesis via activation of the kynurenine pathway and increased production of neurotoxic kynurenine metabolites (Zunszain et al., 2012).

Recently, sex-specific differences in the effects of inflammation in the context of depression have been described (Lombardo et al., 2021; Lombardo et al., 2024; Lombardo et al., 2022). This, however, has focused on the sex of the adult affected by depression. Importantly, studies investigating the effect of antenatal depression or maternal inflammation on offspring outcomes have highlighted a myriad of sex-specific effects that are relevant to the sex of the foetus (Guma and Chakravarty, 2025; Ardalan et al., 2019; Makinson et al., 2017; Ahun et al., 2021). In a recent review and meta-analysis of clinical studies, it was concluded that maternal depression was associated with poorer cognitive outcomes only in male, but not in female offspring (Ahun et al., 2021). Furthermore, studies have suggested that sex-specific effects may depend on the depression-associated biomarker which is raised in the mother, with studies revealing that prenatal inflammation has adverse effects on male offspring (Makinson et al., 2017; Hunter et al., 2021; Babri et al., 2014; Taylor et al., 2012; Nelson and Lenz, 2017), while the effect of maternal cortisol is greater in female offspring (Freedman et al., 2021; Graham et al., 2019). Such evidence highlights the importance of considering sex as a factor when trying to understand the specific mechanisms underlying the intergenerational transmission of psychiatric risk.

Given the observed sex-specific effects and the fact that only 50 % of offspring exposed to antenatal depression go on to develop adverse outcomes (Biaggi et al., 2025), it is important to investigate the biological factors that may underlie these differences or influence risk. As we are considering these effects of pregnancy, the potential role of sex hormones is particularly important. Oestradiol, the main bioactive compound of the female sex hormone, oestrogen, is a potent neurosteroid (Arevalo et al., 2015) and has been shown to influence mental health, with fluctuations in its levels, particularly during periods such as perimenopause, being linked to increased risk of mood disorders, including depression and anxiety (Behrman and Crockett, 2023). Although maternal oestradiol rises during pregnancy, it is largely prevented from entering the fetal brain due to binding to alpha-fetoprotein. The classical view, based on rodents, holds that only male fetuses generate brain oestradiol via aromatization of gonadal androgens, driving brain masculinization. However, increasing evidence shows that both male and female rodent brains can synthesise oestradiol *de novo* from cholesterol (Naftolin et al., 1971; Diotel et al., 2018; Amateau et al., 2004). Indeed, oestradiol concentrations in the neonatal hippocampus have been shown to be the same in males and females, which, in the absence of the androgen precursor in females, points to *de novo* estradiol synthesis (Amateau et al., 2004; McCarthy, 2009). Indeed, oestradiol and oestrogen receptors are abundant within the fetal brain of both males and females, where they are thought to play essential roles in synaptogenesis, apoptosis and neuronal differentiation (Amateau et al., 2004; Konkle and McCarthy, 2011; González et al., 2007; Baron-Cohen et al., 2020). Clearly, evidence for this is virtually all in rodents, and whether the same is true during human fetal development is unclear (McCarthy, 2008).

It is thought that this locally synthesised oestradiol may, in part, provide neuroprotection, and thus may play a role in modulating risk or resilience to prenatal inflammation (McCarthy, 2009). However, oestradiol's effects are complex, exhibiting both neuroprotective (Clevenger et al., 2018; Hilton et al., 2004) and neurodamaging actions *in vivo* (Nuñez et al., 2005; Nuñez and McCarthy, 2003). Oestradiol can modulate kynurenine pathway activity, where it has been shown to reduce inflammation-associated increases in IDO (Xu et al., 2015), but also inhibit kynurenine aminotransferase (KAT) enzymes responsible for producing neuroprotective kynurenine metabolites (Mason and Gullekson, 1960; Jayawickrama et al., 2017). Sex-specific responses to oestradiol modulation have also been reported (Azcoitia et al., 2019), and it is thought that the sex-dependent vulnerability to neuropsychiatric disorders following immune activation is underscored by a complex interaction with sex hormones (Ardalan et al., 2019; Arambula and McCarthy, 2020; McCarthy, 2019).

Despite the growing body of literature supporting oestrogen's immunomodulatory functions, there remains a lack of clarity regarding its specific effects on NPCs, particularly in the context of inflammatory responses. Furthermore, while sex differences in oestrogen-mediated neuroprotection have been reported in adult models, it is unknown whether these divergences lie at the level of specific neuronal responses. To fill this gap, the current study examines whether oestradiol modulates short- and long-term inflammatory responses in two different fetal NPC lines: one derived from a female donor, and one a male donor. This *in vitro* model provides a controlled system for isolating the direct effects of oestradiol and inflammation on NPCs, offering critical mechanistic insights into intergenerational transmission that are difficult to obtain *in vivo* due to the complex and dynamic physiological changes that occur during pregnancy. Moreover, since maternal immune activation has been shown to prime long-lasting deficits in hippocampal neurogenesis (Couch et al., 2021), modelling NPC responses *in vitro* provides a relevant framework for studying early vulnerability to neurodevelopmental disorders.

2. Materials and methods

2.1. Cell culture

For experiments, two multipotent human HPC lines were used: HPC0A07/03C (female donor, provided by ReNeuron, Surrey, UK) and HIP-009 (male donor, Vesta biotherapeutics). HPC0A07/03C cells were allowed to proliferate in reduced modified media (for details on media

reagents see (Zunszain et al., 2012; Borsini et al., 2017) supplemented with the growth factors epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and 4-hydroxytamoxifen (4-OHT). Cells were grown in 25 cm^2 filtered cap culture flasks (Nunclon, Roskilde, Denmark) and regulatory passaged at 80 % confluence before being transferred to plates. To initiate differentiation, growth factors and 4-OHT were removed. Detailed information on this cell line can be found in our previous publications (Borsini et al., 2017; Anacker et al., 2013; Borsini et al., 2018; Borsini et al., 2020; Borsini et al., 2022; Borsini et al., 2021).

For HIP-009, cells were expanded and differentiated as described in manufacturer's instructions. Briefly, HIP-009 were allowed to proliferate in neural stem cell growth media (Vesta Biotherapeutics), with added growth supplement cocktail (supplied by Vesta Biotherapeutics: Neural StemCell Growth Supplement, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), proprietary factor and laminin) on laminin coated 100 mm dishes. Cells were regularly passaged at 80 % confluence. Prior to initiating differentiation, cells were first grown in Neural transition medium (Vesta Biotherapeutics) with added transition medium supplement cocktail (supplied by Vesta Biotherapeutics: Neural transition base supplement, bFGF and EGF) for 3 days. Following this, neural differentiation medium (Vesta Biotherapeutics) with added differentiation medium supplement cocktail (supplied by Vesta Biotherapeutics: Neural differentiation base supplement, brain derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and laminin) was given. To maintain similar culture conditions, HIP-009 cells were also left to differentiate for 7 days. All cell culture was performed at 37 °C in 5 % CO_2 in a humidified atmosphere.

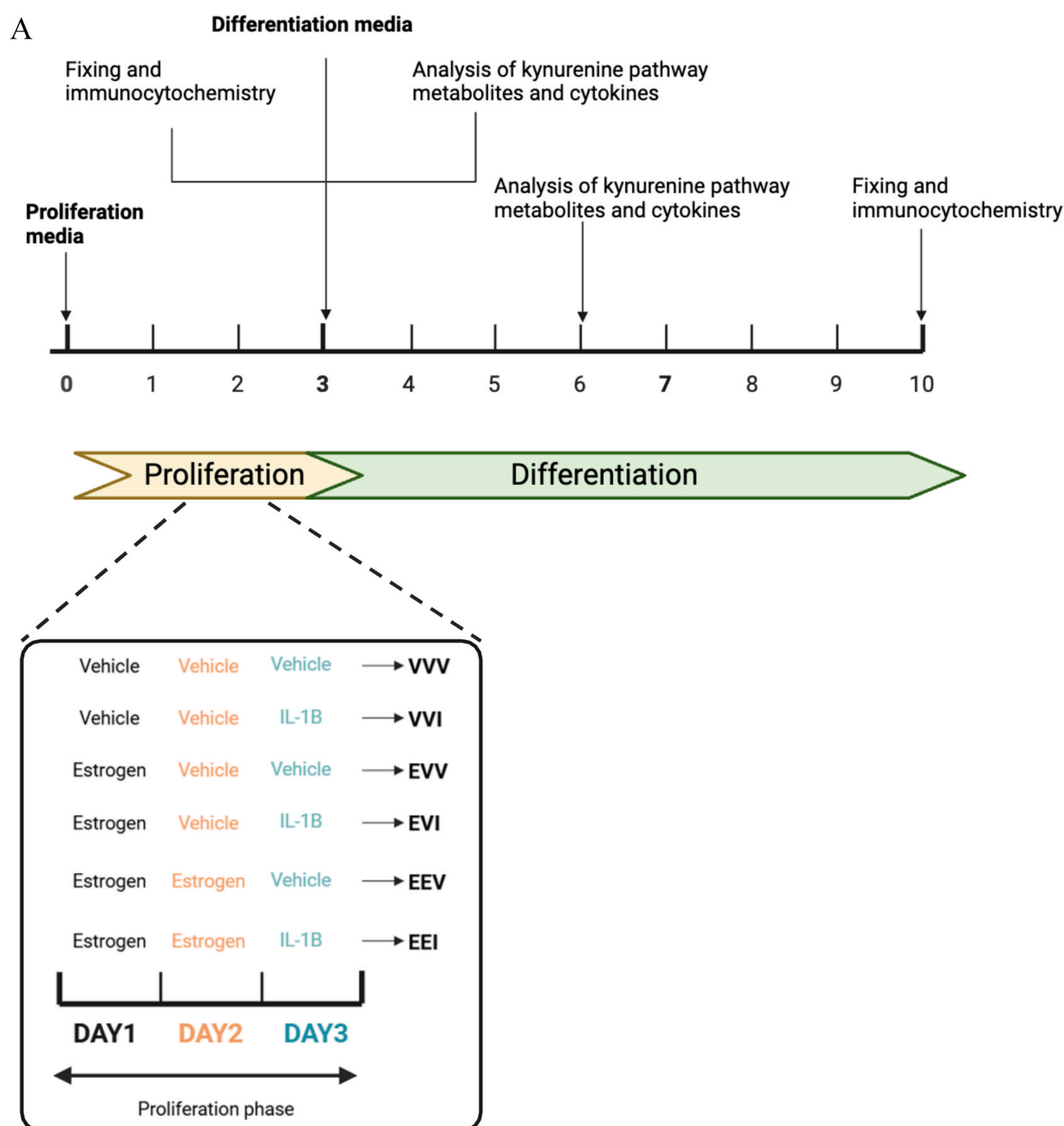

Fig. 1. Experimental timeline and treatment groups used during the proliferation phase – A) Female-derived HPC0A07/03C neural progenitor cells (NPC) were left in proliferating media for three days, followed by one week in differentiating conditions. Male-derived HIP-009 cells were given an additional 3-day transition period between the proliferation and differentiation timepoints (B). Both cell lines underwent the same combination of treatments during the proliferation phase. Treatments consisted of vehicle (standard growth media), oestradiol (10 ng/ml) and interleukin 1-beta (IL-1β). Treatment codes indicate 3 × 24 h sequences: V = vehicle, E = oestradiol, I = IL-1β. VVV represents cells given vehicle for all three days of proliferation; VVI represents cells given vehicle on days 1 and 2, followed by IL-1β on day 3; EVV is cells given oestradiol on day 1 followed by vehicle on days 2 and 3; in EVI cells were given oestradiol on day 1, vehicle on day 2, and IL-1β on day 2; in EEV cells were given oestradiol on days 1 and 2 followed by vehicle on day 3; and finally in EEI cells given oestradiol on days 1 and 2, followed by IL-1β on day three. For both cell lines, fixing and immunocytochemistry was performed at the end of proliferation and at the end of differentiation. Additionally, kynurenine pathway metabolites and cytokines were analysed in the supernatant at the end of proliferation and at day 3 of differentiation.

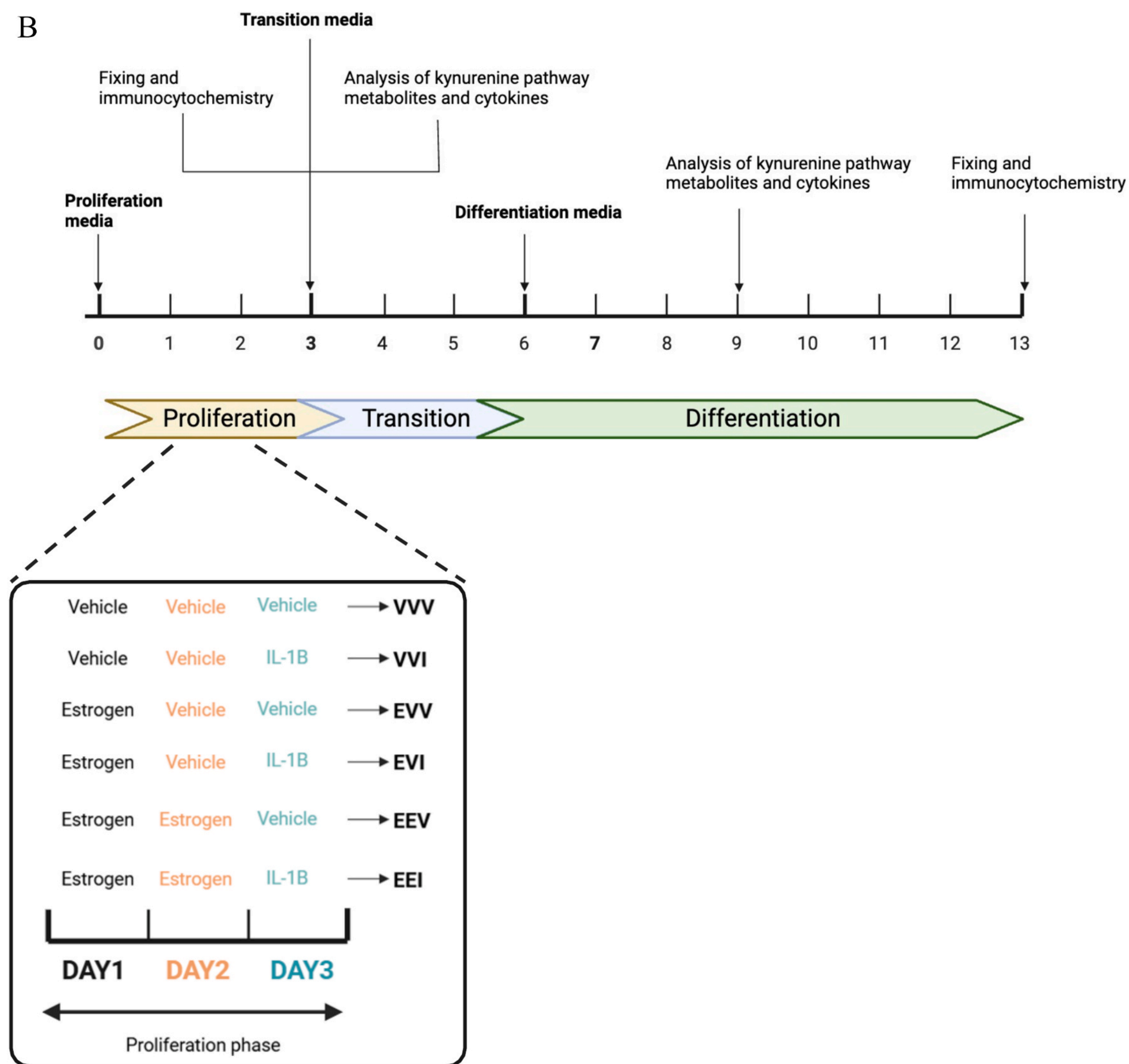

Fig. 1. (*continued*).

2.2. Cellular assays with oestradiol and IL-1β

For both cell lines, proliferating cells were plated in 96-well plates (Nunclon) at a density of 15,000 cells per well and allowed to adhere for 24 h. Cells were treated during the proliferation stage with estradiol (10 ng/ml) or vehicle for 24 h, or for 48 h with the compound added on day 1 and refreshed on day 2, followed by an immune challenge with IL-1β (10 ng/ml) for the final 24 h of proliferation. The timing of oestradiol treatment was chosen to reflect the effects of acute versus repeated oestradiol exposure, since previous studies have highlighted that timing can influence oestrogen's immunomodulatory effects (Straub, 2007; Hoffmann et al., 2023). Additionally, we aimed to model fluctuating oestradiol exposure during the perinatal period. The concentration of oestradiol was selected to align with levels reported in cord blood (25–40 nmol/L; 6.8–10.9 ng/ml) (Hollier et al., 2014; van de Beek et al., 2004), providing a physiologically relevant approximation of direct fetal exposure. An IL-1β concentration of 10 ng/ml was selected based on previous dose–response studies in the HPC0A07/03C cell line, which demonstrated this dose reliably induces a robust inflammatory response (Zunszain et al., 2012; Borsini et al., 2017; Horowitz et al., 2020; Horowitz et al., 2014); the same concentration was applied here to ensure consistency across both cell lines. For the HPC0A07/03C cells, 4-OHT was omitted from the media at the start of experiments due to oestrogenic activity, therefore vehicle treatment is proliferation media without 4-OHT. After the three-day proliferation period, cells were either fixed with 4 % paraformaldehyde (PFA) for immunocytochemical analysis of proliferation, or the respective growth media was removed, and cells were allowed to differentiate. After 7 days of differentiation, cells were fixed with 4 % PFA for immunocytochemistry. For analysis of cytokine production and kynurenine pathway metabolites, cell supernatant was collected at day 3 of proliferation and day 3 of differentiation. See Fig. 1 for an experimental timeline and explanation of treatment group abbreviations.

2.3. Multiplex cytokine assay

The concentration of cytokines in the supernatant was measured after day 3 of proliferation and day 3 of differentiation, for each cell line and treatment group. Supernatants were analysed using the Human Proinflammatory V-Plex Panel 1 Kit from Meso Scale discovery (MSD) (Gaithersburg, MD) according to the manufacturer's instructions. In brief, 50 µl of each diluted sample was added in duplicate to the MSD plate before shaking at 700 rpm for 2 h at room temperature. Subsequently, the plate was washed three times, before addition of 25 µl detection antibody mix to each well and shaking for another 2 h at 700 rpm. Finally, the plate was washed three times and 150 µl of read buffer were added to each well. The plate was analysed using the SECTOR Imager machine to measure a panel of 10 cytokines- IL-1β, IL-2, IL-4, IL-

6, IL-8, IL-10, IL-12, IL-13, TNF-α and IFN-γ. Unless otherwise stated, all cytokines were measurable above the MSD assay detection limit; pooled ranges across all groups for each analyte in each cell line are provided in Supplementary Table 1.

2.4. Liquid chromatography mass spectrometry

Cell's supernatants were defrosted on melting ice protected from light, and then 50 μL of conditioned media was added with an equal volume of ice-cold 1 M perchloric acid ($HClO_4$) fortified with a mix of the following stable isotope labeled internal standard (final concentration 1 μM): D-Glutamic Acid-d_5 (Cayman Chemical Ann Arbor, MI, USA), kynurenine-d4, kynurenic acid-d5, quinolinic acid-d_3 (Buchem BV, Netherlands) and tryptophan-d5 (Merck KGaA, Darmstadt, Germany). Samples were centrifuged (15,000 × g, 15 min), and the supernatants collected for injection into LC-MS/ MS. An Agilent 6410 triple quadrupole-mass spectrometer with an Agilent HP 1200 liquid chromatograph (Agilent, Milan, Italy) was used for experiments. As analytical column, a Discovery HS-F5 column (3 μm particle size, 150 × 2.1 mm, Supelco, Milan, Italy) was used. The duration of each run was 42 min. The analytes were separated by a gradient elution method, including water with 0.1 % formic acid (eluent A), and acetonitrile with 0.1 % formic acid (eluent B) using a linear elution profile of 15 min from 5 % to 90 % of ACN with a flow of 0.3 mL/min. For the LC-MS/MS measurement, an optimized, dynamic, Multiple Reaction Monitoring (MRM) program in ESI^+ was used. The injection volume was 20 μL. The Selected Reaction Monitoring (SRM) pairs were 148 → 84\153 → 135, 205 → 188\210 → 192, 209 → 192\213 → 196, 190 → 144\193 → 147, 138 → 120\171 → 153 for glutamate\glutamic acid-d_5, tryptophan \tryptophan-d_5, kynurenine\kynurenine-d_4, kynurenic acid\kynurenic acid-d_3 and anthranilic acid\quinolinic acid-d_3 respectively. The calibration curves were constructed using calibration standards and were linear over the concentration range of 0.48–500 μM, 0.005–5 μM, 0.097–100 μM and 0.001–1 μM for glutamate, kynurenine, tryptophan and kynurenic acid-anthranilic acid, respectively; with a correlation coefficient (r^2) ≥ 0.98 and an accuracy within acceptable range (100 % ± 20 %). Quinolinic acid, picolinic acid, xanthurenic acid, 3-hydroxyanthranilic acid, and 3-hydroxykynurenine were below the limit of detection (LOD) of the chromatographic method.

2.5. Immunocytochemistry

Immunocytochemistry was performed after day 3 of proliferation and day 7 of differentiation to investigate changes in proliferation, neurogenesis and apoptosis. Briefly, fixed cells were incubated in blocking solution (5 % normal donkey serum, 0.3 % Triton X-100 in PBS) for 1 h at room temperature, prior to incubation with primary antibodies (rabbit anti-doublecortin (DCX, ab18723), 1:500; mouse anti-microtubule-associated protein 2 (MAP2, ab11267), 1:500; mouse anti-Beta-tubulin III (TUJ1, MAB1195), 1:500; rabbit anti-cleaved caspase 3 (CC3, 9664 T), 1:500 and mouse anti-Ki67 (9449S), 1:500) at 4 °C overnight. Cells were incubated sequentially in blocking solution for thirty minutes, secondary antibodies (Alexa 488 donkey anti-rabbit (A21206); 1:1000; Alexa donkey 555 anti-mouse (A31570); 1:1000, Invitrogen) for 2 h, and 4′,6-diamidino-2-phenylindole (DAPI, D9542) dye for 5 min. Cells were imaged using the Opera Phenix and the percentage of Ki67-, DCX-, MAP2, TUJ1, and CC3-positive cells, as well as specific morphological properties (development of characteristic neurites, reflecting a more advanced stage of differentiation, as well as the mean neurite length and number of primary neurites), were assessed using an automated approach using Harmony.

2.6. Statistical analysis

Data are presented as mean ± SEM. All statistical analyses were performed using GraphPad Prism (Version 10.06.0). For each experiment, 3–6 independent biological replicates were analyzed, with at least three technical replicates per biological sample. One-way ANOVA with Bonferroni's post-hoc test was used for multiple comparisons. P-values < 0.05 were considered significant.

3. Results

3.1. Oestradiol prevents IL-1β-induced cellular and cytokine changes in female-derived HPC0A07/03C NPCs during proliferation

We first aimed to determine how oestradiol and Il-1β exposure would influence cellular and protein outcomes during proliferation in female-derived HPC0A07/03C NPCs. For this, cells were fixed after the three-day proliferation period and stained with antibodies for the proliferative marker Ki67, as well as the apoptotic marker CC3. Additionally, supernatant was collected after the 3-day proliferation period and analysed for cytokines and kynurenine metabolites, to understand potential mechanisms.

In cells treated with only the vehicle, the percentage of cells positive for Ki67 was 73 %. Similarly to our previous findings in female-derived HPC0A07/03C NPCs (Zunszain et al., 2012), treatment with IL-1β for the last 24 h of proliferation led to a significant increase in the percentage of cells positive for Ki67 compared to the control group (VVV vs VVI, 73 % vs 81 %, $p < 0.001$, Figs. 2A and 2B). As has been previously demonstrated (Smeeth et al., 2021), oestradiol treatment alone had no effect on proliferation markers. However, in cells treated with oestradiol for 48 h prior to IL-1β, the percentage of cells positive for Ki67 returned to control levels and was significantly decreased compared to that of cells given only IL-1β (VVI vs EEI, 81 % vs 74 %, $p < 0.01$, Figs. 2A and 2B). This effect was not seen with 24 h of oestradiol treatment.

Additionally, treatment with IL-1β for the last 24 h of proliferation led to a small significant increase in CC3+ cells (VVV vs VVI, 3.9 % vs 6.1 %, $p = 0.01$, Figs. 2A and 2C). However, treatment with oestradiol for 24 h prior to IL-1β returned the number of CC3-positive cells to control levels and was significantly decreased compared to that of cells given only IL-1β (VVI vs EVI, 6.1 % vs 4.0 %, $p = 0.03$, Figs. 2A and 2C), an effect not seen with 48 h of oestradiol pre-treatment. Interestingly, 48 h of oestradiol treatment, with no cytokine exposure, increased the number of apoptotic cells compared to the vehicle-only treatment (VVV vs EEV, 3.9 % vs 6.3 %, $p = 0.03$).

Consistent with these cellular outcomes in female-derived NPCs, pre-treatment with 10 ng/ml oestradiol during the first 24 h of proliferation prior to the IL-1β challenge significantly attenuated IL-1β-induced induction of IFN-γ (−16 %, $p = 0.03$, Fig. 3A), IL-2 (−25 %, $p < 0.01$, Fig. 3C), IL-6 (−33 %, $p < 0.001$, Fig. 3E), IL-12 (−29 %, $p < 0.0001$, Fig. 3H), IL-13 (−28 %, $p < 0.01$, Fig. 3I), and TNF-α (−37 %, $p < 0.0001$, Fig. 3J). Additionally, 48 h of oestradiol pre-treatment significantly decreased IL-1β-induced production of IFN-γ (−16 %, $p = 0.04$, Fig. 3A), IL-2 (−24 %, $p = 0.01$, Fig. 3C), IL-6 (−18 %, $p = 0.01$, Fig. 3E), IL-12 (−20 %, $p = 0.02$, Fig. 3H), IL-13 (–22 %, $p = 0.05$, Fig. 3I), and TNF-α (−25 %, $p < 0.01$, Fig. 3J), compared to IL-1β treatment alone. Without oestradiol, IL-1β broadly upregulated cytokine production (Supplementary Fig. 1A-J) Analysis of kynurenine pathway metabolites at this same timepoint revealed that IL-1β treatment for the last 24 h of proliferation induced a significant upregulation of kynurenine (VVV vs VVI, 0.08 μM vs 0.35 μM, $p < 0.01$, Fig. 3K), while no changes were observed in the other measured metabolites (Supplementary Fig. 1 K-M). Oestradiol pre-treatment had no effect on this increase in kynurenine, but we did observe time-dependent increase in glutamate levels by oestradiol treatment, compared to the vehicle-only treatment (Fig. 3L).

In summary, IL-1β enhanced proliferation in HPC0A07/03C NPCs, but this effect was blocked by 48 h of oestradiol pre-treatment. Oestradiol also inhibited the pro-apoptotic effects of IL-1β when administered for 24 h. Acute IL-1β exposure during proliferation induced inflammatory cytokine production and activated the kynurenine

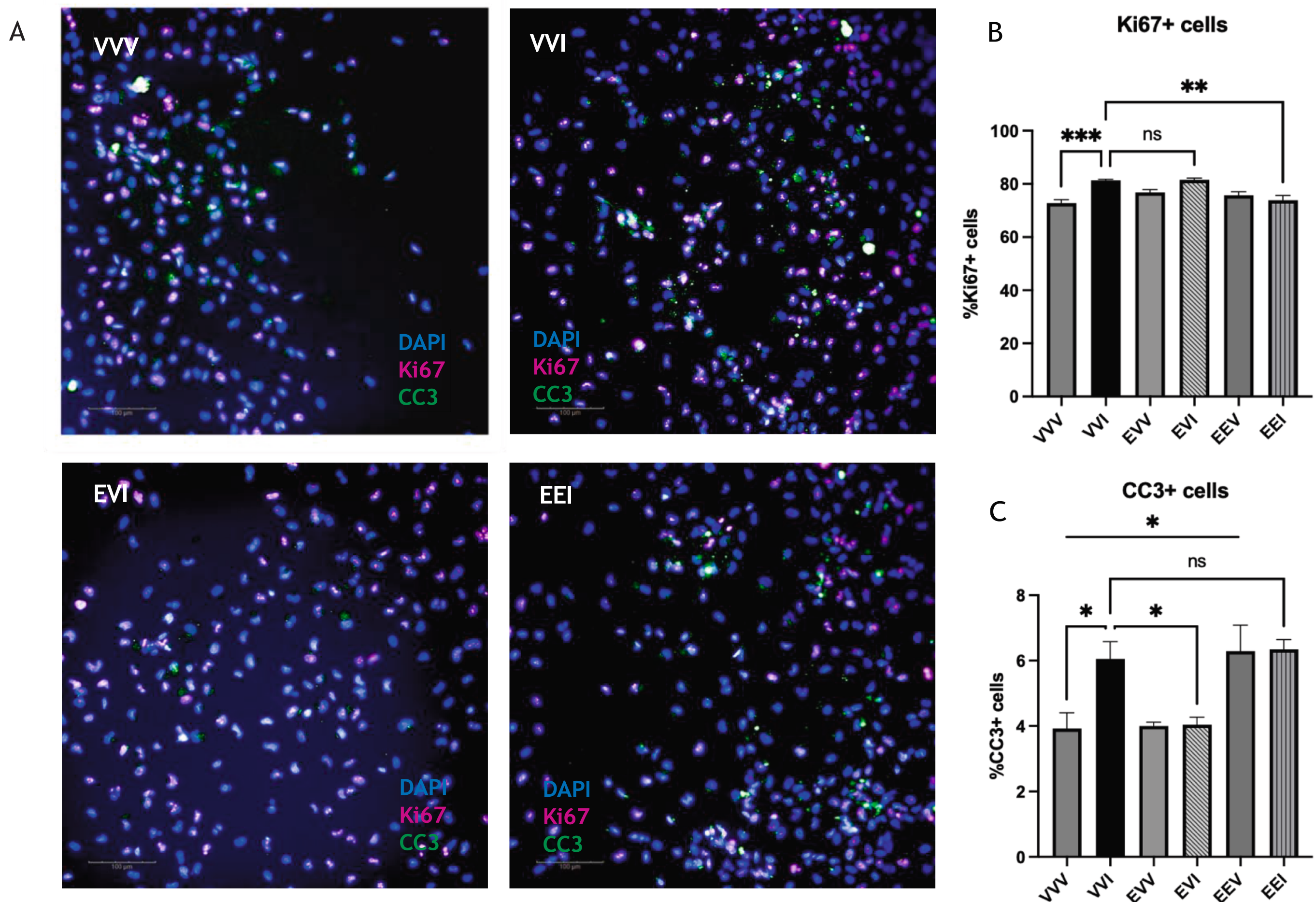

Fig. 2. The effects of oestradiol pre-treatment and IL-1β on cell proliferation and apoptosis in female-derived HPC0A07/03C neural progenitor cells (NPCs) – *(A) Cells were stained for the* proliferative marker Ki67 (pink) and the apoptotic marker cleaved caspase-3 (CC3, green). (B–C) The percentage of Ki67+ (B) and CC3+ (C) cells was quantified after 72 h of proliferation. Treatments were administered in three sequential 24-hour phases, represented by a three-letter code: V = vehicle, E = oestradiol, I = IL-1β. Oestradiol alone (EVV or EEV) did not significantly alter the percentage of Ki67+ cells compared to control (VVV). IL-1β (VVI) increased proliferation, an effect blocked by 48 h of oestradiol pre-treatment (EEI). Conversely, EEV increased apoptosis (CC3+ cells), as did IL-1β (VVI); however, 24-hour oestradiol pre-treatment (EVI) reduced IL-1β-induced apoptosis. Data are presented as mean ± SEM; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Results are from 4 independent biological replicates.

pathway. While oestradiol pre-treatment dampened cytokine production, it did not alter kynurenine pathway activation at this time point.

3.2. Oestradiol does not prevent IL-1β-induced cellular outcomes in male-derived HIP-009 cells during proliferation, but increases cytokine production

We then looked at these same cellular and protein outcomes in male-derived HIP-009 cells given the same treatment during the proliferation period. Similarly to female HPC0A07/03C NPCs, the percentage of cells positive for Ki67 in the HIP-009 vehicle-only group was 80 %. However, in contrast to HPC0A07/03C NPCs, treatment of male HIP-009 cells with IL-1β for the last 24 h of proliferation led to a significant decrease in the number of Ki67 positive cells (VVV vs VVI, 79 % vs 73 %, $p < 0.01$, Figs. 4A and 4B), compared to cells treated with only the vehicle. In this cell line, this effect was maintained even in cells given oestradiol pre-treatment prior to the inflammatory challenge. Furthermore, while treatment with IL-1β for the last 24 h of proliferation did not significantly change the number of CC3+ cells compared to the control group, cells treated with oestradiol for 48 h, followed by the inflammatory challenge with IL-1β, had a significant decrease in CC3-positive cells compared to treatment with IL-1β alone (VVI vs EEI, 25 % vs 16 %, $p = 0.04$, Figs. 4A and 4C).

In contrast to the anti-inflammatory effect observed in female HPC0A07/03C NPCs, acute 24-hour oestradiol pre-treatment of HIP-009 had no effect on cytokine production compared to IL-1β treatment alone Fig. 5A-J). However, notably, 48 h of oestradiol pre-treatment significantly increased IL-1β-induced production of IFN-γ (+11.49 %, $p < 0.01$, Fig. 5A) and IL-6 (+24.43 %, $p < 0.01$, Fig. 5E), when compared with treatment with IL-1β alone. Again, IL-1β induced a broad upregulation of all cytokines measured in this cell line (Supplementary Fig. 2(A-J)). Surprisingly, in these cells, 24 h of IL-1β treatment, either alone or following oestradiol pre-treatment, did not significantly change the concentration of any kynurenine metabolites measured (Fig. 5K and Supplementary Fig. 2K-M). However, as in the HPC0A07/03C NPCs, oestradiol time-dependently increased glutamate production (Fig. 5L).

In summary, IL-1β reduced proliferation in male-derived HIP-009 NPCs, and this effect was not altered by oestradiol pre-treatment. Repeated oestradiol exposure instead appeared to potentiate IL-1β-driven inflammatory signaling, with no corresponding regulation of the kynurenine pathway at this stage.

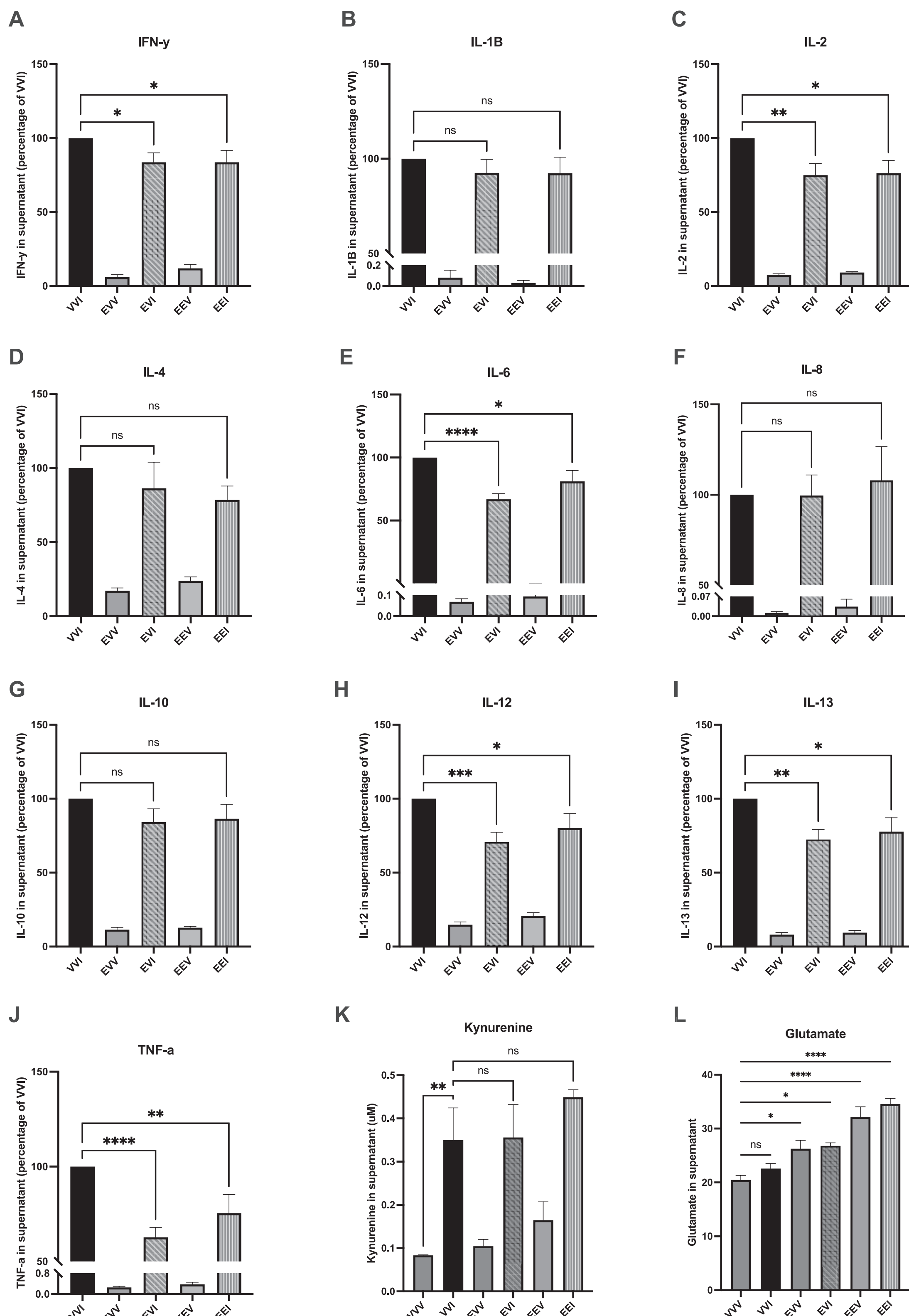

Fig. 3. The effects of oestradiol pretreatment and IL-1β on the level of inflammatory cytokines and kynurenine pathway metabolites in the supernatant of female-derived HPC0A07/03C NPCs after proliferation. – The concentration of cytokines and kynurenine pathway metabolites was measured in the supernatant following a three-day proliferation period under different treatment conditions. Oestradiol pre-treatment for both 24- (EVI) and 48-hours (EEI) significantly decreased the production of IFN-γ (A), IL-2 (C), IL-6 (E), IL-12 (H), IL-13 (I) and TNF-α (J) compared to the treatment with IL-1β alone (VVI). Treatment with IL-1β alone (VVI) significantly increased kynurenine levels compared to vehicle-treated controls (VVV), and this effect was not altered by oestradiol pre-treatment (K). Oestradiol treatment increased glutamate levels in a time-dependent manner (L). Treatment codes indicate 3 × 24 h sequences: V = vehicle, E = oestradiol, I = IL-1β. Data are presented as mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Results are from 3 to 6 independent biological replicates.

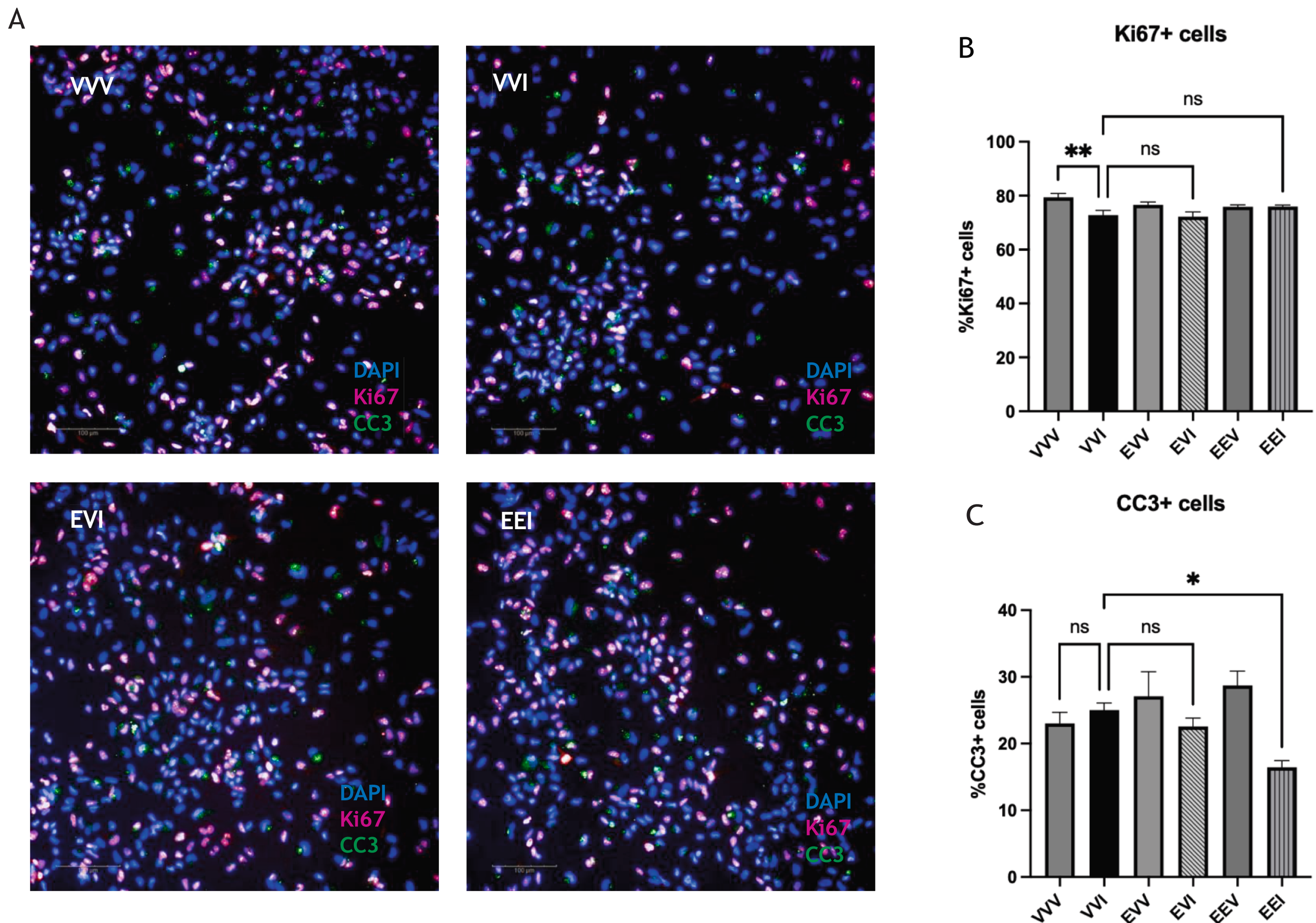

Fig. 4. The effects of oestradiol pre-treatment and IL-1β on cell proliferation and apoptosis in male-derived HIP-009 cells – (A) Cells were stained for the proliferative marker Ki67 (pink) and the apoptotic marker cleaved caspase-3 (CC3, green). (B–C) The percentage of Ki67+ (B) and CC3+ (C) cells was quantified after 72 h of proliferation. Treatments were administered in three sequential 24-hour phases, represented by a three-letter code: V = vehicle, E = oestradiol, I = IL-1β. Oestradiol alone (EVV or EEV) did not significantly alter the percentage of Ki67+ or CC3+ cells compared to control (VVV). IL-1β (VVI) decreased proliferation, however this was not impacted by oestradiol pre-treatment. While IL-1β had no effect on apoptosis, 48-hours of oestradiol pre-treatment, followed by IL-1β (EEI), decreased the number of apoptotic cells. Data are presented as mean ± SEM; $^*p < 0.05$, $^{**}p < 0.01$. Results are from 4 independent biological replicates.

3.3. *Proliferation-phase oestradiol pre-treatment alters neuronal differentiation and enhances IL-1β-induced kynurenine responses during differentiation in female-derived HPC0A07/03C NPCs*

Although oestradiol and IL-1β treatment was limited to proliferation, we aimed to assess whether oestradiol exposure and inflammatory challenge during this early phase would influence long-term cell fate by examining markers of neurogenesis after seven days in differentiation conditions. Furthermore, to analyse potential upstream mediators of these outcomes, cytokines and kynurenine metabolites were measured at day 3 of differentiation (Fig. 1).

We first assessed how treatment impacted the number of cells positive for the early marker of differentiation, TUJ1. In the vehicle only group in female-derived HPC0A07/03C NPCs, the percentage of TUJ-positive (TUJ+) cells was 91 %. Treatment with IL-1β for the last 24 h of proliferation increased the percentage of TUJ+ cells (VVV vs VVI, 91 % vs 99 %, $p < 0.01$); the number of TUJ+ cells which had neurites (VVV vs VVI, 48 % vs 71 %, $p < 0.0001$); the number of primary neurites (VVV vs VVI, 2.3 vs 3.6, $p < 0.0001$); and the mean neurite length (VVV vs VVI, 78 μm vs 94 μm, $p < 0.001$) at the end of the 7 day differentiation period (Fig. 6A and B). None of these outcomes were influenced by oestradiol pre-treatment (Fig. 6A and B). Despite IL-1β treatment seemingly increasing neuronal maturation and complexity, this increase was additionally associated with an increase in the expression of CC3 (VVV vs VVI, 3.4 % vs 11 %, $p < 0.0001$, Fig. 6A and B).

We then assessed the mature neuronal marker, MAP2. In female-derived HPC0A07/03C NPCs, the number of MAP2-positve cells in the vehicle only group was 44 %. Treatment with IL-1β for the last 24 h of proliferation led to a significant increase the in the percentage of MAP2-positive cells (VVV vs VVI, 44 % vs 59 % $p = 0.04$). However, oestradiol pre-treatment for both 24-hour (VVI vs EVI, 59 % vs 35 %, $p < 0.01$) and 48-hours (VVI vs EEI, 59 % vs 33 %, $p < 0.01$) decreased the number of MAP2-positive cells compared to treatment with IL-1β alone (Fig. 6C and D). Treatment with oestradiol alone had no impact on the percentage of MAP2+ cells.

Given these effects on neuronal differentiation, we next examined whether proliferation-phase IL-1β and oestradiol treatments produced lasting inflammatory or metabolic changes during differentiation in female-derived HPC0A07/03C NPCs. Concentrations of IFN-γ, IL-12, and IL-4 remained below the detection limit across all groups (Supplementary Table 1B). Surprisingly, while IL-1β treatment during the final 24 h of proliferation led to a sustained increase in IL-1β, IL-2, IL-6, IL-8, IL-10, IL-13 and TNF-α that persisted at day three of differentiation (Supplementary Fig. 3A-G), oestradiol pre-treatment had no anti-inflammatory effect at this timepoint (Fig. 7A-G). However, interestingly, IL-1β treatment at the end of proliferation led to a sustained increase in kynurenine at this differentiation timepoint (VVV vs VVI, 0.21 μM vs 0.46 μM, $p = 0.04$ vs vehicle only), an effect which was

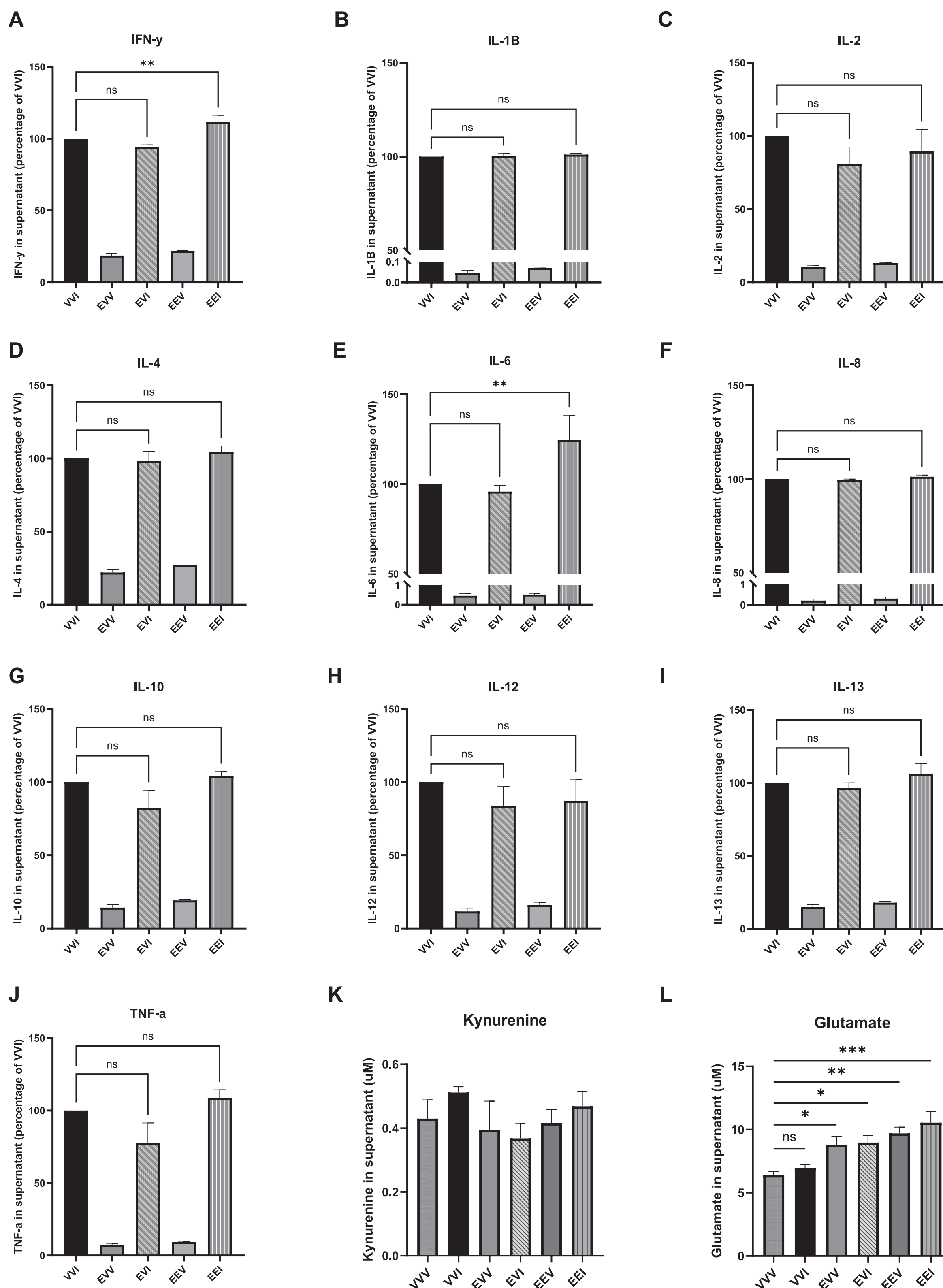

Fig. 5. The effects of oestradiol pretreatment and IL-1β on the level of inflammatory cytokines and kynurenine pathway metabolites in the supernatant of male-derived HIP-009 cells after proliferation. – The concentration of cytokines and kynurenine pathway metabolites were measured in the supernatant following a three-day proliferation period under different treatment conditions. Oestradiol pre-treatment for 48-hours prior to IL-1β (EEI) significantly increased the concentration of IFN-γ (A) and IL-6 (E), compared to IL-1β alone (VVI). Kynurenine levels remained unchanged across all groups (K), but oestradiol treatment increased glutamate levels in a time-dependent manner (L). Treatment codes indicate 3 × 24 h sequences: V = vehicle, E = oestradiol, I = IL-1β. Data are presented as mean ± SEM; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Results are from 6 independent biological replicates.

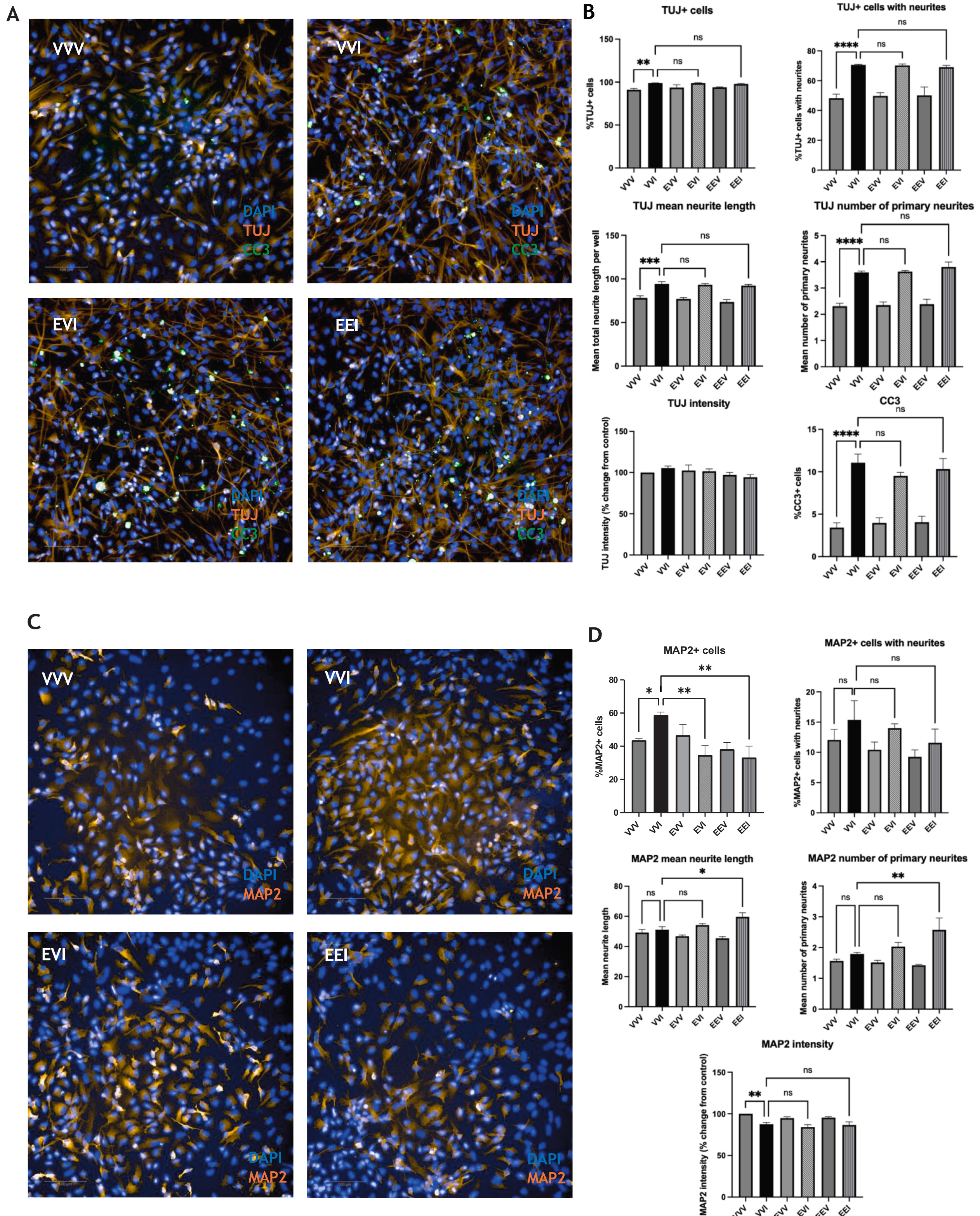

Fig. 6. Lasting effects of oestradiol pre-treatment and IL-1β on cell neurogenesis and apoptosis in female-derived HPC0A07/03C NPCs following 7 days of differentiation – Cells were treated for 72 h under different proliferation conditions, after which all groups were switched to standard differentiation media without additional treatment. After 7 days of differentiation, immunocytochemistry was performed to assess markers of neurogenesis. Treatment codes represent the compounds administered during the 3-day proliferation phase in three consecutive 24-hour intervals during proliferation: V = vehicle, E = oestradiol, I = IL-1β. (A) Cells were stained for the immature neuronal marker, TUJ (orange), as well as the apoptotic marker, CC3 (green). (B) Treatment with IL-1β for the last 24 h of proliferation (VVI) increased the number of TUJ+ cells, neurite complexity and apoptosis. This was not impacted by oestradiol pre-treatment. (C) Cells stained with the mature neuronal marker, MAP2 (orange). (D) VVI treatment increased the number of MAP2+ cells, but both 24- (EVI) and 48-hours (EEI) of oestradiol pre-treatment decreased the number of MAP2 positive cells. Data are presented as mean ± SEM; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Results are from 4 independent biological replicates.

potentiated by 48 h of oestradiol pre-treatment prior to the inflammatory challenge (VVI vs EEI, 0.46 μM vs 0.74 μM, p = 0.04, Fig. 7H). Similarly to during proliferation, oestradiol treatment time-dependently increased glutamate production (Fig. 7I). No other metabolites were influenced by any treatment combination (Supplementary Fig. 3H-J).

In summary, acute IL-1β exposure during proliferation has lasting effects on differentiation in female-derived HPC0A07/03C NPCs, namely increased neuronal maturation and elevated cell death. Oestradiol pre-treatment prior to the inflammatory challenge reduced neuronal maturation but did not protect against cell death. While oestradiol suppressed IL-1β-induced cytokine production during proliferation, this effect was not sustained during differentiation. Additionally, acute IL-1β exposure induced a sustained increase in kynurenine during differentiation, which was further amplified by oestradiol pre-treatment.

3.4. *Proliferation-phase oestradiol pre-treatment alters neuronal differentiation and enhances IL-1β-induced cytokine and kynurenine responses during differentiation in male-derived HIP-009 NPCs*

Again, we then assessed these same cellular and protein outcomes of the differentiation phase in HIP-009 cells. Similarly to HPC0A07/03C NPCs, the percentage of TUJ- and MAP2-positive cells in the vehicle-only group of HIP-009 cells was 99 % and 39 %, respectively (Fig. 8). However, in this cell line, the number of cells positive for TUJ was not affected by any of the treatment groups. When further filtering the cells by morphology, it was observed that treatment with IL-1β for the last 24 h of proliferation led to a significant decrease in the number of TUJ+ cells with neurites (VVV vs VVI, 79 % vs 72 %, $p < 0.0001$); the mean neurite length (VVV vs VVI, 164 μm vs 134 μm, p = 0.01) and the number of primary neurites (VVV vs VVI, 7.1 vs 6.1, p = 0.04) (Fig. 8A and B). Further, IL-1β treatment during proliferation increased the number of apoptotic cells during differentiation (VVV vs VVI, 33 % vs 43 %, p = 0.01, Fig. 8A and B). Similarly to the results seen in HPC0A07/03C NPCs, oestradiol pre-treatment had no effect on any of these TUJ or CC3 parameters in male-derived HIP-009 cells.

In contrast to the findings in female-derived HPC0A07/03C NPCs, in HIP-009 cells treatment with IL-1β for the last 24 h of proliferation did not significantly change the number of MAP2+ cells, although it did induce a significant decrease in the mean neurite length (VVV vs VVI, 88 μm vs 72 μm, p = 0.03, Fig. 8C and D). However, treatment with oestradiol for either 24- (VVI vs EVI, 40 % vs 28 %, $p < 0.01$) or 48-hours (VVI vs EEI, 40 % vs 28 %, $p < 0.01$) prior to IL-1β led to a significant decrease in the number of cells positive for MAP2 compared to IL-1β treatment alone (Fig. 8C and D).

Building on the observed effects on differentiation in HIP-009 cells, we next examined whether proliferation-phase IL-1β and oestradiol treatments produced lasting inflammatory and metabolic changes during differentiation. Analysis of day 3 supernatants revealed that IL-1β exposure during the final 24 h of proliferation induced a sustained increase in production of IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13 and TNF-α, while IL-12 remained below detection and IL-4 levels were similar to controls (Supplementary Fig. 4A-I, Table 1B). Notably, 48 h of oestradiol pre-treatment further enhanced IL-1β-induced production of IFN-γ (+111 %, $p < 0.001$, Fig. 9A), IL-1β (+33 %, $p < 0.001$, Fig. 9B), IL-6 (+43 %, $p < 0.01$, Fig. 9D), IL-10 (+51 %, $p < 0.01$, Fig. 9F), IL-13 (+38 %, $p < 0.01$, Fig. 9G), and TNF-α (+43 %, $p < 0.01$, Fig. 9H) at day 3 of differentiation, compared to IL-1β treatment alone, while 24 h of oestradiol pre-treatment had no effect.

Furthermore, at this timepoint, HIP-009 cells given IL-1β treatment for the last 24 h of proliferation presented with significant increases in the concentration of kynurenine (VVV vs VVI, 0.08 μM vs 0.56 μM, p = 0.001, Fig. 9I) and kynurenic acid (VVV vs VVI 0.0003 μM vs 0.002 μM, $p < 0.001$, Fig. 9J). Oestradiol pre-treatment for both 24- (VVI vs EVI, 0.56 μM vs 0.91 μM, p = 0.02) and 48-hours (VVI vs EEI, 0.56 μM vs 0.94 μM, $p < 0.01$) prior to the inflammatory challenge led to a significant increase in the concentration of kynurenine compared to Il-1β treatment alone (Fig. 9I). Similar observations were observed for kynurenic acid (24 h pretreatment: VVI vs EVI, 0.002 μM vs 0.003 μM, $p < 0.001$; 48 h pre-treatment: VVI vs EEI, 0.002 μM vs 0.004 μM, $p < 0.01$) (Fig. 9J). Again, oestradiol upregulated glutamate production compared to control (Fig. (9 K).

In summary, in male-derived HIP-009 cells, IL-1β exposure during proliferation decreased TUJ neurite complexity and increased apoptosis by the end of differentiation, while oestradiol pre-treatment reduced MAP2-positive neurogenesis. IL-1β also induced sustained increases in cytokine and kynurenine production during differentiation, which were further amplified by oestradiol pre-treatment.

4. Discussion

There is ample evidence to support the role of inflammation in the mechanisms underlying the intergenerational transmission of psychiatric risk following exposure to antenatal depression (Osborne et al., 2018). However, despite an abundance of research looking at the effects of inflammation on fetal brain development (Couch et al., 2021; Kirkpatrick et al., 2025), it is currently unclear whether there are other factors which may confer risk or resilience to exposed individuals. In the current study, we demonstrate that oestradiol, an important steroid hormone during fetal brain development, can modulate inflammatory responses within fetal HPCs. Importantly, we demonstrate not only differences in the way the two fetal HPC lines respond to oestradiol in the presence of an immune challenge, but also opposite responses to the inflammatory challenge itself.

4.1. *Female-derived HPC0A07/03C and male-derived HIP-009 NPCs express divergent cellular responses to an acute inflammatory challenge*

Studies have consistently demonstrated that exposure to neonatal systemic inflammation or direct exposure of NPCs to an inflammatory challenge can lead to alterations in proliferation and differentiation (Couch et al., 2021; Kirkpatrick et al., 2025). In the present study, we find that while both HPC lines display a robust inflammatory cytokine response to IL-1β and display comparable percentages of specific cell populations in the control group, the cellular outcomes of this treatment differ. In HPC0A07/03C female NPCs, acute IL-1β treatment during proliferation leads to an increase in the number of proliferating cells at the end of the proliferation phase, an effect that we have previously demonstrated with IL-1β treatment in this cell line (Zunszain et al., 2012). One week after the inflammatory challenge, we observed a significant increase in both immature and mature neurons, as well as enhanced neurite complexity. This may appear to be in contrast with our previous findings in this cell line (Zunszain et al., 2012; Borsini et al., 2017), wherein continuous IL-1β treatment for 10 days during both the proliferation and the differentiation decreases neuronal maturation. However, it is important to note that in the present study we look at the effects of an acute 24-hour proliferation phase treatment on subsequent differentiation outcomes 7 days later. Notably, this increase in neuronal maturation that we find here was accompanied by a significant increase in the number of apoptotic cells, suggesting this may reflect a dysregulated differentiation response. In contrast, in HIP-009 male cells, we observe that acute cytokine treatment leads to a decrease in proliferation and a decrease in immature neurons and neurite complexity, but no changes in the number of mature neuronal cells. These results highlight that there is an intrinsic difference in the way these two cell lines respond to an inflammatory challenge.

Indeed, previous literature demonstrates variability in the response to IL-1β, where it has been shown to both decrease (Veerasammy et al., 2020; Green et al., 2012; Koo and Duman, 2008; Crampton et al., 2012; Wang et al., 2007) and increase proliferation (Zunszain et al., 2012). Studies most consistently demonstrate that inflammatory challenges decrease neurogenesis (Kirkpatrick et al., 2025; Zunszain et al., 2012;

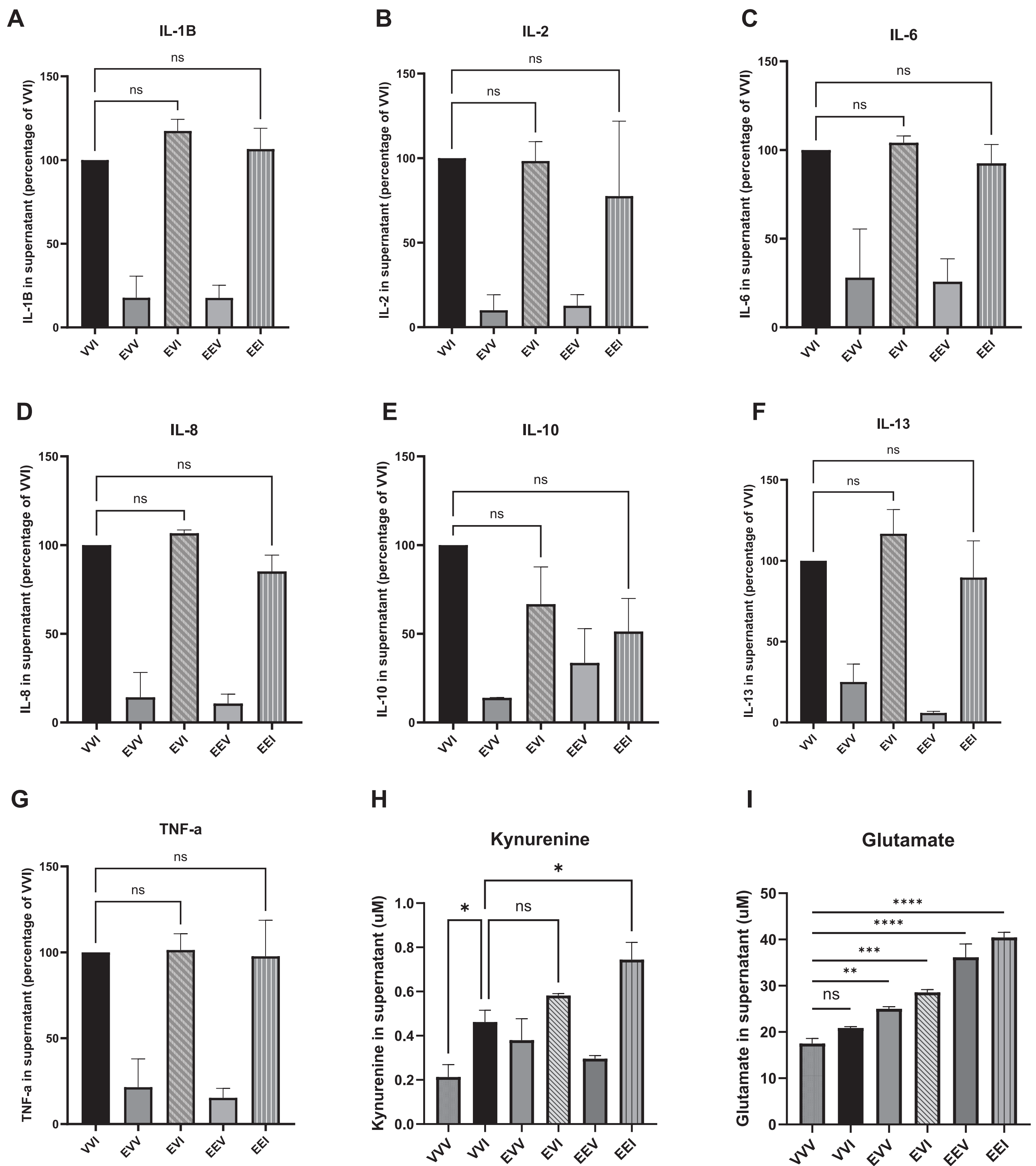

Fig. 7. Effects of oestradiol pre-treatment and IL-1β on cytokine and kynurenine metabolite production in female-derived HPC0A07/03C NPCs at day 3 of differentiation – Cells were treated for 72 h under different proliferation-phase conditions, followed by culture in standard differentiation media without further treatment. Cytokine and kynurenine metabolite concentrations were measured in the culture supernatant on day 3 of differentiation. Oestradiol pre-treatment (EVI or EEI) did not change the concentration of cytokines compared to IL-1β alone (VVI) (A-G). Treatment with IL-1β alone during proliferation (VVI) significantly increased kynurenine levels at day 3 of differentiation compared to vehicle-treated controls (VVV). This effect was increased by 48-hours of oestradiol pre-treatment (EEI) (H). Oestradiol treatment during proliferation increased glutamate levels at day 3 of differentiation in a time-dependent manner (I). Treatment codes represent compounds administered during the 3-day proliferation phase in three consecutive 24-hour intervals during proliferation: V = vehicle, E = oestradiol, I = IL-1β. Data are presented as mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$. Results are from 3 independent biological replicates.

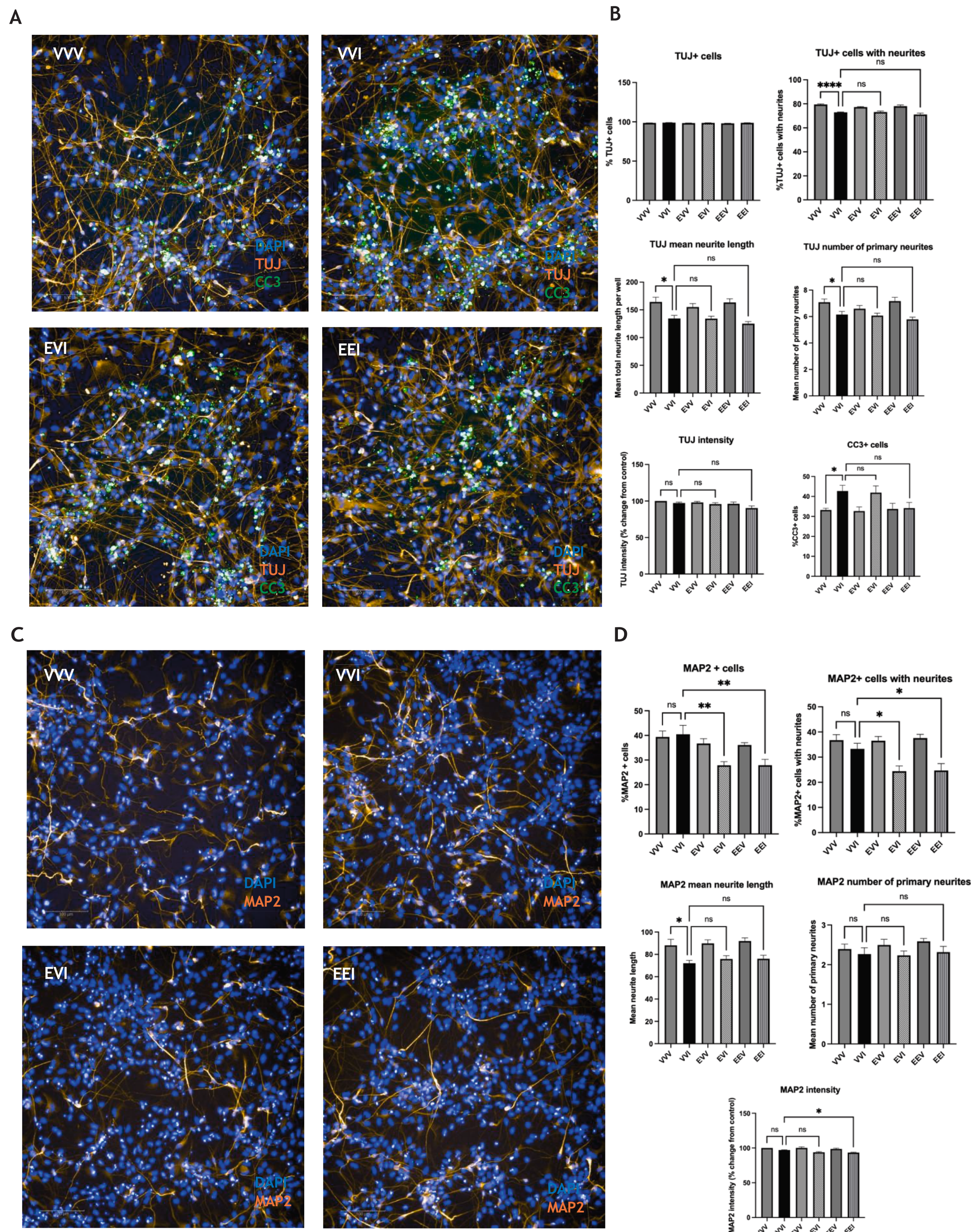

Fig. 8. Lasting effects of oestradiol pre-treatment and IL-1β on cell neurogenesis and apoptosis in male-derived HIP-009 following 7 days of differentiation – Cells were treated for 72 h under different proliferation conditions, after which all groups were switched to standard differentiation media without additional treatment. After 7 days of differentiation, immunocytochemistry was performed to assess markers of neurogenesis. Treatment codes represent the compounds administered during the 3-day proliferation phase in three consecutive 24-hour intervals: V = vehicle, E = oestradiol, I = IL-1β. (A) Cells were stained for the immature neuronal marker, TUJ (orange), as well as the apoptotic marker, CC3 (green). (B) Treatment with IL-1β for the last 24 h of proliferation (VVI) decreased the number of TUJ+ cells, neurite complexity and apoptosis. This was not impacted by oestradiol pre-treatment. (C) Cells stained with the mature neuronal marker, MAP2 (orange). (D) VVI treatment during proliferation did not change MAP2+ cells, but both 24- (EVI) and 48-hours (EEI) of oestradiol pre-treatment decreased the number of MAP2 positive cells. Data are presented as mean ± SEM; *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$. Results are from 4 independent biological replicates.

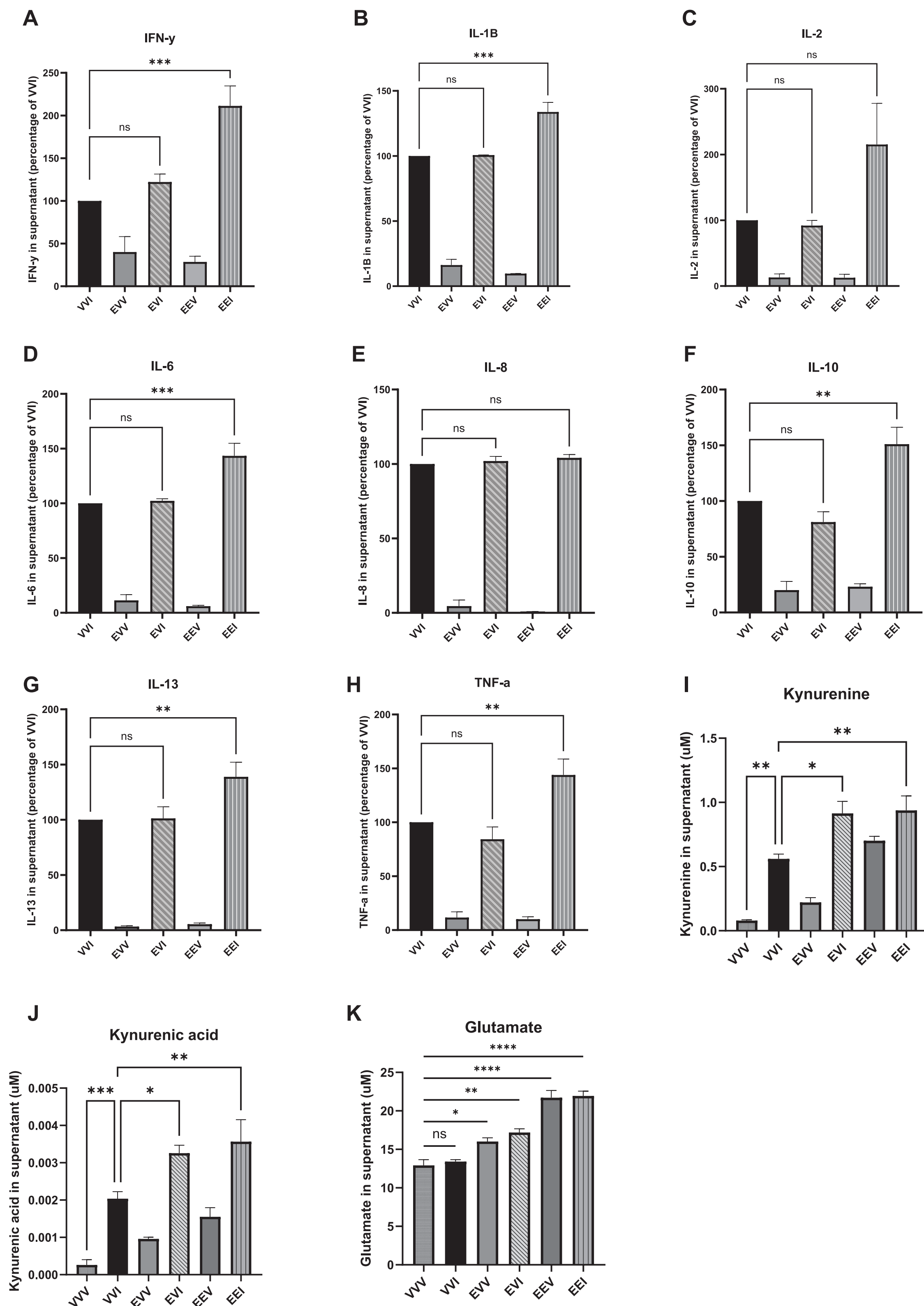

Fig. 9. Lasting effects of oestradiol pre-treatment and IL-1β on cytokine and kynurenine metabolite production in male-derived HIP-009 cells at day 3 of differentiation – Cells were treated for 72 h under different proliferation-phase conditions, followed by culture in standard differentiation media without further treatment. Cytokine and kynurenine metabolite concentrations were measured in the culture supernatant on day 3 of differentiation. Oestradiol pre-treatment for 48 h (EEI) during proliferation increased the concentration of IFN-γ (A), IL-1β (B), IL-6 (D), IL-10 (F), IL-13 (G) and TNF-α (H) at day 3 of differentiation compared to treatment with IL-1β alone (VVI). Treatment with IL-1β alone during proliferation (VVI) significantly increased kynurenine and kynurenic acid levels at day 3 of differentiation compared to vehicle-treated controls (VVV). This effect was increased by 24- (EVI) and 48-hours of oestradiol pre-treatment (EEI) (I and J). Oestradiol treatment during proliferation increased glutamate levels at day 3 of differentiation in a time-dependent manner (K). Treatment codes represent compounds administered during the 3-day proliferation phase in three consecutive 24-hour intervals: V = vehicle, E = oestradiol, I = IL-1β. Data are presented as mean ± SEM; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. Results are from 3 independent biological replicates.

Ardalan et al., 2019; Borsini et al., 2017), however in some cases IL-1β has been shown to increase differentiation and neurite complexity (Park et al., 2018; Temporin et al., 2008), as seen here in HPC0A07/03C NPCs. Variability in reported outcomes is often attributed to differences in treatment timing, dose, and model system (Seguin et al., 2009; Borsini et al., 2023). However, in the present study, both cell lines were exposed to the same concentration and duration of IL-1β, suggesting that intrinsic properties of the cells themselves, such as genetic background or donor sex, may drive the differential outcomes. Individual studies directly comparing sex differences in cellular responses to inflammation in NPCs are limited, however *in vitro* studies have demonstrated that other brain cell types, including astrocytes and microglia, display distinct responses to an inflammatory challenge depending on sex (Santos-Galindo et al., 2011; Loram et al., 2012). Indeed, sex differences in outcomes following prenatal exposure to inflammation are widely reported (Ardalan et al., 2019), thus while we cannot definitively identify sex as a causative factor in the present design, the observed divergence is broadly consistent with prior work describing sex-linked variation in neuroimmune signalling.

4.2. Oestradiol modulation of inflammatory responses is distinct in female-derived HPC0A07/03C NPCs versus male-derived HIP-009

A growing number of studies demonstrate the neuroprotective actions of oestradiol (Arevalo et al., 2015). In the present study, we demonstrate that in female-derived HPC0A07/03C NPCs, oestradiol prevents the pro-proliferative and pro-apoptotic effects of IL-1β, in a time-dependent manner. Furthermore, at the end of proliferation, female-derived HPC0A07/03C NPCs pre-treated with oestradiol exhibited a significantly reduced inflammatory response. This finding aligns with prior research demonstrating that oestradiol can attenuate pro-inflammatory cytokine production in astrocytes (Cerciat et al., 2010), microglia (Zhao et al., 2016), as well as neuronal cells (Yun et al., 2018). Additionally, oestradiol has been demonstrated to inhibit IL-1β-induced caspase activation in mouse embryonic hippocampal NPC cultures (Kajta et al., 2006). Mechanistically, this effect has been attributed in part to the inhibition of Nf-KB, an important transcription factor involved in cytokine regulation (Azcoitia et al., 2019). Bai et al. (2020) further elucidated this pathway by showing that G-protein coupled oestrogen receptor (GPER) activation in hippocampal neurons induces robust expression of the interleukin-1 receptor antagonist (IL-1RA), providing a plausible mechanism by which oestradiol may counteract IL-1β-induced effects (Bai et al., 2020).

However, much of the research highlighting these potent anti-inflammatory effects of oestradiol have been conducted in either female animals or in mixed sex NPCs. In contrast to our observations in female-derived HPC0A07/03C NPCs, oestradiol does not mitigate the anti-proliferative effects of IL-1β in the male-derived HIP-009 cell line. Notably, a 48-hour pre-treatment with oestradiol prior to the inflammatory challenge not only fails to confer protection but also amplifies cytokine production. These contrasting responses may point toward a sex- or cell line-dependent effect of oestradiol on inflammatory signalling. Supporting this possibility, Loram et al (2012) demonstrated that cultured neonatal microglia display sex-specific inflammatory modulation by oestradiol, wherein oestradiol decreases IL-1β in response to LPS in male microglia but increases it in females (Loram et al., 2012). Although the direction of the observed sex-specific responses in rodent microglia differs from what we observe here, this may reflect differences in species (rat vs. human), cell type (microglia vs. HPCs), treatment duration (4 h vs. 24–48 h), and timing of oestradiol exposure (co-treatment vs. pre-treatment). However, this result nonetheless highlights the potential for oestradiol to exert divergent modulation of cellular inflammatory responses depending on sex. Indeed, in *in vivo* models, sex differences in the neuroprotective effects of oestradiol are widely reported, with divergent responses arising depending on the model (Azcoitia et al., 2019; Gillies and McArthur, 2010). For example, oestradiol protects only females from striatal lesions (Gillies and McArthur, 2010), but only males from experimental stroke (Liu et al., 2012). These divergent neuroprotective effects, seen both *in vivo* and in the current study, are hypothesized to arise from differences in oestrogen receptor distribution and signalling (Llorente et al., 2020; Oberlander and Woolley, 2016; Koss et al., 2018), epigenetic modifications (Schwarz et al., 2010), sex chromosome-linked factors, and variations in endogenous brain-derived hormone levels (Azcoitia et al., 2019; Bowers et al., 2010). While we did not directly assess oestrogen receptor expression or signalling in the HPC0A07/03C and HIP-009 cell lines, previous studies indicate that ERα, ERβ, and GPER are all expressed in HPC0A07/03C cells (Smeeth et al., 2021; Mandal et al., 2025), and future experiments measuring their levels could clarify the molecular basis for the divergent immunomodulatory responses observed here.

4.3. Long term cellular outcomes of acute oestradiol and IL-1β exposure

While oestradiol has been reported to influence NPC growth and differentiation across various models (Sahab-Negah et al., 2020; Barha et al., 2009), in the current study, oestradiol alone did not affect the proliferation or differentiation of either male-derived HIP-009 or female-derived HPC0A07/03C NPCs. This observation is consistent with earlier findings using the HPC0A07/03C NPCs, which reported no significant changes in the expression of proliferation, apoptosis, or differentiation markers following oestradiol treatment (Smeeth et al., 2021). The lack of a cellular response to oestradiol alone in these cell lines is likely underscored by concentration and timing-dependent effects.

Interestingly, in female-derived HPC0A07/03C NPCs, oestradiol pre-treatment prior to IL-1β exposure led to a decrease in MAP2 expression compared to IL-1β treatment alone. This finding was surprising considering the observed protective effects of oestradiol against both cellular and cytokine outcomes during proliferation. Since IL-1β alone induced an increase in neuronal maturation that was accompanied by elevated cell death, this reduction in MAP2 following oestradiol pre-treatment may reflect a normalisation or alleviation of an aberrant, dysregulated differentiation trajectory. Indeed, oestrogen is thought to be important in maintaining the balance between proliferation and differentiation in the brain (Bustamante-Barrientos et al., 2021).

Alternatively, these findings may indicate that, while acute oestradiol exposure induces short-term protection, its long-term effects could be detrimental. This interpretation is supported by our observation that, during the differentiation phase, cytokine profiles in the oestradiol pre-treatment groups resembled those of the IL-1β-only groups, indicating a loss of oestradiol's anti-inflammatory effect present during proliferation. Concurrently, 48 h of oestradiol pre-treatment led to a marked increase in kynurenine levels at this stage. In our previous study in HPC0A07/03C NPCs, we demonstrated that reductions in neurogenesis induced by IL-1β treatment were underscored by activation of the kynurenine pathway, highlighting that this upregulation of kynurenine may be the mechanism underlying the observed decrease in MAP2 (Zunszain et al., 2012).

There is a paucity of research specifically addressing the long-term consequences of acute oestradiol treatment. However, studies have highlighted that some of the neuroprotective and anti-inflammatory actions of oestradiol are underscored by activation of the GPER, which induces rapid membrane-initiated effects, independent of nuclear oestrogen receptor (ER) transcriptional activities (Azcoitia et al., 2019; Bai et al., 2020; Fiocchetti et al., 2012; Xu et al., 2023). Therefore, the immediate protective effects we observe may be underpinned by this rapid GPER signalling, while the delayed detrimental effects, such as increased kynurenine levels and reduced neurogenesis, may involve slower ER transcriptional signalling. Notably, these detrimental effects appear only when oestradiol is administered in the context of concomitant inflammation. Indeed, oestradiol has been shown to inhibit kynurenine transaminase, the enzyme involved in converting

kynurenine to the neuroprotective kynurenic acid (Mason and Gullekson, 1960; Jayawickrama et al., 2017). This suggests that, beyond the acute phase, oestradiol may engage slower, transcriptional signaling through ERα and ERβ, which in the presence of inflammatory cues may synergistically enhance kynurenine pathway activation. Such a mechanism could account for the observed impairment in neurogenesis at later stages, adding to a growing body of research supporting the context-dependent duality of oestradiol's immunomodulatory actions (Straub, 2007). However, it is worth noting that only 48-hours of oestradiol pre-treatment upregulated kynurenine production, yet both 24- and 48-hours induced reduced MAP2 expression, highlighting that the specific detrimental mechanisms require further investigation.

Similar to the female-derived HPC0A07/03C NPCs, male-derived HIP-009 cells showed reduced MAP2 expression and elevated kynurenine levels following oestradiol pre-treatment and IL-1β exposure, suggesting that the long-term consequences of combined treatment are consistent across both cell lines. However, in male-derived HIP-009 cells, IL-1β alone reduced TUJ1-positive cells but had no effect on MAP2, indicating early differentiation was disrupted without affecting later maturation. The additional reduction in MAP2 following oestradiol pre-treatment likely reflects a further disruption along the maturation pathway, potentially driven by increased kynurenine (Zunszain et al., 2012). Notably, only the 48-hour oestradiol treatment elevated cytokines, yet both 24- and 48-hour treatments reduced MAP2, suggesting that kynurenine pathway activation, rather than cytokine load, may underlie impaired neuronal maturation.

4.4. Limitations and future directions

Although this study is robust in design, we recognise there are potential limitations. Primarily, our use of an *in vitro* model of hippocampal neurogenesis cannot fully replicate the complexity of the *in vivo* environment, particularly the dynamic interactions with other cell types, such as microglia. Nevertheless, many previous findings obtained in our *in vitro* models of stress and inflammation have also been replicated in the brains of animals subjected to stress and in the blood of people suffering from stress-related mental health disorders (Borsini et al., 2022; Anacker et al., 2013; Cattaneo et al., 2018). Of course, multiple factors may underlie the distinct responses observed between the two cell lines, such as subtle variations in genotype (Lee et al., 2021); and additional research is needed to determine whether the observed differences in cell line responses are truly driven by intrinsic sex-based factors, potentially involving GPER-dependent signalling pathways. We acknowledge that, with only one female and one male line, the current study cannot definitively separate sex-dependent effects from cell line-specific effects, and the findings should therefore be interpreted as differences between two individual lines rather than conclusive sex-specific responses. Definitive attribution would require either isogenic cell lines differing only in sex chromosome complement or multiple independent male and female lines to account for line-to-line variability, which represents an important direction for future work. Nevertheless, both the HPC0A07/03C NPCs and HIP-009 cell line are human fetal hippocampal progenitor cell lines derived from comparable developmental stages. In our experiments, both lines produce glutamate, and oestradiol enhances glutamate production in each, supporting the evidence that they share key functional characteristics. Consistent with this, baseline expression of proliferation (Ki67) and neuronal differentiation markers (MAP2 and TUJ1) were comparable between the two lines, further supporting their use as parallel models of neurodevelopment. Previous studies characterizing the HIP-009 cell line (Fukushima et al., 2016; Fukushima et al., 2014) also support that they express neurogenic genes similar to those found in HPC0A07/03C NPCs (Powell et al., 2017). Taken together, these data strengthen the rationale for comparing these cell lines, while also highlighting that any differences observed likely reflect sex differences.

4.5. Conclusion

In conclusion, the results of the present study demonstrate that acute oestradiol exposure during the proliferation phase, in the context of an inflammatory challenge, exerts divergent immunomodulatory effects in two fetal NPC lines: reducing inflammatory responses in female-derived HPC0A07/03C cells, but increasing or having no effect in male-derived HIP-009 cells. Because only one line of each sex was examined, these differences should be interpreted primarily as cell line–specific; however, the divergent responses align with prior evidence that male and female neural cells and immune systems can exhibit distinct responses to hormonal and inflammatory stimuli, suggesting that sex-linked mechanisms may contribute to the observed patterns. Notably, analysis of downstream effects revealed that this acute treatment led to inhibition of MAP2 expression and increased kynurenine production in both cell lines, suggesting potentially detrimental long-term consequences for neuronal development. These findings contribute to a growing body of evidence implicating hormonal–immune interactions in the biological mechanisms underlying the intergenerational transmission of risk for neuropsychiatric conditions, highlighting the complex interplay that may influence vulnerability and resilience in exposed offspring.

CRediT authorship contribution statement

Madeline Kirkpatrick: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Silvia Alboni:** Writing – review & editing, Methodology, Data curation. **Federico Imbeni:** Methodology, Data curation. **Nicole Mariani:** Writing – review & editing, Methodology. **Nuriza Tukiran:** Writing – review & editing, Methodology. **Erin Mason-White:** Writing – review & editing, Methodology. **Carmine M. Pariante:** . **Alessandra Borsini:** Writing – review & editing, Supervision, Methodology, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr Alessandra Borsini and Professor Carmine M. Pariante have received research funding also from Johnson & Johnson for research on depression and inflammation which included cellular work (2012–2018), but this work is unrelated to that funding; moreover, less than 10 % of Professor Pariante's support in the last 10 years derives from commercial collaborations, including: a strategic award from the Wellcome Trust (Neuroimmunology of Mood Disorders and Alzheimer's Disease (NIMA) Consortium, grant 104025), in partnership with Janssen, GlaxoSmithKline, Lundbeck and Pfizer; and consultation and speakers fees from Boehringer Ingelheim, Eli Lilly, Compass, Eleusis, GH Research, Lundbeck, and Värde Partners. Alboni, Mariani, Tukiran, Imbeni, Mason-White and Kirkpatrick have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2025.106212.

Data availability

Data will be made available on request.

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