An Examination of The Effects of The Environment on Transplanted Human Interneuron Progenitors in Temporal Lobal Epilepsy

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Abstract

Loss or dysfunction of GABAergic inhibitory neurons is involved in many neurological disorders, including epilepsy. Transplantation of GABAergic neuron progenitors in rodent models of epilepsy significantly reduces seizure occurrence and duration. Therefore, transplantation of GABAergic neuron progenitor of human origin is being considered as a potential therapy for drug resistant epilepsy.

The microenvironment plays an important role in regulating survival, proliferation and differentiation of neuronal stem cells. Evidence shows an inflamed microenvironment with significant upregulation and secretion of inflammatory cytokines after seizures. Whilst the major emphasis to date has been on generating the optimal cell types for cell therapy in epilepsy, there has been little attention paid to the effects of the neuroinflammatory environment into which cells will be delivered.

In this study, it is found that in a 3D model of human adult neural tissue excised at epilepsy surgery, the inflamed microenvironment reduced the survival, impaired neurite growth, and differentiation of *in-vitro* transplanted hESC-derived MGE progenitors. IL-1β was recognized as an important pro-inflammatory cytokine which affected the survival and differentiation of hESC-derived MGE progenitors in this model. Restored differentiation and promotion of neurite growth was observed in MGE progenitors by blocking the IL-1R.

Further experiments in monolayer cultures on electrophysiology found MGE-like neurons were less excitable after acute exposure to IL-1β. The mechanism was due to reduction in sodium current rather than alternation in channel activation or inactivation kinetics. And this effect was not persistent when level of IL-1β returned to normal concentration. But when repetitively treated by IL-1β for a week, MGE-like neurons became unhealthy and their excitability reduced even more.

In conclusion, the inflamed microenvironment, especially IL-1β is detrimental for survival, differentiation and electrophysiological activities of transplanted MGE-like neurons in this in-vitro primary human tissue model. The results suggest anti-inflammatory treatment could be beneficial to improve efficiency of neuron transplantation therapy. The results also potentially

imply that neuroinflammation may cause a reduced excitability of interneurons as a potential mechanism for epilepsy.

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List of abbreviations

AP	action potential
BDNF	brain-derived neurotrophic factor
CCK	cholecystokinin
CGE	caudal ganglionic eminence
CNS	central neuron systerm
CR	calretinin
DCX	doublecortin
DG	dentate gyrus
GAD	glutamic acid decarboxylase
GCD	granule cell dispersion
GE	ganglionic eminence
GFP	green fluorescent protein
GWAS	genome-wide association studies
HD	Huntington's disease
hESC	human embryonic stem cell
HPSC	human pluripotent stem cell
HS	hippocampal sclerosis
IL-1R	interleukin-1 receptor
IL-1RA	interleukin-1 receptor antagonist
IL-1α	interleukin-1α
IL-1β	interleukin-1β
IL-6	interleukin-6
LGE	lateral ganglionic eminence
MAPK	mitogen-activated protein kinase
MFS	mossy fiber sprouting
MGE	medial ganglionic eminence
NPY	neuropeptide Y
NSFA	non-stationary fluctuation analysis
PD	Parkinson's disease
PI	propidium iodide
PKA	protein kinase A
PKC	protein kinase C
PNS	Peripheral nervous system
PV	parvalbumin
RA	retinoid acid
RMP	resting membrane potential
SCM	stem cell conditioned medium
SGZ	sub granule zone
SHH	sonic hedgehog
STT	somatostatin
SVZ	sub ventricular zone
TLE	temporal lobe epilepsy
TLR	toll like receptor
TNF-α	tumor necrosis factor α
TTX	tetrodotoxin
VGSC	voltage-gated sodium channel
VIP	vasoactive intestinal polypeptide

1. Introduction

1.1 Neuropathology in epilepsy

1.1.1The etiology of epilepsy

Epilepsy affect millions of people in the world and half of patients with chronic epilepsy suffer from cognitive or psychiatric disorders such as learning and memory deficits, anxiety and depression. Epilepsy can be classified into two categories by etiology. One is acquired epilepsy which caused by stroke, brain trauma, infection, medication etc. The other is idiopathic epilepsies caused by genetic mutations or undiscovered genetic deficits. Thanks to fast development of gene sequencing, so far, the researchers have identified 977 genes related with epilepsy (Wang, Lin et al. 2017). The most common epilepsy genes are mutations in ion channels, synaptic receptors and enzyme/enzyme-modulators. Several decades of exploration have established the idea that the mutation which cause an imbalance between inhibitory and excitatory conductance lead to seizures. Functional shifts in the balance between inhibition and excitation arising from abnormal synaptic plasticity could transform high but normal levels of network activity into epileptic events (Staley 2015). The long-term histological and cellular change of circuity can gradually lead to the imbalanced shifts. Therefore, the pathological changes in neurons, circuits and microenvironment together lead to epileptogenesis and the subsequent development of the epileptic state.

1.1.2 Pathology of Temporal Lobe Epilepsy (TLE) in hippocampus

Temporal lobe epilepsy (TLE) is one of the most common forms of epilepsy. All patients with TLE suffer from chronic seizures. TLE usually arises from the hippocampus or hippocampal formation, where chronic abnormalities are detected in histology, and at cellular and molecular levels (Vezzani, Aronica et al. 2013).

1.2.2.1 Hippocampus sclerosis

Hippocampus sclerosis (HS) is the pathological in size and volume of hippocampus resulted from seizure induced severe neuron loss, abnormal neurite growth and distribution and gliosis. It has

been observed in about 90% of operated cases in patients with chronic TLE (de Lanerolle, Kim et al. 1989, Thom, Sisodiya et al. 2002).

The hippocampus is composed of 4 subfields: CA1, CA2, CA3, and CA4, including the dentate gyrus (DG). DG consists of three layers: the molecular layer, the granular layer, and the polymorphic layer (hilus) and is the only region, except for sub ventricular zone (SVZ), which supports adult neurogenesis (Goncalves, Schafer et al. 2016). Researchers have found three main pathological changes of DG in HS: loss of neurons; impaired neurogenesis (Bengzon, Kokaia et al. 1997, Gray and Sundstrom 1998, Barkas, Redhead et al. 2012) and formation of new recurrent excitatory circuits (Dudek and Sutula 2007, Goldberg and Coulter 2013). What's more, the histological are not restricted to DG, the neuron loss and gliosis also exist in the subfields of CA1 to CA3, and even extend to extrahippocampal tissues, such as the amygdala and para-hippocampal gyrus (Thom 2014). Two third of the patients with TLE recovered become seizure free after surgical removal of HS (Salanova, Markand et al. 2002), suggesting the critical role of hippocampal sclerosis in generating epileptic seizures.

Granule cell dispersion (GCD)

GCD in the dentate gyrus is a frequent feature and occurs in 45.5% of hippocampal specimens from 66 patients with TLE (da Costa Neves, Jardim et al. 2013).

In normal hippocampus, the cell bodies of granule cells located in the granule layer form a uniform pattern. In HS, the bodies of granule cells form a thicker layer with disrupted order in clusters or bi-laminar structures in the granule layer and appear in ectopic regions such as the molecular layers and hilus (Parent, Yu et al. 1997). A study based on resected hippocampus from patients with TLE showed that GCD in sclerotic hippocampus is caused by an abnormal migration of mature granule cells along a radial glial scaffold (Fahrner, Kann et al. 2007). Similarly, in the kainic acid (KA) induced epilepsy model, the time lapse uncovered the surviving cell changed the dendrite organization and translocated the cell body into an apical dendrite, expending its innervation territory as the compensation for neuron loss (Chai, Munzner et al. 2014).

Except for aberrant migration, the differentiation of granule cells is also affected during GCD. Calbindin is expressed by mature granule cells, it is essential for long-term potentiation and synaptic plasticity which are the cellular basis of learning and memory (Abraham, Veszpremi et

al. 2009). Loss of calbindin expression in granule cells has been found in samples of most TLE patients (Magloczky, Halasz et al. 1997), and clinical evidence support its association with memory dysfunction in TLE patients (Karadi, Janszky et al. 2012).

Granule cells participate in spatial memory formation by receiving synaptic input from the entorhinal cortex via the perforant path and play an important role in long-term potentiation and long-term depression by delivering synaptic output to CA3 and CA1. Clinical research shows that the presence of GCD in right sided hippocampal sclerosis is correlated with worse visuospatial memory performance (Neves, de Souza Silva Tudesco et al. 2012).

Mossy fiber sprouting (MFS)

In normal hippocampus, the dendrites of granule cells are in the molecular layer, and the cell bodies form the granule cell layer. The axons of granule cells go through the hilus, forming synaptic connection with mossy cells, CA3 pyramidal neurons and interneurons. The axon fiber gives rise to large presynaptic swellings at its terminal, which look like giant mossy boutons as seen by electron microscopy, thus called mossy fibers.

In sclerotic hippocampus, the mossy fibers extend into the molecular layer and granule layer, forming exclusively asymmetric, putatively excitatory synapses with dendritic spines and the soma of granule cells, which create a recurrent excitatory input with a potential to stimulate synchronized hyperactivity of neuronal networks (Frotscher, Jonas et al. 2006).

The MFS is triggered by seizure activity and neuron loss. The mature granules cells contribute to MFS at early stage, 4-9 days after induced seizures in mice (Mello, Cavalheiro et al. 1993), but the new born neurons from SGZ also give rise to similar mossy fibers when they are functionally excitable (Restivo, Niibori et al. 2015). The development of MFS could be suppressed by constant application of rapamycin before and after induced seizures (Buckmaster, Ingram et al. 2009, Zeng, Rensing et al. 2009). Application of rapamycin after induced seizures, reduced seizure frequency (Zeng, Rensing et al. 2009). In contrast, preventing MFS by cycloheximide did not work on spontaneous recurrent seizures (Longo and Mello 1999). Mice model showed that MSF reached a plateau 100 days after induced seizures (Mello, Cavalheiro et al. 1993). As for human, MFS become a permanent change even after seizure were well controlled (Thom, Martinian et al.

2009). So, it is still hard to decide whether MFS promotes epileptic seizure in all TLE cases (Buckmaster 2012).

Seizure-generated cells

The new born neuron generated after seizure adopt abnormal identity, morphology and electrophysiological functions. Neuronal hyperactivity induced by seizures activate the radial glia cells the majority of which differentiate towards reactive astrocyte instead of neurons (Sierra, Martin-Suarez et al. 2015, Shtaya, Sadek et al. 2018). And even the seizure-born neurons have distinct excitability performance due to the heterogenous morphology. The majority new-born neurons have reduced spine number, suggesting reduction in excitability. A significant subset shows enlarged cell bodies, long basal dendrites and received more mossy fiber input, implying those cells robustly integrated into the pathological circuit (Murphy, Pun et al. 2011).

Given that abnormal neurogenesis produces either defects in newly born granule cell neurons or glial cells, suppressing abnormal neurogenesis turns out to be a therapeutic target for epilepsy. Conditional ablation of neuron stem cells in the hippocampus before pilocarpine-induced acute seizures reduced the frequency of seizure and restored cognition deficiencies in mice (Cho, Lybrand et al. 2015). In chronic epilepsy, neurogenesis is reduced in contrast to rapid increase in neurogenesis in the acute phase (Shtaya, Sadek et al. 2018). Overproduction of seizure-born cells in the acute phase depletes the stem cell niche in the SGZ, eventually leading to memory loss and cognitive deficit (Sierra, Martin-Suarez et al. 2015).

1.2.2.2 Selective Interneuron loss and dysfunction

Early research has shown significant reduction of interneurons and principal neurons in sclerotic hippocampus of TLE patients (Dam 1980, de Lanerolle, Kim et al. 1989), especially in the dentate hilus. The sub granular population of Somatostatin (STT) expressing interneurons is nearly completely lost in TLE patients (de Lanerolle, Kim et al. 1989).

Neuron loss is caused by seizure induced neuron necrosis and apoptosis. The necrosis is mediated by excitotoxicity due to high glutamate levels, excessive Na^{+} and Ca^{2+} and cellular free-radical production and inflammatory mediators in the microenvironment (Aktas, Ullrich et al. 2007, Vezzani, Aronica et al. 2013, Vezzani and Viviani 2015). The apoptosis is caused by intrinsic mitochondrial dysfunction induced activation of Bcl-2 and Caspase families and the extrinsic

activation of cell surface receptors which deliver the programmed cell death signaling (Henshall 2007).

Hippocampal interneurons originate from the same region as cortical interneurons, the MGE and CGE area of ventral telencephalon. The inducible fate mapping of MGE and CGE derived interneuron revealed interneuron progenitors migrate to hippocampus from E14.5–E18. CGE-derived interneurons (CR-, CCK- and VIP-expressing) primarily distribute in superficial layers of the hippocampus especially in stratum lacunosum moleculare and deep stratum radiatum, while MGE-derived interneurons (PV-, STT-and NPY-expressing) are distributed through all layers (Thom, Martinian et al. 2009).

Interneuron migration significantly decreases after birth (Thom, Martinian et al. 2009). Therefore, unlike granule cells loss being potentially replaced by newly born neurons, the loss of interneuronS is likely permanent. The GABAergic interneurons have diverse functions correlated with distinct subtypes in hippocampal networks, including regulating network excitability, controling network synchronization, modulating neurogenesis (proliferation, migration and maturation) and supporting memory consolidation. The functional alterations in relation to cellular, network changes and epileptogenesis, can be closely correlated with histological changes in interneuron subgroups (Thom 2014).

STT-expressing interneurons

In DG, STT positive interneurons take up 50% of total interneuron population in hilus, innervating the most prominent territory in the molecular layer of DG, where the fibers of the perforant path terminate (Katona, Acsady et al. 1999). Serving as modulators between perforant path and granule cells, STT positive neurons also have a feedback circuit with granule cells (Katona, Acsady et al. 1999). Selective loss of STT positive interneurons has been observed in both animal models of TLE and HS in patients with TLE, implying the STT positive interneurons are vulnerable in response to pathological microenvironment of sclerotic hippocampus (Sloviter 1987, de Lanerolle, Kim et al. 1989, Buckmaster and Dudek 1997). Meanwhile, the surviving STT positive interneurons have enlarged cell bodies with extended innervation territory formed by overgrowth of dendrites and axons and more synaptic connections with granule cells, as a possible compensation for neuron loss (Zhang, Yamawaki et al. 2009).

PV-expressing interneuron

The PV-expressing interneurons are usually in the granule cells, forming synaptic input with somata, proximal dendrites, and axon initial segments of granule cells. Although the PV-expressing neurons are lower in number compared with STT-expressing neurons in hippocampus and have no significant reduction in number after seizures, when STT-expressing neurons were lost in rat model with kainate induced epilepsy, PV-expressing neurons provide robust inhibitory synaptic output which compensate the loss of STT-expressing neurons (Buckmaster and Dudek 1997). Therefore, the PV-expressing neurons are the major type of interneuron suppressing the hyperexcited neuron circuit. Besides, peri somatically targeted PV-expressing neurons are essential for generating gamma oscillations which is critical for cognition functions including memory formation and sensory processing (Bartos, Vida et al. 2007), implying the dysfunction of PV expressing neurons may lead to cognitive and psychiatric disorders found in TLE. Except for the inhibitory effect, the PV-expressing neurons have a special function of regulating adult neurogenesis in hippocampus. Activation of PV-expressing interneurons promotes survival and proliferation of newborn neurons at critical stage of adult hippocampal neurogenesis in sub granule zone (SGZ) (Song, Sun et al. 2013, Zaben et al 2009). Although the PV-expressing neurons form direct synaptic contacts with new born neurons, the effect could come through circuity modulation (via neuropeptide VIP mediated neurotransmission (Zaben et al 2009) or releasing growth factors other than GABA (Song, Sun et al. 2013).

Calretinin and reelin-expressing interneuron

Calretinin and reelin-expressing interneurons are all derived from CGE origin and distribute mainly in the superficial layer. The calretinin expressing interneurons target not only the principal neurons but also the interneuron population. The disinhibition function of calretinin expressing interneurons is essential for synchronization within interneuron population. The number of calretinin-expressing interneurons significantly decreased in relation with the principal cell loss in excised HS tissue from patients with TLE (Toth, Eross et al. 2010). Besides, surviving calretinin expressing interneurons in HS formed dramatical had less connections with other calretinin expressing interneurons (Toth, Eross et al. 2010). The dysfunction of interneuron synchronization due to calretinin expressing interneuron loss and reorganization could lead to pro-epileptic activities.

Reelin protein in extracellular matrix maintains the layered structure of hippocampus and controls neuronal migration partially by acting on the radial glial scaffold required for neuronal grafting (Frotscher, Haas et al. 2003). KA application caused decreased expression of reelin, particularly in hilar interneurons (Chai, Munzner et al. 2014). Reelin distribution affects the migration of both newly born neurons and the mature granule cells. In the absence of neurogenesis, dysfunction in reelin cause displacement of mature neurons which is relevant to the development of Granule Cell Dispersion. While exogenous application of reelin prevented abnormal migration in GCD (Haas and Frotscher 2010).

In summary, all types of hippocampal interneurons contribute to the delivery of inhibitory synaptic output and to maintaining the balance between excitation and inhibition. Meanwhile, dysfunction and/or loss of certain subtypes is correlated with distinct pathology encompassing abnormal neurogenesis, ectopic migration and network synchronization, in the sclerotic hippocampus.

1.1.3 Neuroinflammation in TLE

1.1.3.1 Inflammation in CNS

With increasing understanding of the immune response towards injury, infection and neurological diseases in CNS, the inflammatory microenvironment has been found to affect cell survival, neurogenesis, neuronal integration and migration, which are relevant to the development of brain diseases in CNS (Toulmond, Parnet et al. 1996, Belarbi and Rosi 2013).

Microglia are the resident cells contributing most to the inflamed microenvironment. In response to pathogen-associated or damage-associated molecules, microglia are activated and transformed to different phenotypes, secreting inflammatory mediators including pro-and anti-inflammatory cytokines, complement and reactive oxygen species (Allan, Tyrrell et al. 2005, Belarbi and Rosi 2013). They also recruit inflammatory cells like monocytes and macrophages from the circulation to injured brain area. Apart from microglia, nearly all endogenous brain cells in CNS can release inflammatory mediators, especially in chronic phases even the neurons and astrocytes participate in maintaining the inflammatory microenvironment (Allan, Tyrrell et al. 2005).

It is hard to determine if the function of inflammatory cytokines are beneficial or detrimental in CNS. At the acute phase after brain injury cell of the innate and adaptive immune systems are activated and release inflammatory mediators to remove lethal pathogens and damaged tissue. At later stages, activated microglia can acquire distinct phenotypes, which either support or interfere with processes of neuron stem cell renewal needed for neurogenesis. In the persistent and chronic stage, the inflamed microenvironment usually undermines recovery of brain damage (Vezzani, Aronica et al. 2013, Xanthos and Sandkuhler 2014). Neuroinflammation also involves cooperation between local and peripheral immune cells and complex stimulating or inhibiting downstream signals received from various cytokines. A lot of effort has been made to elucidate the mechanism of neuroinflammation in different rodent models and cell culture models, the results are various due to applying timing, dosage and duration of inflammatory stimulators (Strijbos and Rothwell 1995, Vezzani, Conti et al. 1999, Diem, Hobom et al. 2003, Fogal, Hewett et al. 2005, Hewett, Jackman et al. 2012, Dey, Kang et al. 2016). The inflammatory cytokines such as TNF-α, IL-1β, IL-16 etc which are significantly upregulated in pathological conditions have been heavily studied as the potential anti-inflammatory targets for CNS diseases. Although the research has demonstrated positive outcomes in rodent models of CNS diseases, due to the complex mechanism of neuroinflammation the clinical translation of anti-inflammatory therapy for CNS diseases is still under investigation.

1.1.3.2 Neuroinflammation and abnormal neurogenesis in TLE.

Hippocampal neurogenesis is consistent throughout life (Shors, Miesegaes et al. 2001). In adult humans, 700 new neurons are added per day and the vast majority of dentate gyrus neurons in hippocampus are subject to exchange postnatally (Spalding, Bergmann et al. 2013). The neuron stem cells exist in the SGZ of the DG. These stem cells can divide asymmetrically to produce one daughter progenitor cell and one stem cell. The progenitor cell can then divide asymmetrically and give birth to daughter cells that differentiate into either astrocytes or neurons (Kohman and Rhodes 2013). Hippocampal neurogenesis is regulated by the microenvironment in the neurogenic niche. The immune system is the most important factor in the microenvironment which regulates neurogenesis in neurogenic niche by releasing different inflammatory cytokines. Evidence suggests that in healthy hippocampus the inflammatory cytokines secreted by immune cells participate in maintaining normal neurogenesis (Kokaia, Martino et al. 2012). However, in

the pathologically inflamed brain microenvironment, neurogenesis is suppressed and is related with memory loss, cognition deficiency and behavior abnormalities. For example, reduced proliferation of neural progenitor cells and accumulated non-migratory neuroblasts have been observed in the neurogenic niche of SVZ in persistent brain inflammation (Pluchino, Muzio et al. 2008).

Abnormal hippocampal neurogenesis has been observed in epilepsy (Cho, Lybrand et al. 2015, Shtaya, Sadek et al. 2018). Accumulated evidence suggests cytokines which are upregulated in TLE, such as IL-1β, TNF-α, INF-γ (Butovsky, Ziv et al. 2006), could increase proliferation of neuron progenitor cells. But increased proliferation in acute phase of neuroinflammation does not necessarily compensate neuron loss in epilepsy. Because in the inflamed hippocampus, the proportion of new-born radial-glia-like NPCs instead of neuroblasts significantly increases in the SGZ of the dentate gyrus (Giannakopoulou, Grigoriadis et al. 2013), therefore the differentiation of neuronal cells decreases despite an increase in cell proliferation. And not all new-born neurons are able to develop into mature neurons and cooperate in the local network, around 70% new-born neurons will be eliminated (Chesnokova, Pechnick et al. 2016). So early increase in proliferation will deplete storage of neuron stem cells in SGZ and suppress hippocampal neurogenesis in chronic epilepsy. Evidence suggested that administration of LPS into rat hippocampus activated microglia and strongly impaired basal hippocampal neurogenesis (Ekdahl, Claasen et al. 2003). While inflammatory blockade prevented reduction of neurogenesis in adult hippocampus (Monje, Toda et al. 2003). Therefore, understanding the relationships between the inflamed microenvironment and abnormal neurogenesis will be critical to develop therapeutic strategies.

1.1.4 IL-1 family and signaling pathway

Interleukin-1 (IL-1) is a large family of cytokines which mediate innate immune responses during acute brain injury as well as chronic neurological and neurodegenerative diseases. The IL-1 family has 11 family members (IL-1F1 to IL-1F11), and they are encoded by 11 distinct human genes (Figure 1-1 A). Among all IL-1 family members, IL-1α (IL-F1) and IL-1β (IL-1F2) has been heavily investigated due to their importance in inflammatory diseases. They have similar biological effects by activating the IL-1 receptor-mediated signalling pathway. But IL-1α is only released after cell death, while IL-1β is secreted into the bloodstream by living cells (Dinarello 2009). IL-1RA (IL-

1F3) is an antagonist for IL-1α and IL-1β, but in most inflammatory syndrome, IL-1RA is usually produced at the later stage and has an anti-inflammation effect by antagonizing IL-1α or IL-1β. Other members such as IL-18 (IL-1F4), IL-33 (IL-1F11), IL-36 (IL-1F6) have a similar proinflammatory function as IL-1α and IL-1β. The different members of the IL-1 family play an essential role in many autoinflammatory diseases.

Correspondingly, the IL-1R family has also expanded to 9 distinct genes and includes coreceptors (IL-1RAcP), decoy receptors (IL-1R2), and inhibitory receptors (IL-1RAcPb) (Figure 1-1 A). Receptor IL-1R1 is the primary receptor which delivers a downstream signal when binds with its ligands. IL-1RAcP is the coreceptor which is recruited after IL-1β or IL-1α binding with IL-1R1. IL-1R2 is lack of intercellular domain, therefore, is incapable of activating the downstream signal. In the brain and spinal cord, the variant IL-1RAcPb can form the heterodimeric complex with IL-1or IL-1 and IL-1RI, but this complex fails to recruit MyD88, and there is inhibition of the IL-1 signal (Figure 1-1 A).

The most important pathway follows activation of IL-1 receptors is similar as activation of Toll-like receptor (TLR) which recognise a diverse number of bacterial products, nucleic acids, and possibly some endogenous lipoproteins (Figure 1-1 B). Because the intercellular segment of IL-1 receptor shares similar structure as the TLR receptor, which is also called Toll-IL-1 receptor (TIR), both receptor recruit MyD88 as the first step after activation (Dinarello 2011). At its downstream, tumour necrosis factor-associated factor 6 (TRAF6) is activated. Then TRAF6 and phosphorylated IRAK1 and IRAK2 dissociate and migrate to the membrane to associate with TGF-β-activated kinase 1 (TAK1) and TAK1-binding proteins TAB1 and TAB2. And This is the critical point where the inflammatory signalling pathway coming from TNF-α and TGF-β are merged with the IL-1 pathway. Phosphorylated TAK1 activated the inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) and activated IKKβ phosphorylates the nucleus factor kappa-B inhibitor (IκB) which gets degraded, so that nucleus factor kappa-B kinase (NF-κB) is released and migrates to the nucleus. TAK1 can also activate mitogen-activated kinases (MAPK) p38, c-Jun N-terminal kinases (JNK) and extracellular signal-regulated kinases (ERK) by activating MAP kinase (MKK) proteins (Acuner Ozbabacan, Gursoy et al. 2014) (Figure 1-1 B). Except for this popular pathway, there is also evidence suggesting TIR receptor actives PI3K-Akt pathway (Figure 1-1 B) which is

also at the downstream of IL-6, another important cytokine massive releases in inflammatory syndrome (Diem, Hobom et al. 2003).

The activation of transcription in the nucleus will produce many bioactive proteins which have multiple effects on survival, differentiation, growth, secretion and functional state of the cells. Meanwhile, the production of IL-1β can further increase as one of the consequences (Figure 1-1 C). The IL-1β precursor is first released from nucleus to cytoplasm in a non-functional state. Then IL-1β precursor is cleaved by caspase-1 which is one of the inflammasomes also produced from nucleus transcription in secretory lysosome (Weber, Wasiliew et al. 2010, Grishman, White et al. 2012). In this way, the active IL-1β finally be transported outside the cells. Immune cells such as monocytes and macrophages contribute to the early production of IL-1β. All endogenous brain cells including microglia, astrocytes and neurons could secrete IL-1β at a later stage after brain insults (Allan, Tyrrell et al. 2005). Therefore, elevated levels of IL-1β can accumulate in chronic epilepsy. Also, the function of IL-1β is not merely restricted by itself. For example, HMGB1 which is secreted at the later stage after brain injury could enhance the effect of IL-1β through the formation of complexes with IL-1β (Wähämaa, Schierbeck et al. 2011). In this scenario, the strength of IL-1 receptor activation can increase about 100-fold.

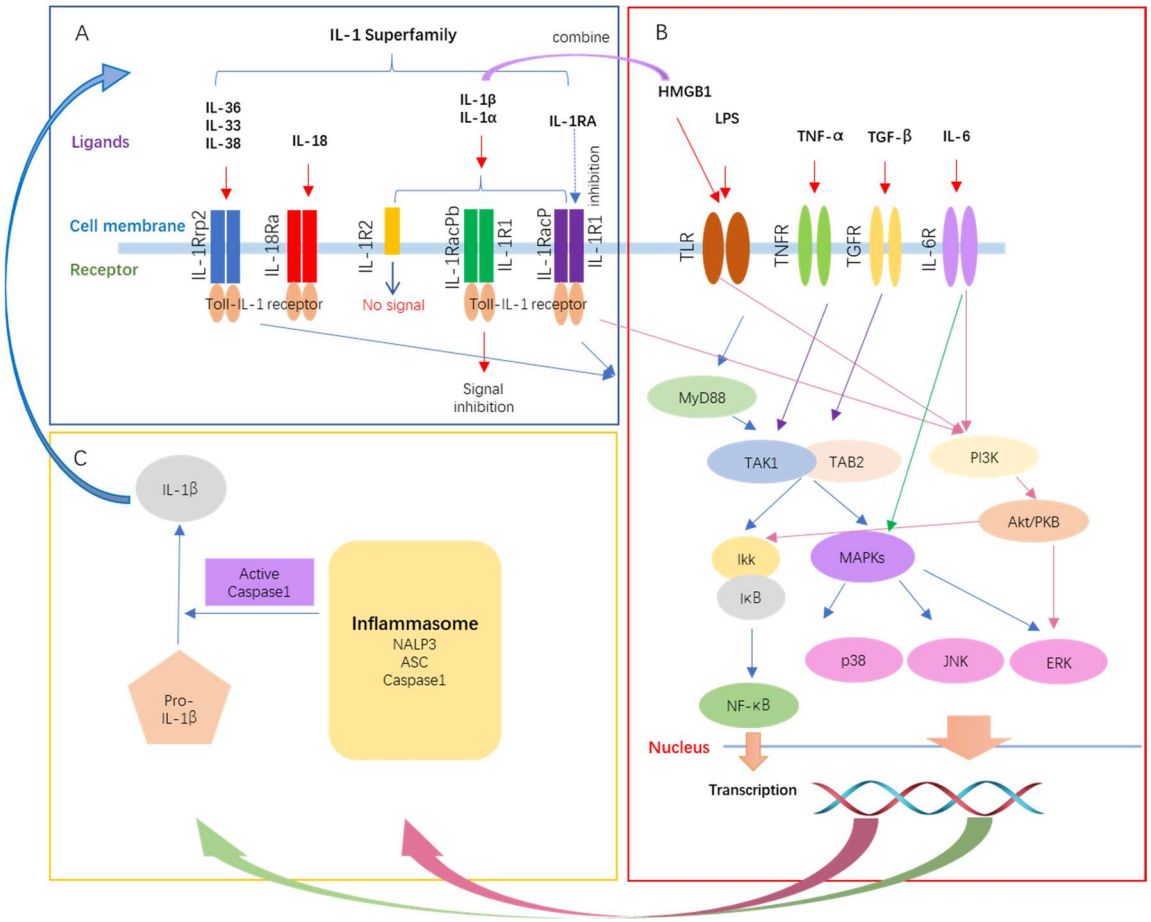

Figure 1-1 IL-1 family and signaling pathway

A) IL-1 family has 11 family members, including pro- inflammatory cytokines (IL-1α, IL-1β) and anti-inflammatory cytokine (IL-1RA). Correspondingly, the IL-1R family has also expanded to 9 distinct genes and includes coreceptors (IL-1RAcP), decoy receptors (IL-1R2), and inhibitory receptors (IL-1RAcPb). B) The most important pathway follows activation of IL-1 receptors is similar as activation of Toll-like receptor (TLR), because the intercellular segment of IL-1 receptor shares similar structure as the TLR receptor, which is also called Toll-IL-1 receptor (TIR). Once activated, the downstream of IL-1 pathway converges with signal coming from other pro-inflammatory cytokines, such as TNF-α TGF-β and IL-6. C) The production of IL-1β can further increase as one of the consequences when the transcription is activated in nucleus. The IL-1β precursor is first releaseo from nucleus to cytoplasm in a non-functional state. Then IL-1β precursor is cleaved by caspase-1 which is one of the inflammasomes also produced from nucleus transcription in secretory lysosome.

1.2 Cortical interneurons

Cortical interneurons comprise 20 - 30% of the total neuron population in the central neuron system (CNS). Unlike the principal neurons which propagate the excitatory signals, most interneurons connect with the surrounding neurons, releasing inhibitory neurotransmitters such

as GABA, modulating the transmission of excitatory signals and regulating the balance of the neuronal circuit activities. Cortical interneurons also serve a vital role in neurogenesis which modulate neuronal proliferation and differentiation during pre-and post-natal CNS development (Le Magueresse and Monyer 2013).

1.2.1 The heterogeneity of cortical interneurons

Most cortical interneurons are born in the subpallial ganglionic eminence (GE) during prenatal CNS development (Anderson, Eisenstat et al. 1997, Hansen, Lui et al. 2013). Distinct subtypes of cortical interneurons originate from discrete areas of the subpallial GE (Gelman, Martini et al. 2009, Miyoshi, Hjerling-Leffler et al. 2010, Kepecs and Fishell 2014). The heterogeneity of interneurons is achieved by the acquisition of different properties such as morphology, molecular targeting, synaptic transmission and physiological characters. (De Marco Garcia, Karayannis et al. 2011). Dysfunction or loss of specific types of interneuron is closely related to different CNS disorders, including epilepsy, autism and schizophrenia (Nakazawa, Zsiros et al. 2012, Le Magueresse and Monyer 2013, Hunt and Baraban 2015).

During last decades, a lot of effort has been made to elucidate the relationship between heterogeneity of cortical interneurons and their correlated morphology, molecular and electrophysiological properities (Petilla Interneuron Nomenclature, Ascoli et al. 2008, Kepecs and Fishell 2014).

1.2.2 The genetic development of cortical interneurons

The subtypes of cortical interneurons are initially being determined by intrinsic expression of regional gene markers at temporal developmental stages when the cells are born in ventral telencephalon; which consists of three distinct progenitor zones, the lateral, medial, and caudal ganglionic eminences (LGE, MGE, and CGE). MGE has been recognized as the specific region where around 70% to 80% GABAergic interneurons are produced. Genetic fate mapping studies revealed that the CGE also contributes to 30% of cortical interneurons in the adult mouse (Miyoshi, Hjerling-Leffler et al. 2010). LGE has been considered as the factory for striatal interneurons and only gives rise to a small number of cortical interneurons (Wonders and Anderson 2006).

Nkx 2.1 is most representative regional transcriptional markers for MGE and ventral CGE. It is the initial specification gene which promote the differentiation of parvalbumin (PV), somatostatin

(STT) and neuropeptide Y (NPY) expressing interneurons (Butt, Sousa et al. 2008). Nkx 2.1 also inhibits the possibility of becoming dorsal CGE-like interneurons which are dominated by expression of Coup TF II at the progenitor stage and eventually become Calretinin, Vasoactive Intestinal Polypeptide (VIP) and Reelin expressing interneurons (Fogarty, Grist et al. 2007). Other genetic makers such as homeobox gene DLX1/2, Gsh2, Olig2, Mash1 are pan-GE makers and participate in encoding the GABAergic lineage together with the specific regional makers. When interneuron progenitors are fully developed within MGE and CGE, the cells exit the cell cycle and start to migrate under control of DLX1/2, towards their destined layers in neocortex and hippocampus. The genetic subgroups of interneurons have been determined by the location and birth date at progenitor stage before the cells migrate to achieve morphological and physiological maturation. Even the interneuron progenitors which do not express specific genetic makers when they become post mitotic neurons, taking several weeks for maturation during migration, their fate does not change by the factors encountered in migration (Wonders and Anderson 2006). Therefore, it become critical to understand the regulation genes and biochemicals which contribute most to specify the subtypes of cortical interneuron during embryonic development

Conditional knockdown and fate mapping of specific genes uncovered the genetical development of cortical interneurons in sequence (Anastasiades and Butt 2011). The proneuronal gene Mash 1 in ventral telencephalon induces the expression of DLX1/2 which marks the origin of GABAergic lineage (Anderson, Eisenstat et al. 1997, Castro, Martynoga et al. 2011). Expression of Nkx 2.1 restricts the future regional identity of cells within MGE origin (Butt, Sousa et al. 2008). The post-mitotic marker Lhx6 is expressed under control of Nkx2.1 and finally gives rise to PV and STT positive interneurons (Flandin, Zhao et al. 2011). In Lhx6 absent regions, or with conditional knock down of Lhx6, calretinin expressing interneurons become the major subtype. Sox6, the downstream transcriptional factor of Lhx6 is also involved in subtype specification when the interneuron progenitors exit the cell cycle (Batista-Brito, Rossignol et al. 2009). Loss of Sox6 does not affect the total number of GABAergic neurons but results in a significant reduction of MGE-derived early born interneurons (PV and STT expressing) and correspondingly causes a significant increase of NPY expressing neurons, which are born later during embryonic development (Azim, Jabaudon et al. 2009).

As for CGE-derived lineage, the ventral CGE shares similar regional specification genes with MGE. But dorsal CGE has distinct regional gene markers. Coup TFII is preferentially expressed in dCGE, regulating together with Sp8 which is expressed in one fifth of cortical interneurons, promote the growth of Reelin, VIP, NPY, and Calretinin expressing interneurons (Miyoshi, Hjerling-Leffler et al. 2010, Ma, Zhang et al. 2012, Reinchisi, Ijichi et al. 2012, Hansen, Lui et al. 2013).

The genetic subtypes of cortical interneuron are closely related with their laminar positions in cortex. The MGE-derived PV/STT positive neurons are usually found in layer IV to VI, while the dCGE-derived calretinin, NPY and reelin positive neuron prefer to stay in the superficial layer I to III (Flandin, Zhao et al. 2011, Ma, Zhang et al. 2012). Likewise, the synaptic function, morphological and electrophysiological properties of different cortical interneuron subgroups are also partly determined by the genetic features. PV-expressing interneurons which are also called basket or chandelier cells based on their morphology, target the soma of projection neurons and are usually fast spiking neurons (Cauli, Audinat et al. 1997); STT-expressing interneurons also called Martinotti cells, target the dendrite of projection neurons and are usually late or regular spiking neurons (Kawaguchi and Kubota 1996, Tamas, Lorincz et al. 2003); VIP and Calretinin expressing interneurons which demonstrate bipolar shape and burst spiking electrophysiological activity have a specially disinhibition function by suppressing activity of other interneurons (Kawaguchi and Kubota 1996); nNOS expressing interneurons also called neurogliaform cells are late spiking neurons and are responsible for the slow GABAergic inhibition of projection neurons and interneurons (Tamas, Lorincz et al. 2003, Karagiannis, Gallopin et al. 2009, Kepecs and Fishell 2014). While NPY expressing interneurons have been classified into three major subgroups, neurogliaform-like neurons, Martinotti-like cells and a fast-spiking subgroup which co-express PV (Karagiannis, Gallopin et al. 2009). But due to the diversity of interneurons, there is no classification system which can exclusively anticipate the function of a certain interneuron phenotype. For example, there are at least 3 type of PV expressing interneurons, and at least two different subgroups of STT expressing interneurons (Kepecs and Fishell 2014). Recent research has found another specialized GABAergic neuron subtype called “rosehip” cells (GAD1+CCK+) (Boldog, Bakken et al. 2018). Those rosehip cells are in layer 1 and target apical dendritic shafts of layer 3 cortical projection neurons (Boldog, Bakken et al. 2018). With the development of an understanding of interneurons subtypes, the function of interneurons in different genetic subtypes will be clearer in the future.

1.2.3 The function of cortical interneurons in CNS

The major function of inhibitory interneurons is regulating neuronal network activity by releasing inhibitory neurotransmitter GABA. GABA is synthesized by glutamic acid decarboxylase (GAD) which is present only in GABAergic interneurons not excitatory neurons. Pre-synaptic release of GABA activates ionotropic post synaptic GABA type A ($GABA_A$) receptors to increase permeability of chloride and bicarbonate ions in the mature neurons.

Apart from regulating neuronal network excitability, the cortical interneurons also play an important role in pre-and post-natal neurogenesis, neuron migration and maturation. GABA release controls the proliferation and differentiation of neuron precursors (Represa and Ben-Ari 2005). During cortical neurogenesis, GABA can activate GABAA receptors to prevent the proliferation of cortical progenitors (LoTurco, Owens et al. 1995, Antonopoulos, Pappas et al. 1997), leading to an increased number of differentiated neurons (Antonopoulos, Pappas et al. 1997). GABA also promotes the migration of glutamatergic neurons and GABAergic interneurons during embryonic development and directs the cells to stop migration at their destined laminar positions in cortex by hyperpolarization (Bortone and Polleux 2009, Inamura, Kimura et al. 2012).

In the mature CNS, cortical interneurons are also critical for memory consolidation. The negative regulation of GABA signaling which inhibits the proliferation of newborn synapsis while promoting maturation of remaining nascent synapse, is a vital process for memory formation (Wu, Fu et al. 2012). Repression of the activity of PV positive interneurons in the hippocampus impaired augmented sleep-associated oscillations and stabilization of functional connectivity patterns in CA1 neurons and causes deficits in memory consolidation (Ognjanovski, Schaeffer et al. 2017).

Since the inhibitory interneurons consistently and dynamically affect neuronal network activity, the CNS development and memory formation, dysfunction or loss of GABAergic neurons will lead to a range of neurological and neurodegenerative disorders.

1.3 Interneuron transplantation for epilepsy

Despite rapid development of anti-epilepsy drugs, 20-30% patients are poorly responsive to drug treatment (Loscher, Klitgaard et al. 2013). Moreover, the gradual development of drug resistance in some patients further undermines the efficiency of seizure control. Surgical removal of sclerotic hippocampus has proved to be more effective than long-term medication treatment, more than two thirds of patients with TLE were seizure free or have significant less seizure events after surgery, but the adverse effects due to surgery can't be ignored in around 10% of patients, including verbal memory deficiency and infection (Wiebe, Blume et al. 2001). Therefore, novel therapeutic methods are required for improve outcome of patients with drug-resistance epilepsy.

1.3.1 Cell replacement therapy for brain diseases

The possibility of cell replacement therapy for neuron loss or dysfunction in CNS has been investigated for more than two decades. A lot of progress based on in vitro and in vivo neuron transplantation studies has been made since the concept was proposed. Evidence showed the transplanted neurons could integrate into local neuronal circuitry and improve the outcome in animal models of Parkinson's disease (PD), Huntington's disease (HD), epilepsy and stroke (Freed, Greene et al. 2001, Bliss, Guzman et al. 2007, Maucksch, Vazey et al. 2013, Southwell, Nicholas et al. 2014, Hunt and Baraban 2015). And the clinical trials of neuron replacement therapy have been approved for PD and HD which have the precise brain regions to target and cell type to replace, and strong evidence from relevant rodent and primate models suggests high efficiency of improvement (Olanow, Kordower et al. 1996, Bachoud-Levi, Bourdet et al. 2000, Freed, Greene et al. 2001, Olanow, Goetz et al. 2003). As for epilepsy, the consequence of pathology varies from different models and patients, the mechanism of how neuron transplantation works is still unclear, more evidence is required to optimize the strategy and support future clinical trials.

Neuron progenitors with committed lineage identity are best-suited for cell replacement therapy. Undifferentiated neuron stem cells proliferate quickly and hold the potential to become any type of cells, which increases the incidence of teratoma formation after transplantation (Conti and Cattaneo 2010). Furthermore, neuron stem cells without a committed lineage identity are not designed to supplement dysfunction or neuron loss in certain CNS disorders (Conti and Cattaneo 2010, Steinbeck and Studer 2015). Alternatively, specified post mitotic neurons have a very limited

ability for migration and fail to establish reciprocal connectivity with existing neuron network, and are therefore, unable to provide functional improvement.

Transplanted neuron progenitors also have other physiological functions rather than just reestablishment of the impaired neuron circuit. They could secrete neurogenic or neurotrophic factors which affect entire microenvironment, modulating neuron proliferation, differentiation and maturation. So, it is essential to understand how transplanted neurons work in pathological conditions before clinical use.

1.3.2 GABAergic neuron transplantation in epilepsy

Epilepsy is not a singular disease as in PD or HD in which predominant loss of dopaminergic neurons or medium spiny neurons is the direct cause of the diseases. In epilepsy, neuron loss, dysfunction is accompanied with aberrant neurogenesis, and the neuropathological in principal cells, mossy cells, interneurons and glia cells all contribute to shifted balance between excitation and inhibition. It is unlikely any single factors could be identified to explain and reverse all pathological changes.

Various types of neuronal cells have been selected to test the cell replacement therapy including fetal brain tissue from hippocampus, neocortex, and subcortical structures, mouse and human neural stem cells, and genetically engineered neurons or glia designed to express putative anticonvulsant substances (Goldberg and Coulter 2014). Although all those cell replacement therapies have positive outcomes and special benefits, the GABAergic neuron progenitors are the best candidate so far compared to the other types of cell. Despite the complexity and variety of the neurophysiology, GABAergic interneuron is the major type of neuron which delivers inhibitory signals and contribute most to suppress the over activated neuronal circuit in epilepsy.

The first GABAergic neuron progenitor transplantation was conducted in Kv1.1 mutation induced mouse seizure model. The bilateral transplantation of MGE graft derived from donor embryo into postnatal cortex efficiently reduced the duration and frequency of spontaneous seizures (Baraban, Southwell et al. 2009). Following that breakthrough, primary cultured mouse MGE cells have been transplanted into mice after pilocarpine induced epileptic status. Similar to the previous study, the MGE progenitors can migrate widely following transplantation into the adult hippocampus, differentiate into mature GABAergic neurons and form synaptic integration with local projection

neurons (Hunt, Girskis et al. 2013). After transplantation, the mice receiving exogenous MGE progenitors had 92% reduction in spontaneous seizures as well as a reduction in aggressive behavior and improvement in short-time spatial memory (Hunt, Girskis et al. 2013). In agreement with this finding, another research group confirmed significant reduction of seizure frequency and seizure duration after receiving bilateral transplantation of primary culture of mouse MGE cells in similar pilocarpine induced mouse model of epilepsy. This research has also showed that transplanted cells formed mature inhibitory synaptic complexes at their terminal and apposed onto the somas, apical dendrites, and axon initial segments of dentate granule cells, suggesting enhanced synaptic inhibition from transplanted MGE cells (Henderson, Gupta et al. 2014). Those studies provide promising evidence for using MGE progenitors as a treatment for human epilepsy.

For the potential future clinical application in TLE patients, the GABAergic neuron progenitors from fetal tissue maybe the ideal cell type, at least theoretically, for cell transplantation, as transplanted human fetal tissues derived neuron progenitors have been proved to be safe in PD and HD patients and one is replacing cells with their immediate progenitors. However, the limitation in tissue availability and ethical concerns become the major obstacles in addition to scientific utility. To provide reproducible, reliable and constant neuron progenitors for cell transplantation, many researchers have investigated the potential of using hESC, human pluripotent stem cells (hPSC) or induced hPSC to generate linage specific neuron progenitors. By precisely controlling the temporal and regional patterning signals to simulate human telencephalon development, the GABAergic neuron progenitors with committed linage identity could be generated from hPSCs and ihPSCs (Hunt, Girskis et al. 2013, Liu, Weick et al. 2013, Cunningham, Cho et al. 2014, Kim, Yao et al. 2014). Meanwhile, the possibility of using MGE-like interneurons generated from hESCs and hPSCs has also been under investigation for treatment of epilepsy. In one study, mice given hESC-derived MGE cells showed improvements in learning and memory deficits after receiving induced learning and memory loss by P75-SAP (Liu, Weick et al. 2013). However, unlike primary MGE cells, the hESC-derived MGE cells show limited grafts in hippocampus and only 45% cells become GABAergic neurons 6 months after transplantation (Liu, Weick et al. 2013). Similar results have been reported by another study in which 50% GAD expressing neurons was identified 3 months after transplantation into TLE model of mouse (Maisano, Litvina et al. 2012). In another recent study, transplanting hPSC-derived MGE-like progenitors showed similarly anti-epileptic effects as primary culture of mouse MGE cells in

pilocarpine induced mouse model of epilepsy (Cunningham, Cho et al. 2014). But the electrophysiological data showed the transplanted hPSC-derived MGE-like neurons did not reach the same maturation state as mature adult neurons (Cunningham, Cho et al. 2014). This suggests that grafted neurons were capable of suppressing seizures even before acquiring full electrophysiological maturation.

But some scientific concerns still need to be investigated especially in terms of long-term efficacy. A recent study found that the effectiveness of transplanted GABAergic progenitors did not extend to long-term epileptic seizure control (Henderson, Gupta et al. 2014). So, the former short –term EEG recordings and behavioral improvement may overestimate the efficiency of MGE grafts. Therefore, it is important to determine the exact mechanism of how GABAergic progenitors suppress seizures. The research also demonstrated the GABAergic neuron progenitor did not improve pathological alterations in sclerotic hippocampus like MFS (Maisano, Litvina et al. 2012, Hunt, Girskis et al. 2013). Moreover, not all transplanted MGE cells of human original differentiated into neurons, there were small populations of astrocytes and oligodendrocytes (Liu, Weick et al. 2013, Cunningham, Cho et al. 2014). And although GABAergic neurons modulate neurogenesis and during brain development and in normal adult neurogenesis, so far, no direct evidence showed they could modulate aberrant neurogenesis in the sclerotic epileptic hippocampus. It might be due to distinct function of interneuron subtypes correlated with heterogeneity in genetic development, as only certain type of interneurons (PV- and NPY-expressing) have been suggested to regulate neurogenesis in brain development (Howell, Doyle et al. 2005, Howell, Silva et al. 2007, Song, Sun et al. 2013). Indeed, in transplanted MGE-like cells, the population of PV or NPY expressing interneurons is less than 10% (Cunningham, Cho et al. 2014). In addition, a large population of injected hESC or hPSC derived MGE-like cells stay within the injection site and the differentiation of mature human interneurons takes a long time (Maisano, Litvina et al. 2012, Liu, Weick et al. 2013, Cunningham, Cho et al. 2014). So, it is hard to predict how long it will take for the transplanted cells to show their effects in patients with TLE.

Microenvironment is completely different in sclerotic hippocampus. Inflammatory cytokines such as TNF-α, IL-1β, IL-16 etc are enriched in sclerotic hippocampus, (Jimenez-Pacheco, Diaz-Hernandez et al. 2016). They are involved in neuron death, abnormal neurogenesis and electrophysiological dysfunctions of neurons (Schafers and Sorkin 2008, Vezzani, Balosso et al.

2008, Belarbi and Rosi 2013, Vezzani and Viviani 2015). Many research studies suggest that pathological levels of inflammatory cytokines are detrimental for neuronal survival and neurogenesis (Toulmond, Parnet et al. 1996, Aktas, Ullrich et al. 2007, Vezzani, French et al. 2011, Kokaia, Martino et al. 2012). Recently Genome-wide association studies (GWAS) and exome sequencing has been used to recognize the major detrimental inflammatory mediators in different relevant animal models and patients with brain diseases (Johnson, Behmoaras et al. 2015). IL 1R–TLR (Toll like receptor) signaling is thought to be pivotal for initiating the inflammatory brain response following seizures or epileptogenic brain insults. And the release of IL-1β caused neuron injury, hyperexcitability which further aggravate occurrence of seizures.

Interleukin-1 (IL-1) is one of most powerful pro-inflammatory cytokines which contributes to a variety of neurological and neurodegenerative diseases in CNS (Toulmond, Parnet et al. 1996, Allan, Tyrrell et al. 2005). Researches have shown that IL-1β produced by activated microglia affects the generation, differentiation and survival of neural stem cells (Green, Treacy et al. 2012, Belarbi and Rosi 2013). Immune cells such as monocytes and macrophages contribute to early production of IL-1β, all endogenous brain cells including microglia, astrocytes and neurons could secrete IL-1β at later stage after brain insults (Allan, Tyrrell et al. 2005). Therefore, IL-1β can accumulate in chronic epilepsy. It is critical to understand how persistent inflamed microenvironment of epilepsy affect neurogenesis in human.

So far, the researchers are focused on the effect of inflammatory microenvironment on local brain cells. It remains to be known about the cross- talk between exogenous neurons and local microenvironment in brain diseases. Especially transplanted human neurons will take several months for neuronal differentiation before incorporating with the local neuronal circuit. Therefore, during that long period how transplanted exougenous neuron progenitors adapt to the pathological microenvironment is still unclear. There is also a lack of evidence on whether chronic pathologic microenvironment of epilepsy will affect the survival, differentiation and electrophysiological activity of transplanted GABAergic interneuron progenitors.

1.4 Aim

This project will investigate how transplanted cells behave in the chronic pathological environment and how the inflamed microenvironment affects the survival, proliferation,

functional maturation and electrophysiological activities of transplanted MGE progenitors. In addition, hESC derived 3D neuronal culture which can recreate a modifiable microenvironment will be established to observe the effect of inflammatory cytokines on neuronal cells

Objective 1, Generation and characterization of cortical neuron progenitors, cortical GABAergic neuron progenitors and astrocytes from hESC

Objective 2, Integration of GABAergic neurons into human epileptic hippocampal Hi-Spot Cultures and an examination of their effects compared to cultures derived from “normal” or non-epileptic cortex.

Objective 3, Investigate the effect of IL-1β on cell survival and differentiation of transplanted MGE-like neuron progenitors.

Objective 4 Generation and characterization of hESC-derived three-dimensional neuronal cultures.

Objective 5 Investigate the effect of IL-1β on the functional electrophysiological properties of MGE-like neuron progenitors

2. Methods and Materials

2.1 Cell culture

The cell incubator (Galaxy 170 R, New Brunswick) was used to maintain the cell culture at 37°C and 5% CO2. The laminar flow hood (Maxisafe 2020, Thermo Scientific) was used for the cell culture work under sterile conditions. The cell culture-treated plastic multi-well plates (Thermo Scientific) or glass coverslips (VWR) with specific coating were used for cell growing.

2.1.1 Human ESC culture and maintain

H7 hESC line (Passage number 10 to 35) or their tau-GFP-transfected counterparts (TG-H7, from Zoe Noakes) (Passage number 4 to 25) were used for the all stem cell experiments. Multi-well plates were coated with Matrigel (Corning) diluted 1 in 100 with DMEM/F-12 (Gibco) medium for at least 1 hour at 37°C. H7 or TG-H7 cells were seeded at the density approximately 50000 to 150000 cells/cm^2 and maintained in TeSR-E8 medium (Stem Cell Technlogies) in 6-well plates. The medium was changed every day or every other day depending on the confluence of the cells. The cells were split as follows when they reached 80-90% confluence. The cells were washed once with D-PBS (Gibco) before adding EDTA (Sigma) or gentle cell dissociate reagent (Stem Cell Technlogies) for 3 to 5 mins at 37°C. EDTA or gentle cells dissociate reagent was aspirated and substituted with E8 medium. A serological pipette was used to mechanically scratch and manually dissociate the cells by titrating into small clusters. Cell suspension was centrifuged at 1150 rpm for 3 minutes (5810 R, Eppendorf) to create a cell deposit. Discarded the supernatant and replaced with fresh E8 medium. The cells were then re-suspended and seeded at gradient ratios (1: 2, 1:3, 1:6).

2.1.2 Human ESC freezing and thawing

H7 and TG-H7 cells split at low passage number were stored in liquid nitrogen for future use. The stem cells for storage were kept in large clusters when dissociated as described above. Cells were harvested when the cells reached 80% to 90% confluence in muti-well plates. The cell suspension was then transferred in to cryogenic vials with E8 medium containing 10% DMSO. The cryogenic vials were then placed inside a cell freezing container which helped achieve gradient

temperature decrease in -80°C freezer. The cells were kept in -80°C for at least 24 hours before eventually transferred into the liquid nitrogen tank.

The cryogenic vials with cells for thawing were transferred in dry ice from liquid nitrogen. The cryogenic vials were put into 37°C water bath as quickly as possible. Shake the cryogenic vial while thawing the cells. Removed the vials from the water bath when only a small ice pellet left in the vial. Transferred the cell suspension at the ratio of 1 in 10 into the pre-warmed E8 medium and centrifuged at 1150 rpm for 3 min. Discard the supernatant, re-suspended the cells with E8 medium and seeded the cells into a single well of a 6-well plate.

2.1.3 Human hHA culture and maintain

The human hippocampal astrocytes (hHA) were purchased from ScienCell Research lab (#1830). (Lot number 1670) Muti-well plates or glass coverslip were coated with poly-D-lysine (10ug/ml diluted in D-PBS) for at least one hour in room temperature, rinsed by D-PBS and left to dry. The hHAs were seeded at 5000 cells/cm^2 and maintained in conditioned medium (DMEM/F12 100ml, Neurobasal 50ml, FBS 5%). The culture medium was aspirated every two or three days. The cells were passaged when reached 80% to 90% confluency. The cells at low passage number were stored in liquid nitrogen for future use. The cells at passage 3 to passage 6 were used to support neuron growth for electrophysiological experiment.

2.1.4 Human ESC differentiation

The 12- well plate were coated with Growth Factor Reduced Matrigel (Corning) diluted 1:15 in DMEM/F12 Cells for 1 hour at 37°C. hESC were harvested from the 6-well plate and placed into the prepared 12-well plate. When the cells grew to 90% confluency, the medium was change from E8 to defined N2B27 medium (100 ml DMEM-F12, 50 ml Neurobasal, 1 ml N2 supplement, 1 ml B27 without retinoic acid, 2 mM L-glutamine, penicillin-streptomycin, 0.1 mM β-mercaptoethanol, all from Gibco). And that day was defined as day 0.

To obtain MGE progenitors, LDN-193189 (LDN;100nM, Tocris), SB431542 (SB;10uM, Tocris) and XVA939 (XVA;2uM, Tocris) were given from day 0 to day 9. The 1st passage was taken at day 9-10. The cells were pre-incubated with ROCK inhibitor for at least 1 hour at 37°C and dissociated with EDTA as described above. Gently titrated the cell suspension and kept the cells in large cluster for good survival. The cells were then split at a 2: 3 ratios and seeded into the 12-well

plate coated with fibronectin (15 µg/ml, 2 hours at 37°C; Millipore). Sonic hedgehog (SHH;200ng/ml, R&D) and Purmorphamine (Pur,1uM; Millipore) were given from day 10 to day 18. The 2nd passage was taken after day 20. The 12-well plate were pre-coated with poly-D-lysine (PDL, Sigma;10 µg/ml in D-PBS, was added for 1 hour at room temperature, rinsed once with D-PBS) and laminin (Sigma;10 µg/ml in D-PBS, incubated overnight at 37°C after PDL coating.) The cells were dissociated with EDTA as described above, vigorous titrated into small clusters and spilt at a ratio of 1:3 to 1:4. When the cells started to grow processors at around day 25, the supplement B27 with Retinol acid (RA) were given to replace the previous supplement B27 without RA. The medium was also supplemented with BDNF and GDNF (10 ng/ml; Peprotech) which promoted neuron differentiation and maturation.

To obtain cortical progenitor, LDN-193189 (LDN;100nM, Tocris), SB431542 (SB;10uM, Tocris) were given from day 0 to day 12. The 1st passage was taken at day 9-10. The cells were pre-incubated with ROCK inhibitor for at least 1 hour at 37°C and dissociated with EDTA as described above. Gently titrated the cell suspension and kept the cells in large cluster for good survival. The cells were then split at a 2: 3 ratios and seeded into the 12-well plate coated with fibronectin (15 µg/ml, 2 hours at 37°C; Millipore). When the cells started to grow processors at around day 25, the supplement B27 with Retinol acid (RA) were given to replace the previous supplement B27 without RA. Half medium were replaced every other day.

The neuron progenitors generated from h-ESC were used for astrogila differentiation. The MGE or cortical progenitors were cultured in N2B27 medium until differentiation day 20 with the method described above. The cells were then dissociated and passaged at the density of 10000~20000/cm2 onto poly-d-lysine (10ug/ml) and Laminin (10ug/ml) coated plate. Switch to medium composed of DMEM/F12, Neurobasal, 10%FBS, FGF2(20ng/ml), 1%L-glutamine, Mycozap (Lonza), β-mercaptoethanol thereafter. The cells were passaged weekly when they reached 80% to 90% confluency. The medium was changed every two to three days.

2.2 Hi-spot culture

2.2.1 Brain tissue preparation

The cortical and hippocampal tissue was obtained from the patients undergoing epilepsy or brain tumor surgery. The brain tissue was collected with sterile falcon tubes filled with 10-20ml of Gey's

balanced salt solution (Sigma) supplemented with MK-801(Tocris), then transferred into a 10-cm petri-dish and washed 3 times with D-PBS. The tissue was then placed onto a plastic flask inside the tissue chopper (McIlwain) and mechanically chopped into 1mm^3 size. The tissue was dissociated in papain (Sigma) for 30min at 37℃. Once dissociated, the cells were vigorously titrated and flittered twice by 100um mesh cell strainers (Millipore). The viable cell number was estimated using the trypan blue (Sigma) exclusion assay.

2.2.2 human Hi-spot generation and maintain

The hi-spot culture dishes were prepared under sterile condition in advance as follows. 1ml hi-spot medium composed of 62% DMEM/F12, 20% FBS, 10% Ham's F-12 Nutrient Mix, 5% horse serum 1% pen-strep-glutamine, 1% hepes, 1% GlutaMAX supplement (all form life tech) was added into each well of 6-well plates. The Millipore membrane inserts (Millicell® PCF ,30mm, 0.4 µm, polycarbonate) were then placed into each well. 3 to 4 Teflon membrane discs (pore size 0.4 µm with a thickness of ~30 µm; purchased from Biocell Interface, SA) were taken by forceps and carefully placed onto the Millipore membrane inserts. The prepared 6-well plates were left at 37°C overnight.

The GFP-MGE progenitor (differentiation from TG-H7 cell line) around differentiation day 20 or differentiation day 45 were dissociated by accutase (Sigma) for 10 min at 37 °C and vigorous titrated into single cell suspension. The viable cell number was estimated by trypan blue.

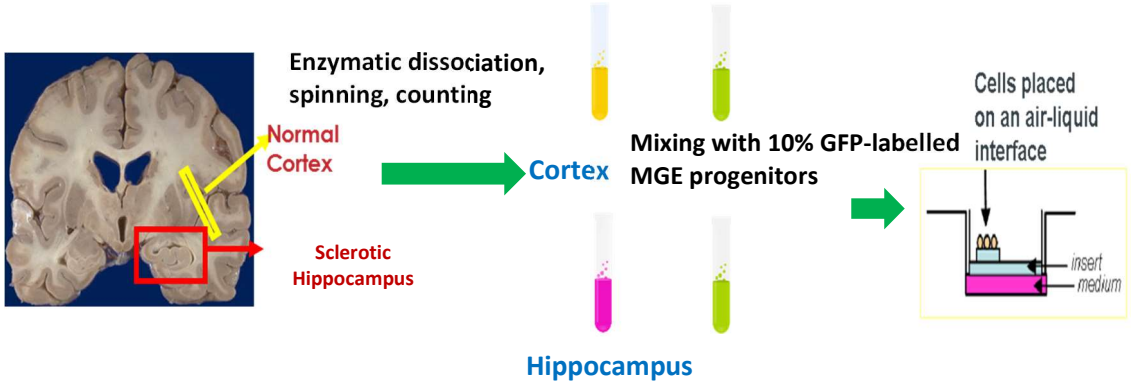

Figure 2-1 Diagram for generating hi-spot from human brain tissue

10% GFP-MGE progenitors were well mixed with 90% previous prepared viable human brain cells (2.2.1) in suspension. The mixed cell suspension was centrifuged at 1200 rpm for 5 mins. Discard the supernatant and replaced with certain amount of hi-spot medium (1ul per 50,000 cells). Re-suspended the cell pellet and pipetted 5ul high-density cell suspension in a single drop and placed on the top of each Teflon membrane disc. (Figure 2-1)(Bailey, O'Connor et al. 2011). The medium was replaced twice a week

2.2.3 ES hi-spot generation and maintain

The human ES-derived cortical progenitors, astrocytes and MGE progenitors were dissociated by accutase for 10min at 37℃.The viable cell number was estimated using the trypan blue exclusion assay. The astrocytes, cortical progenitors and the MGE progenitors were mixed at the ratio of 90:9:1. The cell suspension was centrifuged at 1150rmp for 5 min and the cell rich pellet was re-suspended in hi-spot medium (described in 2.2.2). Pipetted 5ul high-density cell suspension in a single drop and placed on the top of each Teflon membrane disc. The medium was replaced twice a week.

2.2.4 Experimental Groups and time points

For each experiment, the sclerotic hippocampal tissue was used to simulate epileptic microenvironment while neocortical tissue from the same patient was considered to simulate non-inflamed microenvironment. To block the effect of IL-1β, IL-1RA (100ng/ml; PeproTech EC Ltd), the antagonist of IL-1R1 was added to the medium of sclerotic hippocampal and cortical hi-spots from day 1 to day 28. Same concentration of IL-1RA was given to the cultures when change the medium every three days. To observe the effect of IL-1β in non-pathological environment, IL-1β (10ng/ml) was add to the culture medium of non-epileptic hippocampal and cortical hi-spot culture from day 1 to day 28. Same concentration of IL-1β was given to the cultures when change the medium every three days the hi-spots were fixed at day 14 and day 28 for immunochemistry.

2.3 Cell and hi-spot analysis

2.3.1 Immunochemistry

The cells in culture plate or on coverslip were washed once with D-PBS and fixed in 3.7% PFA for 15 minutes at room temperature. For nuclear and cytoplasmic makers, the cells were washed three times with PBS-T (PBS+0.3% Triton-X-100), 10 mins each, for permeabilization.

For cell surface maker (IL-1R1 receptor), the cells were washed three times with PBS only.

For hi-spots, PBS-T with 0.5% to 1% Triton-X-100 was given for thorough permeabilization.

The cells or hi-spots were incubated with 2% BSA (Sigma) and 3% donkey serum (Merck-Millipore) for 20-30 minutes at room temperature for blocking. Primary antibodies listed in the **Table 1** were diluted in PBS-T with 3% donkey serum at given concentration and left overnight at 4°C. The cells and hi-spots were rinsed three times, 10 mins each, with PBS-T the next day and incubated in secondary antibodies diluted with PBS-T (1:1000 for cells and 1:500 for hi-spots, AlexaFluor anti-donkey 488, 555, 594, 647; Life Technologies) for 1 hour at room temperature in dark. After that, DAPI (1:3000 in PBS; Molecular Probes) was added for 3 mins at room temperature. Stained cells or hi-spots were rinsed 3 times with PBS and mounted in Mowiol 4-88 (Sigma) and fixed on to glass slides (VWR).

Image analysis was performed using Leica DM6000B upright (for slides) or Leica DMI6000B inverted (for plates) fluorescent microscopes at 10X, 20X or 40X magnification.

2.3.2 Annexin V/Propidium Iodide (PI) Apoptosis Assay

The cells in culture plate or on coverslip were washed once with fresh medium. The cells were then incubated with PI (BD Pharminge,1:500) and Annexin V (BD Pharminge ,1:1000) at 37C^{o} for 30mins. The cells were rinsed one time with PBS. DAPI was given to the cells for another 10mins at 37C^{o}. The coverslips were rinsed one times with PBS and mounted in Mowiol 4-88 and fixed on to glass slides. Image was taken using Leica DM6000B upright fluorescent microscopy.

2.3.3 Cell quantification

Cell counting was performed with images taken from at least four random fields of a coverslip or a hi-spot. The number of marked cells were manually counted by using Fiji (Image J). The density

and the area of fluorescent were automatically measure by image J. The average density was calculated by density/area.

2.3.4 Sholl analysis

Sholl analysis is widely used to evaluate morphological complexity of neurons (Sholl 1953). Sholl profile is described by plotting the number of neurite intersections against the gradient radial

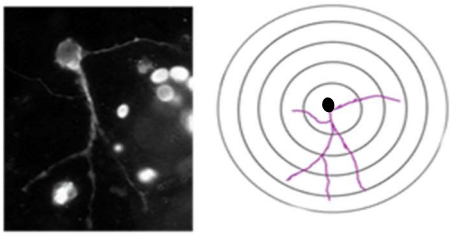

Figure 2-2 Diagram for Sholl analysis

distance from the soma (Figure2-2). The area under the Sholl curve can be used as a single measure of dendritic complexity.

The labelled neurons for Sholl analysis were placed around the centre of the view when taking photos. The pictures were then imported into Fiji for Sholl analysis. The scale of image was adjusted by the scale bar. The cells were firstly processed by the plugin - Simple Neurite Tracer. The estimated geometric centre of the cell body was marked as the starting point for the processors. The processors were semi-automatically traced one by one from the starting point to the visible end. The secondary and above branches were traced from the connection point at certain primary or above branches. The detailed information about branch length and branch number was automatically obtained after tracing the neurons. The short cut 'Ctrl+Shift+A' were applied to calculate the number of intersections with digitally tracing rings at different distances form the soma centre. The area under the intersection-distance relation curve was measured by Graphpad Prism for individual neurons.

2.4 Electrophysiological analysis

The whole cell patch clamp recording was performed at room temperature (20 to 22°C) , using Multiclamp 700B amplifier and Digidata 1550B analogue to digital converter with pClamp 10 software (all Molecular Devices, USA). Glass pipettes (4-6 MΩ) were pulled from borosilicate

capillary glass (Sutter Instruments, USA) and filled with fresh internal solution before patching. The GFP-labelled neurons were visualized and selected at 40X magnification under the fluorescent microscope. The external solution in the recording chamber was cycled by the pump at the flow rate of 2-2.5ml/mins. Data was sampled at 20kHz with a 6 kHz Bessel filter. Recording traces were extracted by Clampfit 10.7 software (Molecular Devices) exported to Excel and plotted using Graphpad Prism.

2.4.1 Cell preparation

The 24-well plate with 13 mm glass coverslips were coated with PDL as described above. hHA (P 3 to P6) were passaged onto the glass coverslip. The hHA were kept in conditioned medium (2.1.3) until 90% to 100% confluent. The culture medium was then gradually replaced by SCM medium (25 ml DMEM-F12, 25 ml Neurobasal A, 1 ml B27 with RA, 2 mM L-glutamine, 100 µl Mycozap, 0.1 mM β-mercaptoethanol, 2 µM PD0332991, 3 µM CHIR 99021, 0.3 mM CaCl2, 200 µM ascorbic acid, 10 ng/mL BDNF, 1 µM LM22A4)(Telezhkin, Schnell et al. 2016). The GFP-MGE progenitors (generated from TG-H7 cell line) around differentiation day 20 were dissociated by accutase (Sigma) for 10 min at 37 °C. The cells were then vigorous titrated into single cells and seeded on the top of hHA at the density of 10,000 to 25,000 cells/cm^2. The astrocyte-supported neuronal differentiation was kept in SCM medium for up to 6 weeks. The medium was changed every three days.

In IL-1β acute exposure experiment, (IL-1β 10ng/ml was given to the cells for 24 hours before recording. In IL-1β chronic exposure experiment, IL-1β (10ng/ml) was give twice in 7 days. In IL-1β chronic repetitive exposure experiment, IL-1β (10ng/ml) was given to the cells every day to replace the old medium for 7 days.

2.4.2 Whole cell current clamp

The current clamp was used to record resting membrane potential (RMP) and action potentials (AP). Data were sampled at 20kHz with a 6 kHz Bessel filter. A coverslip of astrocytes supported neuronal culture (described above) was transferred into the recording chamber perfused with external solution. The external solution was composed of 135mM NaCl, 5mM KCl, 1.2mM MgCl2, 1.25mM CaCl2, 10mM D-glucose, 5mM N-2-265 hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (all from Sigma), and pH was titrated to 7.4 by NaOH. The internal solution filled in

the glass pipettes was composed of 117mM KCl, 10mM NaCl, 11mM HEPES, 2mM Na2-ATP, 2mM Na-GTP, 1.2mM Na2-phosphocreatine, 2mM MgCl2, 1mM CaCl2 and 11mM ethylene-glycol-tetra-acetic acid (EGTA) (all from Sigma), and pH was titrated to 7.2 by NaOH. The resistance of a patch pipette filled with intracellular solution was about 3–9 MΩ. The series resistance component was fully compensated using the bridge balance function of amplifier. The RMP of cells was recorded immediately after breaking into the cells in gap free mode. A systematic current injection protocol (duration, 1 s; increment, 20 pA; from -100pA to 200pA) was applied to the neuron held at -70mv to evoke APs. Further analysis for action potentials were carried out by clampfit 10.7.

2.4.3 Whole cell voltage clamp

The whole cell voltage clamp was used for voltage-dependent sodium current recordings. Data were sampled at 20 kHz with a 6 kHz Bessel filter. To isolate pure ionic currents, P/6 leak subtraction was applied in recording protocol. The external solution was composed of 60mM NaCl, 80mM TEA-Cl, 5mM KCl,1.8mM $CaCl_2$, 0.8mM $MgCl_2$, 10mM D-glucose,10mM HEPES, 1mM $NiCl_2$, 1mM $CdCl_2$, 1uM TTA-P2, 50um Nifedipine, and pH was titrated to 7.4 by KOH. The internal solution filled in the glass pipettes was composed of 20mM NaCl ,20mM TEA-Cl,100mM CsCl,1mM $CaCl_2$,2mM Mg-ATP,0.3mM Na_2-GTP,10mM EGTA, 10mM HEPES, and pH was titrated to 7.4 by CsOH. The predicted liquid junction potential is -5 mV. The resistance of a patch pipette filled with intracellular solution was about 3–9 MΩ. The cells were holding at -40mv when break into. The series resistance was compensated by 70%. To record well controlled Na^+ currents in neurons with complex processes, a pre-pulse (duration 10ms, 50mV) was used to eliminate the uncontrolled currents from axon(Milescu, Bean et al. 2010). （Figure 2-3）

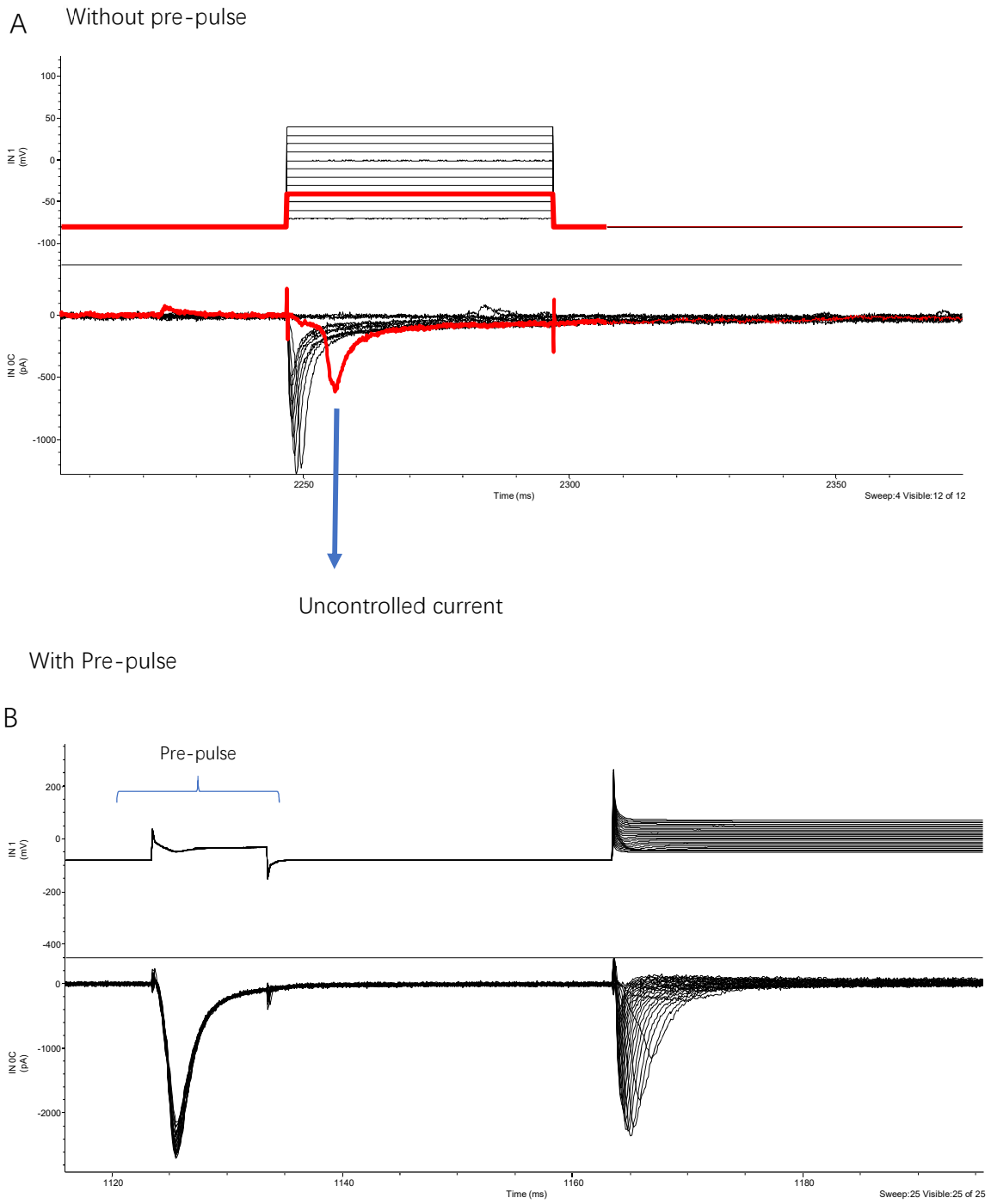

Figure 2-3 Pre-pulse eliminates uncontrolled currents from axons

A) Na^+ channels are displayed in high density at the initial segment of axons. Without applying pre-pulse, the late onset Na current (red) was coming from activation of Na+ channels on axon rather than somatic Na+ channels. B) The N^{a+} channels in axon were activated during the pre-pulse therefore stay inactivated during somatic sodium current recording.

2.4.2.1 Steady state I_{Na} activation

The cells were held at -60mv, hyperpolarized to -80mV and stimulated by a range of systematic depolarizing voltages (duration 50ms, 10mV incriminate, -60mV to 80mV) were given to evoke Na+ current. For voltage-dependent activation curve, conductance (g) through Na+ channels were calculated using the equation:

$$g = I_{Na} / (V - Er)$$

where INa is the peak Na+ current, V is the test membrane potential, and Er is the observed reversal potential when the no net flow of I_{Na}.

For activation curve, the data for I-V relationship was fitted with a Boltzmann equation:

$$g/gmax = 1/ (1 – \exp [(V50 – V)/k])$$

Where gmax is the peak conductance, V is the membrane potential under which the Na+ current develops, and V50 is the half-maximal activation potential, k is the slope factor.

2.4.2.2 Steady state I_{Na} inactivation

The neurons were held for a range of hyperpolarizing voltage (duration 1s, 10mV incriminate, from -130mV to 0mV) and depolarized to 10mV.

For voltage-dependent inactivation curve, the data were fitted with a Boltzmann equation:

$$I/I_{max} = 1/ (1 – \exp [(V_{50} – V)/k])$$

Where I_{max} is the peak current, V is the membrane potential under which the Na^+ current develops, and V_{50} is the half-maximal inactivation potential, k is the slope factor.

2.4.4 Non- stationary fluctuation analysis (NSFA)

NSFA is used to analyze microscopic currents from single ion channel (i) and the number of total channels on cell membrane (N)(Heinemann and Conti 1992, Alvarez, Gonzalez et al. 2002). The data were recorded in whole cell voltage clamp mode. The internal solution and external solution were the same as described in 2.3.3. Date were sampled at 40 kHz with a 10 kHz Bessel filter. To isolate pure ionic currents, P/4 leak subtraction was applied in recording protocol. The series resistance was compensated by 70%. The cells were held at -60mv at the beginning of the recording. The cells were then hyperpolarized to -80mv for 200ms followed by depolarizing stimulation from -80mv to -10mv for 50ms. The recording protocol was repeated for at least 60

times for each cell. The first ten sweeps of each set of data were neglected to minimize the contamination of variance coming from gradual activation of sodium channels. The rising phase of sodium current were used for variance-mean plot. To correct the variance coming from time-dependent channel rundown, variance is calculated from the ensemble average of the squared differences of successive records, i.e.

$$y_i = \frac{1}{2}(x_i - x_{i+1})$$

Where y_i is differences of successive points, x_i and x_{i+1} indicate the point of the same time along successive sweep(Heinemann and Conti 1992).

The variance could be calculated by equation as follow,

$$\sigma^2 = \frac{1}{n-1}\sum_{1}^{n}(y_i - \bar{y})^2$$

Where σ^2 is squared differences from pairs of successive points (y_i), n is the number of sweeps. I represents the averaged current from recording

Values for the total number of channels (N) and the single-channel current (i) were estimated by fitting the functional relationship between the variance - mean current.

$$\sigma^2 = \mathrm{iI} - \frac{1}{N}I^2$$

The channel open possibility $P_{(o)}$ was calculated by

$$P_O = iN/I_{max}$$

2.5 Statistical analysis

All data shown are expressed in terms of Mean ± SEM. Statistical analyses were performed using GraphPad Prism software (version 7.03) or SPSS (Version 22). In instances where single measurements are taken from two groups, a two-tailed t-test was used to compare means. In instances where the data are compared at multiple level, a one-way or two-way analysis of variance (ANOVA) was applied to test for significant differences between groups, with Tukey's post-hoc or Sidak's multiple comparisons test for multiple pairwise comparisons. In instances where the data represent a mixture of between-group and repeated-measures variables, mixed design of repeated measures ANOVA was applied to test significant differences between

matched data in different groups. In instances where difference of the sample frequency was compared between groups, chi-squared test or Fisher's exact test ($n<5$) was used comparing the distribution of samples. The null hypothesis was rejected at 0.05 for t-test, ANOVAs and post hoc tests.

The statistical results for analysis which showed significant difference between groups were listed in appendix

Table 1 List of antibodies used for immunostaining

Nkx 2.1	rabbit	Abcam	1:1000
Mash1	mouse	Abcam	1:1000
Pax 6	mouse	DSHB	1:1000
Nestin	mouse	BD	1:1000
Tuj1	rabbit	Biolegend	1:500
GABA	rabbit	Sigma	1:500
GAD 67	mouse	Millipore	1:500
GFAP	rat	Invitrogen	1:1000
Calbindin	rabbit	Swant	1:500
Calretinin	rabbit	Swant	1:500
STT	rat	Millipore	1:100
GFP	rabbit	Invitrogen	1:500
GFP	mouse	Invitrogen	1:500
Trb2	rabbit	Abcam	1:500
IL-1R1	rabbit	Abcam	1:100
GLAST	rabbit	Abcam	1:500
S100β	mouse	Sigma	1:1000
Nav-pan	rabbit	Alomone lab	1:200
Nav1.1	rabbit	Alomone lab	1:200
Nav1.2	rabbit	Alomone lab	1:200
Nav 1.6	rabbit	Alomone lab	1:200
Navβ2	rabbit	Alomone lab	1:200

3. The Epilepsy microenvironment compromises survival and differentiation of transplanted human ES-derived MGE progenitors in vitro

3.1 Introduction

During last two decades, cell replacement therapy attracted more and more attention for treating neurological and neurodegenerative disorders such as Parkinson disease (PD), Huntington's disease (HD) and epilepsy (Bjorklund and Lindvall 2000, Roper and Steindler 2013). Since adult neurogenesis occurs only in restricted brain areas with limited capacity for brain repair (Danzer 2012), transplantation of exogenous neuron progenitors of human origin could potentially restore brain function caused by neuron loss and dysfunction.

Inhibitory interneurons are critical for the stability of neural network function. Genetic deficits or dysfunction of inhibitory GABAergic neurons are involved in development of many types of seizures, including focal and absence seizures (Powell, Campbell et al. 2003, Yu, Mantegazza et al. 2006, Yalçın 2012, Powell 2013). The imbalance between neural excitation and inhibition due to interneuron loss and dysfunction will accelerate progress of seizures (Liu, Yu et al. 2014). The literature suggests that signaling imbalance can be restored by replacing lost GABAergic interneurons, and transplanted interneurons have been shown to modify inhibitory signaling and disease phenotypes in epilepsy (Hunt, Girskis et al. 2013, Cunningham, Cho et al. 2014, Henderson, Gupta et al. 2014). Therefore, the GABAergic interneuron-based cell transplantation has great therapeutic potential for the treatment of epilepsy (Goldberg and Coulter 2014). The seizure suppressing effect of transplanted interneurons is thought to rely on their ability to restored synaptic inhibition (Henderson, Gupta et al. 2014). The intrinsic potentially excitatory abnormalities such as mossy fiber sprouting didn't alter after receiving transplantation of MGE progenitors in mouse model of TLE (Hunt, Girskis et al. 2013). However, the seizure suppressing effect of transplanted interneurons takes several weeks to be confirmed and their therapeutic

effects over extended periods is still doubtful (Henderson, Gupta et al. 2014). One possibility is that the survival of transplanted cells is reduced longer term (Hunt, Girskis et al. 2013). So, an optimized strategy is needed to improve survival and promote maturation of transplanted interneurons.

Inflammatory cytokines such as TNF-α, IL-1β, IL-16 etc are enriched in sclerotic hippocampus (Jimenez-Pacheco, Diaz-Hernandez et al. 2016). They are involved in neuron death, abnormal neurogenesis and electrophysiological dysfunctions of neurons (Schafers and Sorkin 2008, Vezzani, Balosso et al. 2008, Belarbi and Rosi 2013, Vezzani and Viviani 2015). Many studies suggest that pathological levels of inflammatory cytokines are detrimental for neuronal survival and neurogenesis (Toulmond, Parnet et al. 1996, Aktas, Ullrich et al. 2007, Vezzani, French et al. 2011, Kokaia, Martino et al. 2012). So far, there is a lack of evidence on whether the chronic pathologic microenvironment of epilepsy will affect the survival or differentiation of transplanted GABAergic interneuron progenitors.

To address these issues, in this chapter I generated MGE progenitors from hESC and transplanted the cells into 3D hi-spot cultures generated from brain tissue of patients undergoing epilepsy surgery to observe how "transplanted" cells behave in a relevant pathological microenvironment. The results showed the inflamed microenvironment caused reduced survival, impairment in morphological maturation and functional differentiation of transplanted hESC-derived MGE progenitors.

3.2 Results

3.2.1. Generation and characterization of Nkx 2.1+ progenitor cells from hESC

Nkx2.1 is one of most specific markers of ventral forebrain. Nkx2.1^{+} cells derived from medial ganglionic eminence (MGE) contribute to about 70% of total cortical interneuron population (Butt, Sousa et al. 2008, Miyoshi, Hjerling-Leffler et al. 2010). To generate MGE progenitors from hESC ,dual SMAD inhibitors (SB-451542 and LDN-193189) were applied from day 0 to day 10 to improve neural induction(Chambers, Fasano et al. 2009). Meanwhile, Wnt inhibitor XVA939 were supplemented to help the cells achieve anterior telencephalic identity (Nicoleau, Varela et al. 2013). Subsequent addition of sonic hedgehog (Shh) and Purmorphumine (Pur) from day 10 to day 18 promoted ventralization of early neuroectoderm and directed the cells towards MGE

lineage (Maroof, Keros et al. 2013) (Figure 3-1A). The average expression of Nkx2.1 on day 25 post-differentiation was estimated over 90% (Figure 3-1B). Other ventral telencephalic progenitor markers Olig2 and Ascl1 (Mash1) also expressed at the same period (Figure 3-1B). In contrast, Pax6, a marker for dorsal telencephalic neural progenitors, was not detected (Figure 3-1B). Therefore, the hESC-derived NKX2.1+ cells represented a ventral telencephalic MGE-like neuronal lineage.

The maturation of hESC-derived neuron progenitors occurs over a long-term in vivo. The cells were treated with retinoid acid (RA) from day 20 and BDNF for further maturation after day 25. The expression of GABAergic neuron markers was inspected by Immunostaining on differentiation day 60. GAD 67 synthesizes GABA which is the major inhibitory neuron transmitter produced by cortical interneurons (Chattopadhyaya, Di Cristo et al. 2007). About 60% cells expressing GAD 67 on differentiation day 60 suggesting that most cells were becoming GABAergic neurons (Figure 3-1C). The interneuron subtype marker calbindin, calretinin and somatostatin (Monyer and Markram 2004) were also detected as the same time. About 60% cells express calretinin ,50% express calbindin, only 5% express STT and no PV positive cells were detected (Figure 3-1C).

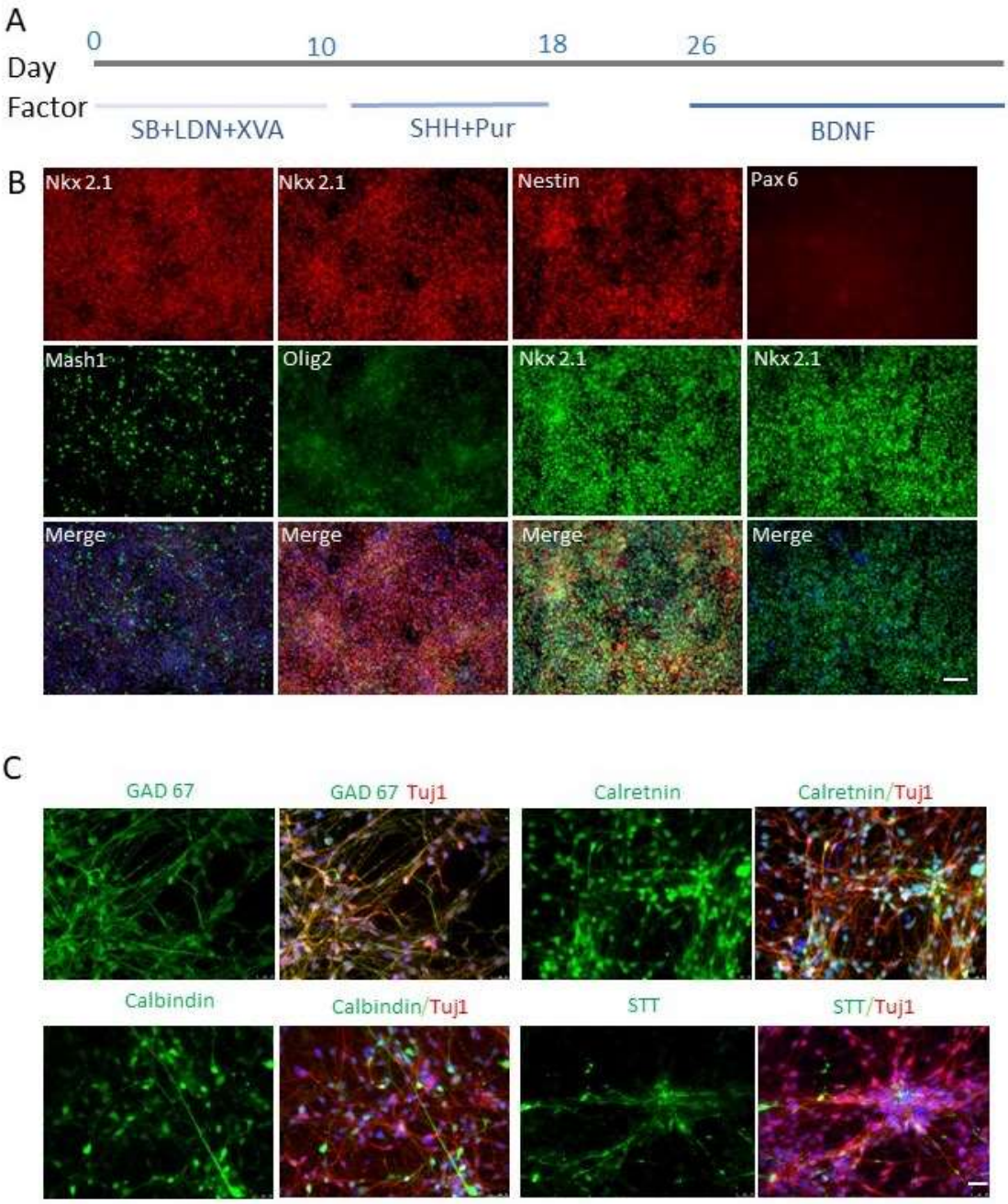

Figure 3-1 Generating MGE cells from hESC

A) Schematic timeline of MGE progenitor differentiation from H7 cell line. SB451542, inhibitor of the TGFb1 activin receptor-like kinases; LDN193189, inhibitor of bone morphogenetic protein (BMP) type I receptors; XVA939, wnt signaling inhibitor; Shh, Sonic hedgehog; Pur, Purmorphamine; BDNF, brain-derived neurotrophic factor. B) On differentiation day 20 the cells robustly expressed NKX2.1, nestin, Olig 2 and nestin and were negative for PAX6. Scale bar 100um.C) On differentiation day 60, the cells expressed post mitotic marker tuj1+; GABAergic marker GAD 67 and interneuron subtype marker Calbindin, Calretinin, Somatostatin. (Scale bar 50um)

3.2.2. Characterizing MGE progenitors for transplantation

H7 hESCs were labelled with GFP by transfecting a plasmid containing expression of tau-GFP fusion protein (TG4). TG4-H7 cell line was used for generating MGE progenitor using the protocol described above. Time dependent maturation of MGE progenitors was examined by comparing expression of the post-mitotic neuron marker Tuj1 between post differentiation day 20 and day 45. The result revealed on differentiation day 20, the cells grew in rosette and only 7% ±1.4% cells expressed Tuj1. By differentiation day 45, the cells migrated away from the rosette and the ratio of tuj1+ cells significantly increased to 37.6%±3.5% (n=4 from the same differentiation), two-tail unpaired t-test, t=8.041, df=6, P=0.0002) (Figure 3-2A, C.) Subsequently result showed 41.6% cells express GABA and 32.3% express GAD 67 on post-differentiation day 45(Figure 3-2 B, D).

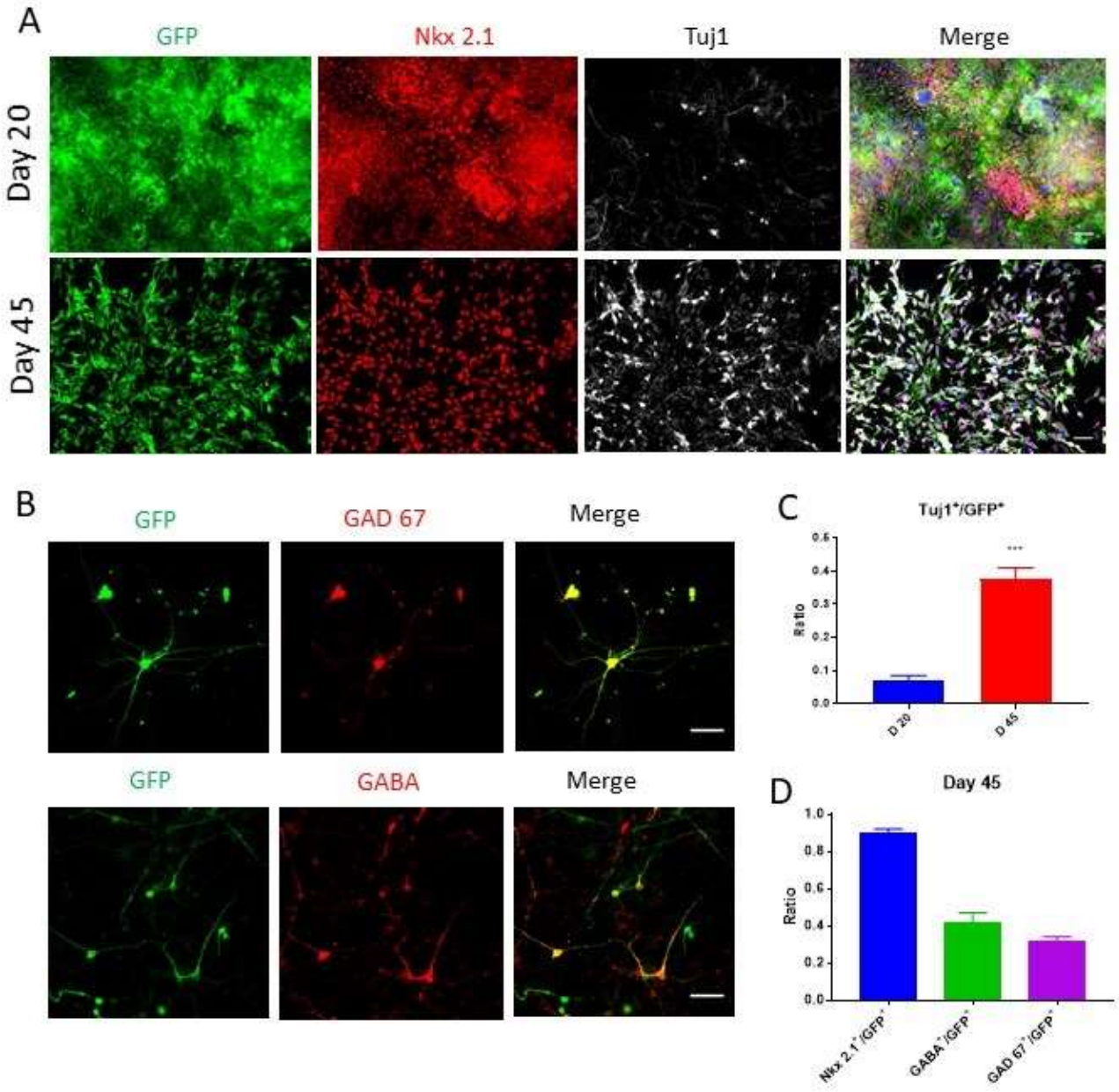

Figure 3-2 Characterizing MGE progenitors during differentiation

*A) TG4-H7 cell line was used for MGE differentiation with the protocol described above. The negative control which applied only secondary antibodies showed no staining. The immunostaining showed expression of Nkx2.1 and Tuj1 on differentiation day 20 and day 45. Scale bar 50um B) The ratio of cells expressed Tuj1 was significantly increased on differentiation day 45 compared to differentiation day 20. (Data show mean± SEM, n=4, t-test, ***P<0.001) (C-D) The ratio of Nkx 2.1, GAD 67 and GABA positive cells respectively on differentiation day 45 (Data show mean± SEM, n=4)*

3.2.3. hESC-derived MGE progenitors survive and differentiate in hi-spot culture

Hi-Spot culture is a new cell culture method developed for culturing postnatal brain tissue (Bailey, O'Connor et al. 2011). With this method, the brain tissue is dissociated and reorganized as a self-organised, dense, organotypic-like culture in air-liquid surface. The hi-spot culture allows cells to grow in 3D which simulate the construction and the microenvironment of postnatal brain rather than conventional 2D monolayer culture. Previous data from our lab show the inflamed microenvironment and major cellular components in human brain tissue are preserved in hi-spot culture (Figure 3-3, Zaben & Gray). Nearly all relevant cells in brain tissue can be detected in hi-spots derived from human hippocampus, including immature hippocampal neurons, mature neuron, microglia, astrocytes, neuron stem cells and endothelial cells (Figure 3-3 A-D). In addition, the level of IL-1β in the medium of sclerotic hippocampus is self-maintained in higher level compared to non-sclerotic cortical hi-spots (Figure 3-3E). It suggests the pathological inflamed microenvironment of sclerotic hippocampus can be maintained in hi-spot culture.

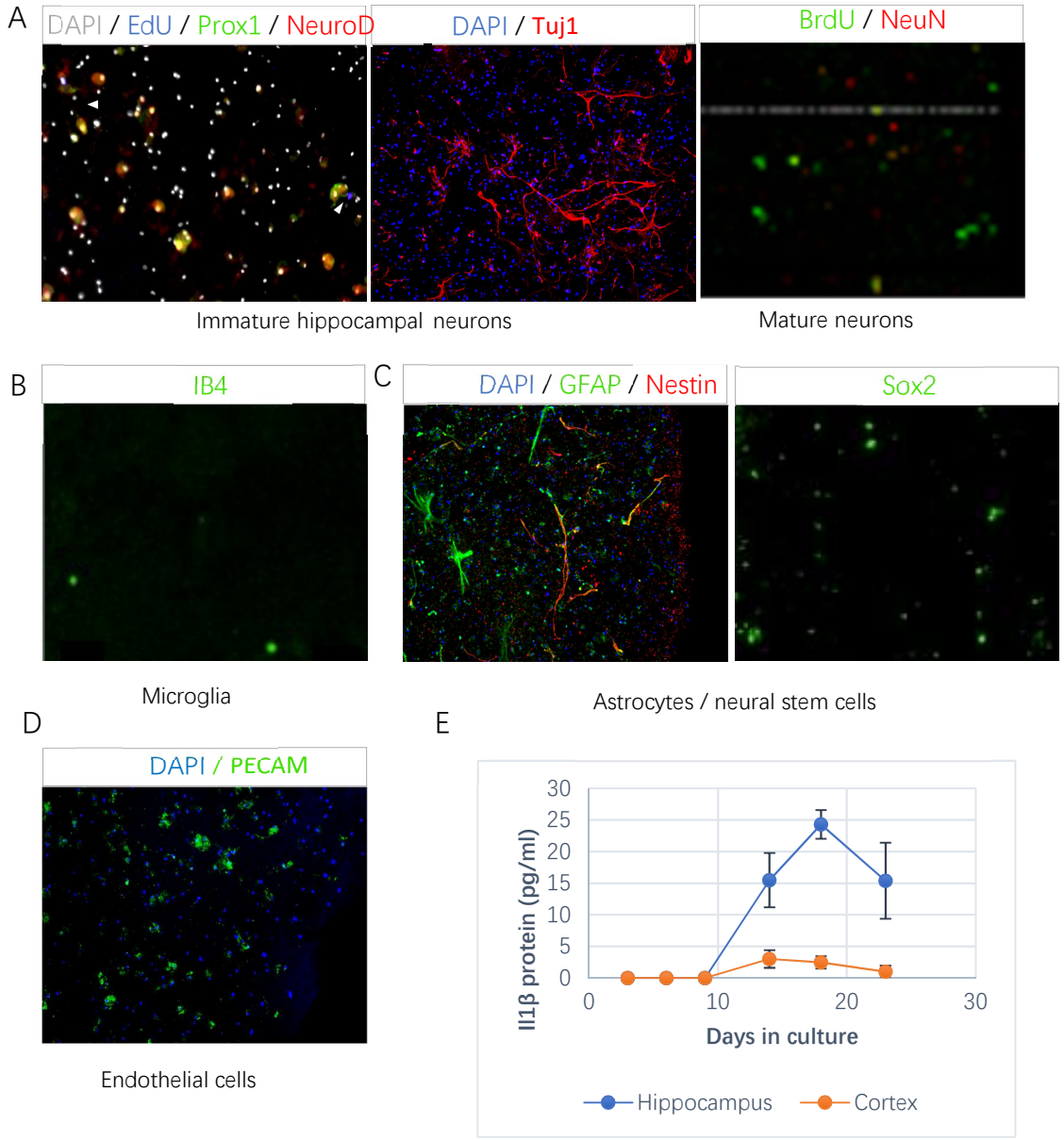

Figure 3-3 Human hi-spots contain all relevant brain cells and mimic pathological microenvironment. (Zaben & Gray)

A-D) The immunostaining showed relevant cells survived in hi-spots generated from sclerotic hippocampal tissue resected from patient undergoing epilepsy surgery. E) The line graph showed the level of IL-1β in the medium of sclerotic hippocampal hi-spot significantly increased from day 10 compared to non-sclerotic cortical Hi-spots

To set up and test the culture model for observing the effect of microenvironment on transplanted MGE progenitors in vitro, 10% GFP expressing MGE progenitors were mixed with 90% dissociated human brain tissue removed from patients undergoing epilepsy surgery to generate “transplanted ”hi-spot cultures (Figure 3-4 A-B).

First, the survival and morphological change of MGE progenitors in hi-spot culture were inspected by observing live imaging of surviving GFP expressing MGE progenitors at different time points. The live imaging showed the MGE progenitors displayed round cell body and evenly distributed inside the hi-spots on day 1. The majority of MGE progenitors were lost by day 7. The remaining MGE progenitors started to grow primary processors and form small colonies on day 14. Cells displayed secondary processors and migrated away from the cluster on day 28(Figure3-4 C). The gradual changes in number and morphology of MGE progenitors suggest part of the initial cell population survive and differentiate inside the hi-spot cultures.

Next, the hi-spots were fixed at post culture day 14 and 28 to investigate the phenotype of surviving MGE progenitors. The immunostaining showed Nkx 2.1 was co-stained with GFP. Similar to the phenotypes of MGE progenitor before transplantation (Figure 3-2A), the populations of $Nestin^{+}$ and $Tuj1^{+}$ cells could be both observed among $Nkx2.1^{+}$ GFP^{+} cells on day 14. On day 28, the population of $GFP^{+}Tuj1^{+}$cells co-expressed $GABA^{+}$ or GAD 67^{+} could be observed in hi-spots, suggesting the transplanted MGE progenitors differentiated into functional GABAergic neurons (Figure 3-4D). Together, these results demonstrated that although there was distinct maturation kinetics among MGE progenitors due to the heterogeneity of the cells during differentiation, part of MGE progenitor population can survive and differentiate into morphologically identifiable GABAergic neurons in hi-spot culture.

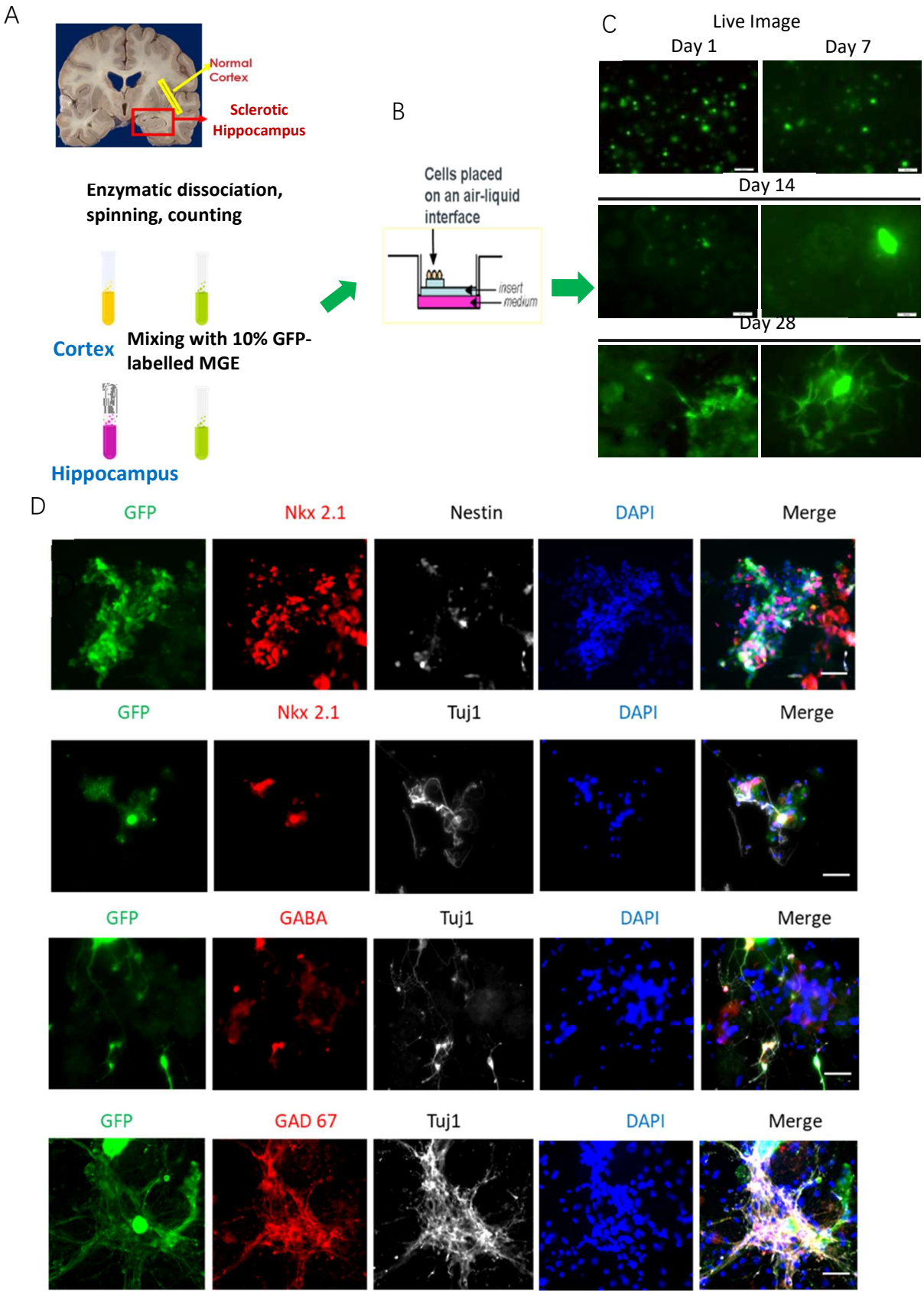

Figure 3-4 Transplanted MGE progenitors into 3D hi-spot culture derived from human brain tissue show evidence of survival and GABAergic differentiation

A) The diagram of 3D hi-spot culture. The human brain tissue resected from patients during epilepsy surgery were dissociated with papain and mixed with 10% TG4-H7 derived MGE progenitors to generate hi-spots B) The cells were centrifuged into high density and placed as 3D hi-spot culture in air-liquid surface. C) The live image of MGE progenitors in hi-spot culture at different time points showed time-dependent morphological maturation. Scale bar 50um D) Neuronal subsets co-expressed Nkx 2.1 and Nestin or Nkx2.1 and Tuj1 in GFP$^+$ hESC-derived MGE progenitors on day 14, and Neuronal subsets co-expressed GABA and Tuj1 or GAD 67$^+$ and Tuj1c in GFP$^+$ hESC-derived MGE progenitors on day 28.

3.2.4. The epileptic microenvironment caused reduction in survival of MGE progenitors

The epileptic microenvironment affects survival, proliferation and differentiation of new-born neurons in epilepsy (Barkas, Redhead et al. 2012, Danzer 2012, Cho, Lybrand et al. 2015). There is lack of evidence to elucidate whether transplanted neuron progenitors will be affected by the epileptic microenvironment. Previous research in our lab demonstrates pathological level of inflammatory cytokine IL-1β is maintained in the medium of sclerotic hippocampal hi-spots not in cortical hi-spots (Figure 3-3E). Therefore, in this research, hi-spot culture generated from sclerotic hippocampal tissue was used to simulate the epileptic microenvironment of chronic epilepsy. Meanwhile the hi-spots generated from cortical tissue removed from the same patient were used to simulate non-epileptic microenvironment.

To decide on the most suitable MGE progenitors for hi-spot culture, the MGE progenitors of different differentiation days (differentiation day 20 and differentiation day 45) were transplanted in hi-spot culture(Figure3-5A).

First, the number of surviving young MGE progenitors (Diff 20) and old MGE progenitors (diff 45) in hippocampal and cortical hi-spot were compared at different time points by live imaging (n=12 hi-spots coming from two patients in each condition). On Day 1 the number of surviving diff 20 and diff 45 MGE progenitors were similar in both cortical and hippocampal hi-spots (two-way ANOVA, P>0.05) (Figure 3-5B), suggesting initial number of transplanted MGE progenitors was similar in hi-spots. However, on day 7, the number of surviving diff 20 MGE progenitors was significantly reduced in both cortical and hippocampal hi-spots compared to the number of surviving diff 45 MGE progenitors in cortical and hippocampal hi-spots respectively (two-way ANOVA, $F(1, 70) = 11.78$ $P=0.001$) (Figure 3-5C). On day D14, diff 20 MGE progenitors were invisible in live imaging, only 3 cells were identified later in cortical hi-spot by immunostaining (not shown), while all were lost in hippocampal hi-spots. Mmeanwhile, diff 45 MGE progenitors

were still detectable with live imaging in both cortical and hippocampal hi-spots. These results suggest diff 20 MGE progenitors cannot survive as long in hi-spots compared to diff 45 MGE cells, irrespective of the microenvironment.

Therefore, the hi-spot transplanted with Diff 45 MGE progenitors were used to investigate the effect of the inflamed microenvironment on cell survival at indicated time points (Figure 3-5D) The bar graph revealed that the number of surviving diff 45 MGE cells showed similar pattern of significant time dependent reduction in cortical and hippocampal hi-spots (n=12 hi-spots coming from three patients in each condition), two-way ANOVA. $F(1,66) = 42.35$, $P<0.0001$). Overall, the number of surviving MGE cells was significantly higher in cortical hi-spot compared to the number in hippocampal hi-spot. (two-way ANOVA. $F(2,66) = 832.5$, $P<0.0001$). Sidak's multiple post-hoc comparison revealed the number of surviving MGE progenitors was significantly lower in hippocampal hi-spot compared to cortical hi-spots on post culture day 7 and day 14. (276.98 ± 9.68 vs 397.6 ± 12.41, $P<0.0001$; 181.72 ± 11.76 vs 86.59 ± 7.53, $P<0.0001$). (Figure 3-5B). The results suggest that the epileptic microenvironment of sclerotic hippocampus is detrimental to survival of MGE progenitors.

Last, the phenotypes of surviving MGE progenitors were compared between cortical and hippocampal hi-spots (Figure3-5G). Nkx 2.1 is significantly downregulated at post-natal stage compared to pre-natal in populations of interneurons (Butt, Sousa et al. 2008). Previous data (Figure 3-4D) suggests Nkx 2.1 is rigidly expressed in transplanted MGE progenitor. In this experiment, the result revealed the proportion of $Nestin^+$ $Nkx2.1^+$ cells was significantly decreased in hippocampal hi-spots compare to cortical hi-spots (Figure 3-5E) (n=6 hi-spots coming from one patient in each condition, $17\%\pm3\%$ vs $36\%\pm3\%$, unpaired t-test, $P<0.01$). In contrast, the proportion of $Tuj1^+$ $Nkx2.1^+$ cells among population of $Nkx2.1^+$ cells was similar between cortical and hippocampal hi-spots ($39\%\pm5\%$ vs $29\%\pm10\%$ two-way t-test, $P>0.05$) (Figure 3-5F). These results indicate $Nestin^+$ young MGE progenitors are more vulnerable than $Tuj1^+$ immature MGE-like neurons in epileptic microenvironment. It implies that the epileptic microenvironment is having different effects on MGE progenitors with different phenotypes.

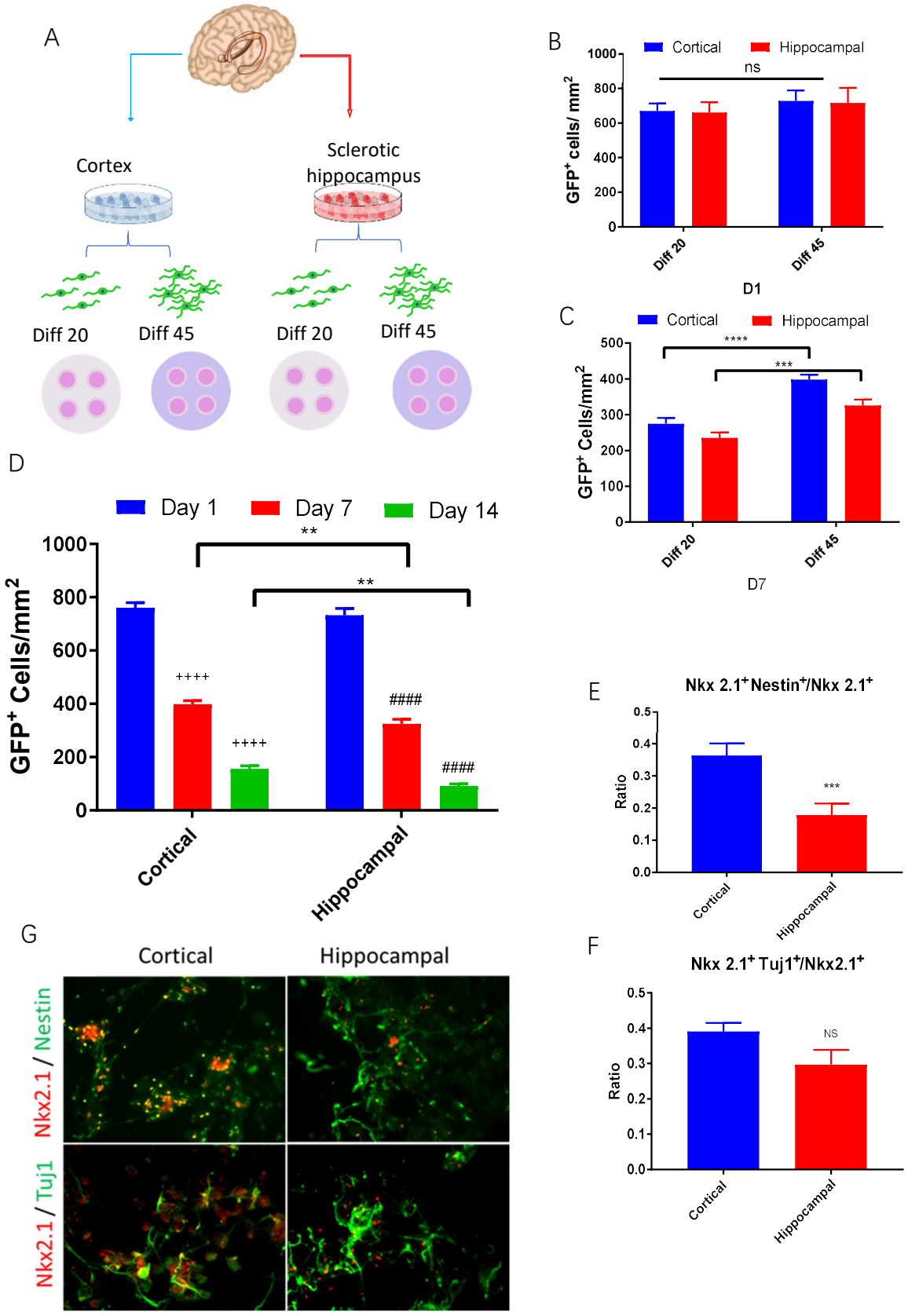

Figure 3-5 The survival of MGE progenitors in Hi-spot culture.

*A) The illustration of experiment groups. The sclerotic hippocampal tissue simulated epileptic microenvironment while the cortical tissue simulated non-epileptic microenvironment. The survival of MGE progenitors of differentiation day 20 or day 45 were quantified in cortical and hippocampal hi-spots. B) The bar graph showed the initial number of differ 20 and differ 45 MGE progenitors in hi-spot cultures on day 1. C) The bar graph showed the surviving number of differ 45 MGE progenitors was significantly higher in both cortical and hippocampal hi-spots compared to the number of diff 20 MGE progenitors on day 7(Data show mean± SEM, n=12,two-way ANOVA with, Sidak's multiple comparisons test * denote significant differences between the conditions ***P<0.001; ****P<0.0001).D) Significant time dependent reduction of GFP-MGE progenitors in both cortical and hippocampal hi-spots. The surviving number of GFP-MGE progenitors was lower on D7 and D14 in hippocampal hi-spots compared with the number in cortical hi-spots. (Data show mean± SEM, n=8, two-way ANOVA with Tukey's post-hoc multiple comparisons test, * and # denote significant differences between the conditions and the time points, respectively. ##, **P<0.01; ####,****P<0.0001) E-F) The bar graph showed the ratio of Nestin$^+$ Nkx2.1$^+$and Tuj1$^+$ Nkx2.1$^+$ among population of Nkx2.1$^+$ cells in cortical and hippocampal hi-spots (Data show mean± SEM, n=4, t-test, ns P>0.05, ***P<0.001) G) The immunoassaying compared population of Nkx2.1$^+$Nestin$^+$ and Nkx2.1$^+$ Tuj1$^+$ in hippocampal and cortical hi-spot cultures fixed on day 14. (Scale bar 50um)*

3.2.5. The epileptic environment impaired maturation of MGE progenitors

Next, the effect of epileptic microenvironment on morphological growth of MGE progenitors was investigated by comparing the cells in hippocampal hi-spots to cortical hi-spots. GFP density represents the number and morphological growth of the transplanted MGE progenitors. In cortical hi-spots, the overall MGE-like cells were more complex in morphology and covered larger area than the cells in hippocampal hi-spot. (n=10 cells coming from two patients in each condition) (Figure3-6 A, C). To further compare the morphological complexity and branch growth of MGE-like cells in cortical and hippocampal hi-spots, Sholl analysis was performed to single MGE progenitor in hippocampal and cortical hi-spots (Figure3-6 D). The repeated measures revealed that there was no significant variance among the MGE cells in hi-spots coming from two different patients. The area under curve which represents neurite complexity revealed impaired neurite complexity of MGE progenitors in hippocampal hi-spots compared to cortical hi-spots (Repeated measures ANOVA, F (1,8) =8.15, P=0.021) (Figure3-6 E). The branch of the cells in cortical hi-spots covered much longer distance (200um to 300 um) compared to the cells in hippocampal hi-spots. Correspondingly, total branch length (400±36 um vs 216±25 um, n=10, Repeated measures ANOVA, F (1,8) =28.493, P=0.001) and maximum branch length (210±22 um vs 120±10 um, n=10, Repeated measures ANOVA, F (1,8) =15.174, P=0.005) of MGE progenitor were significantly shorter in hippocampal hi-spots compare to cortical hi-spots (Figure3-6G-H).

On the other hand, cells in hippocampal hi-spots had similar number of branches compared to cells in cortical hi-spot. (two-way unpaired t-test, t=1.464, df=18, P=0.16) (Figure3-6F). These results suggest inflamed microenvironment interfered with branch elongation of MGE-like cells.

Last, the differentiation of MGE progenitors in hippocampal and cortical hi-spot was compared by the ratio of Tuj1 and GAD 67 expressing MGE cells among the population of surviving MGE progenitors. The result showed that the proportion of $Tuj1^+$ MGE cells was significantly increased on day 28 compared to day 14 in both types of hi-spots (n=12 hi-spots coming from three patients in each condition, two-way ANOVA, F (1,44) =17.74, P=0.0001). And the Sidak's post-hoc multiple comparison showed the ratio of $Tuj1^+$ MGE-like cells in hippocampal hi-spots was significantly lower compared to the ratio in cortical hi-spots on day 28 (0.76±0.03 vs 0.57±0.05 P=0.0014) but not on day 14 (0.32±0.06 vs 0.45±0.02 P=0.051) (Figure3-6I) Similarly, the population of $GAD67^+$ MGE cells among the population of surviving MGE cells in hippocampal hi-spot was also significantly lower than that in cortical hi-spot on day 28 (Figure 3-6J). Together these results suggest that the epileptic microenvironment impaired neuronal differentiation of MGE progenitors in over 28 days in culture.

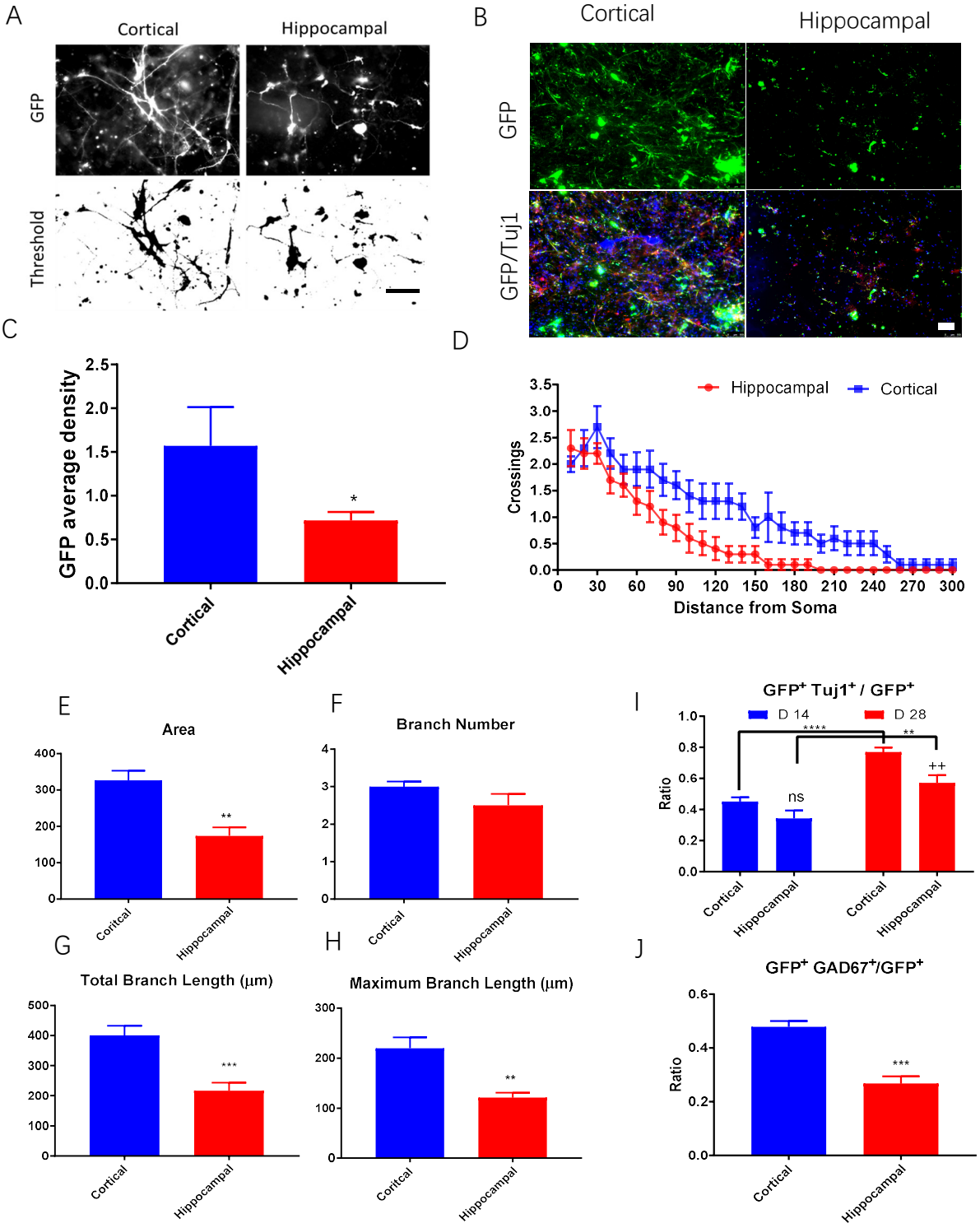
A
Cortical
Hippocampal
GFP
Threshold
B
Cortical
Hippocampal
GFP
GFP/Tuj1
C
GFP average density
2.5
2.0
1.5
1.0
0.5
0.0
*
Cortical
Hippocampal
D
Hippocampal
Cortical
Crossings
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
30
60
90
120
150
180
210
240
270
300
Distance from Soma
E
Area
400
300
200
100
0
**
Coritcal
Hippocampal
F
Branch Number
4
3
2
1
0
Cortical
Hippocampal
I
GFP+ Tuj1+ / GFP+
D 14
D 28
****
**
++
ns
Ratio
1.0
0.8
0.6
0.4
0.2
0.0
Cortical
Hippocampal
Cortical
Hippocampal
G
Total Branch Length (μm)
500
400
300
200
100
0
***
Cortical
Hippocampal
H
Maximum Branch Length (μm)
300
200
100
0
**
Coritcal
Hippocampal
J
GFP+ GAD67+/GFP+
Ratio
0.6
0.4
0.2
0.0
***
Cortical
Hippocampal

Figure 3-6 The morphology and differentiation of MGE progenitors in hi-spot culture.

*A) The cortical and hippocampal hi-spots was fixed on day 14. The images of MGE progenitors processed by image J was shown in "Threshold". Scale bar 50um B) The immunostaining showed the population of MGE cells co-expressed Tuj1 in cortical and hippocampal hi-spots on day 14. Scale bar 100um C) The bar graph showed average GFP density in cortical and hippocampal hi-spots. (Data show mean± SEM, n=4, t-test, *P<0.05) D) Cells were selected for Sholl analysis in image J. The number of crossings was counted at different distances. Data show mean± SEM, n=10 for each condition. E-H) The area under curve, the number of branches, the total length of branch and the maximum length of branch were compared between cells in cortical and hippocampal hi-spots. (Data show mean± SEM, n=10 for each condition, t-test, ***P<0.001). I) The bar graph showed the ratio of Tuj1+ MGE cells within total MEG cells at indicated time points and conditions. Data show mean± SEM, n=8, two-way ANOVA with Tukey's post-hoc multiple comparisons * and # denote significant differences between the time points and the conditions, respectively. ##,**P<0.01;****P<0.0001) J) The bar graph showed the ratio of GAD 67+ MGE cells within total MGE cells on Day 28 (Data show mean± SEM, n=8, t-test, ***P<0.0001).*

3.3 Discussion

In this chapter, MGE progenitors, which can particularly give rise to inhibitory interneurons, were generated from hESCs according to the protocol published previously (Maroof, Keros et al. 2013). Next, a novel 3D hi-spot culture model which is composed of human brain tissue coming from patients undergoing epilepsy surgery to recreate the pathological microenvironment of chronic epilepsy has been established. With this model, it is possible to investigate the effects of epileptic microenvironment on human transplanted MGE-progenitors. The following results revealed that the epileptic microenvironment of TLE is detrimental for survival, neurite growth and differentiation of transplanted hESC-derived MGE progenitors in vitro.

Transplanting MGE progenitors has been investigated as a cell replacement therapy for TLE in the last two decades. By simulating the microenvironment of telencephalon development in the embryonic stage, MGE progenitors can differentiate from hESCs (Liu, Liu et al. 2013). The former study suggests Wnt signaling and the SHH signaling pathway are the critical factors which direct the cells to achieve ventral–anterior telencephalic identity during brain development (Li, Zhang et al. 2009, Maroof, Keros et al. 2013). Early inhibition of the Wnt signaling pathway promotes hESC- derived neural progenitors to achieve anterior forebrain identity. SHH is one of the best-known ventral pattern factors working throughout the neuron tube. In the absence of SHH signaling, the hESC-derived neural progenitors acquire a default dorsal identity (Nicoleau, Varela et al. 2013). The timing and degree of SHH activation modulates the ventralization of telencephalic cells (Maroof, Keros et al. 2013) (Liu, Liu et al. 2013, Nicoleau, Varela et al. 2013). The data showed homogeneous and effective production of MGE-like Nkx2.1+ neuron progenitors with time-controlled activation of SHH signaling. However, PV-expressing cells were absent at the later stage of differentiation, possibly because the cells in differentiation exhibit the identity of dorsal MGE not ventral MGE, which is where most PV cells arise from (Reinchisi, Ijichi et al. 2012, Tyson, Goldberg et al. 2015). The other reason could be that PV is expressed at a later stage which requires extended time for differentiation in vitro.

To observe the survival and differentiation of hESC-derived MGE-like progenitors in the epileptic microenvironment after neuron transplantation, 3D hi-spot cultures were used to simulate the pathological microenvironment in chronic TLE. Only a small proportion of MGE-like progenitors were found to survive in human hi-spots. About 60% to 80% of MGE progenitors died in hi-spots

culture during the first week in both cortical and hippocampal hi-spots. The exact reason for early death of MGE progenitors in still unknown. One possibility is the hi-spots generated from adult brain tissue are lacking neurogenic factors which are essential to support survival of neuronal stem cells (Bjornsson, Apostolopoulou et al. 2016). So far only two regions of the hippocampus, SVZ and DG, have been proven to support neurogenesis in an adult rodent model(Jin, Minami et al. 2001). So, transplantation of MGE progenitors (around differentiation day 20) usually targets the DG in adult rodent models (Henderson, Gupta et al. 2014, Kepecs and Fishell 2014, Kim, Yao et al. 2014, Wang, Hong et al. 2016). The cortical tissue and the hippocampal tissue removed from patients may lack the neurogenic niche to support the survival of MGE progenitors. Secondly, the young progenitors require survival signals from each other by direct contact (Southwell, Paredes et al. 2012). The immature nestin+ cells which survive in hi-spot usually form small clusters. The mature tuj1+ cells were more independent and tended to migrate away from the cluster. Similar phenomena were seen in animal MGE transplantation experiments, where more than half of the transplanted MGE cells which stay in the injection site were immature cells, but only a small proportion of MGE cells became mature neurons and migrated (Hunt, Girskis et al. 2013, Cunningham, Cho et al. 2014). This suggests mechanical isolation of MGE progenitors when generating hi-spots might cause cell death due to lack of survival signals from each other in hi-spots where they are distributed uniformly at least initially. Thirdly, the brain microenvironment strictly selects the new born neurons and only a small proportion of new born neurons are finally qualified to develop into functional neurons (Southwell, Paredes et al. 2012). Therefore, transplanted MGE progenitors could be eliminated by intrinsic factors during early stages of development.

Unlike old MGE progenitors (diff 45), young MGE progenitors (Diff 20) are less likely to survive in hi-spot culture. Possibly because survival of young neuron progenitors relies on neurotrophic factors, such as BDNF, NGF. Evidence suggests neurotrophic factors are critical to promote proliferation and survival of neuron stem cells (Chao 2003, Jiao, Palmgren et al. 2014). It is necessary to further characterize the hi-spot culture to figure out the levels of major detrimental cytokines and beneficial growth factors in hi-spots. It will be also interesting to investigate whether survival of MGE progenitors can be improved by treatment with neurotrophic factors.

A GWAS and exome sequencing based on human sclerotic hippocampus reported upregulation of TLR-signaling and various cytokine genes which cause neuron death and abnormal

neurogenesis (Johnson, Behmoaras et al. 2015). The levels of IL-1β, TNF-α and IL-6 increased within 24 h after epilepsy in patients and experimental models (Uludag, Duksal et al. 2015, de Vries, van den Munckhof et al. 2016, Alapirtti, Lehtimaki et al. 2018). The cytokines secreted after acute injury could lead to excitotoxicity, oxidative stress, and neuron apoptosis (Lorigados Pedre, Morales Chacon et al. 2018). Several studies suggested attenuated acute inflammatory response prevented neuron injury and reduced seizures in experimental models of epilepsy (Vezzani and Granata 2005). In chronic epilepsy, neuroinflammation could last for years. Cytokines produced by immune cells and the local brain cells regulate neurogenesis (Kokaia, Martino et al. 2012). The chronic inflamed microenvironment aggravates abnormal neurogenesis in the hippocampus(Belarbi and Rosi 2013). Previous experiments in our lab found that the pro-inflammatory cytokines were enriched and maintained at a high concentration for weeks in hi-spots generated from human sclerotic hippocampus (Zaben &Gray). So, the hi-spot culture makes it possible to observe the survival and differentiation of hESC-derived MGE cells in a human brain-based organ-like structure with a preserved pathological microenvironment.

Consistent with previous research which found that pathological levels of pre-inflammatory cytokines produced in neurodegenerative diseases and brain injury induce neuron death (McCoy and Tansey 2008, Glass, Saijo et al. 2010), the results demonstrated that the inflamed microenvironment of the hippocampus led to reduced surviving number of transplanted exogenous MGE progenitors. The possibility that an inflamed microenvironment prevents proliferation of MGE progenitors should be taken in to consideration as well. Evidence suggested systematic inflammation acutely prevented proliferation of neuron precursors in DG (Fujioka and Akema 2010). But in the experiment, the number of surviving MGE progenitors in hi-spot culture didn't significantly increase on post culture day 28 compared to post culture day 14 in both cortical and hippocampal hi-spots. This suggests MGE progenitors may not proliferate in hi-spot culture. Therefore, the reduced number of surviving MGE progenitors is mainly caused by cell death rather than reduced cell proliferation.

There are two possible reasons for the reduced number of mature neurons ($Tuj1^+$ and $GAD\ 67^+$ cells) in sclerotic hippocampal hi-spots, one is altered neuronal differentiation; the other is delayed neuronal differentiation in pathological microenvironment. Some research support the idea that pro-inflammatory cytokines, such as IL-1β and TNF-α, promote astroglial differentiation instead of neuronal differentiation(Peng, Whitney et al. 2008, Green, Treacy et al.

2012), while there is also evidence to support the later assumption. BDNF is an environmental neurotrophic factor that promotes neuronal differentiation and improves neuron survival(Jiao, Palmgren et al. 2014). IL-1β has been reported to suppress BDNF signal via activating P38-MAPK pathway(Tong, Balazs et al. 2008, Tong, Prieto et al. 2012). It is possible that IL-1β prolongs neuronal differentiation of MGE progenitors in inflamed microenvironment by interfering with the BDNF neurotrophic signal. This hypothesis needs to be investigated in the future.

In general, the results suggest that the epileptic microenvironment can cause reduced efficiency of cell replacement treatment. There is still much work needed to recognize the main inflammatory cytokines which are detrimental for survival and differentiation of transplanted neuron progenitors. Hi-spots culture is a method to predict how inflammation affects the transplanted neuron progenitors, therefore providing an optimized strategy for future clinical neuron transplantation therapy.

4. IL-1RA restored differentiation of transplanted MGE progenitors in inflamed microenvironment.

4.1 Introduction

Cytokines, in physiological levels, play an important role in regulating cell proliferation, survival, and neuronal differentiation during CNS development (Mehler and Kessler 1997). The inflammatory milieu is entirely different after seizures and in chronic epilepsy where significant upregulation and secretion of inflammatory cytokines by peripheral immune cells, local microglia, astrocytes and neurons are observed (Vezzani, Balosso et al. 2008, Uludag, Duksal et al. 2015, Vezzani and Viviani 2015). To a physiological extent, cytokines are necessary for activating the growth and differentiation of neuronal stem cells which might replace the lost neurons and facilitate neuronal circuit repair (Kokaia, Martino et al. 2012, Belarbi and Rosi 2013). On the contrary, evidence showed prolonged and intensive stimulation of inflammatory cytokines lead to neuron death and abnormal neurogenesis in epilepsy and brain injury (Belarbi and Rosi 2013, Webster, Sun et al. 2017). The detrimental effects of inflammatory cytokines further increase seizure susceptibility in epilepsy (Ravizza, Gagliardi et al. 2008, Maroso, Balosso et al. 2011). However, it is not always clear what the significant and key mediators of neuroinflammation are in epilepsy or indeed the many brain diseases for which cell therapy might be applicable.

Interleukin-1 (IL-1) is one of most powerful pro-inflammatory cytokines which contributes to a variety of neurological and neurodegenerative diseases in the CNS (Toulmond, Parnet et al. 1996, Allan, Tyrrell et al. 2005). IL-1R has two different ligands, IL-1α and IL-1β. They have similar biological effects caused by the activation of the IL-1 receptor mediated signaling pathway. IL-1α is only released after cell death, while IL-1β is secreted into the blood stream by living cells (Dinarello 2009). Researches reveal that IL-1β produced by activated microglia affects the generation, differentiation and survival of neural stem cells (Green, Treacy et al. 2012, Belarbi and Rosi 2013). IL-1β is also one of the major pro-convulsant inflammatory factors released in both seizure generation and epileptogenesis. Its pro-convulsant effect is involved in regulating neuronal network excitability, turnover and release of various neurotransmitters, expression of

neuropeptides and neurotrophic factors, and altering synaptic transmission and ionic currents (Vezzani, Moneta et al. 2000, Friedman 2001, Allan, Tyrrell et al. 2005, Johnson, Behmoaras et al. 2015)..

Immune cells such as monocytes and macrophages contribute to the early production of IL-1β. All endogenous brain cells including microglia, astrocytes and neurons could secrete IL-1β at a later stage after brain insults (Allan, Tyrrell et al. 2005). Therefore, elevated levels of IL-1β can accumulate in chronic epilepsy. In the previous chapter, it was found that the epileptic microenvironment reduced cell survival and compromised branch growth and differentiation of hESC-derived MGE progenitors. It is interesting to know whether the detrimental effect of the epileptic microenvironment could be reversed by modifying the environment. In established hi-spot cultures generated from sclerotic hippocampus, IL-1β is continuously secreted and maintained in high concentration (Zaben&Gray). So, it is worth to figure out, whether the increased level of IL-1β affects survival, branch growth and differentiation of MGE progenitors in the pathological microenvironment of epilepsy.

In this chapter, IL-1RA, a competitive antagonist of IL-1R1 was applied to epileptic hippocampal hi-spots to investigate whether there was any potential benefit in improving the efficiency of MGE transplantation therapy for epilepsy. The results showed an improvement in branch growth and a restored ratio of mature MGE-like neurons but no significant increase in cell survival in IL-1RA treated hi-spots. To investigate whether IL-1β plays a dominant role in inflamed microenvironment, IL-1β was applied to non-sclerotic hippocampal hi-spots to test its effects on survival and differentiation of MGE progenitors. The result showed IL-1β caused reduced survival and branch growth but didn't affect differentiation of MGE-like cells. The data provide evidence that IL-1β is one of the important targets to restore the detrimental effects of pathological microenvironment on MGE progenitors.

4.2 Results

4.2.1 IL-1RA didn't improve the survival of MGE cells in an inflamed environment.

Having found the detrimental effects of the epileptic microenvironment on the survival and differentiation of MGE-like cells, this experiment tried to modify the inflamed microenvironment by applying IL-1RA and testing its effects (see method 2.2.4). The surviving number of MGE-like

cells in IL-1RA treated hi-spots were compared to untreated hi-spots on post culture day 14 and day 28 (Figure 4-1A). The results revealed the number of surviving MGE-like cells did not significantly improve in IL-1RA treated hippocampal hi-spot compared to untreated at either time point (n=12 hi-spots coming from three patients for each condition, two-way ANOVA, F (1,44) =1.459, P=0.23) (Figure 4-1B). Likewise, in cortical hi-spots, the number of surviving MGE-like cells wasn't changed by the application of IL-1RA. (n=12 hi-spots coming from three patients for each condition, two-way ANOVA, F (1,44) =0.465, P=0.49) (Figure 4-1C). Besides, in both cortical hi-spots (two-way ANOVA, F (1,44) =0.823, P=0.40) and hippocampal hi-spots (two-way ANOVA, F (1,44) =2.819, P=0.1) the number of surviving MGE-like cells on post culture day 14 was similar to post culture day 28. These results suggest IL-1RA did not improve the survival of MGE progenitors in the epileptic microenvironment.

4.2.2 IL-1RA promoted neurite growth and restored differentiation of MGE progenitors in epileptic microenvironment.

Next, Sholl analysis was used to compare the morphological complexity of MGE-like cells in IL-1RA treated hippocampal hi-spots to the cells in untreated hi-spots (Figure 4-1F). Interestingly, maximum branch lengths were improved in IL-1RA treated cells than untreated (164.1±13.0um vs 120.9±10.3um, Repeated measures ANOVA, F (1,8) =6.707, P=0.032 (Figure 4-1J). The cells in IL-1RA treated hi-spots showed longer extensions over 160um compared with cells in untreated hi-spots. Correspondingly, the area under the curve for the sholl analysis was in IL-1RA treated cells compared to untreated (229.5±14.0 vs 173.5±23.2, n=10 for each condition, Repeated measures ANOVA, F (1,8) =7.379, P=0.026) (Figure 4-1G) However, the number of branches was similar between IL-1RA treated and untreated cells () (Figure 4-1H). These results suggest IL-1RA partially restored neurite elongation but didn't improve neurite sprouting of MGE progenitors in epileptic microenvironment.

Lastly, the differentiation of MGE progenitors was examined by comparing the expression of Tuj1 and GAD 67 in MGE-like cells in IL-1RA treated hippocampal and cortical hi-spots to untreated. The results revealed the ratio of $Tuj1^+$ MGE-like cells (n=12 hi-spots coming from three patients for each condition, two-way ANOVA, F (1,44) =5.081, P=0.029) and GAD 67^+ MGE-like cells (n=12 for each condition, two-way ANOVA, F (1,44) =7.655, P=0.0082) were significantly higher in IL-1RA treated hi-spots compared to untreated (Figure 4-1D-E). Tukey's multiple comparison

revealed the ratio of Tuj1^{+} MGE-like cells (0.71±0.03 vs 0.57±0.05, P=0.032) and GAD 67+ MGE-like cells (0.43±0.03 vs 0.27±0.02, P=0.001) were significantly improved in hippocampal hi-spots but not in cortical hi-spots by the application of IL-1RA (Figure 4-1D-E). This result suggests increased neuronal differentiation of MGE progenitors in epileptic microenvironment of the epileptic hippocampus only

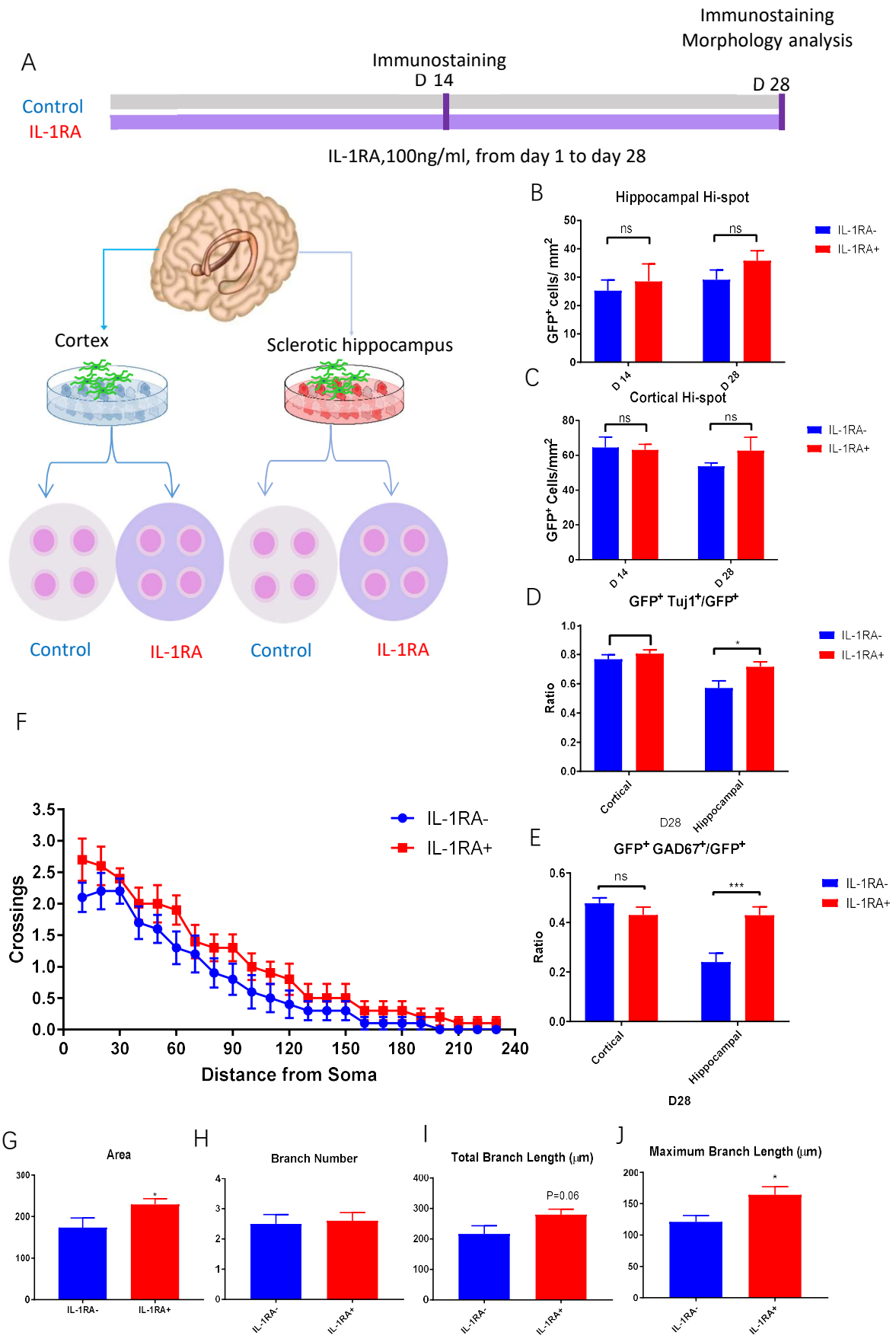

Figure 4-1 The effect of IL-1RA on MGE progenitors in the inflamed microenvironment

*A) The illustration of experimental groups. Brain tissue removed from patients with TLE was used in the experiment. IL-1RA was added into culture medium from the beginning. The hi-spots from different conditions were fixed on day 14 and day 28. B-C) The bar graph showed no significant changes on day 14 and day 28 in survival of MGE progenitors which received IL-1RA treatment. Data show mean± SEM, Patient =2, n=4 for each condition, two-way ANOVA with Tukey's post-hoc test, ns P>0.05. D-E) The bar graph showed on day 28 the ratio of Tuj1+ and GAD 67+ cells in MGE progenitors were improved after IL-1RA treatment. Data show mean± SEM, Patient =2, n=4 for each condition, two-way ANOVA with Tukey's post-hoc test, *P<0.05, ***P<0.001. F) Cells in hippocampal hi-spots were selected for sholl analysis in image J. The number of crossings were counted at different distances. Data show mean± SEM, n=10 for each condition. G-J) The area under curve, the number of branches, the total length of branch and the maximum length of branch were compared between MGE-like cells with or without treatment of IL-1RA in hippocampal hi-spots.*

4.2.3 IL-1β caused acute death and altered branch growth of MGE-like cells in a non-epileptic environment

Previous results showed the detrimental effects of the epileptic microenvironment on transplanted MGE progenitors were partly reversed by applying IL-1RA. In the following experiment, the effect of IL-1β alone on the survival and differentiation of MGE progenitors was inspected in the non-epileptic hippocampus.

The hi-spots were generated from a unique patient with non-sclerotic hippocampal and cortical tissue removed from patient undergoing lesional brain tumor surgery. First, the surviving number of MGE-like cells in IL-1β (10ng/ml) treated hi-spots were compared to untreated hi-spots on post culture day 14 and day 28 (Figure 4-2A). The results showed the number of surviving MGE-like cells was significantly reduced in IL-1β treated hippocampal hi-spots compared to untreated (n=8, two-way ANOVA, F (1,28) =14.59, P=0.0007) (Figure 4-2 B). Moreover, Tukey's post-hoc comparison revealed the number of surviving MGE cells was significantly lower in IL-1β treated hippocampal hi-spots on day 14 (66.8±7.8 vs 139.3±11.7, P=0.0012) but not on day 28 (57.9±5.7 vs 77.7±18.8, P=0.65) (Figure 4-2B-C). In addition, the number of surviving MGE cells didn't reduce from post culture day 14 to day 28 in IL-1β treated hi-spot, (Tukey's multiple comparison, P=0.95). while in untreated hi-spots, the number was significantly lower on post culture day 28 compared to day 14 (Tukey's multiple comparison, P=0.0062) (Figure 4-2 B-C). On the contrary, the surviving number of MGE-like cells didn't change in IL-1β treated cortical hi-spots compared to untreated at both time points. (n=8 for each condition, two-way ANOVA, F (1,28) =0.7555, P=0.39) (Figure 4-2 B). The results suggest IL-1β temporarily reduced the initial survival of MGE progenitors but didn't affect cell survival in the longer-term perspective between days 14-28.

Next, Sholl analysis was used to inspect the effect of IL-1β on the morphological development of MGE-like cells in non-epileptic hippocampal hi-spots. As shown in the graph, MGE-like cells in IL-1β treated hi-spots showed fewer crossings than cells in untreated hi-spots from 0 to 120um (Figure 4-2 E). Consistently, the area under the curve was significantly smaller in cells treated with IL-1β compared to untreated (224.0±17.4 vs 306.5±17.7 n=10 for both conditions, two-way unpaired t-test, t=3.315, df=18, P=0.0035) (Figure 4-2 F). The number of branches was lower but not significantly so, in cells treated with IL-1β compared to untreated (2.3±0.3 vs 3.3±0.4, t=2.013, df=18, P=0.0593) (Figure 4-2 G). There were no significant changes in total branch length (P=0.11) or maximum branch length (P=0.49) (Figure 4-2 H-I).

Lastly, the effect of IL-1β on differentiation of MGE progenitors was investigated by comparing the ratio of $Tuj1^+$ MGE-like cells in IL-1β treated to untreated hi-spots. The result showed the ratio of $Tuj1^+$ MGE-like cells was similar between IL-1β treated and untreated hi-spots (two-way ANOVA, F (1,28) =0.3821, P=0.54) (Figure 4-2D). Together, these results suggested IL-1β alone had limited effect on survival and branch growth in non-epileptic hippocampal hi-spots but did affect differentiation of MGE progenitors.

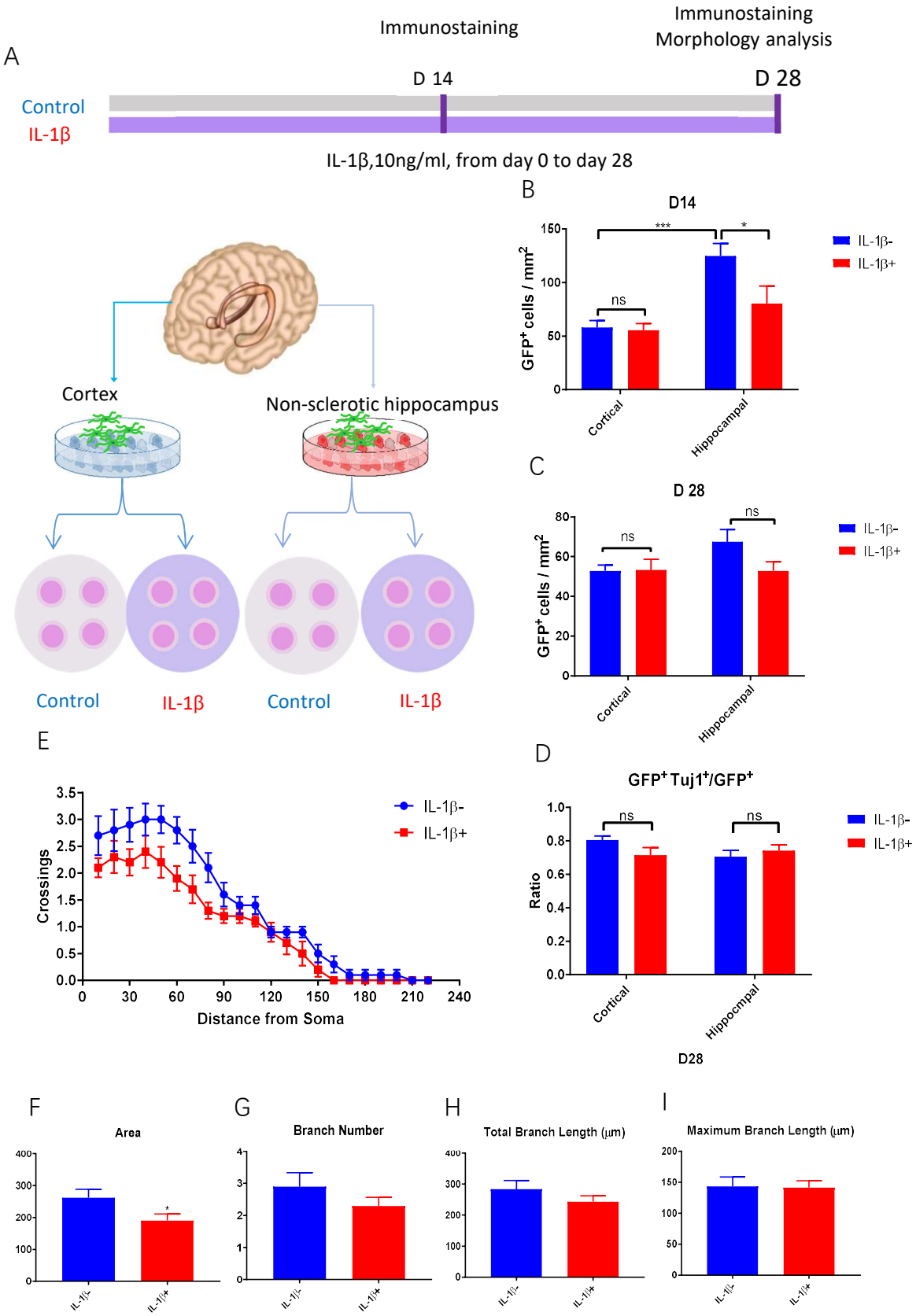

Figure 4-2 The effect of IL-1β on MGE progenitors in non-inflamed microenvironment

*A) The illustration of experimental groups. Non-epileptic brain tissue removed from patients was used in the experiment. IL-1β was added into culture medium from the beginning. The hi-spots from different conditions were fixed on day 14 and day 28. B-C) The bar graph showed survival of MGE progenitors on day 14 and day 28 in IL-1β treated and untreated cortical and hippocampal hi-spots. Data show mean± SEM, Patient =1, n=4 for each condition, two-way ANOVA with Tukey's post-hoc test, ns P>0.05. D) The bar graph showed on day 28 the ratio of Tuj1+ in MGE progenitors were similar in IL-1β treated and untreated hi-spots, (two-way ANOVA with Tukey's post-hoc test, *P<0.05, ***P<0.001. E) Cells in hippocampal hi-spots were selected for sholl analysis in image J. The number of crossings were counted at different distances. Data show mean± SEM, n=10 for each condition. F-I) The area under curve, the number of branches, the total length of branch and the maximum length of branch were compared between cells with or without treatment of IL-1β in hippocampal hi-spots. Data show mean± SEM, n=10 for each condition, t-test, *P<0.05)*

4.3 Discussion

The results in this chapter demonstrate that applying IL-1β alone temporarily reduced the survival of transplanted MGE progenitors and delayed their morphological maturation in epileptic hippocampal hi-spots. By blocking IL-1β activation of the ILR1 receptor, the detrimental effects of microenvironment on neurite growth and differentiation of MGE progenitors were partially reversed. These findings highlight the potential importance of an optimal microenvironment for achieving optimal outcomes from cell transplantation for chronic epilepsy and identify the IL-1β/IL-1R1 pathway as an important component of the pathological microenvironment relevant to cell replacement therapy in human TLE.

This research found that IL-1β reduced survival of MGE progenitors when applied to non-epileptic hippocampal hi-spots. However, when the IL-1β/IL1-R1 pathway was blocked in epileptic hippocampal hi-Spot tissue, the survival of transplanted MGE progenitors didn't improve. Moreover, in non-epileptic cortical hi-spots, addition of IL-1β didn't affected cell survival at all. There are several possibilities underlie the conflicting results. Firstly, IL-1β seems to exert complex effects by interacting with, and being influenced by, multiple cell types, such as microglia and astrocyte, in the brain (Allan, Tyrrell et al. 2005). It is important to remember that in epileptic hippocampal hi-spots, immune cells and microglia have been recruited and the inflammatory cytokines have been accumulated before generation of the hi-Spot. Therefore, other signalling pathways which cause cell death have been already activated before giving IL-1RA. On the contrary, in non-epileptic hi-spots, only inactivated local immune cells exist, so adding IL-1β merely evokes immune response locally and is not strong enough to cause similar detrimental effect as the inflammatory cytokines in epileptic hi-spots do. Although microglia are

present in all major divisions of the brain, the distribution of microglia is much denser in hippocampus compared to cortex(Lawson, Perry et al. 1990) and therefore the immune responses is milder in cortex than hippocampus. That may explain why there is no significant death of MGE progenitors in cortical hi-spots treated with IL-1β. Similar to my result, an in vivo study shows injection of IL-1β (20ng) into normal cortex fails to cause any brain damage (Stroemer and Rothwell 1998). Secondly, although IL-1/TLR is the most common and classic cell signalling pathway activated by IL-1β, mice lacking IL-1 receptors have exacerbated ischemic brain damage caused by IL-1β (Touzani, Boutin et al. 2002). So, it is possible that IL-1β can activate other receptors which are independent of the IL-1 receptor. This may be another reason that cell survival didn't improve by treatment of IL-1RA. Thirdly, evidence suggests IL-1β has contradictory effects on neurons in vitro corresponding to its concentration and duration of treatment. For example, IL-1β shows a protective effect for cortical neurons incubated with excitatory amino acids when applied for 24hr, but resulted in neurotoxicity when the application is extended to 72hr (Strijbos and Rothwell 1995). In addition, IL-1β cooperates with other cytokines and damage signals, because IL-1β by itself is not neurotoxic in some cell cultures (Fogal, Hewett et al. 2005). For example, HMGB1 which is secreted at the later stage after brain injury could enhance the effect of IL-1β through formation of complexes with IL-1β(Wähämaa, Schierbeck et al. 2011). Moreover, combined treatment with TNFα, IL-1β, and IFNγ in astrocytes results in distinct upregulation of several genes for cytokines and growth factors which can't be induced by applying IL-1β alone (Meeuwsen, Persoon-Deen et al. 2003). Therefore, in healthy microenvironment, where other cytokines are absent, the effect of IL-1β may be limited.

It is also worth to notice that in health hippocampal hi-spots, MGE progenitors survive better than cortical hi-spots; while in sclerotic hippocampal hi-spots, the survival of MGE progenitors is reduced compared to cortical hi-spots. It is possibly due to that the dentate gyrus of the hippocampus is the region for adult neurogenesis and was chosen for MEG progenitor injection site in many rodent experiments (Henderson, Gupta et al. 2014, Kepecs and Fishell 2014, Kim, Yao et al. 2014, Wang, Hong et al. 2016). Thus, health hippocampus could be more supportive of the survival of MGE progenitors. But due to the limitation of human tissue, it is impossible to compare the survival of MGE progenitors in normal to sclerotic hippocampus from the same patient. And the composition of cortical tissue is not the same as hippocampal tissue even from

the same patient. Therefore, it will be important to validate the assumption with rodent model in the future.

Although the surviving number of MGE cells is important in cell transplantation, the surviving number of functional inhibitory neurons is more critical for restoring the overexcited neuronal network of epilepsy. In this experiment, the proportion of MGE cells expressing Tuj1 or GAD 67 were recovered by applying IL-1RA in hippocampal hi-spots. In line with the results, previous research shows that during neuronal differentiation, IL-1β has an anti-neurogenic but pro-gliogenic effect on neuron progenitor cells mediated by IL-1R1 (Green, Treacy et al. 2012), and restored neuronal differentiation of hippocampal neuron progenitors can be achieved by IL-1RA (Zhang, Xu et al. 2013). While there is also evidence supports the other assumption. BDNF is an environmental neurotrophic factor that promotes neuronal differentiation and improves neuron survival(Jiao, Palmgren et al. 2014). IL-1β has been reported to suppress BDNF signal via activating P38-MAPK pathway(Tong, Balazs et al. 2008, Tong, Prieto et al. 2012). It is possible that IL-1β prolongs neuronal differentiation of MGE progenitors in inflamed microenvironment by interfering with the BDNF neurotrophic signal. In addition, IL-1RA also partly restored branch growth of MGE cells in the epileptic microenvironment. So far, there is a lack of evidence for the direct effect of IL-1RA on improving branch growth. However, evidence showed an IL-1RA activated specific phenotype of microglia which has repair and regenerative functions (Chhor, Le Charpentier et al. 2013). The growth factors released by those microglia may help to restore neurite growth of MGE-like neurons.

Although previous researches show chronic application of IL-1β supresses hippocampal neurogenesis in mice and reduces proliferation of hippocampal neuron progenitors (Goshen, Kreisel et al. 2008, Ryan, O'Keeffe et al. 2013), there is no evidence in hi-spots that the proliferation of MGE cells was affect by IL-1β in hi-spot cultures, as the number of surviving MGE cells were similar on day 28 compared to day 14. The reason might be the brain tissue used for hi-spots might not form the neurogenic niche which is necessary for cell proliferation. In addition, alterations in neuronal differentiation via IL-1β (Peng, Whitney et al. 2008, Zhang, Xu et al. 2013) were not observed in MGE progenitors cultured in hi-spots. This is also in contradiction with the result that IL-1RA restored neuronal differentiation of MGE progenitors in the epileptic microenvironment. It implies IL-1β alone is not enough to cause alterations in the neuronal

differentiation of MGE progenitors but collaboration with other cytokines is necessary to trigger the regulatory signal downstream.

Despite the area under the curve of Sholl analysis showed a significant difference between IL-1RA treated and untreated MGE-like cells in sclerotic hippocampal hi-spots, the complexity of MGE-like cells was not different, as the branch number was similar, and the highest number of crossings showed at the initial segment were similar between treated and untreated cells. This result represented that the MGE-like neurons displayed simple morphology with predominantly primary branches in hippocampal hi-spots. As one of the limitations for hi-spot cultures, the hi-spots can't be kept for longer than a month, which is not long enough for complex neurite formation of hESC derived MGE-like cells. Even in health hippocampal hi-spots, the average number of branches is about only 3, which is similar to the number in untreated sclerotic hippocampal hi-spots. So, restricted by the culture model, it is not enough the draw a conclusion that IL-1RA or IL-1β can affect nascent neurite formation. But the Sholl analysis also presented information about neurite elongation. Neurite initiation and elongation simultaneously occur during neurite differentiation, but the mechanism is not exactly the same. The initiation of a nascent neurite starts with the formation of an actin rich filopodia from the neuronal cell body, followed by a broadening of the filopodia into a neurite. While neurite elongation is due to the extension of microtubules into actin rich filopodia (Sainath and Gallo 2015, Athamneh, He et al. 2017). My results suggested neurite elongation not neurite formation is partially revered by apply IL-1RA in sclerotic hippocampal hi-spots. Similar to my observation, IL-1β reduces neurite growth of rat hippocampal precursor cells when being applied for 7 days (Green, Treacy et al. 2012). And in KA model of epilepsy, the morphological complexity of PV and CCK expressing interneurons was significantly reduced (Khan, Shekh-Ahmad et al. 2018). However, in addition to IL-1β, TNF-α is also upregulated in chronic epilepsy, and can directly inhibit neurite formation by activating GTPase RhoA (Neumann, Schweigreiter et al. 2002). This additional signalling may explain why IL-1RA can't completely reverse the detrimental effect of the inflamed microenvironment on neurite growth.

In general, blockage of the function of IL-1β is beneficial for MGE progenitors in an epileptic microenvironment. The restored differentiation of MGE progenitors could be critical in long term restoration of imbalance between inhibition and excitation in epilepsy. The results suggest that

combined anti-inflammatory therapy may improve the outcome of MGE progenitor transplantation in epilepsy.

5. IL-1β mediated modulation of voltage-gated sodium currents reduces cellular excitability in HESC-derived MGE-like neurons

5.1 Introduction

Previous studies suggest that neuronal activity is compromised by inflammatory cytokines released after brain injury, infection and seizure. Increasing evidence supports the idea that cytokines can interact with receptor and voltage-gated ion channels to regulate neuronal excitability, synaptic plasticity and neuron transmitter release (Schafers and Sorkin 2008, Vezzani and Viviani 2015). The disrupted neuronal activity in the inflamed environment is closely related to ictal discharges and the development of epileptiform activity (Librizzi, Noe et al. 2012, Webster, Sun et al. 2017).

IL-1β is one of the predominant cytokines released after seizures. In the previous chapter, the effect of IL-1β on survival and differentiation of inhibitory neuron progenitors was discussed. This chapter will present the results of electrophysiological experiments performed to determine the effects of IL-1β on neuronal excitability. Several studies suggest that a pathological concentration of IL-1β in the CNS and PNS can affect excitability of principle neurons (Diem, Hobom et al. 2003, Liu, Yang et al. 2006, Schafers and Sorkin 2008, Zhou, Qi et al. 2011, Vezzani and Viviani 2015). Furthermore, IL-1β also significantly reduces GABAA-R mediated currents in the hippocampus and the temporal cortex of patients with TLE (Roseti, van Vliet et al. 2015). However, the effects of inflammatory cytokines, including IL-1β, on the function of inhibitory GABAergic neurons remain unknown.

Although inhibitory neurons make up only 20% of the neuronal population in the cortex, they provide input to a large group of surrounding excitatory neurons to coordinate network activities, and their dysfunction in seizures and epilepsy is well known. Thus, this work investigated the acute and chronic effects of IL-1β on the electrophysiological activities of hESC-derived MGE-like inhibitory neurons. The experiments found that acute exposure to IL-1β caused a reduction of voltage-gated sodium channel (VGSC) currents which lead to reduced excitability in MGE neurons. Chronic exposure to IL-1β causes apoptosis of astrocytes which indirectly affects the

viability of MGE neurons. These results suggest the function of inhibitory neurons is compromised in the inflamed microenvironment which may contribute to insufficient inhibitory output in epilepsy.

5.2 Results

5.2.1 MGE progenitors co-cultured with human astrocytes develop mature electrophysiological properties

To investigate the electrophysical activity of hESC-derived neurons ex vivo, the neuron progenitors were usually cultured with mouse astrocytes or conditioned medium to accelerate the electrophysiological maturation of neuron progenitors (Maroof, Keros et al. 2013, Bardy, van den Hurk et al. 2016, Telezhkin, Schnell et al. 2016). Since astrocytes of different origins are diverse in phenotype and function (Song, Stevens et al. 2002, Roy, Cleren et al. 2006) and to simulate the microenvironment of the human hippocampus where the MGE transplantation is targeted, human hippocampal astrocytes from foetal tissue were used to support the functional maturation of MGE cells in conditioned medium (See Method 2.3.1). HESC-derived MGE progenitors after differentiation day 20 were collected and plated on the top of coverslips covered with human hippocampal astrocytes. The standard culture medium (see 2.1.4 Human ESC differentiation) was replaced with conditioned medium (SCM) which contained a combination of neurotrophic factors to promote neuronal maturation (Telezhkin, Schnell et al. 2016) (2 weeks: N = 14, 6 weeks: N = 10) (Figure 5-1C-D)

Whole cell patch clamp recordings were performed in the current clamp (I_{clamp}) mode to measure the excitability of MGE-like cells as they developed in vitro (2 weeks: N = 14, 6 weeks: N = 10) (Figure 5-1C-D)

Spontaneously occurring and current-step evoked over-shooting (full action potentials were defined as spikes whose peak crossed 0mV) and action potentials (APs) were measured after 2 and 6 weeks of astrocyte co-culture. Patch clamp recordings performed at 2 weeks post co-culture demonstrated presence of APs (9 from 14) in MGE-like cells. Six weeks after co-culture, the cells showed more repetitive APs than after two weeks (2 weeks: N = 14, 6 weeks: N = 10) (Figure 5-1C-D)

Consistent with functional development of neurons in vitro previously described by others (Nicholas, Chen et al. 2013), these recordings showed the resting membrane potential (RMP)($P<0.05$, unpaired t-test) and input resistance (R_{IN}) ($P<0.01$, unpaired t-test) of MGE-like neurons were significantly reduced after 6 weeks of astrocyte co-culture versus two weeks co-culture (2 weeks: N = 14, 6 weeks: N = 10) (Figure 5-1C-D)

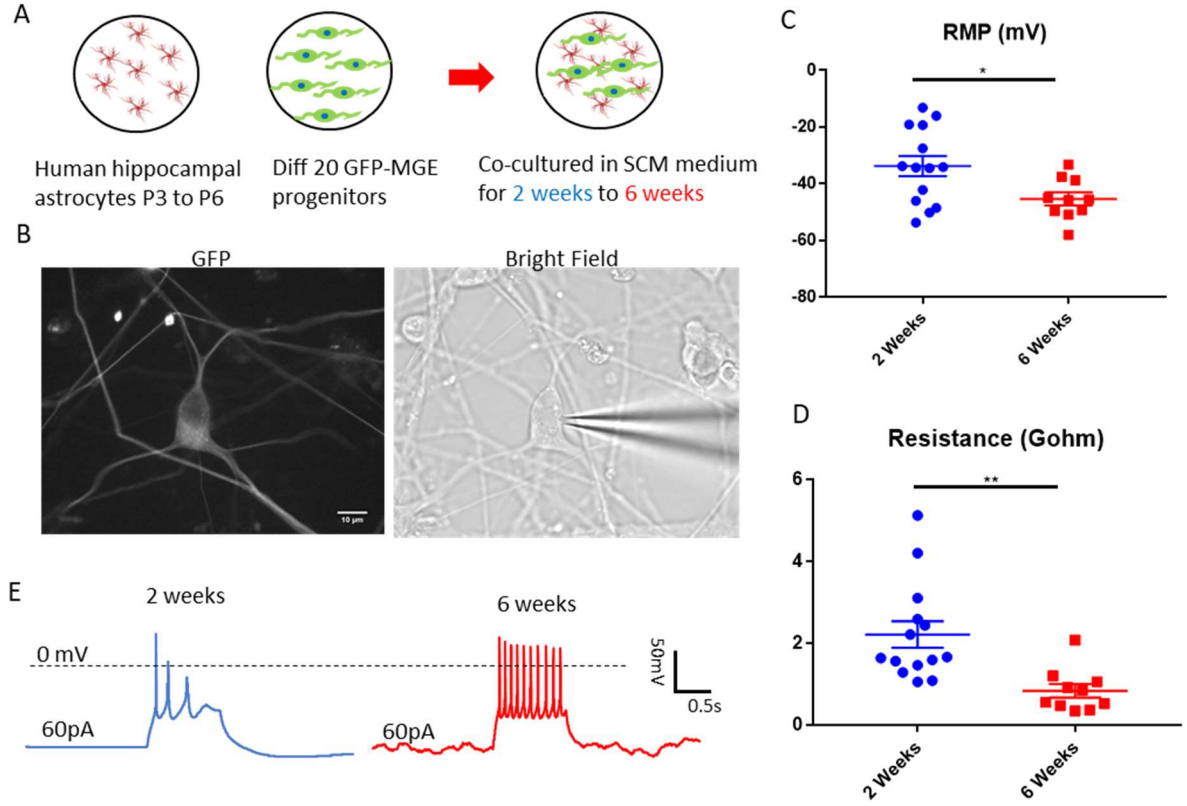

Figure 5-1 MGE progenitor co-cultured with astrocyte developed functional electrophysiological activities

A) Schematic representation of the astrocyte-neuron co-culture protocol. TG4-H7 cell line derived MGE-like progenitors (Diff day 20) line were cultured with human hippocampal astrocyte in SCM medium. Electrophysiological data were collected at 2 and 6 weeks after co-culture. B) MGE-like progenitors were labelled with Tau-GFP. Neurons with large smooth cell bodies were selected for patch clamp recording. 'wide-field fluorescence image on a neuron expressing GFP' and on the right 'a differential interference contrast image of the cell showing the whole-cell recording electrode C-D) RMP was significantly more hyperpolarized and RIN were significantly lower in MGE-like neurons co-cultured with astrocytes for 6 weeks compared to 2 weeks. E) The graph showed traces of evoked action potentials of MGE-like neurons 2w and 6w after co-culture.

5.2.2 MGE progenitors showed reduced excitability after acute exposure to IL-1β

Having established that MGE-progenitors can develop mature functional neuronal properties when co-cultured with human astrocytes in vitro, the next experiment tested the effects of an inflammatory environment on neuronal excitability by the addition of the pro-inflammatory cytokine IL-1β to the cell culture media. IL-1R1 is the major functional receptor for IL-1β. The expression of IL-1R1 was confirmed by immunostaining. In the co-culture system, The IL-1R1 was found to be expressed on both ages of MGE neurons, as can be seen by its co-localization with GFP and astrocytes (Figure 5-2D). After six weeks astrocyte co-culture, MGE neurons were exposed to IL-1β (10ng/ml) for 24 hours before electrophysiological recordings were performed (Figure 5-2A). Current clamp recordings showed that the RMP and R_{IN} were not significantly altered in MGE-like neurons acutely exposed to IL-1β compared to control cells (Control, N=27; IL-1β treated, N=33; unpaired t-test, P>0.05). (Figure 5-2B-C)

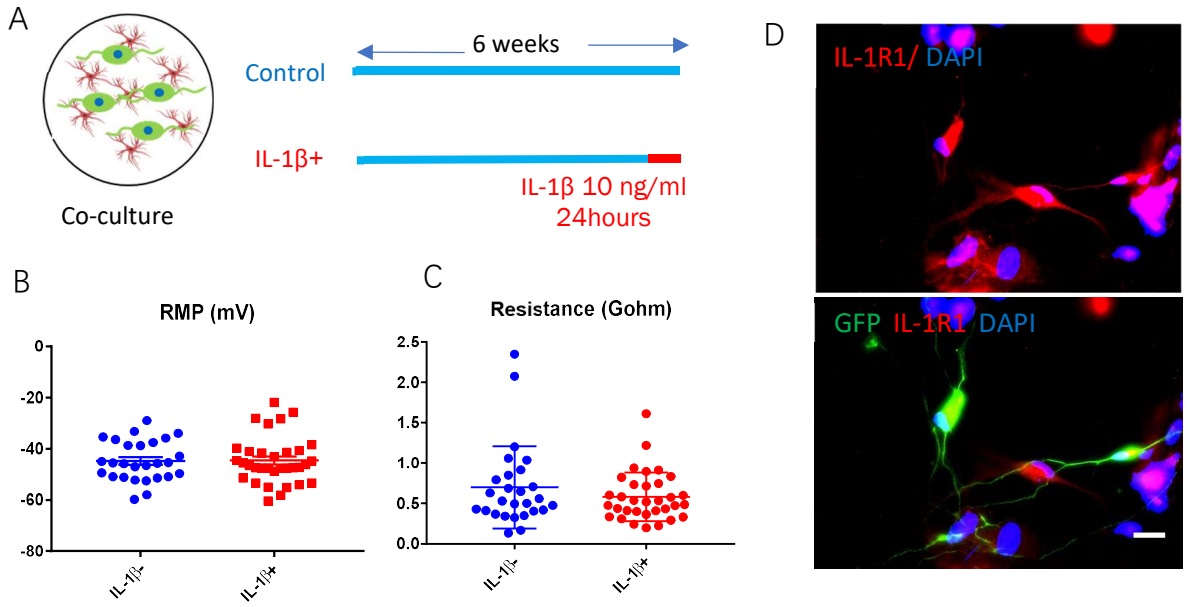

Figure 5-2 Acute exposure of IL-1β didn't change RMP and input resistance in MGE-like cells

A) Schematic representation of the experimental protocol. MGE progenitors after 6 weeks co-culture with human astrocytes were treated with IL-1β (10ng/ml) for 24 hours before patch clamp recording. G-H) The acute application of IL-1β didn't change the resting membrane potential and input resistance in MGE progenitors. D) The immunostaining showed expression on IL-1 receptor (IL-1R1) on MGE cells and astrocytes. Scale bar 50um.

The effects of IL-1β on neuron excitability were tested by comparing the ability of treated and untreated neurons to fire APs. Current-clamped cells were held at -70 mV using d.c. injection.

The cells were held at -70mV to measure action potentials evoked by current steps and APs evoked using depolarizing current steps (duration, 1 s; increment, 20 pA; from -100pA to 200pA). The neurons in control and IL-1β treated cells were classified into three groups based upon their cellular excitability.

Group 1 cells fired single immature ('Attempted') APs that did not overshoot 0 mV;

Group 2 cells fired single mature over-shooting APs;

Group 3 cells were capable of firing trains of 2 or more overshooting APs (Figure 5-3A). As shown in Figure 5-3B, the number of recorded group 3 neurons, with the ability to fire AP trains, was reduced in IL-1β treated cells compared to control cells (27% vs 63%). Correspondingly, the number of group 3 neurons capable of firing only single APs was higher in IL-1β treated cells than control cells (67% vs 36%) indicating reduced excitability following IL-1β treatment. 6% cells showed attempt action potential in IL-1β treated group while all cells displayed proper action potentials in control group (Figure 5-3A-B). Then, the excitability of control and IL-1β treated cells in Group 3 were compared by plotting the number of evoked APs against the amplitude of the current injection step. Figure 5-3C-D shows a plot of the number of APs evoked by each current step revealing that IL-1β treated MGE-like neurons displayed fewer action potentials in response to current steps than control. The total number of APs recorded in response to all current injections steps (0-200 pA) was significantly lower in IL-1β treated cells compared to control cells (n=27 control; n=29 IL-1β treated; unpaired t-test, **P<0.01) (Figure 5-3D-E).

Subsequently, to further examine the effects of IL-1β on MGE derived neuron excitability, the rheobase of cells in Group 2 and Group 3 was compared respectively in control and IL-1β treated. Rheobase is the minimal stimulation current of infinite duration (in practice about 300 ms or longer) that results in the AP threshold potential being reached and a spike fired (Figure 5-3 F-H). Consistent with reduced excitability following IL-1β treatment, the result showed that rheobase current was significantly higher in IL-1β treated cells compared to control (Control 82.96 ± 6.97 pA, n=27; IL-1β treated 119.3 ± 7.458 pA, n=30; two-way ANOVA, $F(1,48) = 7.196$, $P=0.01$), especially among Group 2 cells (Sidak's post-hoc comparison $t=2.58$ $df=28$ $P=0.026$) (Figure 5-3 I). Together, these results suggest that the impaired excitability of MGE-like cells after acute exposure to IL-1β may be caused by the alterations in ion channels.

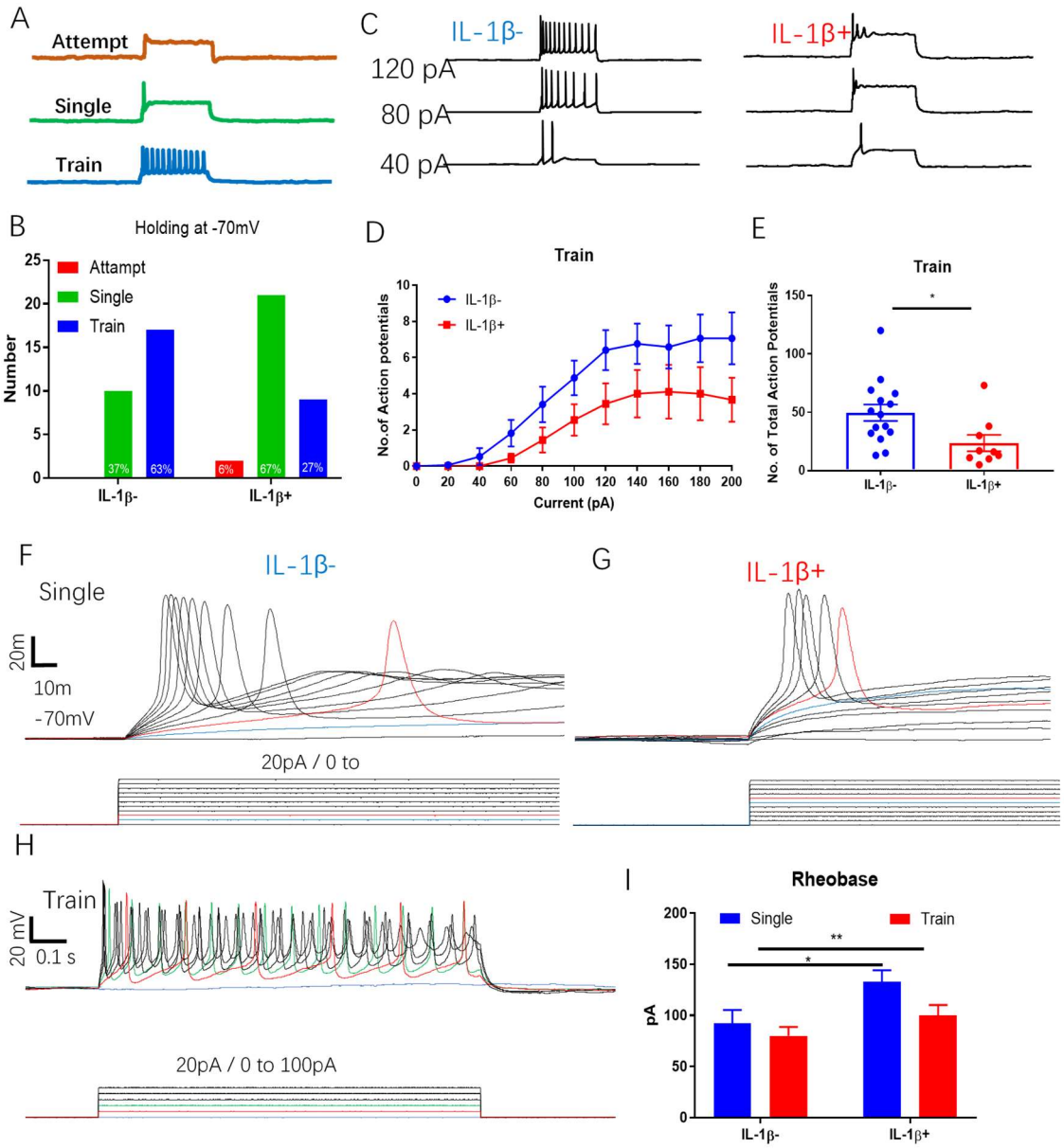

Figure 5-3 MGE-like neurons were less excitable after acute exposure to IL-1β

A) The neurons were classified by their cellular excitability into three groups. The graph represented traces for neurons of different groups. B) The distribution of MGE-like neurons with different cellular excitability in control and IL-1β treated cells. C) The graph represented example traces for number of APs in response to depolarizing current steps in control and IL-1β treated cells D) The number of APs evoked by each depolarization current step in control and IL-1β treated cells. E) The total number of APs recorded in response to all current injection steps (0-200 pA) in control and IL-1β treated cells. F-I) The rheobase current was higher in IL-1β treated cells compared to control cells, especially among Group 3 cells. J-L) The analysis of singular AP was based on the first mature over shooting AP recorded from Group 2 and Group 3 cells. The bar graph showed increased threshold voltage and decreased amplitude in IL-1β treated cells compared to control.

5.2.3 Acute exposure to IL-1β reduced sodium currents in MGE-like cells

Voltage gated sodium channels (VGSCs) play a critical role in generating and propagating action potentials. The previous findings show that IL-1β treatment reduces the excitability of MGE-like neurons, elevates AP threshold potential and decreases AP amplitude. For an AP, the initial trigger and the rising phase of action potential is generated by sodium influx. So, the previous results suggest that reduced cellular excitability in IL-1β treated MGE-like cells might be due to a reduction in sodium currents. Whole-cell voltage clamp and immunohistochemistry were used to test the effects of IL-1β exposure on sodium channel expression and sodium currents in MGE-like cells. Immunostaining revealed expression of several TTX sensitive sodium channel isoforms on MGE cells (Figure 5-4A). Pan-Nav and Nav1.1 were expressed on cell body, proximal and medial neurites. Nav 1.2 and Nav 1.6 were expressed on cell body, medial and distal neurites. Navβ2 were expressed on the cell body of both neuron and astrocytes.

Next, voltage clamp was applied to compare steady -state sodium channel activation and inactivation in control and IL-1β treated cells. Sodium currents recorded from MGE-like cells were nearly blocked by TTX, which suggests that recorded sodium currents were mainly TTX-sensitive sodium currents (Figure 5-4 B). The example traces also showed a reduction of sodium currents in response to command voltage steps in IL-1β treated cells compared with control cells. The I-V relation graph based on steady-state sodium current recording revealed the maximum current density was significantly reduced by nearly 40% in IL-1β treated cells compared to control (n=17 control, n=13 IL-1β treated, n=10 IL-1β+IL-1RA treated, one-way ANOVA ,P=0.03) (Figure 5-4 D). To further examine the specificity of IL-1β signalling in sodium current reduction, the IL-1β antagonist IL-1RA was applied to the cell cultures at the same time as IL-1β. The results show that the reduction of sodium current was prevented by IL-1RA in IL-1β treated MGE-like cells (P>0.05). (Figure 5-4D)

To further investigate the properties of VGSCs, sodium channel activation -voltage and inactivation -voltage relation were plotted according to recorded sodium current from command voltage steps (Figure 5-4 B,I). The data for I-V relationship was fitted with a Boltzmann equation. The results show sodium channel activation and inactivation curve didn't shift in IL-1β treated cells compared to control. Likewise, the V_{50} calculated from Boltzmann fit for sodium channel activation and inactivation in IL-1β treated cells were similar to control cells (P>0.05). The results

also show addition of IL-1RA didn't affect sodium channel activation and inactivation curves or V_{50} for activation and inactivation in IL-1β treated cells compared with control ($P>0.05$) (n=17 control, n=13 IL-1β treated, n=10 IL-1β+IL-1RA treated, one-way ANOVA) (Figure 5-4E-H). These results suggest the reduction of sodium current is not caused by alterations in the properties of VGSCs.

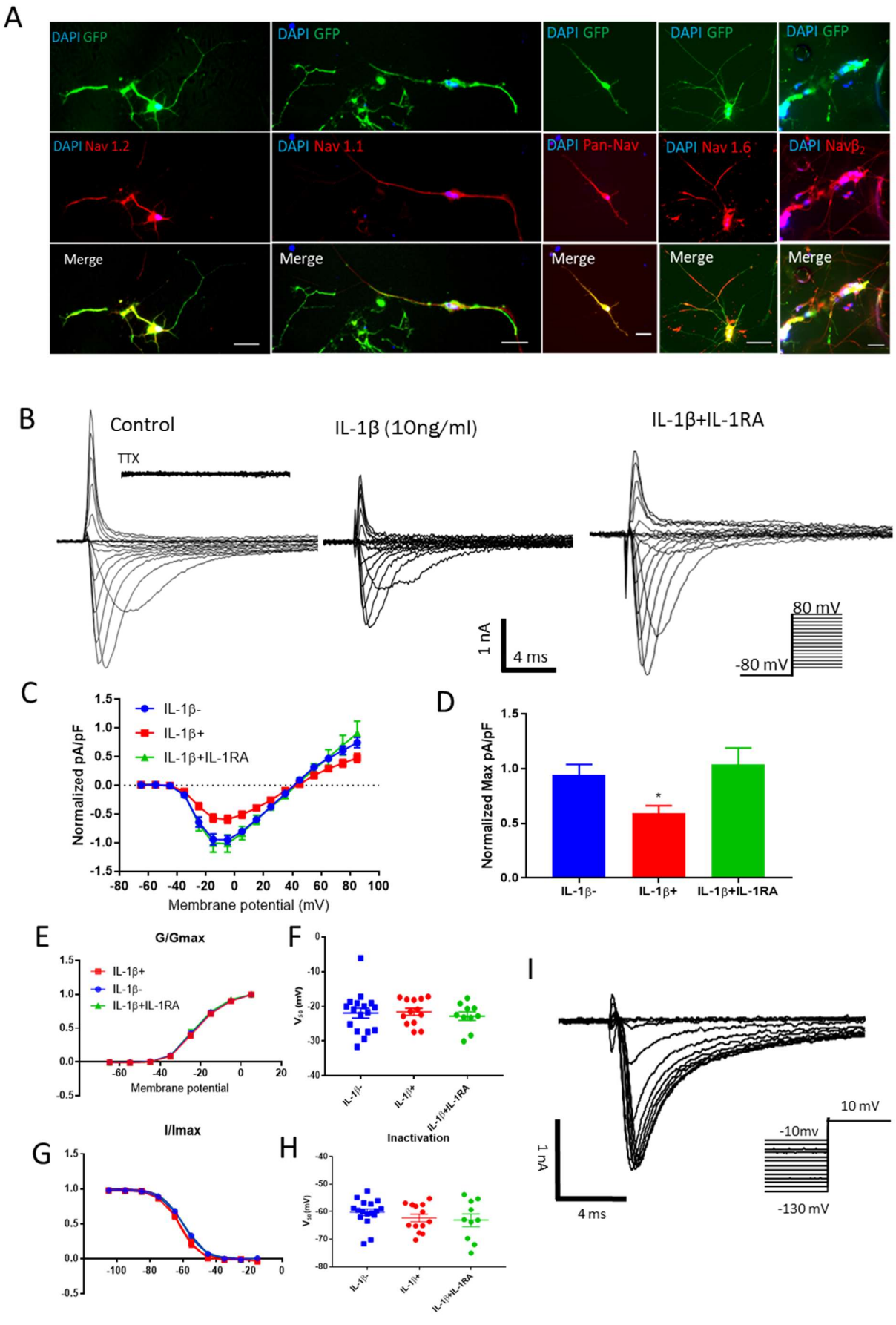
A
DAPI GFP
DAPI Nav 1.2
DAPI Nav 1.1
DAPI Pan-Nav
DAPI Nav 1.6
DAPI Navβ2
Merge
B
Control
TTX
IL-1β (10ng/ml)
IL-1β+IL-1RA
1 nA
4 ms
80 mV
-80 mV
C
IL-1β-
IL-1β+
IL-1β+IL-1RA
Normalized pA/pF
Membrane potential (mV)
D
Normalized Max pA/pF
IL-1β-
IL-1β+
IL-1β+IL-1RA
E
G/Gmax
IL-1β+
IL-1β-
IL-1β+IL-1RA
Membrane potential
F
Inactivation
G
I/Imax
H
I
1 nA
4 ms
10 mV
-10mv
-130 mV

Figure 5-4 Acute exposure to IL-1β caused reduction of sodium currents in MGE-like cells

A) The expression of TTX-sensitive sodium channels Pan-Nav, Nav 1.1, Nav 1.2, Nav 1.6 and Navβ2 in MGE-like neurons. Scale bar 50mm B) The whole cell voltage clamp showed voltage-dependent and tetrodotoxin (TTX) sensitive sodium current in control, IL-1β treated and IL-1β+IL-1RA treated cells C-D) Significant reduction of sodium current density in MGE-like cell treated with IL-1β compared to control and IL-1β+IL-1RA treated cells. E-F) The voltage dependence for steady-state activation profile didn't change in IL-1β treated MGE-like cells compared to control and IL-1β+IL-1RA treated. G-H) The voltage dependence for steady-state inactivation profile didn't change in IL-1β treated MGE-like cells compared to control and IL-1β+IL-1RA treated. I) The example trace recorded from sodium channel steady-state inactivation.

5.2.4 The reduction of sodium current in IL-1β treated MGE-like cells was due to reduction in single channel current of sodium channels

The influx of sodium current (I) is determined by single channel current amplitude (i) multiplied by the number of opening sodium channels (N) ($I = i * N$)(Sigworth 1980, Alvarez, Gonzalez et al. 2002). Therefore, either a reduction in i or N causes the reduction of sodium current in IL-1β treated cells. To determine the direct cause of sodium current reduction, Non-stationary fluctuation analysis (NSFA) (See method 2.3.4) was used to calculate i and N according to the variance (σ^2) from repeated recording against the mean current (I) and compare i and N between IL-1β treated and control cells (Alvarez, Gonzalez et al. 2002). The recording was performed in voltage clamp as described above. The cells were hyperpolarized to -80mv (200ms), then depolarized to -10mv (50ms) where the maximum currents were recorded in previous experiment. The recording was repeated at least 50 times for each cell. The rising phase of the sodium current was used for analysis. The data for variance against mean current was fitted with equation

$\sigma^2 = i * I - I^2/N$ (Figure 5-5 A). The different colors in Figure 5-5 B&C represent a set of data recorded from an individual cell in IL-1β treated or control condition. The fitted data in which R^2 is less than 0.8 was discarded (4 out of 19 in control cells, 5 out of 20 in IL-1β treated cells). The results revealed the single channel current was reduced around 30% in IL-1β treated cells compared to control (control n=16, IL-1β treated, 6.94±0.69 vs 4.76±0.55, two-tailed unpaired t-test, t=2.44, df=29, P=0.021) (Figure 5-4 D). Meanwhile, the number of opening sodium channels was similar between control and IL-1β treated cells. (207.9±27.87 vs 269.2±46.91 P=0.26) (Figure 5-4E) Since N represents only opening ion channels, the channel open possibility (P_O) was

calculated by equation$P_O = i * N / I_{max}$ .The result revealed the P_o in IL-1β treat cells was lower but not significant compared to control (0.93±0.02 vs 0.88±0.03, P=0.18) (Figure 5-4F). Together, the results demonstrate IL-1β mediated reduction in amplitude of single sodium channel current caused reduction of total sodium current in IL-1β treated MGE-like cells.

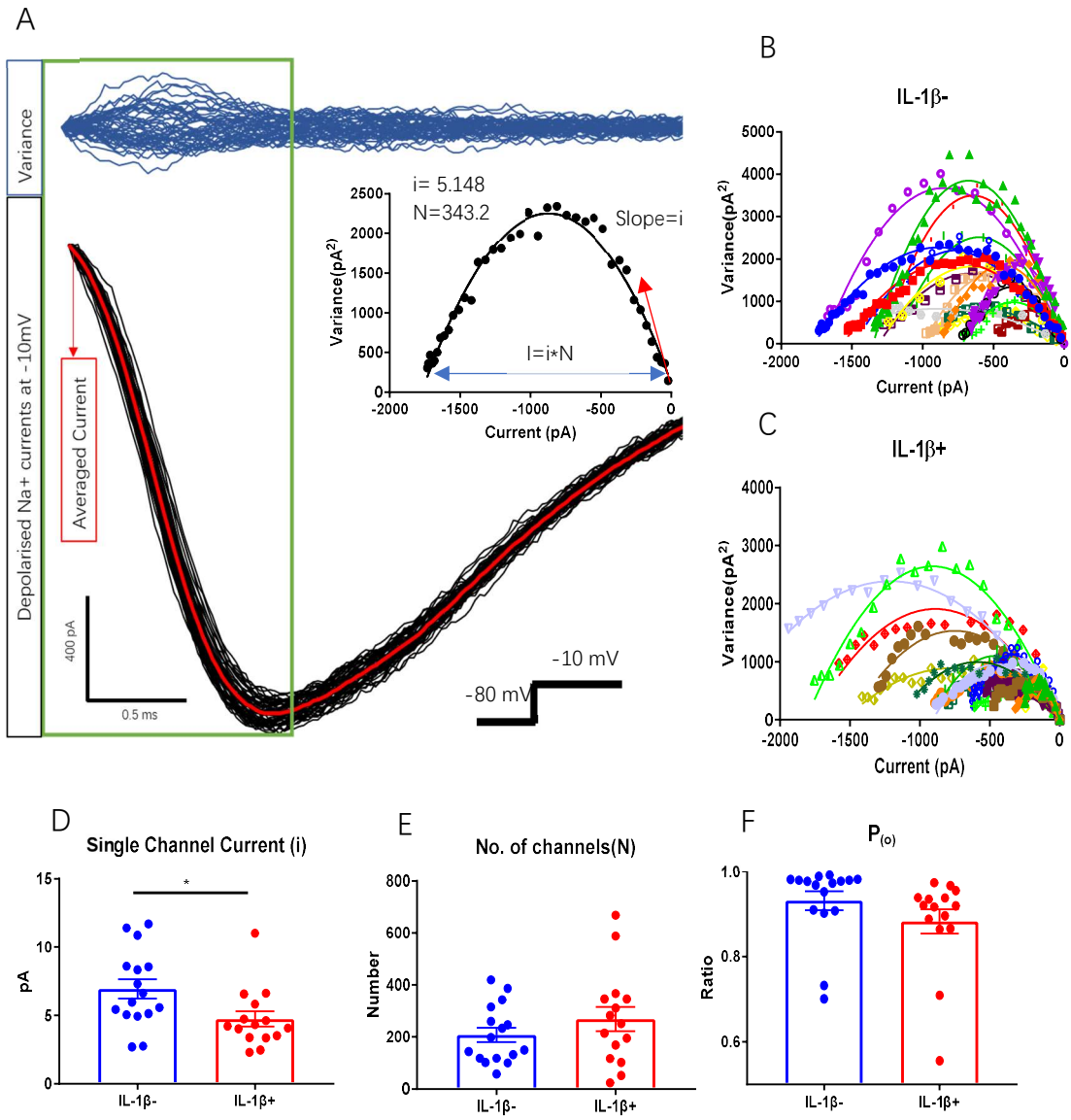

Figure 5-5 Acute exposure of IL-1β caused Reduction in single sodium current

A) The diagram for NSFA recording with voltage clamp. The cells were holding at -80mv before depolarization to -10mv. Each set of recording contains at least 50 repeats. The variance against averaged current in rising phase was fitted by equation $\sigma^2 = i*I - 1/N * I^2$. The parabola on the left side represent the date recorded on the right. The slope i represents the amplitude of single channel current, N represents the number of opening channels. B-C) Different color lines in the graph represent a set of data recorded from an individual cell in control or IL-1β treated MGE-like cells. D) The amplitude of single sodium channel current was significantly reduced in IL-1β treated cells compared with control.

E) The number of opening channels was similar between IL-1β treated cells and control. F) the opening possibility of sodium channels didn't change in IL-1β treated cells compared with control.

5.2.5 Intermittent exposure to IL-1β didn't change excitability in MGE-like cells

Previous experiments revealed that the cellular excitability of MGE-like cells was undermined by acute application of IL-1β. Whether this effect of IL-1β on MGE-like cells was transient or permanent was still unknown. In the following experiment, the MGE-like neurons were intermittently treated by IL-1β every three days from week 5 to week 6 before performing patch clamp recording (Figure 5-6 A). However, the data showed RMP and R_{in} didn't change in IL-1β treated cells (n=16 control, n=15 IL-1β treated -42.93±2.18mv vs -37.89±2.55 mV; 0.71±0.06 Gohm vs 0.69±0.09 Gohm; umpired t-test, P>0.05) (Figure 5-6B-C). Intermittent application of IL-1β did not change cell excitability either. The results showed that all cells in the IL-1β treated and untreated groups could fire overshooting APs upon current injection. The distribution of group 2 cells (7 vs 5) and group 3 neurons (9 vs 10) were similar in IL-1β treated and untreated cells (Figure 5-6D). Likewise, the number of APs in response to injected current steps and total number of APs corresponding to all current injection steps did not change in IL-1β treated cells compared to control (n=15 control, n=16 IL-1β treated, unpaired t-test, P>0.05) (Figure 5-6E-F). Subsequent analysis on the properties of single action potentials revealed no significant changes in rheobase, threshold voltage or amplitude of action potentials in IL-1β treated cells compared with control (two-way unpaired t-test, P>0.05) (Figure 5-6 G-I). These results suggest Intermittent application of IL-1β did not affect the excitability of MGE-like cells.

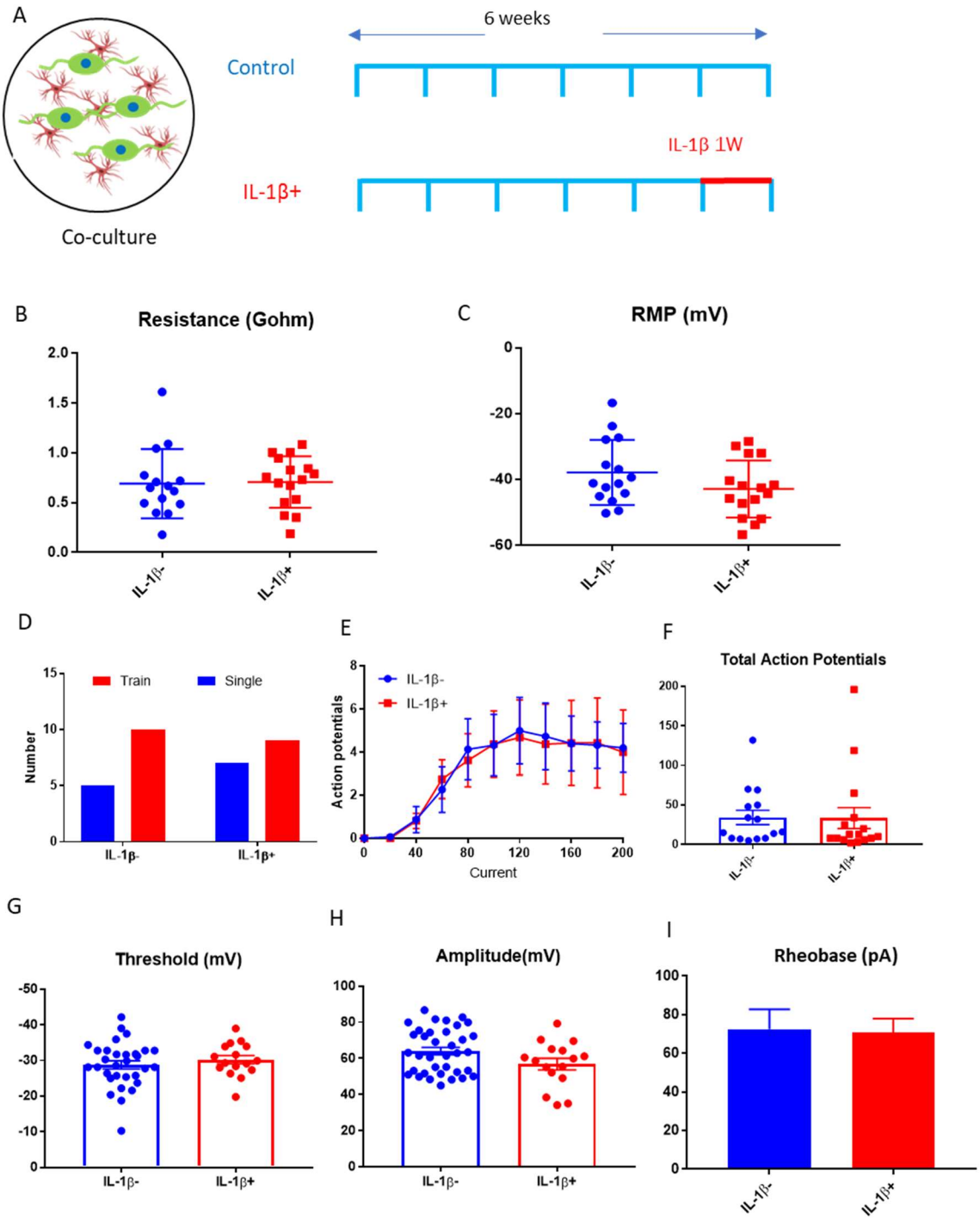

Figure 5-6 Intermittent treatment of IL-1β did not change the excitability of MGE-like cells

A) IL-1β was added every three days in to culture medium from week 5 to week 6 in neuron-astrocyte co-culture. B-C) The input resistance and RMP of MGE progenitors did not alter in IL-1β treated cells compared with control. D) The number of MGE cells showing single or train action potentials with and without IL-1β treatment E-I) The number of action potentials in response to current steps, the total number of action potentials, the properties of action potential and the threshold current were not affected by chronic exposure to IL-1β. (n=15 for control n=16 for IL-1β treated)

5.2.6 Intermittent exposure to IL-1β promoted branch sprouting

The effect of IL-1β on branch growth of electrophysiological functional MGE neurons was also investigated. Alexafluor 555 was added into the internal solution and the images of recorded MGE-like neurons were taken after patch clamp recording. Sholl analysis was used to compare the morphological difference between IL-1β treated MGE cells and control cells (Figure 5-7A). As shown from Figure 5-7 B, the curve showed an increased number of crossings from distance 50um to 200um in IL-1β treated MGE cells compared to control, suggesting increased branch complexity near the soma, but no changes in complexity and branch length in the distal area. The area under the curve was larger but not significant in IL-1β treat MGE cells compared with control (n=17, each group. Unpaired t-test, $P>0.05$) (Figure 5-7 C). In line with the Sholl analysis, the number of secondary and above branches were significantly higher in IL-1β treated MGE cells. The numbers of primary and total branches were not affected($P<0.05$) (Figure 5-7 D-F). The number of junctions indicates the intersection points between branches. IL-1β treated cells have more junctions than control cells ($P<0.05$) (Figure 5-7 G). On the other hand, the size of cells and soma long diameter did not change in IL-1β treated cells compared to control ($P>0.05$) (Figure 5-7 H-I). Together, the results demonstrate that IL-1β promoted branch complexity but did not change the morphological maturation of MGE-like neurons.

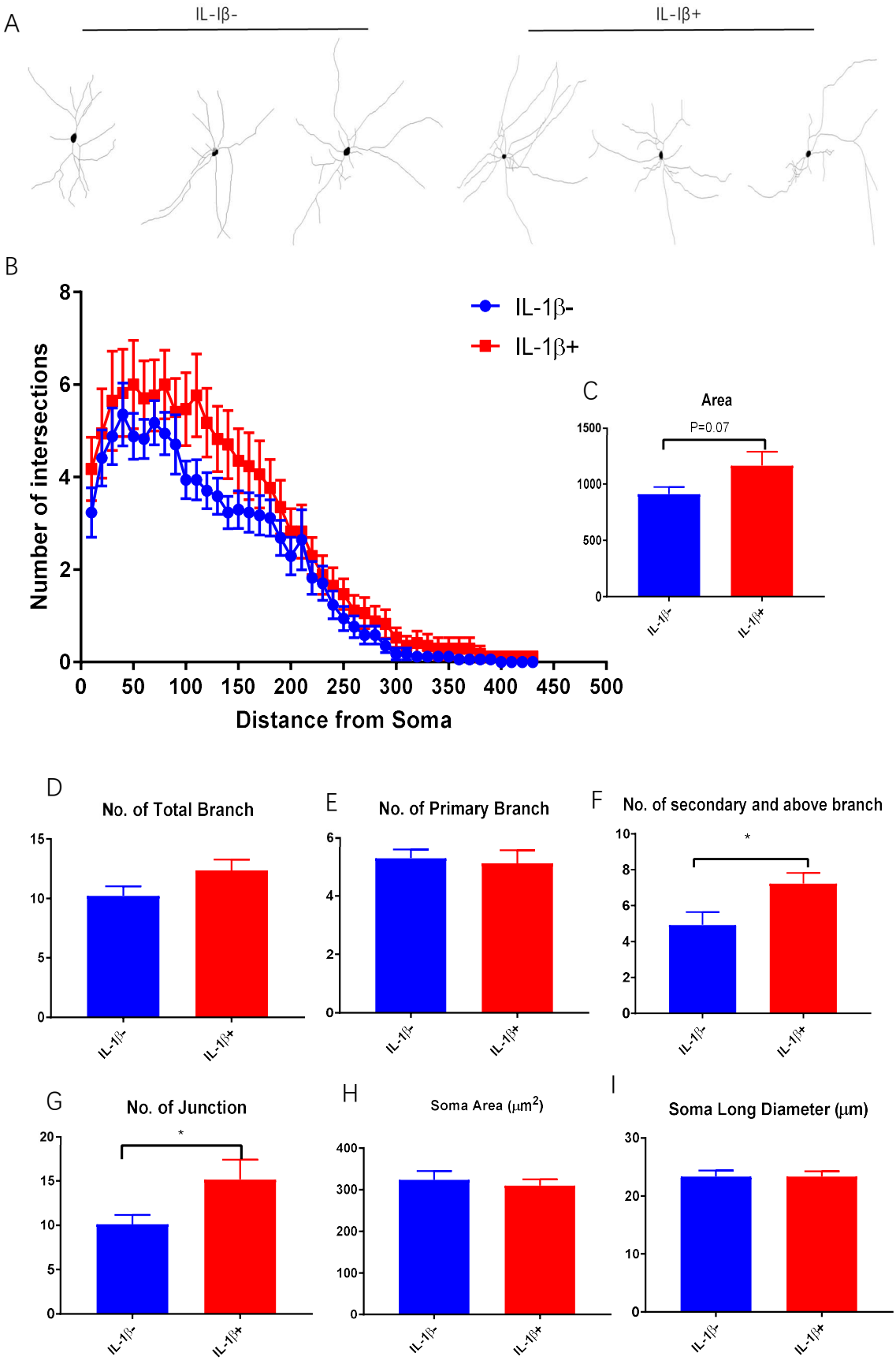

Figure 5-7 Chronic exposure to IL-1β promotes neurite growth in MGE cells

A) The morphology of represented neurons for patch clamp recording. B) The Sholl analysis compared the morphology of neurons with and without treatment of IL-1β. C-I) The area under the curve, the number of branches (total, primary, secondary and above), the number of junctions, soma area, soma long diameters were compared between cells with or without treatment of IL-1β. (N=15 for each condition, Data show mean± SEM, t-test, ∗P<0.05.)

5.2.7 Repetitive application of IL-1β reduced the excitability of MGE-like neurons

The previous experiment found that in contrast to 24hr treatment of IL-1β, one week's intermittent treatment of IL-1β did not reduce the excitability of MGE-like neurons. The contradictory results could be due to an inconsistent concentration of IL-1β in culture medium when applied for a week. The half-life for IL-1β is 2.5hr in vivo. So, the given concentration of IL-1β could be reduced over time in culture medium by metabolism. To investigate the time-dependent metabolism of IL-1β in the co-culture, the medium was sampled and measured by ELISA at 0h, 3h, 6h, 24, 48h and 72h after applying IL-1β (Figure5-8B). As shown in Figure 5-8 C, the concentration of IL-1β was maintained at around the initial level (10ng/ml) 24 hours after application and was significantly reduced to 2.5±0.3 ng/ml after 48 hours (n=8, one-way ANOVA, $F(6.49) = 155.9$, $P<0.0001$). At 72 hours, the concentration of IL-1β returned to baseline. These results suggested the effects of IL-1β on the excitability of MGE-like neurons was related to the its high concentration

Next, the long-term effects of a high concentration of IL-1β on MGE-like cells was investigated. As shown in the diagram (Figure 5-8A), MGE-like cells were cultured in two conditions. In condition 1, IL-1β was intermittently applied (every 3 days) to cells for a week. In condition 2, IL-1β was repeatedly applied (every 24 hours) to the cells for a week. Current clamp was used compare RMP, R_{in} and proportion of MGE-like cells fire attempt, single and train APs in condition 1 and 2 to control. Interestingly, the proportion of MGE-like cells which fired trains of APs was similar in condition 1 (63% vs 67%), but much lower in condition 2 compared to control (17% vs 67%). Moreover, in condition 2, 50% of examined cells fired only a single AP and 33% of cells couldn't fire an over shooting AP (Figure5-8D). The results also revealed no significant changes in RMP (n=15 control, n=16 condition 1, n=12 condition 2; one-way ANOVA, P=0.2) and R_{in} (n=15 control, n=16 condition 1, n=11 condition 2; one-way ANOVA, P=0.26) in condition 1 and condition 2 compared to control (Figure5-8E-F). Subsequent experiment using voltage clamp revealed that repetitive treatment of IL-1β induced 80% reduction of sodium current which could be prevented by IL-1RA (Figure 5-8 G-I) (n=10 for each condition, one-way ANOVA, $P<0.01$).

Together, the results suggest repetitive treatment of IL-1β further reduces excitability of MGE-like neurons.

5.2.8 Repetitive application of IL-1β induced death of astrocytes and affects the viability of MGE-like neurons

In previous experiment, reduced cell density could be observed in cultures treated by IL-1β. The immunostaining revealed that the number of $GFAP^+$ astrocytes significantly reduced in IL-1β treated condition (Figure 5-9 A, B). Therefore, the viability of MGE-like cells in different conditions was then inspected by PI-Annexin V staining (Figure 5-9 C). Propidium iodide (PI) is used in conjunction with Annexin V to determine if cells are viable, apoptotic, or necrotic. In the cell culture, the Annexin V^+ cells were co-localized PI^+, suggesting the cells were either necrotic (PI^+) or late apoptotic (PI^+ $Annexin^+$) (Figure 5-9 D). Only a few PI^+ GFP^+ cells could be observed in all three conditions (Figure 5-9 E). But the number of PI^+ and PI^+ Annexin V^+ cells significantly increased in condition 1 and condition 2 compared with control. Especially in condition 2, the cells were aggregated together, PI^+ and PI^+ Annexin V^+ cell could be observed predominantly in the centre of the cluster. (Figure 5-9 C). These results demonstrate the survival of co-cultured astrocytes was significantly reduced by the application of IL-1β.

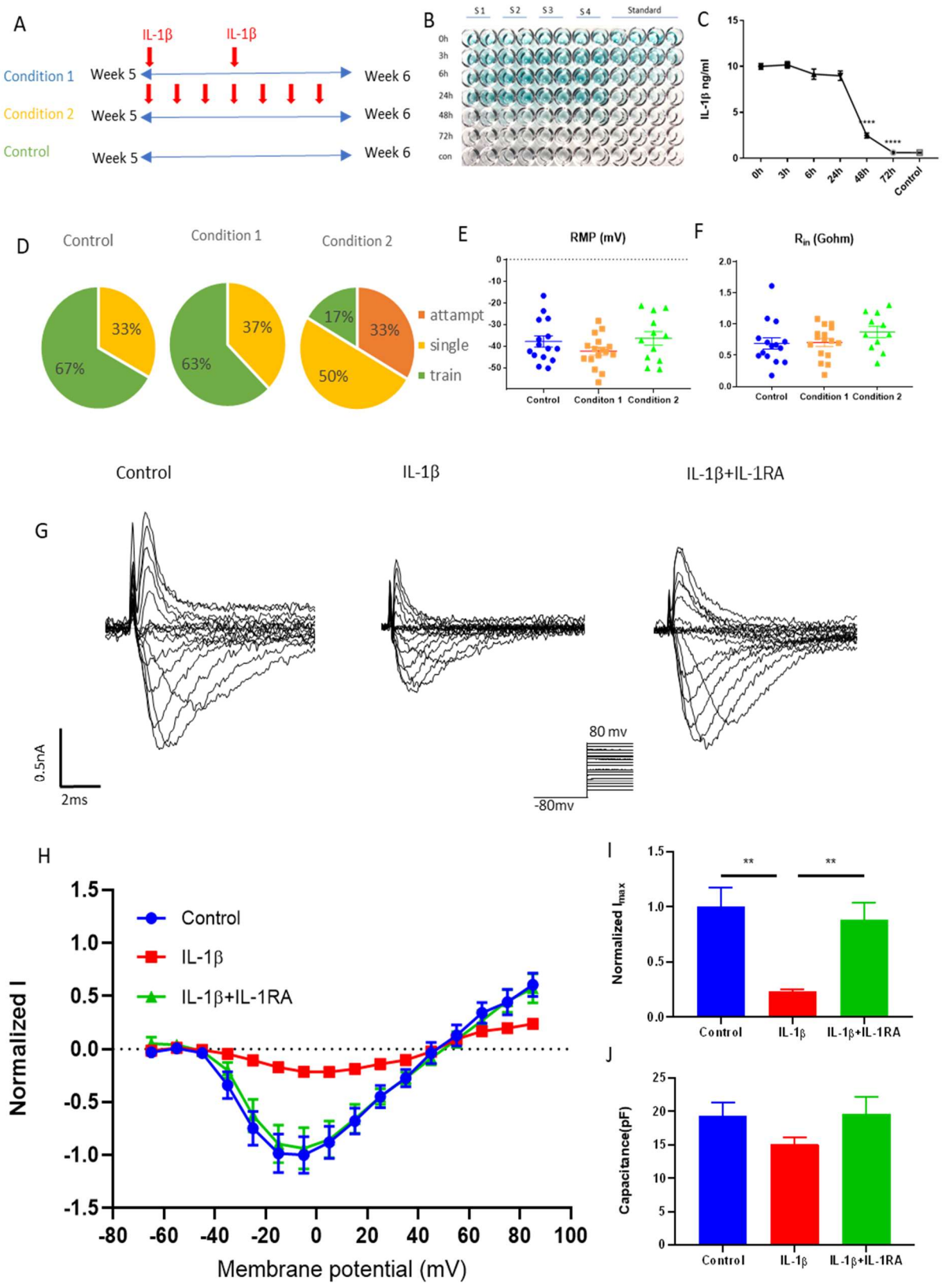

Figure 5-8 Repetitive application of IL-1β further reduced excitability of MGE-like neurons

A) The illustration of experimental conditions. In condition 1, the cells were intermittently treated with IL-1β for a week. In condition 2, the cells were continuously treated with IL-1β for a week. B-C) The ELISA showed the concentration of IL-1β in culture medium significantly reduced after 24 hours (n=4). D) The proportion of cells with attempt, single and train action potentials in each condition. E-F) The Ri_n and RMP of MGE progenitors were not altered in condition 1 and condition 2 compared with control. G-I) Sodium current recording revealed that repetitive treatment of IL-1β induced 80% reduction of sodium current which could be prevented by IL-1RA. J) The capacitance didn't change in IL-1β treated and IL-1β+IL-1RA treated cells.

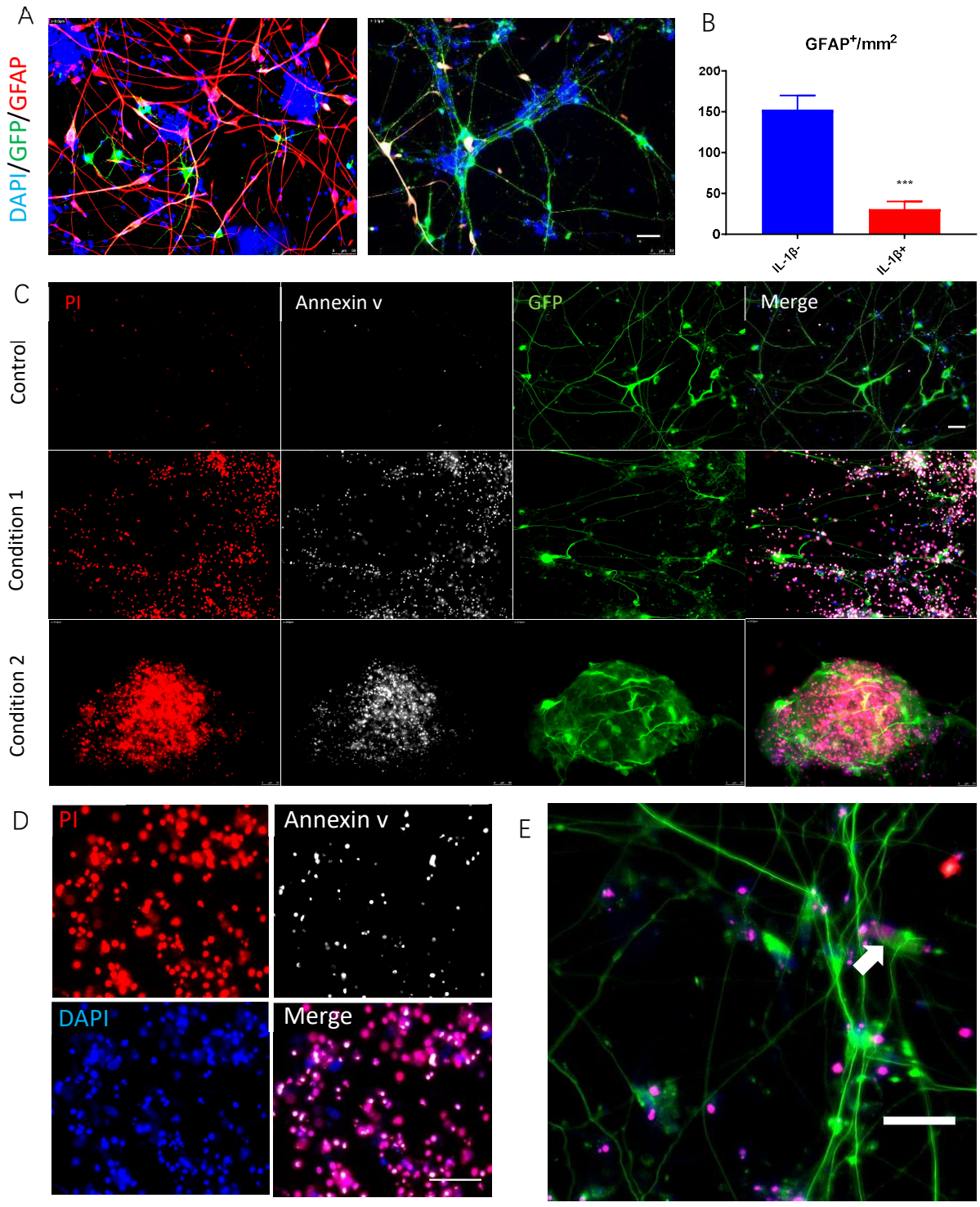

Figure 5-9 Repetitive application of IL-1β caused death of astrocytes and reduced viability of MGE-like neurons

A-B) IL-1β induced significant reduction of GFAP$^+$ astrocytes in MGE-like neuron-astrocyte co-culture. C) The PI/Annexin staining showed increased number of cells stained with PI and Annexin V in condition 1 and condition 2. And the cells were aggerated in clumps in condition 2. Scale bar 50um. D) Annexin V$^+$ cells were co-localized with PI$^+$. E) Only a few PI$^+$ GFP$^+$ cells (yellow arrow) could be observed in the culture

5.3 Discussion

Electrophysiological activity is a key indicator of functional mature MGE-like neurons. The MGE progenitors co-cultured with hippocampal astrocytes demonstrated overshooting APs as early as 2 weeks after co-culture (differ day 35), but it took 6 weeks for the majority of MGE progenitors to acquire mature firing properties. It is consistent with previous research that over shooting APs are observed at around differentiation day 40 to 60 in iPSC and hESC-derived MGE neurons (Nicholas, Chen et al. 2013, Kim, Yao et al. 2014). The RMP and R_{in} are corelated with the expression of ion channels. During neuronal differentiation, as more and more ion channels develop on the cell membrane, the RMP becomes more hyperpolarised and R_{in} is reduced. In this experiment, there was huge diversity among MGE-like neurons in terms of RMP and R_{in} at week 2. This suggests that electrophysiological maturation kinetics vary among MGE-derived neurons. 6 weeks after co-culture, RMP was around -45mV and R_{in} was around 1Gohm in recorded MGE cells. The results are similar to the RMP and R_{in} recorded from hESC or iPSC-derived MGE neurons in vitro (Nicholas, Chen et al. 2013) and early stage transplanted MGE progenitors in vivo (Cunningham, Cho et al. 2014). But it is worth noticing that although it may take several months after transplantation for differentiation of fully functional GABAergic neurons, the inhibitory output could be observed when the transplanted cells showed electrophysiological activity (Cunningham, Cho et al. 2014).

Increasing evidence supports a modulatory effect of IL-1β on different voltage gated ion channels, including K^+, Ca^+ and Na^+ channels in the CNS and PNS (Vezzani and Viviani 2015). In the experiment above, acute treatment with IL-1β caused a reduction in sodium currents in MGE-like neurons which led to attenuated excitability. In line with these results, IL-1β caused a 40% reduction in voltage-gated sodium current and 10mV reduction in the amplitude of the action potential in rat cortical neurons, and an 80% reduction of the sodium current in retinal ganglion cells (Diem, Hobom et al. 2003, Zhou, Qi et al. 2011). In contrast, IL-1β did not change sodium currents in rat hippocampal neurons and even led to a 67% increase of sodium current in trigeminal nociceptive neurons when also applied for 24hr (Liu, Yang et al. 2006). The concentration of IL-1β used in these experiments is much higher (ng/ml) than detectable concentration in human blood (pg/ml). Therefore, pathologically high levels of IL-1β have distinct effects on different types of neurons.

Sodium currents play a vital role in evoking and propagating action potentials. The reduction of sodium currents in inhibitory neurons is closely related to the occurrence of seizures (Lossin, Rhodes et al. 2003, Vanoye, Lossin et al. 2006, Yu, Mantegazza et al. 2006, Tai, Abe et al. 2014). Mutations in the gene encoded Nav1.1 (SCN1A) induced a reduction in sodium currents only in inhibitory neurons rather than excitatory neurons in mice, which caused hyperexcitability and led to spontaneous seizures (Yu, Mantegazza et al. 2006, Tai, Abe et al. 2014). The iPSC-derived inhibitory neurons generated from patients with Dravet Syndrome with a mutation in Nav 1.1 showed deficits in sodium currents and action potential firing (Sun, Pasca et al. 2016). Meanwhile, the cortical neurons generated from iPSCs of the same patient did not show alterations in sodium currents or firing frequency. These studies indicate that reduced excitability of inhibitory neurons accounts for epileptogenesis. Therefore, compromised electrophysiological activities of MGE-like neurons could interfere with the benefits of transplanting MGE progenitors in epilepsy.

The mechanism of how IL-1β affects sodium current is controversial. Although most studies suggest IL-1β works by activating its cognate receptor on cell membrane, a previous study suggested that the reduction of sodium current is independent of IL-1R1 receptor in RGC (Diem, Hobom et al. 2003). Our research found that co-application of the IL-1β antagonist IL-1RA blocked the reduction in sodium current caused by IL-1β. It indicated the effect of IL-1β was mediated by the IL-1R1 receptor. IL-1β activates its downstream signals as quick as 5 mins after binding to IL-1R1 receptors and 2-3% occupation of receptors is enough for biological effects (Dinarello 2009). Its rapid effect may elicit fast post-translational alterations in expression or structure changes in VGSCs. Sodium channel consist of a pore-forming α subunit, and a β subunit. Based on isoforms of the functional α subunit, the VGSCs are named Nav1.1 through Nav1.9, among which NaV1.1, NaV1.2, and NaV1.6 isoforms are highly expressed in the CNS (Maurice, Tkatch et al. 2001). Consistently, expression of Pan-Nav, NaV1.1, NaV1.2, NaV1.6 and Navβ2 were observed in MGE-like cells. So far, only one piece of published research shows a 40% reduced expression of the α1 subunit in mRNA levels after 2h treatment with IL-1β in cortical neurons (Liu, Yang et al. 2006). It is unclear whether other types of sodium channel could be modulated by IL-1β treatment. In addition, NSFA analysis suggested the reduction of sodium current is due to a reduction in single channel current rather than sodium channel expression.

Although the results from NSFA showed significant reduction in channel conductance in IL-1β treated compared to control, it may not be sufficient to draw a conclusion. One issue in the

acquired data is that the variation within groups of recorded cells is large, possibly due to the variety of interneuron phenotypes and their different maturation kinetics(Kepecs and Fishell 2014). Secondly, the single sodium current calculated from NSFA represents macroscopic current which is sum of currents from several sodium channels rather than the unitary sodium current (Lingle 2006, Nekouzadeh and Rudy 2007). Therefore, the data of single sodium current in average from my experiment is significantly larger than previous reports which used excised recording (Wartenberg and Urban 2004, Vanoye, Lossin et al. 2006, Chatelier, Zhao et al. 2010). In addition, in my experiment, the number of recording sweeps may not be sufficient to accurately predict the variance. Based on computer simulation, the variance will be more precisely recorded when one increases the number of recording sweeps (Lingle 2006). However, in my experiment, the hESC-derived neurons were too fragile to endure repetitive simulation for extended long time recording. So, the error of the data can affect the accuracy of calculation for number of channels and single channel current. Further experiments using excised patch clamp recording will help to confirmed whether conductance of sodium channel is affected by IL-1β.

Sodium channel current is regulated by phosphorylation sites in segments of the pore forming α unit. Those phosphorylation sites are regulated by protein kinase A (PKA) or protein kinase C (PKC) in different types of neurons (Marban, Yamagishi et al. 1998, Scheuer and Catterall 2006, Scheuer 2011). A single site phosphorylation is sufficient to cause a reduction in the amplitude of sodium currents without affecting the activation and inactivation kinetics of sodium channels (Smith and Goldin 1997). In rat striatal neurons, the activation of PKA raised the threshold for firing and was associated with a 35% reduction in sodium currents. Likewise, the activation of PKC also reduces the sodium current in cholinergic interneurons (Maurice, Mercer et al. 2004), hippocampal neurons (Numann, Catterall et al. 1991) and other brain neurons (Numann, Catterall et al. 1991). There are also several reports which suggest IL-1β can regulate the phosphorylation of ion channels. In mouse heart cells, IL-1β increased PKC translocation and led to a reduction of calcium current (El Khoury, Mathieu et al. 2014). In rat cortical neurons, activation of PKC by IL-1β caused a 37% inhibition of sodium current (Zhou, Qi et al. 2011). Therefore, it is highly likely that IL-1β regulates sodium currents of MGE-like cells through activation of a phosphorylation protein. Further experiments are required for confirmation.

Pro-inflammatory cytokines are preserved at a high level for several days after seizures and brain injury. IL-1β was intermittently or continuously applied to MGE-like neurons to simulate the

chronic inflamed environment. The results were interesting. 1 week's intermittent application of IL-1β didn't change excitability of MGE cells due to concentration of IL-1β returned to baseline after 72 hours, but 1 week's continuous application of IL-1β caused further reduced excitability of MGE-like neurons, produced severe death of astrocytes and eventually affected the viability of MGE-like neurons. Together, the results suggest the excitability of MGE-like cells could recover when IL-1β was reduced to a non-pathological concentration. On the other hand, when IL-1β was maintained in high concentration, MGE-like cells lost the essential support from astrocytes which affected their functionality and viability. During the sodium current recording, the unhealth cells which showed high leaky current and low sodium current were excluded from the results. Therefore, the effect of repetitive treatment of IL-1β on MGE-like neurons could be underestimated. Consistent with the results, a study showed 48 to 72 h incubation of IL-1β didn't affect the viability of pure cortical neuron cultures but caused caspase induced cell death in glia-neuron cocultures and pure astrocyte cultures (Thornton, Pinteaux et al. 2006). So, it is more likely the neurotoxic effect of IL-1β on MGE-like cells is mediated by astrocytes. Although MGE-like cells and hippocampal astrocytes both expressed the IL-1 receptor, evidence showed IL-1β activated distinct signal pathways in neurons and astrocytes. It can activate the p38 mitogen-activated protein kinase (MAPK) signalling pathway in hippocampal neurons while activating NF-κB in hippocampal astrocytes (Srinivasan, Yen et al. 2004). This may be the reason for different reaction of MGE-like neurons and astrocytes after IL-1β treatment.

In contrast to previous findings in hi-spot culture that IL-1β reduced the branch growth of MGE-like cells, this experiment showed that intermittent treatment of IL-1β promoted neurite growth of MGE-like neurons. This contradictory effect could be due to differences in duration for IL-1β exposure and culture context. In the present experiments, the cells were co-cultured with astrocytes and IL-1β treatment for only 24h. Several studies suggested IL-1β promotes axon growth and modulates neurite growth and regeneration when applied in a healthy microenvironment. The effect is mediated by facilitating the production of growth factors or neurotropic factors from astrocytes or microglia (Parish, Finkelstein et al. 2002, Boato, Hechler et al. 2011). In an inflamed microenvironment, IL-1β dominantly demonstrated its detrimental effects. For example, IL-1β exacerbates brain damage and mediates neuron degeneration in acute brain injury and PD respectively (Touzani, Boutin et al. 2002, Koprich, Reske-Nielsen et al. 2008).

In general, acute exposure to IL-1β temporarily compromised activities of MGE neurons. It could be one of the consequences for increased susceptibility of seizures. Blocking the effect of IL-1β after seizure may help to restore the balance of neuron circuits. Chronic exposure to IL-1β would lead to astrocyte death which would further affect the viability of MGE-like neurons. Therefore, a healthy microenvironment is vital to guarantee the functionality of MGE-like neurons.

6. Generating a 3D Hi-Spot culture from hESC derived cells to further study the inflamed environmental effect on MGE derived progenitors

6.1 Introduction

In chapter 3&4, hi-spots generated from human brain tissue have been used to simulate the inflamed microenvironment of sclerotic hippocampus. However, the disadvantage of the model can't be ignored. Firstly, the availability of human brain tissue is always limited. Secondly, the severity of pathological alternations varies between patients, so the concentration and combination of inflammatory cytokines are not always consistent or controllable in primary brain tissue. To further understand the mechanism of how IL-1β affect MGE progenitors, a new 3D culture model which is more consistent and manageable has been established in this chapter.

With developing understanding of immune response towards injury, infection and neurological diseases in CNS, inflammatory cytokines have been discovered to affect cell survival, neurogenesis, neuron excitability which are vital for progress of many brain diseases in CNS (Kokaia, Martino et al. 2012, Vezzani, Aronica et al. 2013). The inflammatory mediators have controversial effects varying from their concentration, and duration. Cells of innate and adaptive immune system are activated and release inflammatory mediators to remove lethal pathogens immediately after insults (Kokaia, Martino et al. 2012, Xanthos and Sandkuhler 2014). But the prolonged and intensive production of inflammatory mediators in certain area may bring more detrimental rather than beneficial effects on CNS (Vezzani, Aronica et al. 2013, Xanthos and Sandkuhler 2014).

The microenvironment of CNS is constituted by two parts; the regulatory chemicals released by immune cells and the crosstalk via cell-to-cell connection. Conventional single level monolayer culture creates a lack of complexity and tissue-organization from different levels of cells. Systematic time-consuming animal models produce conflicting and inconsistent results to clinical studies due to difference in genetic context between human disease and animal models. Human

embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) derived neuronal cells has been widely used to study the pathology and physiology of CNS (Choi, Kim et al. 2014, Yu, Di Giorgio et al. 2014). The neuronal cells of human origin will be more relevant than primary cultures of animal neuronal cells in terms of anticipating therapeutic target and disease modelling (Schwartz, Hou et al. 2015). Self-assembled aggregate cultures generated from pluripotent neuron stem cells, such as encompass spheroids, embryoid bodies and neurospheres could construct cytoarchitecture of cerebellum and cortex (Pasca, Sloan et al. 2015) (Bershteyn, Nowakowski et al. 2017) (Muguruma, Nishiyama et al. 2015). But it will take more than half year for growing the organoids and cell apoptosis usually observed in the centre of the organoid when the organoid grows bigger.

To solve the problem, 3D culture which preserve the complexity of cell to cell interaction is used in some studies. Neurons cultured in special engineered scaffold or nutritious gels matrix have better cell survival and neurite outgrowth than the cells in 2D culture (Navaei-Nigjeh, Amoabedini et al. 2014, Sun, Liu et al. 2016). To simulate brain microenvironment, a recent study constructs the neural cells into tissue-like 3D structure that mimic dynamic mechanical properties and biochemical functionalities of whole living organs (Bailey, O'Connor et al. 2011). **Therefore, 3D culture of human ES-derived neuronal cells could allow us to investigate the effect of microenvironment on CNS in a more relevant manner without needed to rely on primary human tissue cultures.**

To investigate the influence of microenvironment on survival and differentiation of hESC-derived neuron progenitors, a 3D model which include major cellular components with preserved organ-like structure and complexity is required. In this research, a 3D culture model including hESC-derived excitatory cortical progenitors, inhibitory neuron progenitor and astrocyte, which is an important component for neurogenic niche (Ma, Ming et al. 2005) has been established. The results demonstrate three types of cells can proliferate and differentiate in hi-spot culture. Furthermore, similar to the previous results observed in human hi-spot culture, IL-1β caused reduced survival of MGE progenitors and astrocytes in this 3D model. In addition, reduced cell proliferation was also observed in IL-1β treated hi-spots. These results provide evidence for using hi-spots generated from hESCs to test the influence of pathological microenvironment in vitro.

6.2 Results

6.2.1 Generate and characterise telencephalic excitatory neurons and inhibitory neurons from hESC

First, hESCs were differentiated into telencephalic excitatory and inhibitory neurons in parallel. To generate excitatory neuron progenitors, dual SMAD inhibitors (SB-451542 and LDN-193189) were applied from day 0 to day 12 (Figure 6-1 B). The cells were then passaged and plated on poly-D-ornithine and laminin coated plate for post-mitotic differentiation. The excitatory neurons are derived from dorsal telencephalon. Pax6 is the marker for dorsal telencephalic progenitors. The immunostaining revealed that the expression of Pax6 could be observed in neural rosettes at post differentiation day 20 (Figure 6-1C). At post differentiation day 40, the cells expressed cortical layer specific marker Trb2 and immature neuron marker DCX (Figure 6-1D). These results suggested the cells achieved identity of excitatory cortical progenitors.

The protocol for generating inhibitory neurons was described in (3.2.1)

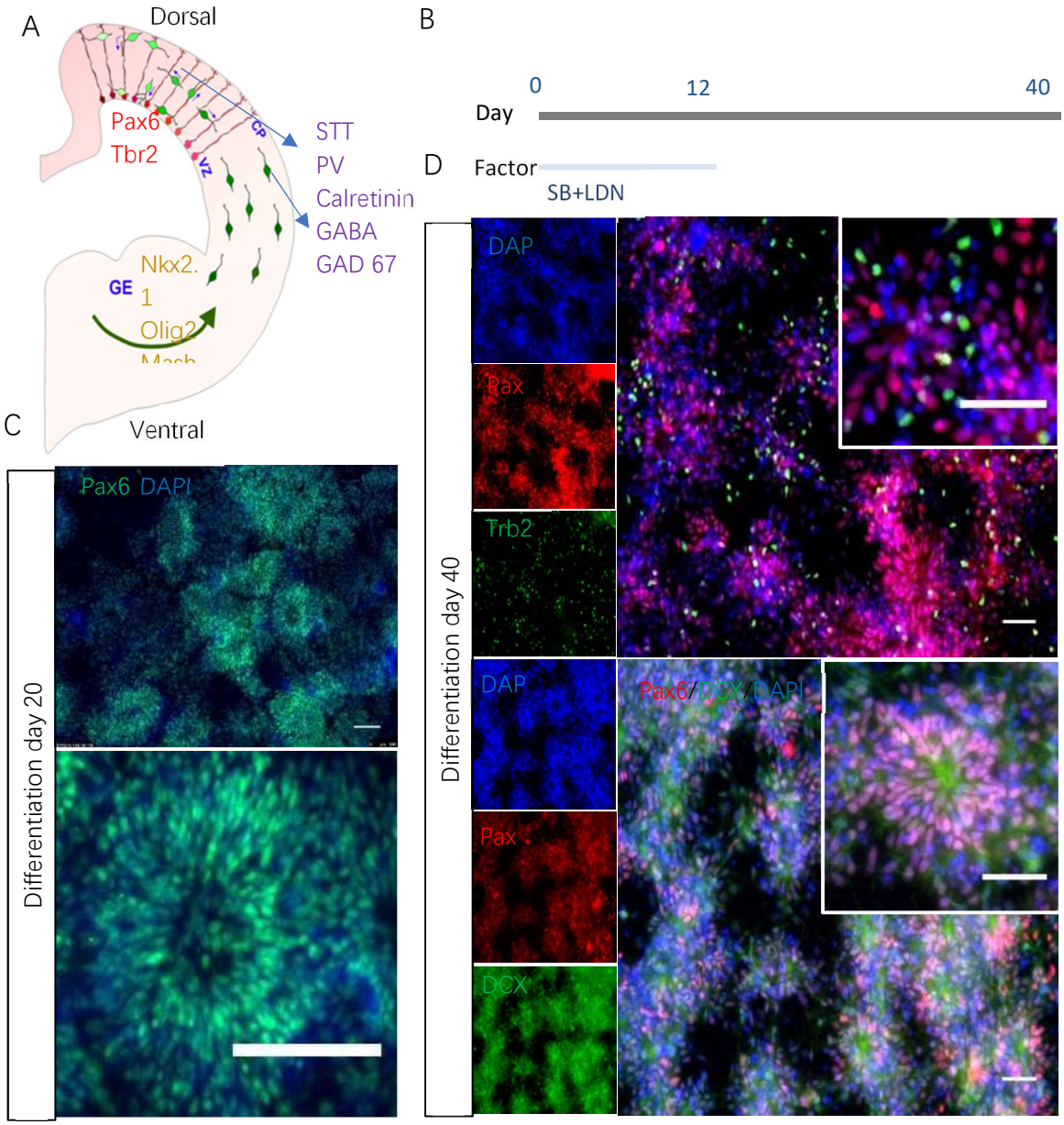

Figure 6-1 Generating cortical neuron progenitors from hESC.

A) The illustration for development of cortical progenitors and inhibitory interneuron progenitors during embryonic development. B) Schematic timeline of cortical progenitor differentiation from H7 cell line. SB451542, inhibitor of the TGFb1 activin receptor-like kinases; LDN193189, inhibitor of bone morphogenetic protein (BMP) type I receptors. C) On differentiation day 20 the cells robustly expressed PAX6 and rosette-like structure Scale bar 100um.D) On differentiation day 40, the cells co-expressed specific cortical neuron marker Trb2, early neuron marker DCX and Pax6. Scale bar 50um

6.2.2 Generating astrocytes from hESC

Astrocytes are born after neurons during embryonic development(Krencik, Weick et al. 2011). Therefore, astroglia differentiation comes after neuron induction. A two-stage protocol was applied to generate astrocytes (Figure 6-2 A). At the first stage, from differentiation day 0 to differentiation day 20, the cells were differentiated into cortical progenitors by the protocol described above. The cells were then dissociated and plated at density of 10000/cm^2 on PDL coated plate. FGF2 (20ng/ml) and 10% fetal bovine serum (FBS) were applied to induce astroglia differentiation from day 21 to day 34(Roybon, Lamas et al. 2013, Shaltouki, Peng et al. 2013, Tcw, Wang et al. 2017). The cells were then fixed for immunostaining. The expression of S100β and GFAP, which are markers for astrocyte(Ludwin, Kosek et al. 1976), significantly increased in FBS and FGF2 treated cells compared to untreated. (Figure 6-2 B, D) (n=4, two-way unpaired t-test, $P<0.001$). In contrast, the expressing of DCX was significantly downregulated in FBS and FGF2 treated cells compared to untreated ($P<0.001$). While, the proportion of $Pax6^+$ cell was similar between FBS and FGF2 treated and untreated (Figure 6-2C, D). These results suggested FBS and FGF2 promote astroglia differentiation without changing the regional identity of where the cells derived from (Krencik, Weick et al. 2011).

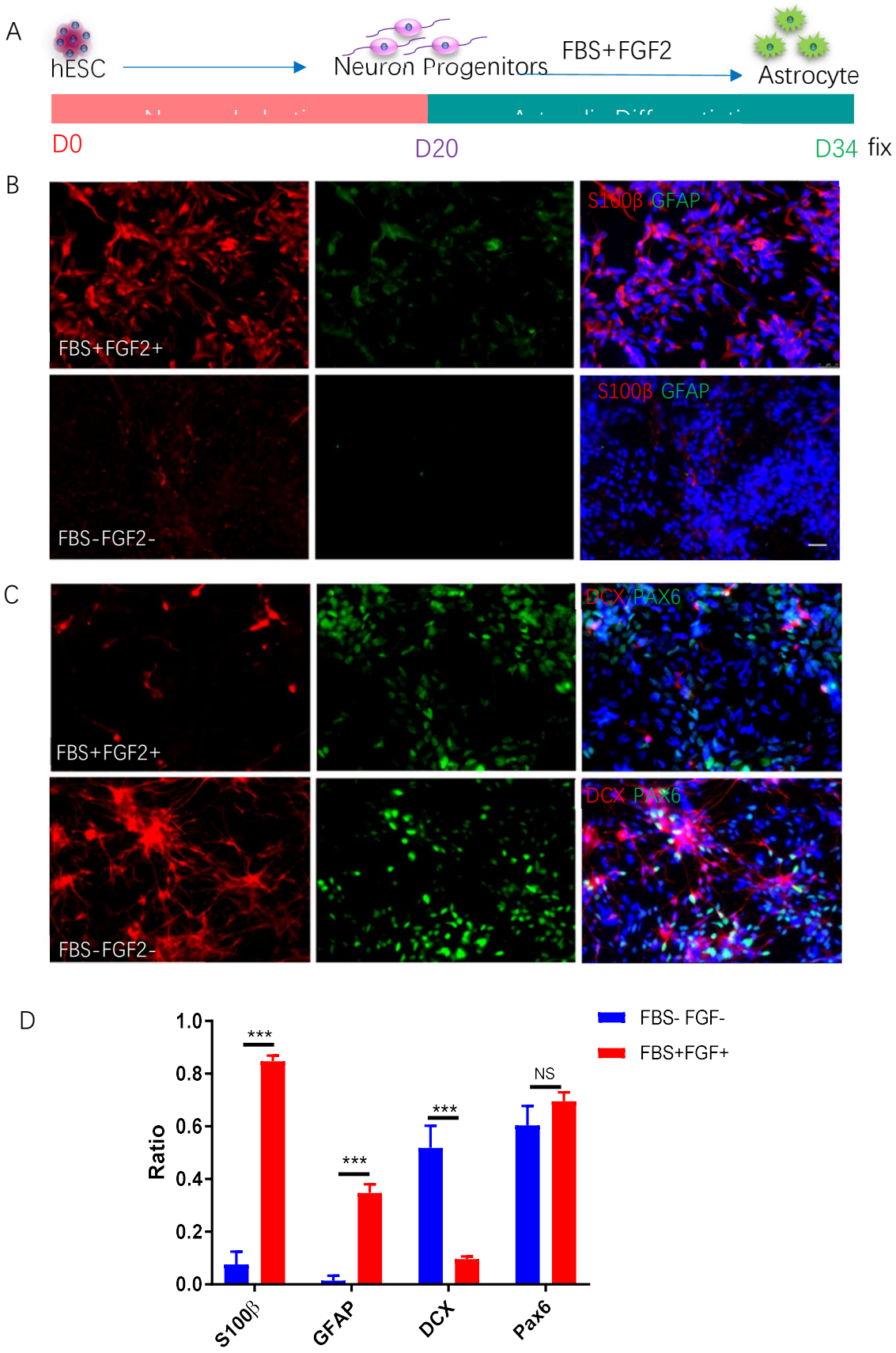

Figure 6-2 Generating astrocytes from hESC

A) Schematic timeline for generating astrocyte from hESC. Neuron progenitors were generated from hESC from day 0 to day 20 with the protocols described previously. The N2B27 medium were replaced by medium containing FBS 10% and FGF2 20ng/ml for 14 days to induce differentiation of astrocytes. B) The immunostaining showed expression of astrocyte marker S100β and GFAP on cells cultured in FBS and FGF2 containing media and control. Scale bar 50um C) The immunostaining showed expression of DCX and Pax6 on cells cultured in FBS and FGF2 containing media and control. Scale bar 50um D) The bar graph compared the expression of S100β, GFAP, DCX and Pax6 in FBS and FGF2 media to control

6.2.3 Astrocytes precursors generated from MGE progenitors demonstrated distinct phenotypes

The astrocytes show distinct phenotypes in different brain area(Olude, Mustapha et al. 2015). To figure out whether astrocytes arise from different origins affect their phenotypes, I first compared morphology of astrocyte precursors derived from MGE progenitors to astrocyte precursors derived from cortical progenitors (Figure 6-3 A). As shown in the graph, cortical progenitor derived astrocyte precursors showed larger soma than MGE progenitor derived astrocyte precursors. Moreover, the cortical progenitor derived astrocyte precursors demonstrated ramified structure while most MGE progenitor derived astrocyte precursors were fusiform. Next, the immunostaining was used to compare expression of astrocyte marker (GFAP and GLAST) and glia marker (nestin) between two types of astrocytes (Figure 6-3 B). The result revealed the expression of GFAP, GLAST and nestin were significantly lower in MGE-derived astrocytes compared to cortical progenitor derived astrocytes (Figure 6-3 C) (n=4, two-way unpaired t-test, $P<0.001$). These results suggested the astrocytes generated from different telencephalic area have different phenotypes.

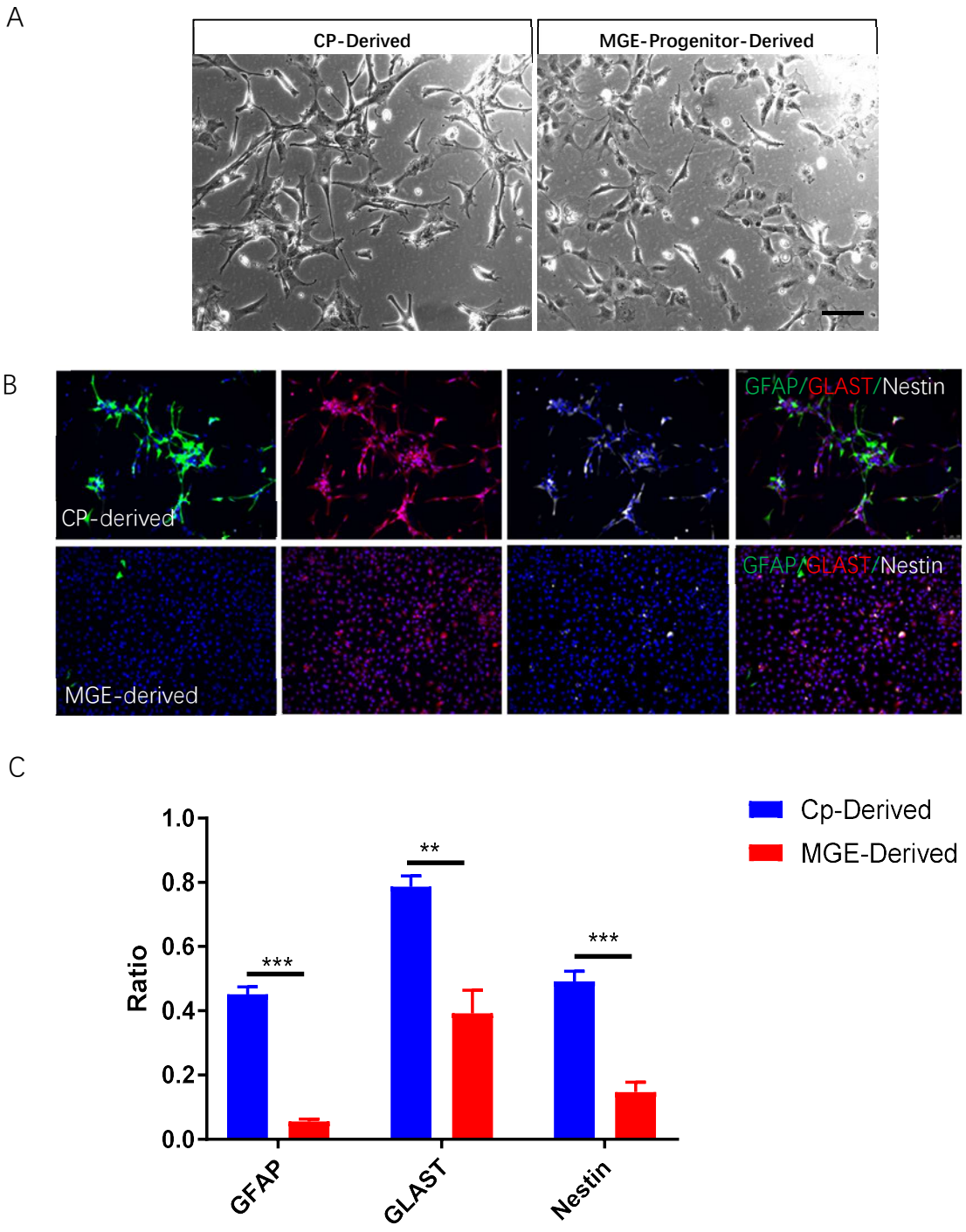

Figure 6-3 Astrocyte generated from cortical and MGE progenitors showed distinct phenotype

*A) The morphology of Cortical progenitor (CP) and MGE progenitors derived astrocytes on differentiation day 35. B) The immunostaining showed expression of GFAP, GLAST and Nestin. Scale bar 50um. C) The bar graph compared the expression ratio of GFAP, GLAST and Nestin between CP and MGE progenitor derived astrocytes. (n=4, two-way t-test, **P<0.01, ***P<0.001)*

6.2.4 Generate hi-spot culture from hESC-derived neural cells

Hi-Spot culture is a new cell culture method developed for culturing postnatal CNS tissue which is hard to maintain in monolayer culture. The brain tissue was reorganized as a self-organised, dense, organotypic culture in air-liquid surface. With hi-spot culture, it is possible to recreate brain microenvironment and observe survival and differentiation of neuronal cells in vitro. The excitatory neuron, inhibitory neuron and astrocyte are the major components of brain tissue. Having established the protocol to generate those cells individually, the three types of cell were mixed together to generate Hi-spot culture. The method was described by illustration (Figure 6-4). 1% MGE progenitors generated from GFP-labelled H7 cell line, 9% cortical progenitors from normal H7 cell line and 90% astrocyte precursors derived from cortical progenitors were mixed to generate hi-spot cultures.

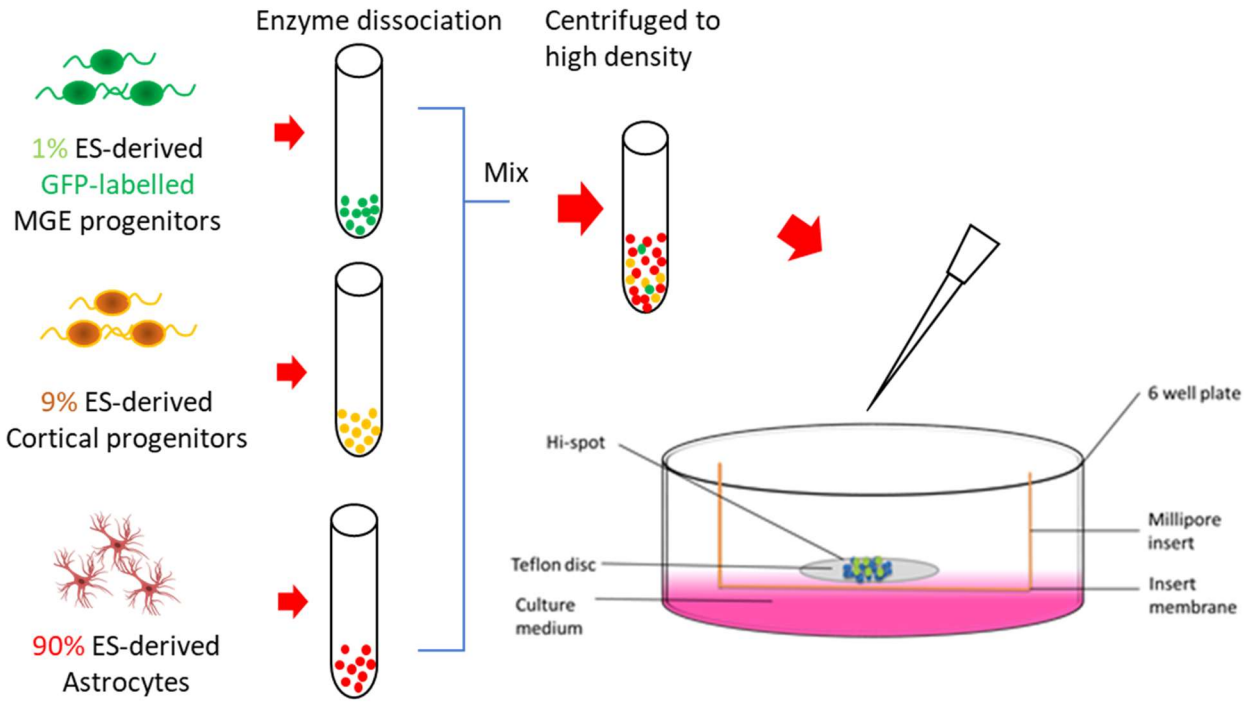

Figure 6-4 The illustration of generating hi-spot culture from hESC-derived neural cells

6.2.5 The influence of culture medium on differentiation of neuron progenitors and astrocytes precursors

After generating hi-spot culture, the influence of culture medium on differentiation of neuron progenitors and astrocyte precursors was inspected initially. Serum-rich medium (5% horse serum + 20% FBS) is used for culturing hi-spots with human brain tissue. N2B27 medium is used for culturing neuronal cells in monolayer culture. Firstly, the number and neurite growth of GFP labelled MGE progenitors were compared at 3 weeks and 6 weeks post hi-spot culture in two culture mediums (Figure 6-5A-B). The results showed that the number of GFP expressing MGE progenitors increased about 50% in both Serum and N2B27 medium 6 weeks post hi-spot culture compared to 3 weeks (n=8 for each condition, two-way ANOVA, $P<0.001$) (Figure 6-5 C). Sidak's multiple comparisons test revealed the number of MGE progenitors was slightly lower but not significant in serum medium compared to N2B27 medium (Figure 6-5 C). Correspondingly, total neurite length of MGE progenitors increased about three times at 6 weeks post culture compared to 3 weeks. And neurite length of MGE progenitors was similar in two media (Figure 6-5D). These results suggested the MGE progenitors grew and survived in hi-spot culture with both culture medium.

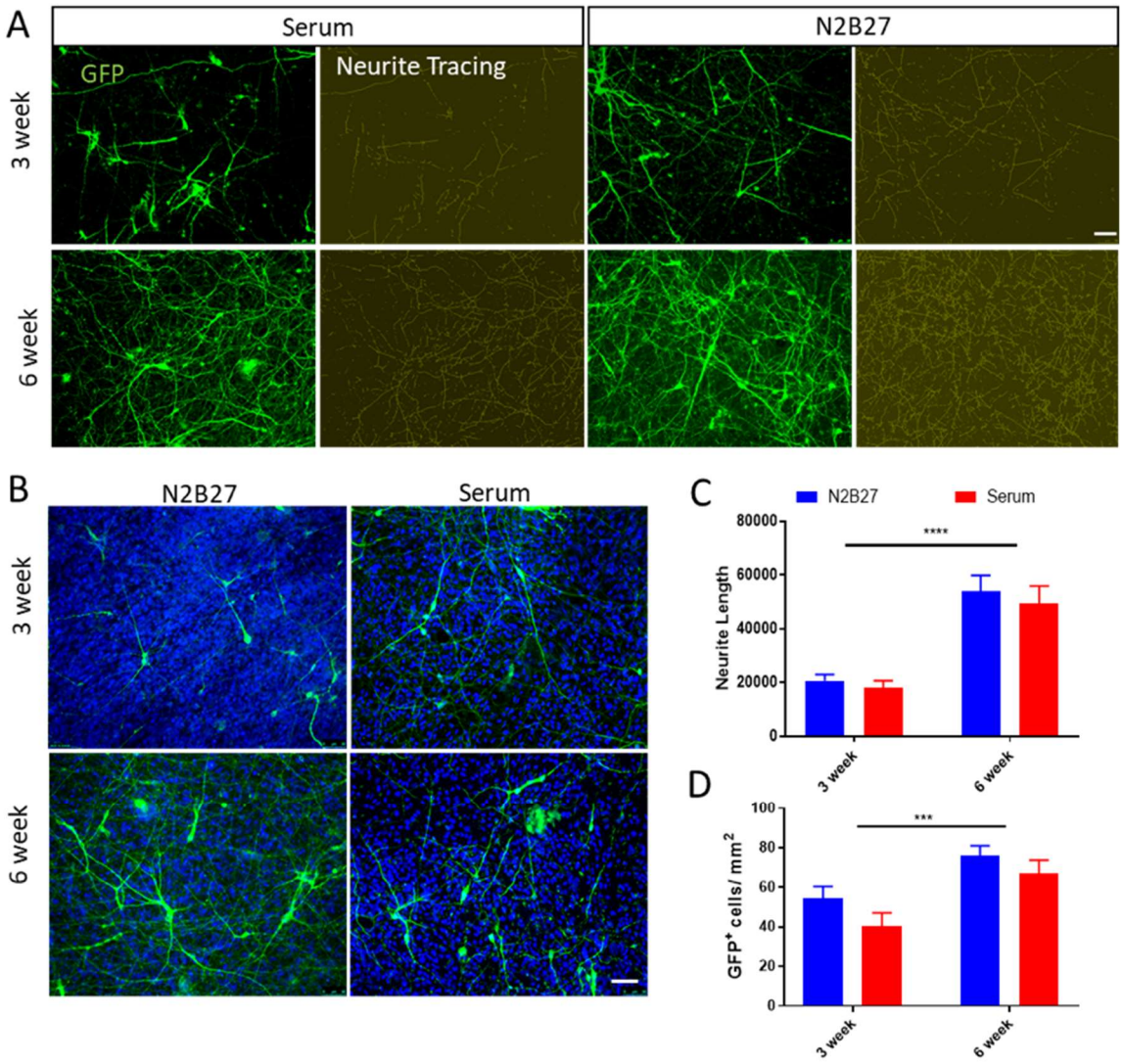

Figure 6-5 Culture medium affects the proliferation and neurite growth of MGE neurons

*A-B) The image showed GFP-labeled MGE neurons in two culture media (Serum vs N2B27) at 3 week and 6 weeks after generating the hi-spot. The neurite tracing image showed the skeleton of processors. Scale bar 50um. C-D) The bar graph compared the number and neurite length of MGE neurons in hi-spot culture for 3 and 6 weeks in N2B27 to the cells in Serum-rich medium. (N=8, two-way anova, ***P<0.001, ****P<0.0001.*

Next, cell proliferation was inspected by comparing the number of ki-67+ cells between two media (Figure 6-6A-B). The result revealed the number of ki-67+ cells was around four times higher in N2B27 medium compared to serum medium (Figure 6-6C) (n=8, two-way t-test, $P<0.001$). Consistently, the number of total cells was significantly higher in N2B27 medium compared to serum medium (Figure 6-6E). Figure 6-6A shows the number of ki-67+ MGE progenitors is quite low and similar between two media. (Figure 6-6A-C) ($P>0.05$). Interestingly, the number of GFAP+ Ki-67+ cells was significantly higher in serum medium compared to N2B27 medium (n=8, two-way t-test, $P<0.001$). And the number of GFAP+ cells was also higher in serum medium compared to N2B27 medium (n=8, two-way t-test, $P<0.001$). About 60% ki-67+ cells were GFAP+ cells in serum medium, but only 5% ki-67+ cells were GFAP+ in N2B27 medium (Figure 6-6C). These results suggested in serum medium, hESC-derived astrocyte precursors were preserved and proliferated, while in N2B27 medium the astrocyte precursors lose its genetic marker.

Finally, we inspected the growth of neuron cells using immunostaining of Tuj1 and compared the growth of processors between two media (Figure 6-6 D). The result showed that the Tuj1+ cells formed complex neural network in hi-spot culture in both medium. But the total length of Tuj1+ processors was significantly higher in cells cultured with N2B27 medium compared to serum medium (Figure 6-6 D-F) (n=8, two-way t-test, $P<0.01$). Together, these results suggest that N2B27 medium is in favour of neuronal differentiation but not astroglia differentiation.

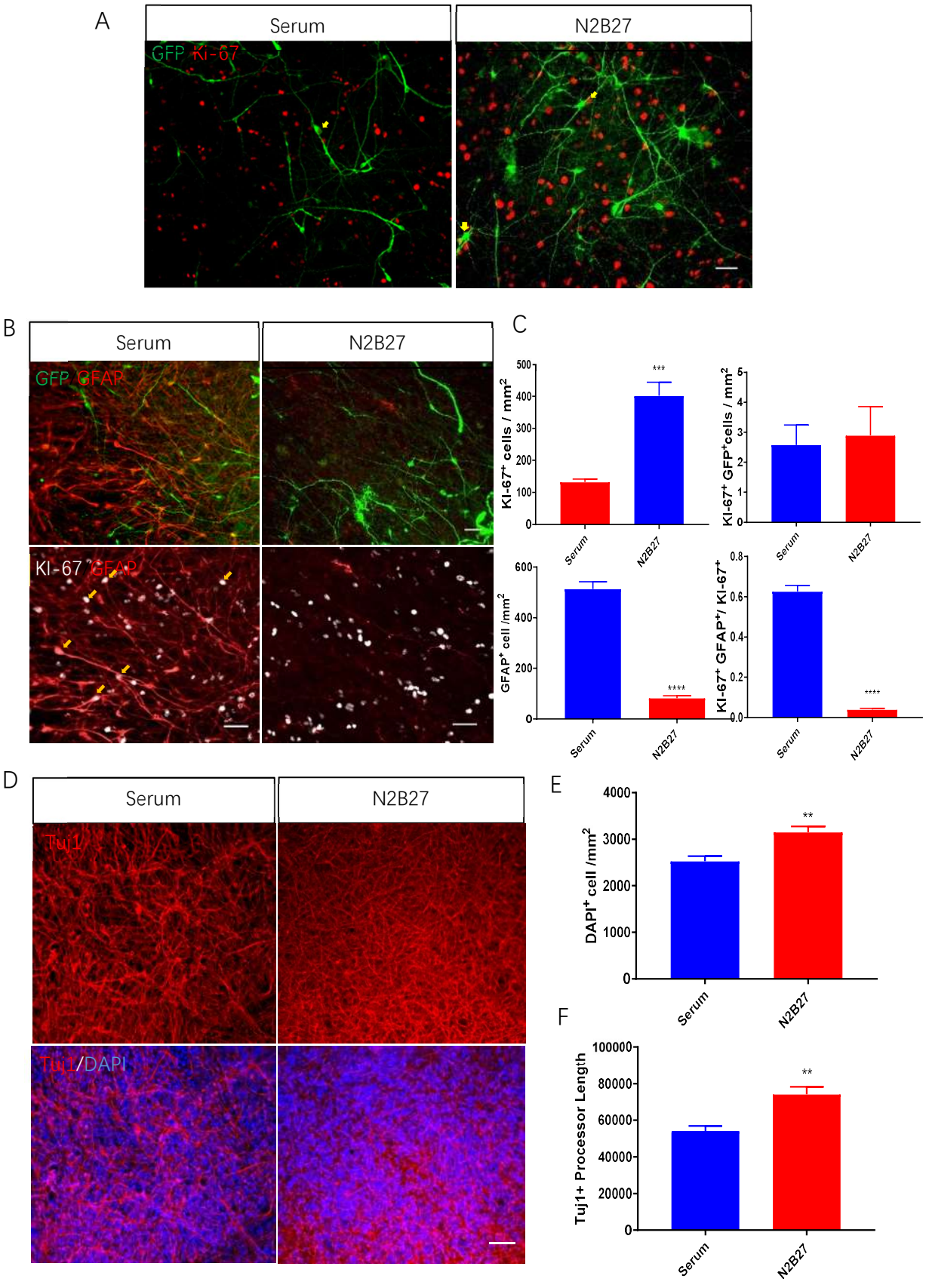

Figure 6-6 Culture medium affect astroglia differentiation and cell proliferation

*A-C) The immunostaining showed only a few MGE progenitors are Ki-67+ (Yellow arrow) in both culture medium. B-C) The immunostaining showed GFAP+ cells were significantly reduced in hi-spots cultured in N2B27 medium compared to serum medium. And in serum medium, some GFAP+ cells also co-stained with Ki-67(Yellow arrow). Scale bar 50um. D-F) The number of cells (DAPI) and total length of Tuj1-expressing neurite were significantly lower in hi-spot cultured in serum medium compared to N2B27 medium. (n=4, two-way t-test, **P<0.01, ***P<0.001, ****P<0.0001)*

6.2.6 Inflamed microenvironment caused reduced cell survival

Having established hESC hi-spot culture, the next step is trying to investigate whether the effect of IL-1β in human hi-spots can be replicated in hESC hi-spots. The hi-spots were cultured in four groups. In IL-1β treated groups, IL-1β was given every three days (Figure 6-7A). First, I compared the survival of MGE progenitors in IL-1β treated to untreated hi-spots. The result revealed the number of MGE progenitors in hi-spots cultured in serum medium was significantly reduced by IL-1β (Figure 6-7B, D) (n=8, two-way anova, Tukey's multiple comparison, $P<0.05$). But in hi-spots cultured in N2B27 medium, the number of MGE progenitors is similar between IL-1β treated and untreated hi-spot (Tukey's multiple comparison, $P>0.05$). Likewise, the neurite length of MGE progenitors cultured in serum medium not N2B27 medium was significantly reduced by IL-1β compared to untreated cells (Figure 6-7C) (two-way ANOVA, Tukey's multiple comparisons, $P<0.05$).

Next, the effect of IL-1β on cell proliferation was evaluated by Ki-67 immunostaining. The result showed the number of ki-67+ cells reduced about 50% in IL-1β treated hi-spot cultured with serum medium (Figure 6-7F) (n=8, two-way t-test, $P<0.01$). While in N2B27 medium, the number of Ki-67+ cell was similar between treated and untreated hi-spots (Figure 6-7E) ($P>0.05$). From previous result, more than half Ki-67+ cells are GFAP+ cells in serum medium. So, I next inspected the number of GFAP+ and GFAP+Ki-67+ cells in hi-spot cultured with serum medium and compared them between IL-1β treat and untreated condition.

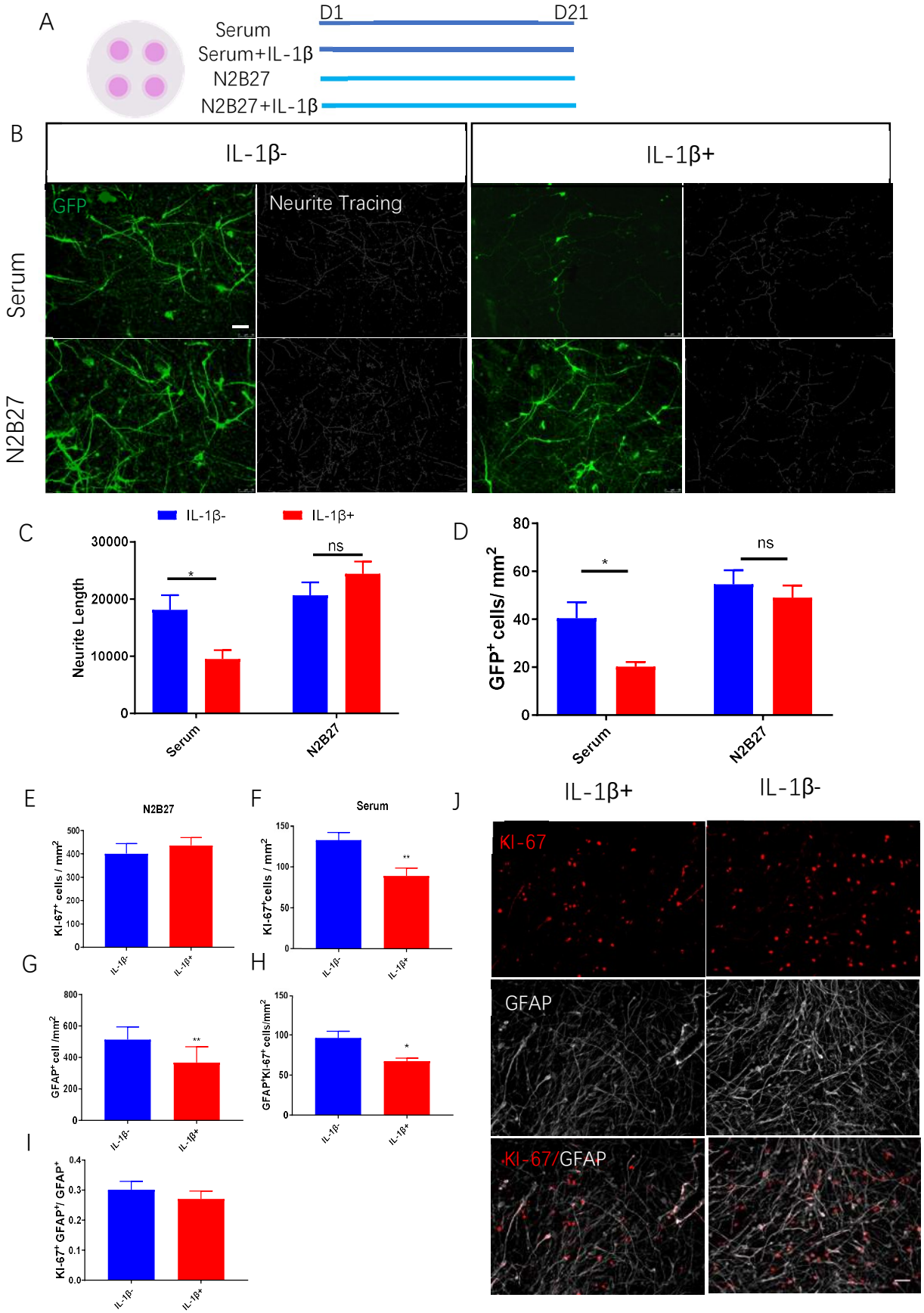

Figure 6-7 IL-1β reduced cell survival and cell proliferation in hi-spot culture

A) The diagram of experimental condition. Hi-spot cultured in serum and N2B27 medium were treated with IL-1β(10ng/ml) for 21 days before fixation. B) The image showed the morphology and neurite growth of MGE cells in 4 different experimental group. C-D) The bar graph compared the number and neurite length of MGE cells and IL-1β treated hi-spots to untreated. E) The bar graph compared the number of ki-67+ cells in IL-1β treated to untreated hi-spots cultured in N2B27 medium. F) The bar graph compared the number of ki-67+ cells in IL-1β treated to untreated hi-spots cultured in serum medium. G-I) The bar graph compared the number of GFAP+, GFAP+Ki-67+ cells and ratio of Ki-67+GFAP+over GFAP+ cell between IL-1β treated and untreated hi-spot cultured in serum medium. J) The immunostaining showed the expression of GFAP and Ki-67 in IL-1β treated and untreated hi-spots cultured in serum medium.

The result revealed the number of $GFAP^+$ cells was significantly lower in IL-1β treated hi-spot compared to untreated (Figure 6-7G) ($P<0.01$). But the ratio of Ki-67+GFAP+ cells over the population of GFAP+ cells was similar between IL-1β treated and untreated (Figure 6-7I) ($P>0.05$). Correspondingly, the number of GFAP+ Ki-67+ cells was significantly lower in IL-1β treated hi-spot compared to untreated (Figure 6-7G) ($P<0.05$). Together, these results indicated IL-1β caused reduced survival of neurons and astrocytes in hi-spot culture in serum medium but not in N2B27 medium.

6.3 Discussion

The microenvironment of the CNS plays an important role in regulating pre- and post-natal neurogenesis. In this research, a 3D hi-spot culture model, aiming to simulate the human brain microenvironment in vitro, was generated from cortical progenitor, MGE progenitor and astrocytes derived from hESCs. First, the hESC hi-spot culture was characterized in serum and N2B27 medium. The result showed serum rich medium didn't alter differentiation of neurons and astrocytes in hi-spot culture. While N2B27 medium only favoured neuronal differentiation but not astroglia differentiation. Furthermore, decreased number of surviving MGE progenitors and astrocytes and reduced cell proliferation was observed when IL-1β was added to the hi-spots cultured in serum medium but not the hi-spot cultured in N2B27 medium.

Serum free medium like N2B27 is usually used for differentiation of neurons. Some research has suggested that at neuron stem cell / progenitor stage, addition of serum in high concentration will interfere with neuronal differentiation (Hu, Ji et al. 2013). As proved by the experiment, cortical progenitors expressed astrocyte marker and lost their neuronal marker when cultured with 10%

FBS for two weeks. On the other hand, serum medium is necessary for survival and maintain astrocytes precursors in vitro. In the experiment, even defined astrocyte precursors lost their genetic marker when cultured in N2B27 medium. So, in monolayer culture, it is hard to maintain both types of cell in the same culture medium for a long time. The 3D hi-spot culture seemed to be a solution for the conflict. As proved by the experiment, neurons and astrocytes simultaneously proliferated and matured in hi-spot culture in serum rich medium.

Astrocytes are a major component of the neurogenic niche, providing structure support, instructive signal for neurogenesis and promote synaptic transmission of neurons (Song, Stevens et al. 2002, Johnson, Weick et al. 2007, Bamba, Shofuda et al. 2014). The heterogeneity of astrocytes makes it hard to classify their phenotypes(Olude, Mustapha et al. 2015). There is evidence suggesting that astrocytes originating from different brain regions have distinct regulatory effects on local neurons. Hippocampal astrocytes, rather than spinal cord- derived astrocytes, instruct the hippocampal neuron stem cell to adopt a neuronal fate (Song, Stevens et al. 2002). Midbrain astrocytes instead of cortical astrocytes promote neuronal progenitors to develop into dopaminergic phenotype (Roy, Cleren et al. 2006). Consistently, I found astrocyte precursors generated from MGE progenitors and cortical progenitors were different in morphology and expression of astrocyte markers, indicating different functions of two type of astrocytes.

The inflamed microenvironment plays an important role in pathology of many neurological and neurodegenerative disorders. IL-1β is one of dominant pro inflammatory cytokines released after brain injury. Some studies suggested IL-1β reduces neuron progenitor cell proliferation and neuronal differentiation and enhances gliogenesis (Crampton, Collins et al. 2012, Zunszain, Anacker et al. 2012). In line with these observation, reduced number of MGE progenitors and decreased cell proliferation was observed in the hi-spot cultured with serum medium. In contrast, there was no evidence for enhanced gliogenesis but reduced number of GFAP+ astrocytes in hi-spot culture. One possibility is that the hi-spots were repetitively treated with IL-1β for several weeks before immunostaining. So, cell apoptosis due to IL-1β is dominant. Interestingly I also found hi-spot cultured with N2B27 medium were immune to the detrimental effect of IL-1β. This may be due to lack of astrocytes in hi-spots cultured with N2B27 medium. As shown in the experiment, the majority of astrocytes induced by FGF2 and FBS in hi-spot culture didn't express astrocyte marker after 3 weeks' culture in serum free N2B27 medium. Evidence suggest the

neurotoxicity of IL-1β is mediated by releasing of free radicals from dying astrocytes (Thornton, Pinteaux et al. 2006). And although neurons and astrocyte both express IL-1 receptor, IL-1β activated different secondary signals when combined with receptors on neuron and astrocyte. Therefore, it is more likely that reduced survival of neurons in serum medium is an astrocyte mediated effect from IL-1β.

In general, the hi-spot culture is an innovative model which simulate brain microenvironment in vitro. There are still room left to optimize the hi-spot culture. The local immune cells like microglia and macrophage of human origin will be introduced to the hi-spot culture in the future. The hi-spot culture is based on human cells, so well-constructed hi-spot culture will include structure and complexity which is more relative for human disease modelling than monolayer culture. The hi-spot culture also provides us with an access to modulate the microenvironment and identify the therapeutic target for drug testing.

7. General Discussion

7.1 Summary of Results

This thesis has reported a 3D hi-spot culture model which is composed of human brain tissue coming from patients undergoing epilepsy surgery to recreate the pathological microenvironment of chronic epilepsy. With this model, it is possible to investigate the effects of epileptic microenvironment on human transplanted MGE-progenitors. In Chapter 3, the results revealed that the inflamed microenvironment of chronic epilepsy is detrimental for survival, neurite growth and differentiation of transplanted hESC-derived MGE progenitors in vitro. As IL-1β is abundant in sclerotic hippocampus we identified it as a potential target for improving the outcome of transplanted progenitors. In Chapter 4, applying IL-1β alone acutely reduced the survival of transplanted MGE progenitors and delayed their morphological maturation in non-inflamed hippocampal hi-spots. By blocking IL-1β activation of the ILR1 receptor, the detrimental effects of microenvironment on neurite growth and differentiation of MGE progenitors were partially reversed. These findings highlight the potential importance of an optimal microenvironment for achieving optimal outcomes from cell transplantation for chronic epilepsy and identify the IL-1β – IL1R1 pathway as an important component of the pathological microenvironment relevant to cell replacement therapy in human TLE.

Next, in chapter 5, to investigate the effect of IL-1β on the functional profile of MGE progenitors, patch clamp recordings were performed on MGE-like neurons which have acquired mature electrophysiological activities when cultured with human hippocampal astrocytes in physiologically and developmentally relevant media. This experiment revealed that IL-1β acutely reduces the excitability of MGE-like neurons by modulating voltage gated Na+ channels (VGSCs), and this effect was reversed by IL-1RA. Chronic application experiments showed that the cellular excitability of MGE-like neurons can recover when the pathological level of IL-1β returns to baseline over 24-72 hours. However, when IL-1β was repetitively applied for a week, MGE-like neurons became unhealthy and their excitability was reduced even more. The results provide evidence that an inflamed microenvironment can reduce the excitability off MGE-like neurons. It

also indicates a potentially novel mechanism of interneuron dysfunction in the inflamed microenvironment of epilepsy.

Finally, the last chapter established a new 3D culture model which is composed of hESC-derived human neurons and astrocytes to simulate the microenvironment of human brain. In line with previous results, the study shows IL-1β in serum-based medium caused a reduction in the survival of hESC-derived MGE progenitors and astrocytes. This model can be refined and extended in the future by introducing microglia, epithelial cells and other immune cells, so it will be possible to simulate and modify the microenvironment of human brain in vitro to recognize elements which affect neuron survival, differentiation and functionality.

7.2 Implications for optimising the microenvironment in neuron transplantation.

Cell replacement therapy for treating neurological and neurodegenerative disorders has been investigated for decades. Linage specified hESCs and ihPSCs show great potential for treating PD, HD and epilepsy. The potential application can also extend to Alzheimer's, stroke and psychiatric disorders in which loss or dysfunction of certain type of neurons are implicated. However, in some neurodegenerative disease, such as epilepsy, PD and Alzheimer's Disease, the microenvironment may completely change due to pathological activity which causes activation of the innate and adaptive immune systems (Vezzani, French et al. 2011, Kokaia, Martino et al. 2012, Meraz-Rios, Toral-Rios et al. 2013, Stojkovska, Wagner et al. 2015). Accumulated inflammatory cytokines are released from local microglia, and monocytes and immune cells recruited from blood stream (Allan, Tyrrell et al. 2005, Aktas, Ullrich et al. 2007, Vezzani, French et al. 2011). Even neurons and astrocytes participate in secreting inflammatory cytokines(Watt and Hobbs 2000, Vezzani, Moneta et al. 2002). In line with many reports demonstrating that inflammatory cytokines cause neuronal injury and degeneration (Toulmond, Parnet et al. 1996, Monje, Toda et al. 2003, Allan, Tyrrell et al. 2005, Aktas, Ullrich et al. 2007, Vezzani and Baram 2007, Hewett, Jackman et al. 2012), my results show for the first time that the inflammatory microenvironment is detrimental for exogenous transplanted MGE progenitors as well. Therefore, it is possible that the benefits coming from neuron transplantation will be attenuated in an acutely or chronically inflamed

microenvironment. By applying IL-1RA, the detrimental effects of IL-1β on transplanted MGE progenitors is partially blocked in inflamed microenvironment of TLE. These results support the hypothesis that optimising the microenvironment can potentially improve the outcome from neuronal transplantation.

Although human hi-spots have provided an opportunity to investigate the pathological microenvironment in vitro, the disadvantage of the model can't be ignored. Firstly, the availability of human brain tissue is always limited. Secondly, in sclerotic hippocampus, there are other inflammatory cytokines which are detrimental to transplanted cells. And it is difficult to control the inflammatory milieu in primary human Hi-Spots. To further understand the mechanism of how inflammatory cytokines work, the hESC-derived hi-spots will be a useful tool which can control the complexity of the model, provide consistent and controllable inflammatory microenvironment to study interactions between inflammatory cytokines in the future.

7.3 Implications for effect of IL-1β in inflamed microenvironment

Despite intense research efforts, the mechanisms of IL-1β in regulating neuron survival, maturation and functionality are not clearly defined. There are conflicting results between in vitro and in vivo findings. For example, some in vitro experiments show that IL-1β has protective effects for decreasing neuron death (Strijbos and Rothwell 1995, Diem, Hobom et al. 2003, Hewett, Jackman et al. 2012). But clinical data and rodent models of epilepsy favour the idea that pathological level of IL-1β increase neuron degeneration which further promote the occurrence of seizures (Vezzani, Conti et al. 1999, Vezzani, Moneta et al. 2000, Aronica and Crino 2011). Temporal factors are important , as when IL-1β is applied before injury, a predominant increase in neuron damage is observed in both in-vivo and in-vitro studies (Vezzani, Conti et al. 1999, Fogal, Hewett et al. 2005). One of the reasons for the contradictory results is that IL-1 seems to exert complex effects by interacting with, and being influenced by, multiple cell types in the brain (Allan, Tyrrell et al. 2005).

7.3.1 Cell survival

When IL-1β was applied to non-inflamed hippocampal hi-spots and hESC-derived hi-spots cultured in serum-based medium the survival of MGE progenitors was reduced. However, when the IL-1β/ IL1-R1 pathway was blocked in epileptic hippocampal Hi-Spot tissue, the survival of transplanted MGE progenitors didn't improve. In non-inflamed cortical hi-spots and hESC-derived hi-spots cultured in N2B27 medium, addition of IL-1β hasn't affected cell survival at all. These apparently conflicting results imply that IL-1β may not directly cause the death of MGE progenitors but does so by interacting directly and indirectly with an inflammatory milieu and other facilitating cells such as astrocytes and microglia, which also release pro and anti-inflammatory cytokines. Evidence shows the effect of IL-1β is amplified by recruiting and activating local microglia, astrocytes and immune cells from blood stream (Basu, Krady et al. 2002, Tichauer, Saud et al. 2007). It is important to remember that in epileptic hippocampal hi-spots, immune cells and microglia have been recruited and the inflammatory cytokines have been accumulated before generation of the hi-Spot. Therefore, other signaling pathways which cause cell death have been already activated before giving IL-1RA. On the contrary, in non-inflamed hi-spots, only inactivated local immune cells exist, so adding IL-1β merely evokes immune response locally and is not strong enough to cause similar detrimental effect as the inflammatory cytokines in epileptic hi-spots do. Although microglia are present in all major divisions of the brain, the distribution of microglia is much denser in hippocampus compared to cortex (Lawson, Perry et al. 1990) and therefore the immune responses are milder in cortex than hippocampus. That may explain why there is no significant death of MGE progenitors in cortical hi-spots treated with IL-1β. Similar to my result, a in vivo study shows injection of IL-1β (20ng) into normal cortex fails to cause any brain damage (Stroemer and Rothwell 1998).

Although microglia haven't been included in hESC-derived hi-spots, hESC-derived astrocytes comprise 90% of the cell population in these hi-spots. A wide variety of cytokines, chemokines, and growth factors are secreted by astrocytes in response to IL-1β (Meeuwsen, Persoon-Deen et al. 2003). There is also research showing that the neurotoxicity of IL-1β is mediated by the release of free radicals from dying astrocytes (Thornton, Pinteaux et al. 2006). Consistent with this discovery, I found a significant decrease in the number of surviving $GFAP^{+}$ astrocytes along with a reduction in MGE progenitors in IL-1β treated hi-spots cultured in serum rich media but not

hi-spots cultured in N2B27 media in which GFAP$^+$ astrocytic cells are not preserved. Similarly, when IL-1β has been repetitive applied to MGE-like neurons and astrocytes co-culture, in a manner that maintains an elevated level, there was significant death of astrocytes with an associated reduction in the viability of MGE-like neurons. Together, these results further support the hypothesis that IL-1β indirectly causes death of MGE progenitors through an astrocytic mediated pathway. An alternative but not necessarily exclusive hypothesis is that IL-1β cooperates with other cytokines and damage signals, because IL-1β by itself is not neurotoxic in some cell cultures (Fogal, Hewett et al. 2005). For example, HMGB1 which is secreted at the later stage after brain injury could enhance the effect of IL-1β through formation of complexes with IL-1β (Wähämaa, Schierbeck et al. 2011). Moreover, combined treatment with TNFα, IL-1β, and IFNγ in astrocytes results in distinct upregulation of several genes for cytokines and growth factors which can't be induced by applying IL-1β alone (Meeuwsen, Persoon-Deen et al. 2003). Therefore, in healthy microenvironment where other cytokines are absent, the effect of IL-1β may be limited.

7.3.2Morphological maturation

Although IL-1R is expressed on the MGE-like neurons (Chapter 5), so far, there is a lack of evidence that IL-1β directly regulates neurite growth. Like its effect on cell survival, IL-1β indirectly regulates neurite growth through activation microglia and astrocytes. Once activated, microglia can promote neuronal injury through the release of low-molecular-weight neurotoxins which compromise neurite growth (Bruce-Keller 1999). In the human hi-spot culture, the results show impaired neurite growth of MGE progenitors mediated by IL-1β, and this effect can be partially reversed by applying IL-1RA. Similar to my observation, IL-1β reduces neurite growth of rat hippocampal precursor cells when being applied for 7 days (Green, Treacy et al. 2012). And in KA model of epilepsy, the morphological complexity of PV and CCK expressing interneurons was significantly reduced (Khan, Shekh-Ahmad et al. 2018). However, in addition to IL-1β, TNF-α is also upregulated in chronic epilepsy, and can directly inhibit neurite formation by activating GTPase RhoA (Neumann, Schweigreiter et al. 2002). This additional signalling may explain why IL-1RA can't completely reverse the detrimental effect of the inflamed microenvironment on neurite growth.

Paradoxically, in MGE-like neuron and astrocyte co-culture, short time exposure to IL-1β slightly increased the morphological complexity of MGE-like neurons. In this scenario, it is likely that IL-1β stimulates the release of neurotropic factors ,such as NGF, IGF and BDNF from astrocytes (Fogal, Hewett et al. 2005), which promoted neurite growth (Parish, Finkelstein et al. 2002, Boato, Hechler et al. 2011, Gougeon, Lourenssen et al. 2013),

The conflicting results in my studies are likely due to the difference in duration of IL-1β treatment and the presence or absence of a pre-inflamed milieu and the tissue cultured in addition to the culture method. In epileptic hippocampal hi-spot, the pathological microenvironment was more faithfully maintained and in the non-epileptic hippocampal hi-spot culture experiments, IL-1β was given repetitively for 4 weeks. While in neuron-astrocyte's co-culture, IL-1β was just given twice for a week, and not so intensively as in the hi-spot cultures. Studies which suggest that IL-1β increases neurite growth mainly use cell culture paradigms with short time exposure to IL-1β (Strijbos and Rothwell 1995, Boato, Hechler et al. 2011).

As most interneurons function locally by providing input to a large group of surrounding excitatory neurons to coordinate network activities, reduced neurite growth of MGE progenitors will affect their innervation territory when these cells become functional interneurons.

7.3.3 Neuronal differentiation

There are two possible reasons for the reduced number of mature neurons ($Tuj1^+$ and $GAD\ 67^+$ cells) in sclerotic hippocampal hi-spots, one is altered neuronal differentiation; the other is delayed neuronal differentiation in inflamed microenvironment. Some researches support the idea that pro-inflammatory cytokines, such as IL-1β and TNF-α, promotes astroglial differentiation instead of neuronal differentiation(Peng, Whitney et al. 2008, Green, Treacy et al. 2012). While there is also evidence to support the later assumption. BDNF is an environmental neurotrophic factor that promotes neuronal differentiation and improves neuron survival(Jiao, Palmgren et al. 2014). IL-1β has been reported to suppress BDNF signal via activating P38-MAPK pathway (Tong, Balazs et al. 2008, Tong, Prieto et al. 2012). It is possible that IL-1β prolongs neuronal differentiation of MGE progenitors in the inflamed microenvironment by interfering with the BDNF neurotrophic signal. This hypothesis needs to be investigated in the future.

On the contrary, when IL-1β was applied to non-epileptic cortical and hippocampal hi-spots, it didn't reduce the proportion of Tuj1 expressing neurons. It is possible that alternations in neuron differentiation requires long lasting / chronic stimulation by IL-1β. In previous research, IL-1β shows anti-neurogenic effects on hippocampal neuron precursors when being repetitively applied for 7 days not for 2 days (Green, Treacy et al. 2012). In sclerotic hippocampal hi-spots, upregulation of IL-1β was long lasting. While in non-epileptic hi-spots, IL-1β was applied every three days. When exogenous IL-1β is rapidly metabolized resulting in lower average or poorly sustained concentrations (Chapter 5), its anti-neurogenic effect may thus be attenuated.

7.3.4 Electrophysiological functionality

In addition to the effects of IL-1β on survival and maturation of MGE progenitors, my work also provided evidence for a direct modulatory effect of IL-1β on the cellular excitability of MGE-like neurons. Many previous studies support the idea that pro-epileptogenic effects of IL-1β is mediated by hyperexcitability and excitotoxicity in hippocampal principle neurons. (Vezzani, Moneta et al. 2002, Viviani, Bartesaghi et al. 2003, Vezzani and Viviani 2015). In a complimentary fashion, my research suggests that IL-1β can reduce cellular excitability in MGE-like interneurons. However, except for the difference in the types of neuron used for the experiments, the IL-1β concentration which increase cell excitability is quite low compared to that used in my research. In previous studies, IL-1β increases neuronal excitability of hippocampal neurons through phosphorylation of Src which increases NMDA-R function and Ca2+ influx (Viviani, Bartesaghi et al. 2003, Yang, Liu et al. 2005). However, a very low concentration of IL-1β (0.01ng/ml), 1000-fold lower than that used to stimulate inflammatory responses, was used. And evidence shows Src phosphorylation can only be observed at low concentrations of IL-1β (0.01ng/ml) but not in any higher concentration in hippocampal neurons (Huang, Smith et al. 2011). This may be one of the reasons that IL-1β shows different modulatory effects on cellular excitability in my research and I did not examine excitability at these very low levels.

In contrast to my result, increased GABAergic inhibition has been shown to be mediated by IL-1β in chronic inflammation. In that study, LPS was given to organotypic brain slices to simulate the release of IL-1β and the recording was taken at day 7 when the concentration of IL-1β returned to baseline (Hellstrom, Danik et al. 2005), whilst in my studies, the acute effect of IL-1β

on cellular excitability was abolished when IL-1β returned to baseline. Besides, apart from presynaptic GABAergic interneuron excitability, postsynaptic GABA receptor responsiveness can affect inhibitory post synaptic potential (IPSP) as well. The contradictory results may be partly due to the concentrations and duration for the treatment, the type of culture models and the method for investigation. In agreement with my results, IL-1β caused a 40% reduction of voltage-gated sodium current and 10mV reduction in amplitude of action potential in rat cortical neurons and 80% reduction of sodium current in retinal ganglion cells (Diem, Hobom et al. 2003, Zhou, Qi et al. 2011). In contrast, IL-1β didn't change sodium currents in rat hippocampal neurons and even led to 67% increase of sodium current in trigeminal nociceptive neurons (Liu, Yang et al. 2006).

Sodium currents are regulated by phosphorylation sites in segments of pore forming α unit and auxiliary β units. Those phosphorylation sites are regulated by either protein kinase A (PKA) or protein kinase C (PKC) in different type of neurons (Marban, Yamagishi et al. 1998, Scheuer and Catterall 2006, Scheuer 2011). A single site phosphorylation is sufficient to cause reduction in amplitude of sodium currents without affecting activation and inactivation kinetics of sodium channels (Smith and Goldin 1997). In rat striatal neurons, activation of PKA raises the threshold for firing and is associated with a 35% reduction in sodium currents. Likewise, activation of PKC also reduces sodium currents in cholinergic interneurons (Maurice, Mercer et al. 2004), hippocampal neurons (Numann, Catterall et al. 1991) and other brain neurons (Numann, Catterall et al. 1991). There are also several reports suggesting that IL-1β can regulate phosphorylation of different ion channel. In mouse heart cells, IL-1β increases PKC translocation and leads to reduction of calcium current (El Khoury, Mathieu et al. 2014). In rat cortical neurons, activation of PKC by IL-1β caused 37% inhibition of sodium currents (Zhou, Qi et al. 2011). Therefore, it is highly likely that IL-1β regulates sodium currents of MGE-like neurons through activation of phosphorylation protein. Further investigations are required to determine whether IL-1β reduces sodium currents in MGE-like neurons by regulating phosphorylation proteins.

In an attempt to explore the mechanism of reduced sodium current in IL-1β treated MGE-like neuron, I used non-stationary fluctuation analysis (NSFA) (Alvarez, Gonzalez et al. 2002) to investigate whether the number of sodium channels or channel conductance was altered by IL-1β treatment. Although the result showed significant reduction in channel conductance in IL-1β treated compared to control, it may not be enough to draw a conclusion. One deficit of acquired

data is that the variation within groups of recorded cells are huge, possibly due to the variety of interneuron phenotypes and their different maturation kinetics (Kepecs and Fishell 2014). Secondly, the single sodium current calculated from NSFA represents a macroscopic current which is the sum of currents from several sodium channels rather than the unitary sodium current (Lingle 2006, Nekouzadeh and Rudy 2007). Therefore, the data of a single sodium current in average from my experiment is significantly larger than previous reports which use excised recording (Wartenberg and Urban 2004, Vanoye, Lossin et al. 2006, Chatelier, Zhao et al. 2010). In addition, in my experiment, the number of recording sweeps may not sufficient to accurately predict the variance. Based on computer simulation, the variance will be more precisely recorded when increase the number of recording sweeps (Lingle 2006). However, in my experiment, the hESC-derived neurons were too fragile to endure repetitive simulation for extended recording time. So, the error of the data can affect the accuracy of the calculation for the number of channels and the single channel current. Further experiments using excised patch clamp recording will help to confirmed whether the conductance of sodium channel is affected by IL-1β.

7.4 Implications for reduced excitability of interneurons in the inflamed microenvironment of epilepsy.

Simplistically, the direct cause of seizures is an imbalance between excitatory and inhibitory activities in neuronal circuits. Genetic mutations which compromise important inhibitory signals would be expected to alter the balance of brain activity. For example, mutation in SCN1A, which reduces excitability of inhibitory interneurons, underlies Dravet syndrome, a serious epileptic encephalopathy that begins in childhood (Lossin, Rhodes et al. 2003, Vanoye, Lossin et al. 2006, Yu, Mantegazza et al. 2006, Tai, Abe et al. 2014). In TLE, interneuron loss is the major reason for compromised inhibitory output. Apart from that, recent research intriguingly shows impairment of cellular excitability in PV expressing inhibitory interneurons in the KA induced rat model of epilepsy (Khan, Shekh-Ahmad et al. 2018) in which the innate immune system is potently activated (Sabilallah, Fontanaud et al. 2016). Moreover, increasing the excitability of interneurons by activating Nav1.1 shows anti - convulsive effect (Frederiksen, Lu et al. 2017). Nav1.1 overexpressing, but not wild-type, MGE progenitors enhance behavior-dependent gamma

oscillatory activity, reduce epileptiform discharges and improve cognitive functions in human amyloid precursor protein (hAPP)-transgenic mice (Martinez-Losa, Tracy et al. 2018). Therefore, my finding of an inflammatory mediated reduced excitability of MGE-like interneurons induced by IL-1β is consistent with findings in previous transplantation models and also raises the possibility that IL-1β in inflamed hippocampus may reduce the excitability of endogenous inhibitory neurons. However, hESC-derived MGE-like neurons can't reach the same maturation state of post-natal inhibitory interneurons in vitro. Moreover, evidence suggests that the dominant isotypes of sodium channel expressed at pre-natal stage are different from the major isotypes expressed at the post-natal / adult stage in mice. Therefore, it is critical to figure out the isotype of sodium channels expressed at different developmental stages of human neurons and to see if they are altered to a developmental phenotype in epilepsy. In addition, interneurons are diverse in terms of their morphology, genetic markers and functionality. So, whether IL-1β broadly affects all types or certain types of interneuron need to be further investigated in animal models. Another important consideration is the effect of other inflammatory cytokines, such as TNF-α, which can affect cell excitability as well (Vezzani and Viviani 2015). Therefore, further experiments are required to investigate the cellular excitability of inhibitory neurons in a complex microenvironment.

However, in neuron-astrocyte's coculture, when IL-1β was applied every three days to the cells for a week, the excitability of MGE progenitors recorded by the end of the week didn't change compared to the control. It is possibly because IL-1β was completely metabolised after 72 hours in the culture medium (Chapter 5). Interestingly, also in that condition, there was significant increase in the number of PI^+ and PI^+ $Annexin^+$ cells compared to the control. Nevertheless, PI^+GFP^+ MGE-like neurons were rarely found and didn't increase compared to the control. It suggested IL-1β caused death of astrocytes but not co-cultured MGE-like neurons. Therefore, it may be controversial that reduced excitability of MGE-like neurons in response to pathological level of IL-1β is beneficial for their survival and potential recovery when IL-1β is metabolized (Diem, Hobom et al. 2003). However, if pathological level of IL-1β lasts, like the condition when the cells were repetitively treated by IL-1β, the excitability of MGE-like neurons has reduced even more along with increased cell death. Together, these results indicate the utility of modulating the inflamed epileptic microenvironment to potentially promote recovery of interneurons.

7.5 Limitations and future work

Firstly, it is hard to guarantee the inflamed microenvironment will be the same among the sclerotic hippocampal tissue from different patients. In the future, it is necessary to investigate the relation of cell survival with the severity of hippocampal inflammation by plotting cell survival against the level and duration of IL-1β in sclerotic hippocampal hi-spots. It will give us a better indication about the timing and duration for anti-inflammatory treatment. Secondly, it is important to include other key inflammatory cytokines, such as TNF-α, HMGB1 into the hi-spot experiment to investigate cooperation between the cytokines. Thirdly, it is critical to understand the mechanism of how IL-1β affects the cellular excitability of MGE-like neurons. Whether similar phenomena can be observed in mature interneurons is also an exciting avenue for further research. In general, my research has found that a non/less-inflamed microenvironment is critical to maintain survival, normal growth, differentiation and electrophysiological activities of exogenous transplanted MGE progenitors. More research is needed to fully understand the mechanism of how IL-1β affects neuron survival and their functionality. hESC-derived hi-spot can be used in the future to explore possible mechanism.

The clinical implications are that we need to consider the inflammatory environment and its possible modulation in order to maximize the survival and optimal differentiation and network integration of interneurons in future cell replacement therapy research.

8. Appendix

Figure 3-5 C

ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	4693	1	4693	F (1, 70) = 1.019	P=0.3161
Row Factor	197419	1	197419	F (1, 70) = 42.88	P<0.0001
Column Factor	54240	1	54240	F (1, 70) = 11.78	P=0.0010
Residual	322252	70	4604		

Figure 3-5 D

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	27470	2	13735	F (2, 66) = 4.892	P=0.0104
Row Factor	4675066	2	2337533	F (2, 66) = 832.5	P<0.0001
Column Factor	118922	1	118922	F (1, 66) = 42.35	P<0.0001
Residual	185320	66	2808		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Cortical - Hippocampal					
D1	28.03	-24.96 to 81.03	No	ns	0.4871
D7	120.7	67.67 to 173.7	Yes	****	<0.0001
D14	95.15	42.15 to 148.1	Yes	***	0.0001

Figure 3-6 I

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.01575	1	0.01575	F (1, 44) = 0.9047	P=0.3467
Row Factor	0.3089	1	0.3089	F (1, 44) = 17.74	P=0.0001
Column Factor	0.9487	1	0.9487	F (1, 44) = 54.49	P<0.0001
Residual	0.7661	44	0.01741		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Cortical - Hippocampal					
D 14	0.1242	-0.0005203 to 0.2489	No	ns	0.0511
D 28	0.1967	0.07194 to 0.3214	Yes	**	0.0014

Figure 4-1 D

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.02521	1	0.02521	F (1, 44) = 1.608	P=0.2114
Row Factor	0.273	1	0.273	F (1, 44) = 17.42	P=0.0001
Column Factor	0.12	1	0.12	F (1, 44) = 7.655	P=0.0082
Residual	0.6897	44	0.01568		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IL-1RA- - IL-1RA+					
Cortical	-0.05417	-0.1725 to 0.06418	No	ns	0.5031
Hippocampal	-0.1458	-0.2642 to -0.02749	Yes	*	0.0131

Figure 4-1E

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.1151	1	0.1151	F (1, 44) = 12.31	P=0.0011
Row Factor	0.1485	1	0.1485	F (1, 44) = 15.89	P=0.0002
Column Factor	0.0475	1	0.0475	F (1, 44) = 5.081	P=0.0292
Residual	0.4113	44	0.009348		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IL-1RA- - IL-1RA+					
Cortical	0.035	-0.05639 to 0.1264	No	ns	0.6157
Hippocampal	-0.1608	-0.2522 to -0.06944	Yes	***	0.0004

Figure 4-2B

ANOVA table	SS (Type III)	DF	MS	F (DFn, DFd)	P value
Interaction	4648	1	4648	F (1, 39) = 3.185	P=0.0821
Row Factor	21991	1	21991	F (1, 39) = 15.07	P=0.0004
Column Factor	5749	1	5749	F (1, 39) = 3.94	P=0.0542
Residual	56903	39	1459		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IL-1β- - IL-1β+					
Cortical	2.366	-38.18 to 42.92	No	ns	0.9885
Hippocampal	44.53	7.45 to 81.62	Yes	*	0.016

Figure 6-7 C

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	424.7	1	424.7	F (1, 28) = 1.956	P=0.1730
Row Factor	3691	1	3691	F (1, 28) = 17	P=0.0003
Column Factor	1328	1	1328	F (1, 28) = 6.117	P=0.0197
Residual	6081	28	217.2		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IL-1β- - IL-1β+					
Serum	20.17	2.763 to 37.58	Yes	*	0.0211
N2B27	5.6	-11.81 to 23.01	No	ns	0.7015

Figure 6-7 D

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	304141730	1	304141730	F (1, 28) = 8.139	P=0.0081
Row Factor	603947636	1	603947636	F (1, 28) = 16.16	P=0.0004
Column Factor	46346229	1	46346229	F (1, 28) = 1.24	P=0.2749
Residual	1046351605	28	37369700		

Sidak's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
IL-1β- - IL-1β+					
Serum	8573	1351 to 15795	Yes	*	0.018
N2B27	-3759	-10981 to 3463	No	ns	0.4056

Figure 3-6 E

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
patient	Linear	20.000	1	20.000	.003	.956	.000
patient * Group	Linear	20.000	1	20.000	.003	.956	.000
Error(factor1)	Linear	50385.000	8	6298.125			

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Patient1	.404	1	8	.543
Patient2	.637	1	8	.448

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Group

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	1474245.000	1	1474245.000	202.385	.000	.962
Group	59405.000	1	59405.000	8.155	.021	.505
Error	58275.000	8	7284.375			

Figure 3-6 G

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Patient	Linear	9919.904	1	9919.904	.735	.416	.084
Patient * Group	Linear	14889.261	1	14889.261	1.103	.324	.121
Error(factor1)	Linear	108021.703	8	13502.713			

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Patient1	1.127	1	8	.319
Patient2	1.310	1	8	.285

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Group

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	1901159.701	1	1901159.701	321.930	.000	.976
Group	168268.595	1	168268.595	28.493	.001	.781
Error	47244.079	8	5905.510			

Figure 3-6 H

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Patient	Linear	1385.463	1	1385.463	.462	.516	.055
Patient * Group	Linear	445.389	1	445.389	.149	.710	.018
Error(factor1)	Linear	23991.450	8	2998.931			

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Patient1	4.067	1	8	.078
Patient2	1.663	1	8	.233

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Group

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	580606.993	1	580606.993	179.623	.000	.957
Group	49046.688	1	49046.688	15.174	.005	.655
Error	25858.853	8	3232.357			

Figure 4-1 G

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Patient	Linear	13781.250	1	13781.250	3.916	.083	.329
Patient * Treatment	Linear	3001.250	1	3001.250	.853	.383	.096
Error(factor1)	Linear	28155.000	8	3519.375			

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Patient1	7.096	1	8	.029
Patient2	.474	1	8	.510

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Treatment

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	712531.250	1	712531.250	269.197	.000	.971
Treatment	19531.250	1	19531.250	7.379	.026	.480
Error	21175.000	8	2646.875			

Figure 4-1 J

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
factor1	Linear	111.656	1	111.656	.099	.761	.012
factor1 * Treatment	Linear	4746.219	1	4746.219	4.228	.074	.346
Error(factor1)	Linear	8979.891	8	1122.486			

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
Patient1	.373	1	8	.558
Patient2	.572	1	8	.471

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Treatment

Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	406091.371	1	406091.371	291.025	.000	.973
Treatment	9359.042	1	9359.042	6.707	.032	.456
Error	11163.062	8	1395.383			

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10. References

Abraham, H., B. Veszpremi, A. Kravjak, K. Kovacs, E. Gomori and L. Seress (2009). "Ontogeny of calbindin immunoreactivity in the human hippocampal formation with a special emphasis on granule cells of the dentate gyrus." Int J Dev Neurosci **27**(2): 115-127.

Aktas, O., O. Ullrich, C. Infante-Duarte, R. Nitsch and F. Zipp (2007). "Neuronal damage in brain inflammation." Arch Neurol **64**(2): 185-189.

Alapirtti, T., K. Lehtimaki, R. Nieminen, R. Makinen, J. Raitanen, E. Moilanen, J. Makinen and J. Peltola (2018). "The production of IL-6 in acute epileptic seizure: A video-EEG study." J Neuroimmunol **316**: 50-55.

Allan, S. M., P. J. Tyrrell and N. J. Rothwell (2005). "Interleukin-1 and neuronal injury." Nat Rev Immunol **5**(8): 629-640.

Alvarez, O., C. Gonzalez and R. Latorre (2002). "Counting channels: a tutorial guide on ion channel fluctuation analysis." Adv Physiol Educ **26**(1-4): 327-341.

Anastasiades, P. G. and S. J. Butt (2011). "Decoding the transcriptional basis for GABAergic interneuron diversity in the mouse neocortex." Eur J Neurosci **34**(10): 1542-1552.

Anderson, S. A., D. D. Eisenstat, L. Shi and J. L. Rubenstein (1997). "Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes." Science **278**(5337): 474-476.

Antonopoulos, J., I. S. Pappas and J. G. Parnavelas (1997). "Activation of the GABAA Receptor Inhibits the Proliferative Effects of bFGF in Cortical Progenitor Cells." European Journal of Neuroscience **9**(2): 291-298.

Aronica, E. and P. B. Crino (2011). "Inflammation in epilepsy: clinical observations." Epilepsia **52 Suppl 3**: 26-32.

Azim, E., D. Jabaudon, R. M. Fame and J. D. Macklis (2009). "SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development." Nat Neurosci **12**(10): 1238-1247.

Bachoud-Levi, A., C. Bourdet, P. Brugieres, J. P. Nguyen, T. Grandmougin, B. Haddad, R. Jeny, P. Bartolomeo, M. F. Boisse, G. D. Barba, J. D. Degos, A. M. Ergis, J. P. Lefaucheur, F. Lisovoski, E. Pailhous, P. Remy, S. Palfi, G. L. Defer, P. Cesaro, P. Hantraye and M. Peschanski (2000). "Safety

and tolerability assessment of intrastriatal neural allografts in five patients with Huntington's disease." Exp Neurol **161**(1): 194-202.

Bailey, J. L., V. O'Connor, M. Hannah, L. Hewlett, T. E. Biggs, L. E. Sundstrom, M. W. Findlay and J. E. Chad (2011). "In vitro CNS tissue analogues formed by self-organisation of reaggregated post-natal brain tissue." J Neurochem **117**(6): 1020-1032.

Bamba, Y., T. Shofuda, D. Kanematsu, M. Nonaka, M. Yamasaki, H. Okano and Y. Kanemura (2014). "Differentiation, polarization, and migration of human induced pluripotent stem cell-derived neural progenitor cells co-cultured with a human glial cell line with radial glial-like characteristics." Biochem Biophys Res Commun **447**(4): 683-688.

Baraban, S. C., D. G. Southwell, R. C. Estrada, D. L. Jones, J. Y. Sebe, C. Alfaro-Cervello, J. M. Garcia-Verdugo, J. L. Rubenstein and A. Alvarez-Buylla (2009). "Reduction of seizures by transplantation of cortical GABAergic interneuron precursors into Kv1.1 mutant mice." Proc Natl Acad Sci U S A **106**(36): 15472-15477.

Bardy, C., M. van den Hurk, B. Kakaradov, J. A. Erwin, B. N. Jaeger, R. V. Hernandez, T. Eames, A. A. Paucar, M. Gorris, C. Marchand, R. Jappelli, J. Barron, A. K. Bryant, M. Kellogg, R. S. Lasken, B. P. Rutten, H. W. Steinbusch, G. W. Yeo and F. H. Gage (2016). "Predicting the functional states of human iPSC-derived neurons with single-cell RNA-seq and electrophysiology." Mol Psychiatry **21**(11): 1573-1588.

Barkas, L., E. Redhead, M. Taylor, A. Shtaya, D. A. Hamilton and W. P. Gray (2012). "Fluoxetine restores spatial learning but not accelerated forgetting in mesial temporal lobe epilepsy." Brain **135**(Pt 8): 2358-2374.

Bartos, M., I. Vida and P. Jonas (2007). "Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks." Nat Rev Neurosci **8**(1): 45-56.

Basu, A., J. K. Krady, J. R. Enterline and S. W. Levison (2002). "Transforming growth factor β1 prevents IL-1β–induced microglial activation, whereas TNFα- and IL-6–stimulated activation are not antagonized." Glia **40**(1): 109-120.

Batista-Brito, R., E. Rossignol, J. Hjerling-Leffler, M. Denaxa, M. Wegner, V. Lefebvre, V. Pachnis and G. Fishell (2009). "The cell-intrinsic requirement of Sox6 for cortical interneuron development." Neuron **63**(4): 466-481.

Belarbi, K. and S. Rosi (2013). "Modulation of adult-born neurons in the inflamed hippocampus." Front Cell Neurosci **7**: 145.

Bengzon, J., Z. Kokaia, E. Elmer, A. Nanobashvili, M. Kokaia and O. Lindvall (1997). "Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures." Proc Natl Acad Sci U S A **94**(19): 10432-10437.

Bershteyn, M., T. J. Nowakowski, A. A. Pollen, E. Di Lullo, A. Nene, A. Wynshaw-Boris and A. R. Kriegstein (2017). "Human iPSC-Derived Cerebral Organoids Model Cellular Features of Lissencephaly and Reveal Prolonged Mitosis of Outer Radial Glia." Cell Stem Cell **20**(4): 435-449 e434.

Bjorklund, A. and O. Lindvall (2000). "Cell replacement therapies for central nervous system disorders." Nat Neurosci **3**(6): 537-544.

Bjornsson, Christopher S., M. Apostolopoulou, Y. Tian and S. Temple (2016). "It Takes a Village: Constructing the Neurogenic Niche." Developmental Cell **32**(4): 435-446.

Bliss, T., R. Guzman, M. Daadi and G. K. Steinberg (2007). "Cell Transplantation Therapy for Stroke." Stroke **38**(2): 817-826.

Boato, F., D. Hechler, K. Rosenberger, D. Lüdecke, E. M. Peters, R. Nitsch and S. Hendrix (2011). "Interleukin-1 beta and neurotrophin-3 synergistically promote neurite growth in vitro." Journal of Neuroinflammation **8**(1): 183.

Boldog, E., T. E. Bakken, R. D. Hodge, M. Novotny, B. D. Aevermann, J. Baka, S. Bordé, J. L. Close, F. Diez-Fuertes, S.-L. Ding, N. Faragó, Á. K. Kocsis, B. Kovács, Z. Maltzer, J. M. McCorrison, J. A. Miller, G. Molnár, G. Oláh, A. Ozsvár, M. Rózsa, S. I. Shehata, K. A. Smith, S. M. Sunkin, D. N. Tran, P. Venepally, A. Wall, L. G. Puskás, P. Barzó, F. J. Steemers, N. J. Schork, R. H. Scheuermann, R. S. Lasken, E. S. Lein and G. Tamás (2018). "Transcriptomic and morphophysiological evidence for a specialized human cortical GABAergic cell type." Nature Neuroscience **21**(9): 1185-1195.

Bortone, D. and F. Polleux (2009). "KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner." Neuron **62**(1): 53-71.

Bruce-Keller, A. J. (1999). "Microglial–neuronal interactions in synaptic damage and recovery." Journal of Neuroscience Research **58**(1): 191-201.

Buckmaster, P. S., E. A. Ingram and X. L. Wen (2009). "Inhibition of the Mammalian Target of Rapamycin Signaling Pathway Suppresses Dentate Granule Cell Axon Sprouting in a Rodent Model of Temporal Lobe Epilepsy." Journal of Neuroscience **29**(25): 8259-8269.

Butovsky, O., Y. Ziv, A. Schwartz, G. Landa, A. E. Talpalar, S. Pluchino, G. Martino and M. Schwartz (2006). "Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells." Mol Cell Neurosci **31**(1): 149-160.

Butt, S. J. B., V. H. Sousa, M. V. Fuccillo, J. Hjerling-Leffler, G. Miyoshi, S. Kimura and G. Fishell (2008). "The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes." Neuron **59**(5): 722-732.

Castro, D. S., B. Martynoga, C. Parras, V. Ramesh, E. Pacary, C. Johnston, D. Drechsel, M. Lebel-Potter, L. G. Garcia, C. Hunt, D. Dolle, A. Bithell, L. Ettwiller, N. Buckley and F. Guillemot (2011). "A novel function of the proneural factor Ascl1 in progenitor proliferation identified by genome-wide characterization of its targets." Genes Dev **25**(9): 930-945.

Cauli, B., E. Audinat, B. Lambolez, M. C. Angulo, N. Ropert, K. Tsuzuki, S. Hestrin and J. Rossier (1997). "Molecular and physiological diversity of cortical nonpyramidal cells." J Neurosci **17**(10): 3894-3906.

Chai, X., G. Munzner, S. Zhao, S. Tinnes, J. Kowalski, U. Haussler, C. Young, C. A. Haas and M. Frotscher (2014). "Epilepsy-induced motility of differentiated neurons." Cereb Cortex **24**(8): 2130-2140.

Chambers, S. M., C. A. Fasano, E. P. Papapetrou, M. Tomishima, M. Sadelain and L. Studer (2009). "Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling." Nat Biotechnol **27**(3): 275-280.

Chao, M. V. (2003). "Neurotrophins and their receptors: A convergence point for many signalling pathways." Nature Reviews Neuroscience **4**: 299.

Chatelier, A., J. Zhao, P. Bois and M. Chahine (2010). "Biophysical characterisation of the persistent sodium current of the Nav1.6 neuronal sodium channel: a single-channel analysis." Pflugers Arch **460**(1): 77-86.

Chattopadhyaya, B., G. Di Cristo, C. Z. Wu, G. Knott, S. Kuhlman, Y. Fu, R. D. Palmiter and Z. J. Huang (2007). "GAD67-mediated GABA synthesis and signaling regulate inhibitory synaptic innervation in the visual cortex." Neuron **54**(6): 889-903.

Chesnokova, V., R. N. Pechnick and K. Wawrowsky (2016). "Chronic peripheral inflammation, hippocampal neurogenesis, and behavior." Brain, behavior, and immunity 58: 1-8.

Chhor, V., T. Le Charpentier, S. Lebon, M.-V. Oré, I. L. Celador, J. Josserand, V. Degos, E. Jacotot, H. Hagberg, K. Sävman, C. Mallard, P. Gressens and B. Fleiss (2013). "Characterization of phenotype

markers and neuronotoxic potential of polarised primary microglia in vitro." Brain, Behavior, and Immunity **32**: 70-85.

Cho, K. O., Z. R. Lybrand, N. Ito, R. Brulet, F. Tafacory, L. Zhang, L. Good, K. Ure, S. G. Kernie, S. G. Birnbaum, H. E. Scharfman, A. J. Eisch and J. Hsieh (2015). "Aberrant hippocampal neurogenesis contributes to epilepsy and associated cognitive decline." Nat Commun **6**: 6606.

Choi, S. H., Y. H. Kim, M. Hebisch, C. Sliwinski, S. Lee, C. D'Avanzo, H. Chen, B. Hooli, C. Asselin, J. Muffat, J. B. Klee, C. Zhang, B. J. Wainger, M. Peitz, D. M. Kovacs, C. J. Woolf, S. L. Wagner, R. E. Tanzi and D. Y. Kim (2014). "A three-dimensional human neural cell culture model of Alzheimer's disease." Nature **515**(7526): 274-278.

Conti, L. and E. Cattaneo (2010). "Neural stem cell systems: physiological players or in vitro entities?" Nat Rev Neurosci **11**(3): 176-187.

Crampton, S. J., L. M. Collins, A. Toulouse, Y. M. Nolan and G. W. O'Keeffe (2012). "Exposure of foetal neural progenitor cells to IL-1beta impairs their proliferation and alters their differentiation - a role for maternal inflammation?" J Neurochem **120**(6): 964-973.

Cunningham, M., J. H. Cho, A. Leung, G. Savvidis, S. Ahn, M. Moon, P. K. Lee, J. J. Han, N. Azimi, K. S. Kim, V. Y. Bolshakov and S. Chung (2014). "hPSC-derived maturing GABAergic interneurons ameliorate seizures and abnormal behavior in epileptic mice." Cell Stem Cell **15**(5): 559-573.

da Costa Neves, R. S., A. P. Jardim, L. O. Caboclo, C. Lancellotti, T. F. Marinho, A. P. Hamad, M. Marinho, R. Centeno, E. A. Cavalheiro, C. A. Scorza and E. M. Targas Yacubian (2013). "Granule cell dispersion is not a predictor of surgical outcome in temporal lobe epilepsy with mesial temporal sclerosis." Clin Neuropathol **32**(1): 24-30.

Dam, A. M. (1980). "Epilepsy and neuron loss in the hippocampus." Epilepsia **21**(6): 617-629.

Danzer, S. C. (2012). "Depression, stress, epilepsy and adult neurogenesis." Exp Neurol **233**(1): 22-32.

de Lanerolle, N. C., J. H. Kim, R. J. Robbins and D. D. Spencer (1989). "Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy." Brain Res **495**(2): 387-395.

De Marco Garcia, N. V., T. Karayannis and G. Fishell (2011). "Neuronal activity is required for the development of specific cortical interneuron subtypes." Nature **472**(7343): 351-355.

de Vries, E. E., B. van den Munckhof, K. P. Braun, A. van Royen-Kerkhof, W. de Jager and F. E. Jansen (2016). "Inflammatory mediators in human epilepsy: A systematic review and meta-analysis." Neurosci Biobehav Rev **63**: 177-190.

Diem, R., M. Hobom, P. Grötsch, B. Kramer and M. Bähr (2003). "Interleukin-1β protects neurons via the interleukin-1 (IL-1) receptor-mediated Akt pathway and by IL-1 receptor-independent decrease of transmembrane currents in vivo." Molecular and Cellular Neuroscience **22**(4): 487-500.

Dinarello, C. A. (2009). "Immunological and inflammatory functions of the interleukin-1 family." Annu Rev Immunol **27**: 519-550.

Dinarello, C. A. (2009). "Immunological and inflammatory functions of the interleukin-1 family." Annu Rev Immunol **27**: 519-550.

Dudek, F. E. and T. P. Sutula (2007). "Epileptogenesis in the dentate gyrus: a critical perspective." Prog Brain Res **163**: 755-773.

Ekdahl, C. T., J.-H. Claasen, S. Bonde, Z. Kokaia and O. Lindvall (2003). "Inflammation is detrimental for neurogenesis in adult brain." Proceedings of the National Academy of Sciences of the United States of America **100**(23): 13632-13637.

El Khoury, N., S. Mathieu and C. Fiset (2014). "Interleukin-1beta reduces L-type Ca2+ current through protein kinase C activation in mouse heart." J Biol Chem **289**(32): 21896-21908.

Fahrner, A., G. Kann, A. Flubacher, C. Heinrich, T. M. Freiman, J. Zentner, M. Frotscher and C. A. Haas (2007). "Granule cell dispersion is not accompanied by enhanced neurogenesis in temporal lobe epilepsy patients." Experimental Neurology **203**(2): 320-332.

Flandin, P., Y. Zhao, D. Vogt, J. Jeong, J. Long, G. Potter, H. Westphal and J. L. Rubenstein (2011). "Lhx6 and Lhx8 coordinately induce neuronal expression of Shh that controls the generation of interneuron progenitors." Neuron **70**(5): 939-950.

Fogal, B., J. A. Hewett and S. J. Hewett (2005). "Interleukin-1beta potentiates neuronal injury in a variety of injury models involving energy deprivation." J Neuroimmunol **161**(1-2): 93-100.

Fogarty, M., M. Grist, D. Gelman, O. Marin, V. Pachnis and N. Kessaris (2007). "Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex." J Neurosci **27**(41): 10935-10946.

Frederiksen, K., D. Lu, J. Yang, H. S. Jensen, J. F. Bastlund, P. H. Larsen, H. Liu, F. Crestey, K. Dekermendjian, L. Badolo, M. Laursen, C. Hougaard, C. Yang, N. Svenstrup and M. Grunnet (2017). "A small molecule activator of Nav1.1 channels increases fast-spiking interneuron excitability and GABAergic transmission in vitro and has anti-convulsive effects in vivo." European Journal of Neuroscience **46**(3): 1887-1896.

Freed, C. R., P. E. Greene, R. E. Breeze, W. Y. Tsai, W. DuMouchel, R. Kao, S. Dillon, H. Winfield, S. Culver, J. Q. Trojanowski, D. Eidelberg and S. Fahn (2001). "Transplantation of embryonic dopamine neurons for severe Parkinson's disease." N Engl J Med **344**(10): 710-719.

Friedman, W. J. (2001). "Cytokines regulate expression of the type 1 interleukin-1 receptor in rat hippocampal neurons and glia." Exp Neurol **168**(1): 23-31.

Frotscher, M., C. A. Haas and E. Forster (2003). "Reelin controls granule cell migration in the dentate gyrus by acting on the radial glial scaffold." Cereb Cortex **13**(6): 634-640.

Frotscher, M., P. Jonas and R. S. Sloviter (2006). "Synapses formed by normal and abnormal hippocampal mossy fibers." Cell Tissue Res **326**(2): 361-367.

Fujioka, H. and T. Akema (2010). "Lipopolysaccharide acutely inhibits proliferation of neural precursor cells in the dentate gyrus in adult rats." Brain Res **1352**: 35-42.

Gelman, D. M., F. J. Martini, S. Nobrega-Pereira, A. Pierani, N. Kessaris and O. Marin (2009). "The embryonic preoptic area is a novel source of cortical GABAergic interneurons." J Neurosci **29**(29): 9380-9389.

Giannakopoulou, A., N. Grigoriadis, C. Bekiari, A. Lourbopoulos, I. Dori, A. S. Tsingotjidou, H. Michaloudi and G. C. Papadopoulos (2013). "Acute inflammation alters adult hippocampal neurogenesis in a multiple sclerosis mouse model." J Neurosci Res **91**(7): 890-900.

Glass, C. K., K. Saijo, B. Winner, M. C. Marchetto and F. H. Gage (2010). "Mechanisms underlying inflammation in neurodegeneration." Cell **140**(6): 918-934.

Goldberg, E. M. and D. A. Coulter (2013). "Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction." Nat Rev Neurosci **14**(5): 337-349.

Goldberg, E. M. and D. A. Coulter (2014). "Seizing the opportunity: stem cells take on epilepsy." Cell Stem Cell **15**(5): 527-528.

Goncalves, J. T., S. T. Schafer and F. H. Gage (2016). "Adult Neurogenesis in the Hippocampus: From Stem Cells to Behavior." Cell **167**(4): 897-914.

Goshen, I., T. Kreisel, O. Ben-Menachem-Zidon, T. Licht, J. Weidenfeld, T. Ben-Hur and R. Yirmiya (2008). "Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression." Mol Psychiatry **13**(7): 717-728.

Gougeon, P. Y., S. Lourenssen, T. Y. Han, D. G. Nair, M. J. Ropeleski and M. G. Blennerhassett (2013). "The pro-inflammatory cytokines IL-1beta and TNFalpha are neurotrophic for enteric neurons." J Neurosci **33**(8): 3339-3351.

Gray, W. P. and L. E. Sundstrom (1998). "Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat." Brain Res **790**(1-2): 52-59.

Green, H. F., E. Treacy, A. K. Keohane, A. M. Sullivan, G. W. O'Keeffe and Y. M. Nolan (2012). "A role for interleukin-1beta in determining the lineage fate of embryonic rat hippocampal neural precursor cells." Mol Cell Neurosci **49**(3): 311-321.

Haas, C. A. and M. Frotscher (2010). "Reelin deficiency causes granule cell dispersion in epilepsy." Exp Brain Res **200**(2): 141-149.

Hansen, D. V., J. H. Lui, P. Flandin, K. Yoshikawa, J. L. Rubenstein, A. Alvarez-Buylla and A. R. Kriegstein (2013). "Non-epithelial stem cells and cortical interneuron production in the human ganglionic eminences." Nat Neurosci **16**(11): 1576-1587.

Heinemann, S. H. and F. Conti (1992). "Nonstationary noise analysis and application to patch clamp recordings." Methods Enzymol **207**: 131-148.

Hellstrom, I. C., M. Danik, G. N. Luheshi and S. Williams (2005). "Chronic LPS exposure produces changes in intrinsic membrane properties and a sustained IL-beta-dependent increase in GABAergic inhibition in hippocampal CA1 pyramidal neurons." Hippocampus **15**(5): 656-664.

Henderson, K. W., J. Gupta, S. Tagliatela, E. Litvina, X. Zheng, M. A. Van Zandt, N. Woods, E. Grund, D. Lin, S. Royston, Y. Yanagawa, G. B. Aaron and J. R. Naegele (2014). "Long-term seizure suppression and optogenetic analyses of synaptic connectivity in epileptic mice with hippocampal grafts of GABAergic interneurons." J Neurosci **34**(40): 13492-13504.

Henshall, D. C. (2007). "Apoptosis signalling pathways in seizure-induced neuronal death and epilepsy." Biochem Soc Trans **35**(Pt 2): 421-423.

Hewett, S. J., N. A. Jackman and R. J. Claycomb (2012). "Interleukin-1beta in Central Nervous System Injury and Repair." Eur J Neurodegener Dis **1**(2): 195-211.

Howell, O. W., K. Doyle, J. H. Goodman, H. E. Scharfman, H. Herzog, A. Pringle, A. G. Beck-Sickinger and W. P. Gray (2005). "Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus." J Neurochem **93**(3): 560-570.

Hu, Y., J. Ji, J. Xia, P. Zhao, X. Fan, Z. Wang, X. Zhou, M. Luo and P. Gu (2013). "An in vitro comparison study: the effects of fetal bovine serum concentration on retinal progenitor cell multipotentiality." Neurosci Lett **534**: 90-95.

Huang, Y., D. E. Smith, O. Ibanez-Sandoval, J. E. Sims and W. J. Friedman (2011). "Neuron-specific effects of interleukin-1beta are mediated by a novel isoform of the IL-1 receptor accessory protein." J Neurosci **31**(49): 18048-18059.

Hunt, R. F. and S. C. Baraban (2015). "Interneuron Transplantation as a Treatment for Epilepsy." Cold Spring Harb Perspect Med **5**(12).

Hunt, R. F., K. M. Girskis, J. L. Rubenstein, A. Alvarez-Buylla and S. C. Baraban (2013). "GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior." Nature Neuroscience **16**(6): 692-697.

Inamura, N., T. Kimura, S. Tada, T. Kurahashi, M. Yanagida, Y. Yanagawa, K. Ikenaka and F. Murakami (2012). "Intrinsic and extrinsic mechanisms control the termination of cortical interneuron migration." J Neurosci **32**(17): 6032-6042.

Jiao, Y., B. Palmgren, E. Novozhilova, U. Englund Johansson, A. L. Spieles-Engemann, A. Kale, S. I. Stupp and P. Olivius (2014). "BDNF increases survival and neuronal differentiation of human neural precursor cells cotransplanted with a nanofiber gel to the auditory nerve in a rat model of neuronal damage." Biomed Res Int **2014**: 356415.

Jimenez-Pacheco, A., M. Diaz-Hernandez, M. Arribas-Blazquez, A. Sanz-Rodriguez, L. A. Olivos-Ore, A. R. Artalejo, M. Alves, M. Letavic, M. T. Miras-Portugal, R. M. Conroy, N. Delanty, M. A. Farrell, D. F. O'Brien, A. Bhattacharya, T. Engel and D. C. Henshall (2016). "Transient P2X7 Receptor Antagonism Produces Lasting Reductions in Spontaneous Seizures and Gliosis in Experimental Temporal Lobe Epilepsy." J Neurosci **36**(22): 5920-5932.

Jin, K., M. Minami, J. Q. Lan, X. O. Mao, S. Batteur, R. P. Simon and D. A. Greenberg (2001). "Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat." Proceedings of the National Academy of Sciences of the United States of America **98**(8): 4710-4715.

Johnson, M. A., J. P. Weick, R. A. Pearce and S. C. Zhang (2007). "Functional neural development from human embryonic stem cells: accelerated synaptic activity via astrocyte coculture." J Neurosci **27**(12): 3069-3077.

Johnson, M. R., J. Behmoaras, L. Bottolo, M. L. Krishnan, K. Pernhorst, P. L. Santoscoy, T. Rossetti, D. Speed, P. K. Srivastava, M. Chadeau-Hyam, N. Hajji, A. Dabrowska, M. Rotival, B. Razzaghi, S. Kovac, K. Wanisch, F. W. Grillo, A. Slaviero, S. R. Langley, K. Shkura, P. Roncon, T. De, M. Mattheisen, P. Niehusmann, T. J. O'Brien, S. Petrovski, M. von Lehe, P. Hoffmann, J. Eriksson, A. J. Coffey, S. Cichon, M. Walker, M. Simonato, B. Danis, M. Mazzuferi, P. Foerch, S. Schoch, V. De Paola, R. M. Kaminski, V. T. Cunliffe, A. J. Becker and E. Petretto (2015). "Systems genetics identifies Sestrin 3 as a regulator of a proconvulsant gene network in human epileptic hippocampus." Nat Commun **6**: 6031.

Karadi, K., J. Janszky, C. Gyimesi, Z. Horvath, T. Lucza, T. Doczi, J. Kallai and H. Abraham (2012). "Correlation between calbindin expression in granule cells of the resected hippocampal dentate gyrus and verbal memory in temporal lobe epilepsy." Epilepsy & Behavior **25**(1): 110-119.

Karagiannis, A., T. Gallopin, C. Dávid, D. Battaglia, H. Geoffroy, J. Rossier, E. M. C. Hillman, J. F. Staiger and B. Cauli (2009). "Classification of NPY-expressing neocortical interneurons." The Journal of neuroscience : the official journal of the Society for Neuroscience **29**(11): 3642-3659.

Katona, I., L. Acsady and T. F. Freund (1999). "Postsynaptic targets of somatostatin-immunoreactive interneurons in the rat hippocampus." Neuroscience **88**(1): 37-55.

Kawaguchi, Y. and Y. Kubota (1996). "Physiological and morphological identification of somatostatin- or vasoactive intestinal polypeptide-containing cells among GABAergic cell subtypes in rat frontal cortex." J Neurosci **16**(8): 2701-2715.

Kepecs, A. and G. Fishell (2014). "Interneuron cell types are fit to function." Nature **505**(7483): 318-326.

Khan, A. A., T. Shekh-Ahmad, A. Khalil, M. C. Walker and A. B. Ali (2018). "Cannabidiol exerts antiepileptic effects by restoring hippocampal interneuron functions in a temporal lobe epilepsy model." Br J Pharmacol **175**(11): 2097-2115.

Kim, T. G., R. Yao, T. Monnell, J. H. Cho, A. Vasudevan, A. Koh, K. T. Peeyush, M. Moon, D. Datta, V. Y. Bolshakov, K. S. Kim and S. Chung (2014). "Efficient specification of interneurons from human pluripotent stem cells by dorsoventral and rostrocaudal modulation." Stem Cells **32**(7): 1789-1804.

Kokaia, Z., G. Martino, M. Schwartz and O. Lindvall (2012). "Cross-talk between neural stem cells and immune cells: the key to better brain repair?" Nat Neurosci **15**(8): 1078-1087.

Koprich, J. B., C. Reske-Nielsen, P. Mithal and O. Isacson (2008). "Neuroinflammation mediated by IL-1beta increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease." J Neuroinflammation **5**: 8.

Krencik, R., J. P. Weick, Y. Liu, Z. J. Zhang and S. C. Zhang (2011). "Specification of transplantable astroglial subtypes from human pluripotent stem cells." Nat Biotechnol **29**(6): 528-534.

Lawson, L. J., V. H. Perry, P. Dri and S. Gordon (1990). "Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain." Neuroscience **39**(1): 151-170.

Le Magueresse, C. and H. Monyer (2013). "GABAergic interneurons shape the functional maturation of the cortex." Neuron **77**(3): 388-405.

Li, X. J., X. Zhang, M. A. Johnson, Z. B. Wang, T. Lavaute and S. C. Zhang (2009). "Coordination of sonic hedgehog and Wnt signaling determines ventral and dorsal telencephalic neuron types from human embryonic stem cells." Development **136**(23): 4055-4063.

Librizzi, L., F. Noe, A. Vezzani, M. de Curtis and T. Ravizza (2012). "Seizure-induced brain-borne inflammation sustains seizure recurrence and blood-brain barrier damage." Ann Neurol **72**(1): 82-90.

Lingle, C. J. (2006). "Empirical considerations regarding the use of ensemble-variance analysis of macroscopic currents." J Neurosci Methods **158**(1): 121-132.

Liu, L., T. M. Yang, W. Liedtke and S. A. Simon (2006). "Chronic IL-1beta signaling potentiates voltage-dependent sodium currents in trigeminal nociceptive neurons." J Neurophysiol **95**(3): 1478-1490.

Liu, Y. Q., F. Yu, W. H. Liu, X. H. He and B. W. Peng (2014). "Dysfunction of hippocampal interneurons in epilepsy." Neurosci Bull **30**(6): 985-998.

Liu, Y., H. Liu, C. Sauvey, L. Yao, E. D. Zarnowska and S. C. Zhang (2013). "Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells." Nat Protoc **8**(9): 1670-1679.

Liu, Y., J. P. Weick, H. Liu, R. Krencik, X. Zhang, L. Ma, G. M. Zhou, M. Ayala and S. C. Zhang (2013). "Medial ganglionic eminence-like cells derived from human embryonic stem cells correct learning and memory deficits." Nat Biotechnol **31**(5): 440-447.

Longo, B. M. and L. E. Mello (1999). "Effect of long-term spontaneous recurrent seizures or reinduction of status epilepticus on the development of supragranular mossy fiber sprouting." Epilepsy Res **36**(2-3): 233-241.

Lorigados Pedre, L., L. M. Morales Chacon, N. Pavon Fuentes, M. L. A. Robinson Agramonte, T. Serrano Sanchez, R. M. Cruz-Xenes, M. L. Diaz Hung, B. Estupinan Diaz, M. M. Baez Martin and S. Orozco-Suarez (2018). "Follow-Up of Peripheral IL-1beta and IL-6 and Relation with Apoptotic Death in Drug-Resistant Temporal Lobe Epilepsy Patients Submitted to Surgery." Behav Sci (Basel) **8**(2).

Loscher, W., H. Klitgaard, R. E. Twyman and D. Schmidt (2013). "New avenues for anti-epileptic drug discovery and development." Nat Rev Drug Discov **12**(10): 757-776.

Lossin, C., T. H. Rhodes, R. R. Desai, C. G. Vanoye, D. Wang, S. Carniciu, O. Devinsky and A. L. George, Jr. (2003). "Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A." J Neurosci **23**(36): 11289-11295.

LoTurco, J. J., D. F. Owens, M. J. S. Heath, M. B. E. Davis and A. R. Kriegstein (1995). "GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis." Neuron **15**(6): 1287-1298.

Ludwin, S. K., J. C. Kosek and L. F. Eng (1976). "The topographical distribution of S-100 and GFA proteins in the adult rat brain: an immunohistochemical study using horseradish peroxidase-labelled antibodies." J Comp Neurol **165**(2): 197-207.

Ma, D. K., G. L. Ming and H. Song (2005). "Glial influences on neural stem cell development: cellular niches for adult neurogenesis." Curr Opin Neurobiol **15**(5): 514-520.

Ma, T., Q. Zhang, Y. Cai, Y. You, J. L. Rubenstein and Z. Yang (2012). "A subpopulation of dorsal lateral/caudal ganglionic eminence-derived neocortical interneurons expresses the transcription factor Sp8." Cereb Cortex **22**(9): 2120-2130.

Magloczky, Z., P. Halasz, J. Vajda, S. Czirjak and T. F. Freund (1997). "Loss of Calbindin-D28K immunoreactivity from dentate granule cells in human temporal lobe epilepsy." Neuroscience **76**(2): 377-385.

Maisano, X., E. Litvina, S. Tagliatela, G. B. Aaron, L. B. Grabel and J. R. Naegele (2012). "Differentiation and functional incorporation of embryonic stem cell-derived GABAergic interneurons in the dentate gyrus of mice with temporal lobe epilepsy." J Neurosci **32**(1): 46-61.

Marban, E., T. Yamagishi and G. F. Tomaselli (1998). "Structure and function of voltage-gated sodium channels." J Physiol **508 (Pt 3)**: 647-657.

Maroof, A. M., S. Keros, J. A. Tyson, S. W. Ying, Y. M. Ganat, F. T. Merkle, B. Liu, A. Goulburn, E. G. Stanley, A. G. Elefanty, H. R. Widmer, K. Eggan, P. A. Goldstein, S. A. Anderson and L. Studer

(2013). "Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells." Cell Stem Cell **12**(5): 559-572.

Maroso, M., S. Balosso, T. Ravizza, V. Iori, C. I. Wright, J. French and A. Vezzani (2011). "Interleukin-1beta biosynthesis inhibition reduces acute seizures and drug resistant chronic epileptic activity in mice." Neurotherapeutics **8**(2): 304-315.

Martinez-Losa, M., T. E. Tracy, K. Ma, L. Verret, A. Clemente-Perez, A. S. Khan, I. Cobos, K. Ho, L. Gan, L. Mucke, M. Alvarez-Dolado and J. J. Palop (2018). "Nav1.1-Overexpressing Interneuron Transplants Restore Brain Rhythms and Cognition in a Mouse Model of Alzheimer's Disease." Neuron **98**(1): 75-89 e75.

Maucksch, C., E. M. Vazey, R. J. Gordon and B. Connor (2013). "Stem cell-based therapy for Huntington's disease." J Cell Biochem **114**(4): 754-763.

Maurice, N., J. Mercer, C. S. Chan, S. Hernandez-Lopez, J. Held, T. Tkatch and D. J. Surmeier (2004). "D2 dopamine receptor-mediated modulation of voltage-dependent Na+ channels reduces autonomous activity in striatal cholinergic interneurons." J Neurosci **24**(46): 10289-10301.

Maurice, N., T. Tkatch, M. Meisler, L. K. Sprunger and D. J. Surmeier (2001). "D1/D5 dopamine receptor activation differentially modulates rapidly inactivating and persistent sodium currents in prefrontal cortex pyramidal neurons." J Neurosci **21**(7): 2268-2277.

McCoy, M. K. and M. G. Tansey (2008). "TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease." J Neuroinflammation **5**: 45.

Meeuwsen, S., C. Persoon-Deen, M. Bsibsi, R. Ravid and J. M. V. Noort (2003). "Cytokine, chemokine and growth factor gene profiling of cultured human astrocytes after exposure to proinflammatory stimuli." Glia **43**(3): 243-253.

Mehler, M. F. and J. A. Kessler (1997). "Hematolymphopoietic and inflammatory cytokines in neural development." Trends Neurosci **20**(8): 357-365.

Mello, L., E. A. Cavalheiro, A. M. Tan, W. R. Kupfer, J. K. Pretorius, T. L. Babb and D. M. Finch (1993). "CIRCUIT MECHANISMS OF SEIZURES IN THE PILOCARPINE MODEL OF CHRONIC EPILEPSY - CELL LOSS AND MOSSY FIBER SPROUTING." Epilepsia **34**(6): 985-995.

Meraz-Rios, M. A., D. Toral-Rios, D. Franco-Bocanegra, J. Villeda-Hernandez and V. Campos-Pena (2013). "Inflammatory process in Alzheimer's Disease." Front Integr Neurosci **7**: 59.

Milescu, L. S., B. P. Bean and J. C. Smith (2010). "Isolation of somatic Na+ currents by selective inactivation of axonal channels with a voltage prepulse." J Neurosci **30**(22): 7740-7748.

Miyoshi, G., J. Hjerling-Leffler, T. Karayannis, V. H. Sousa, S. J. Butt, J. Battiste, J. E. Johnson, R. P. Machold and G. Fishell (2010). "Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons." J Neurosci **30**(5): 1582-1594.

Monje, M. L., H. Toda and T. D. Palmer (2003). "Inflammatory Blockade Restores Adult Hippocampal Neurogenesis." Science **302**(5651): 1760-1765.

Monyer, H. and H. Markram (2004). "Interneuron Diversity series: Molecular and genetic tools to study GABAergic interneuron diversity and function." Trends Neurosci **27**(2): 90-97.

Muguruma, K., A. Nishiyama, H. Kawakami, K. Hashimoto and Y. Sasai (2015). "Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells." Cell Rep **10**(4): 537-550.

Murphy, B. L., R. Y. Pun, H. Yin, C. R. Faulkner, A. W. Loepke and S. C. Danzer (2011). "Heterogeneous integration of adult-generated granule cells into the epileptic brain." J Neurosci **31**(1): 105-117.

Nakazawa, K., V. Zsiros, Z. Jiang, K. Nakao, S. Kolata, S. Zhang and J. E. Belforte (2012). "GABAergic interneuron origin of schizophrenia pathophysiology." Neuropharmacology **62**(3): 1574-1583.

Navaei-Nigjeh, M., G. Amoabedini, A. Noroozi, M. Azami, M. N. Asmani, S. Ebrahimi-Barough, H. Saberi, A. Ai and J. Ai (2014). "Enhancing neuronal growth from human endometrial stem cells derived neuron-like cells in three-dimensional fibrin gel for nerve tissue engineering." J Biomed Mater Res A **102**(8): 2533-2543.

Nekouzadeh, A. and Y. Rudy (2007). "Statistical properties of ion channel records. Part II: estimation from the macroscopic current." Math Biosci **210**(1): 315-334.

Neumann, H., R. Schweigreiter, T. Yamashita, K. Rosenkranz, H. Wekerle and Y.-A. Barde (2002). "Tumor Necrosis Factor Inhibits Neurite Outgrowth and Branching of Hippocampal Neurons by a Rho-Dependent Mechanism." The Journal of Neuroscience **22**(3): 854-862.

Neves, R. S., I. de Souza Silva Tudesco, A. P. Jardim, L. O. Caboclo, C. Lancellotti, T. Ferrari-Marinho, A. P. Hamad, M. Marinho, R. S. Centeno, E. A. Cavalheiro, C. A. Scorza and E. M. Yacubian (2012). "Granule cell dispersion is associated with memory impairment in right mesial temporal lobe epilepsy." Seizure **21**(9): 685-690.

Nicholas, C. R., J. Chen, Y. Tang, D. G. Southwell, N. Chalmers, D. Vogt, C. M. Arnold, Y. J. Chen, E. G. Stanley, A. G. Elefanty, Y. Sasai, A. Alvarez-Buylla, J. L. Rubenstein and A. R. Kriegstein (2013).

"Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development." Cell Stem Cell **12**(5): 573-586.

Nicoleau, C., C. Varela, C. Bonnefond, Y. Maury, A. Bugi, L. Aubry, P. Viegas, F. Bourgois-Rocha, M. Peschanski and A. L. Perrier (2013). "Embryonic stem cells neural differentiation qualifies the role of Wnt/beta-Catenin signals in human telencephalic specification and regionalization." Stem Cells **31**(9): 1763-1774.

Numann, R., W. A. Catterall and T. Scheuer (1991). "Functional modulation of brain sodium channels by protein kinase C phosphorylation." Science **254**(5028): 115-118.

Ognjanovski, N., S. Schaeffer, J. Wu, S. Mofakham, D. Maruyama, M. Zochowski and S. J. Aton (2017). "Parvalbumin-expressing interneurons coordinate hippocampal network dynamics required for memory consolidation." Nat Commun **8**: 15039.

Olanow, C. W., C. G. Goetz, J. H. Kordower, A. J. Stoessl, V. Sossi, M. F. Brin, K. M. Shannon, G. M. Nauert, D. P. Perl, J. Godbold and T. B. Freeman (2003). "A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease." Annals of Neurology **54**(3): 403-414.

Olanow, C. W., J. H. Kordower and T. B. Freeman (1996). "Fetal nigral transplantation as a therapy for Parkinson's disease." Trends in Neurosciences **19**(3): 102-109.

Olude, M. A., O. A. Mustapha, O. A. Aderounmu, J. O. Olopade and A. O. Ihunwo (2015). "Astrocyte morphology, heterogeneity, and density in the developing African giant rat (Cricetomys gambianus)." Front Neuroanat **9**: 67.

Parent, J. M., T. W. Yu, R. T. Leibowitz, D. H. Geschwind, R. S. Sloviter and D. H. Lowenstein (1997). "Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus." J Neurosci **17**(10): 3727-3738.

Parish, C. L., D. I. Finkelstein, W. Tripanichkul, A. R. Satoskar, J. Drago and M. K. Horne (2002). "The role of interleukin-1, interleukin-6, and glia in inducing growth of neuronal terminal arbors in mice." J Neurosci **22**(18): 8034-8041.

Pasca, A. M., S. A. Sloan, L. E. Clarke, Y. Tian, C. D. Makinson, N. Huber, C. H. Kim, J. Y. Park, N. A. O'Rourke, K. D. Nguyen, S. J. Smith, J. R. Huguenard, D. H. Geschwind, B. A. Barres and S. P. Pasca (2015). "Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture." Nat Methods **12**(7): 671-678.

Peng, H., N. Whitney, Y. Wu, C. Tian, H. Dou, Y. Zhou and J. Zheng (2008). "HIV-1-infected and/or immune-activated macrophage-secreted TNF-alpha affects human fetal cortical neural progenitor cell proliferation and differentiation." Glia **56**(8): 903-916.

Petilla Interneuron Nomenclature, G., G. A. Ascoli, L. Alonso-Nanclares, S. A. Anderson, G. Barrionuevo, R. Benavides-Piccione, A. Burkhalter, G. Buzsáki, B. Cauli, J. Defelipe, A. Fairén, D. Feldmeyer, G. Fishell, Y. Fregnac, T. F. Freund, D. Gardner, E. P. Gardner, J. H. Goldberg, M. Helmstaedter, S. Hestrin, F. Karube, Z. F. Kisvárday, B. Lambolez, D. A. Lewis, O. Marin, H. Markram, A. Muñoz, A. Packer, C. C. H. Petersen, K. S. Rockland, J. Rossier, B. Rudy, P. Somogyi, J. F. Staiger, G. Tamas, A. M. Thomson, M. Toledo-Rodriguez, Y. Wang, D. C. West and R. Yuste (2008). "Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex." Nature reviews. Neuroscience **9**(7): 557-568.

Pluchino, S., L. Muzio, J. Imitola, M. Deleidi, C. Alfaro-Cervello, G. Salani, C. Porcheri, E. Brambilla, F. Cavasinni, A. Bergamaschi, J. M. Garcia-Verdugo, G. Comi, S. J. Khoury and G. Martino (2008). "Persistent inflammation alters the function of the endogenous brain stem cell compartment." Brain **131**(Pt 10): 2564-2578.

Powell, E. M. (2013). "Interneuron development and epilepsy: early genetic defects cause long-term consequences in seizures and susceptibility." Epilepsy Curr **13**(4): 172-176.

Powell, E. M., D. B. Campbell, G. D. Stanwood, C. Davis, J. L. Noebels and P. Levitt (2003). "Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction." J Neurosci **23**(2): 622-631.

Ravizza, T., B. Gagliardi, F. Noe, K. Boer, E. Aronica and A. Vezzani (2008). "Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy." Neurobiol Dis **29**(1): 142-160.

Reinchisi, G., K. Ijichi, N. Glidden, I. Jakovcevski and N. Zecevic (2012). "COUP-TFII expressing interneurons in human fetal forebrain." Cereb Cortex **22**(12): 2820-2830.

Represa, A. and Y. Ben-Ari (2005). "Trophic actions of GABA on neuronal development." Trends Neurosci **28**(6): 278-283.

Restivo, L., Y. Niibori, V. Mercaldo, S. A. Josselyn and P. W. Frankland (2015). "Development of Adult-Generated Cell Connectivity with Excitatory and Inhibitory Cell Populations in the Hippocampus." J Neurosci **35**(29): 10600-10612.

Roper, S. N. and D. A. Steindler (2013). "Stem cells as a potential therapy for epilepsy." Exp Neurol **244**: 59-66.

Roseti, C., E. A. van Vliet, P. Cifelli, G. Ruffolo, J. C. Baayen, M. A. Di Castro, C. Bertollini, C. Limatola, E. Aronica, A. Vezzani and E. Palma (2015). "GABAA currents are decreased by IL-1beta in epileptogenic tissue of patients with temporal lobe epilepsy: implications for ictogenesis." Neurobiol Dis **82**: 311-320.

Roy, N. S., C. Cleren, S. K. Singh, L. Yang, M. F. Beal and S. A. Goldman (2006). "Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes." Nat Med **12**(11): 1259-1268.

Roybon, L., N. J. Lamas, A. Garcia-Diaz, E. J. Yang, R. Sattler, V. Jackson-Lewis, Y. A. Kim, C. A. Kachel, J. D. Rothstein, S. Przedborski, H. Wichterle and C. E. Henderson (2013). "Human stem cell-derived spinal cord astrocytes with defined mature or reactive phenotypes." Cell Rep **4**(5): 1035-1048.

Ryan, S. M., G. W. O'Keeffe, C. O'Connor, K. Keeshan and Y. M. Nolan (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.

Sabilallah, M., P. Fontanaud, N. Linck, B. Boussadia, R. Peyroutou, T. Lasgouzes, F. A. Rassendren, N. Marchi and H. E. Hirbec (2016). "Evidence for Status Epilepticus and Pro-Inflammatory Changes after Intranasal Kainic Acid Administration in Mice." PLoS One **11**(3): e0150793.

Salanova, V., O. Markand and R. Worth (2002). "Temporal Lobe Epilepsy Surgery: Outcome, Complications, and Late Mortality Rate in 215 Patients." Epilepsia **43**(2): 170-174.

Schafers, M. and L. Sorkin (2008). "Effect of cytokines on neuronal excitability." Neurosci Lett **437**(3): 188-193.

Scheuer, T. (2011). "Regulation of sodium channel activity by phosphorylation." Semin Cell Dev Biol **22**(2): 160-165.

Scheuer, T. and W. A. Catterall (2006). "Control of neuronal excitability by phosphorylation and dephosphorylation of sodium channels." Biochem Soc Trans **34**(Pt 6): 1299-1302.

Schwartz, M. P., Z. Hou, N. E. Propson, J. Zhang, C. J. Engstrom, V. Santos Costa, P. Jiang, B. K. Nguyen, J. M. Bolin, W. Daly, Y. Wang, R. Stewart, C. D. Page, W. L. Murphy and J. A. Thomson (2015). "Human pluripotent stem cell-derived neural constructs for predicting neural toxicity." Proc Natl Acad Sci U S A **112**(40): 12516-12521.

Shaltouki, A., J. Peng, Q. Liu, M. S. Rao and X. Zeng (2013). "Efficient generation of astrocytes from human pluripotent stem cells in defined conditions." Stem Cells **31**(5): 941-952.

Sholl, D. A. (1953). "Dendritic organization in the neurons of the visual and motor cortices of the cat." J Anat **87**(4): 387-406.

Shors, T. J., G. Miesegaes, A. Beylin, M. Zhao, T. Rydel and E. Gould (2001). "Neurogenesis in the adult is involved in the formation of trace memories." Nature **410**(6826): 372-376.

Shtaya, A., A. R. Sadek, M. Zaben, G. Seifert, A. Pringle, C. Steinhauser and W. P. Gray (2018). "AMPA receptors and seizures mediate hippocampal radial glia-like stem cell proliferation." Glia **66**(11): 2397-2413.

Sierra, A., S. Martin-Suarez, R. Valcarcel-Martin, J. Pascual-Brazo, S. A. Aelvoet, O. Abiega, J. J. Deudero, A. L. Brewster, I. Bernales, A. E. Anderson, V. Baekelandt, M. Maletic-Savatic and J. M. Encinas (2015). "Neuronal hyperactivity accelerates depletion of neural stem cells and impairs hippocampal neurogenesis." Cell Stem Cell **16**(5): 488-503.

Sigworth, F. J. (1980). "The variance of sodium current fluctuations at the node of Ranvier." J Physiol **307**: 97-129.

Sloviter, R. S. (1987). "Decreased hippocampal inhibition and a selective loss of interneurons in experimental epilepsy." Science **235**(4784): 73-76.

Smith, R. D. and A. L. Goldin (1997). "Phosphorylation at a single site in the rat brain sodium channel is necessary and sufficient for current reduction by protein kinase A." J Neurosci **17**(16): 6086-6093.

Song, H., C. F. Stevens and F. H. Gage (2002). "Astroglia induce neurogenesis from adult neural stem cells." Nature **417**(6884): 39-44.

Song, J., J. Sun, J. Moss, Z. Wen, G. J. Sun, D. Hsu, C. Zhong, H. Davoudi, K. M. Christian, N. Toni, G. L. Ming and H. Song (2013). "Parvalbumin interneurons mediate neuronal circuitry-neurogenesis coupling in the adult hippocampus." Nat Neurosci **16**(12): 1728-1730.

Southwell, D. G., C. R. Nicholas, A. I. Basbaum, M. P. Stryker, A. R. Kriegstein, J. L. Rubenstein and A. Alvarez-Buylla (2014). "Interneurons from embryonic development to cell-based therapy." Science **344**(6180): 1240622.

Southwell, D. G., M. F. Paredes, R. P. Galvao, D. L. Jones, R. C. Froemke, J. Y. Sebe, C. Alfaro-Cervello, Y. Tang, J. M. Garcia-Verdugo, J. L. Rubenstein, S. C. Baraban and A. Alvarez-Buylla

(2012). "Intrinsically determined cell death of developing cortical interneurons." Nature **491**(7422): 109-113.

Spalding, K. L., O. Bergmann, K. Alkass, S. Bernard, M. Salehpour, H. B. Huttner, E. Boström, I. Westerlund, C. Vial, B. A. Buchholz, G. Possnert, D. C. Mash, H. Druid and J. Frisén (2013). "Dynamics of hippocampal neurogenesis in adult humans." Cell 153(6): 1219-1227.

Srinivasan, D., J.-H. Yen, D. J. Joseph and W. Friedman (2004). "Cell Type-Specific Interleukin-1β Signaling in the CNS." The Journal of Neuroscience **24**(29): 6482-6488.

Staley, K. (2015). "Molecular mechanisms of epilepsy." Nat Neurosci **18**(3): 367-372.

Steinbeck, J. A. and L. Studer (2015). "Moving stem cells to the clinic: potential and limitations for brain repair." Neuron **86**(1): 187-206.

Stojkovska, I., B. M. Wagner and B. E. Morrison (2015). "Parkinson's disease and enhanced inflammatory response." Exp Biol Med (Maywood) **240**(11): 1387-1395.

Strijbos, P. and N. Rothwell (1995). "Interleukin-1 beta attenuates excitatory amino acid-induced neurodegeneration in vitro: involvement of nerve growth factor." The Journal of Neuroscience **15**(5): 3468-3474.

Stroemer, R. P. and N. J. Rothwell (1998). "Exacerbation of ischemic brain damage by localized striatal injection of interleukin-1beta in the rat." J Cereb Blood Flow Metab **18**(8): 833-839.

Sun, G., W. Liu, Z. Fan, D. Zhang, Y. Han, L. Xu, J. Qi, S. Zhang, B. T. Gao, X. Bai, J. Li, R. Chai and H. Wang (2016). "The Three-Dimensional Culture System with Matrigel and Neurotrophic Factors Preserves the Structure and Function of Spiral Ganglion Neuron In Vitro." Neural Plast **2016**: 4280407.

Tai, C., Y. Abe, R. E. Westenbroek, T. Scheuer and W. A. Catterall (2014). "Impaired excitability of somatostatin- and parvalbumin-expressing cortical interneurons in a mouse model of Dravet syndrome." Proc Natl Acad Sci U S A **111**(30): E3139-3148.

Tamas, G., A. Lorincz, A. Simon and J. Szabadics (2003). "Identified sources and targets of slow inhibition in the neocortex." Science **299**(5614): 1902-1905.

Tcw, J., M. Wang, A. A. Pimenova, K. R. Bowles, B. J. Hartley, E. Lacin, S. I. Machlovi, R. Abdelaal, C. M. Karch, H. Phatnani, P. A. Slesinger, B. Zhang, A. M. Goate and K. J. Brennand (2017). "An Efficient Platform for Astrocyte Differentiation from Human Induced Pluripotent Stem Cells." Stem Cell Reports **9**(2): 600-614.

Telezhkin, V., C. Schnell, P. Yarova, S. Yung, E. Cope, A. Hughes, B. A. Thompson, P. Sanders, C. Geater, J. M. Hancock, S. Joy, L. Badder, N. Connor-Robson, A. Comella, M. Straccia, G. Bombau, J. T. Brown, J. M. Canals, A. D. Randall, N. D. Allen and P. J. Kemp (2016). "Forced cell cycle exit and modulation of GABAA, CREB, and GSK3beta signaling promote functional maturation of induced pluripotent stem cell-derived neurons." Am J Physiol Cell Physiol **310**(7): C520-541.

Thom, M. (2014). "Review: Hippocampal sclerosis in epilepsy: a neuropathology review." Neuropathol Appl Neurobiol **40**(5): 520-543.

Thom, M., L. Martinian, C. Catarino, M. Yogarajah, M. J. Koepp, L. Caboclo and S. M. Sisodiya (2009). "Bilateral reorganization of the dentate gyrus in hippocampal sclerosis A postmortem study." Neurology **73**(13): 1033-1040.

Thom, M., S. M. Sisodiya, A. Beckett, L. Martinian, W. R. Lin, W. Harkness, T. N. Mitchell, J. Craig, J. Duncan and F. Scaravilli (2002). "Cytoarchitectural abnormalities in hippocampal sclerosis." J Neuropathol Exp Neurol **61**(6): 510-519.

Thornton, P., E. Pinteaux, R. M. Gibson, S. M. Allan and N. J. Rothwell (2006). "Interleukin-1-induced neurotoxicity is mediated by glia and requires caspase activation and free radical release." J Neurochem **98**(1): 258-266.

Tichauer, J., K. Saud and R. von Bernhardi (2007). "Modulation by Astrocytes of Microglial Cell-Mediated Neuroinflammation: Effect on the Activation of Microglial Signaling Pathways." Neuroimmunomodulation **14**(3-4): 168-174.

Tong, L., G. A. Prieto, E. A. Kramar, E. D. Smith, D. H. Cribbs, G. Lynch and C. W. Cotman (2012). "Brain-derived neurotrophic factor-dependent synaptic plasticity is suppressed by interleukin-1beta via p38 mitogen-activated protein kinase." J Neurosci **32**(49): 17714-17724.

Tong, L., R. Balazs, R. Soiampornkul, W. Thangnipon and C. W. Cotman (2008). "Interleukin-1 beta impairs brain derived neurotrophic factor-induced signal transduction." Neurobiol Aging **29**(9): 1380-1393.

Toth, K., L. Eross, J. Vajda, P. Halasz, T. F. Freund and Z. Magloczky (2010). "Loss and reorganization of calretinin-containing interneurons in the epileptic human hippocampus." Brain **133**(9): 2763-2777.

Toulmond, S., P. Parnet and A. C. Linthorst (1996). "When cytokines get on your nerves: cytokine networks and CNS pathologies." Trends Neurosci **19**(10): 409-410.

Touzani, O., H. Boutin, R. LeFeuvre, L. Parker, A. Miller, G. Luheshi and N. Rothwell (2002). "Interleukin-1 influences ischemic brain damage in the mouse independently of the interleukin-1 type I receptor." J Neurosci **22**(1): 38-43.

Tyson, J. A., E. M. Goldberg, A. M. Maroof, Q. Xu, T. J. Petros and S. A. Anderson (2015). "Duration of culture and sonic hedgehog signaling differentially specify PV versus SST cortical interneuron fates from embryonic stem cells." Development **142**(7): 1267-1278.

Uludag, I. F., T. Duksal, B. I. Tiftikcioglu, Y. Zorlu, F. Ozkaya and G. Kirkali (2015). "IL-1beta, IL-6 and IL1Ra levels in temporal lobe epilepsy." Seizure **26**: 22-25.

Vanoye, C. G., C. Lossin, T. H. Rhodes and A. L. George, Jr. (2006). "Single-channel properties of human NaV1.1 and mechanism of channel dysfunction in SCN1A-associated epilepsy." J Gen Physiol **127**(1): 1-14.

Vezzani, A. and B. Viviani (2015). "Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability." Neuropharmacology **96**(Pt A): 70-82.

Vezzani, A. and T. Granata (2005). "Brain inflammation in epilepsy: experimental and clinical evidence." Epilepsia **46**(11): 1724-1743.

Vezzani, A. and T. Z. Baram (2007). "New roles for interleukin-1 Beta in the mechanisms of epilepsy." Epilepsy Curr **7**(2): 45-50.

Vezzani, A., D. Moneta, C. Richichi, M. Aliprandi, S. J. Burrows, T. Ravizza, C. Perego and M. G. De Simoni (2002). "Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis." Epilepsia **43 Suppl 5**: 30-35.

Vezzani, A., D. Moneta, M. Conti, C. Richichi, T. Ravizza, A. De Luigi, M. G. De Simoni, G. Sperk, S. Andell-Jonsson, J. Lundkvist, K. Iverfeldt and T. Bartfai (2000). "Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice." Proc Natl Acad Sci U S A **97**(21): 11534-11539.

Vezzani, A., E. Aronica, A. Mazarati and Q. J. Pittman (2013). "Epilepsy and brain inflammation." Exp Neurol **244**: 11-21.

Vezzani, A., J. French, T. Bartfai and T. Z. Baram (2011). "The role of inflammation in epilepsy." Nat Rev Neurol **7**(1): 31-40.

Vezzani, A., M. Conti, A. De Luigi, T. Ravizza, D. Moneta, F. Marchesi and M. G. De Simoni (1999). "Interleukin-1β Immunoreactivity and Microglia Are Enhanced in the Rat Hippocampus by Focal

Kainate Application: Functional Evidence for Enhancement of Electrographic Seizures." The Journal of Neuroscience **19**(12): 5054-5065.

Vezzani, A., S. Balosso and T. Ravizza (2008). "The role of cytokines in the pathophysiology of epilepsy." Brain Behav Immun **22**(6): 797-803.

Viviani, B., S. Bartesaghi, F. Gardoni, A. Vezzani, M. M. Behrens, T. Bartfai, M. Binaglia, E. Corsini, M. Di Luca, C. L. Galli and M. Marinovich (2003). "Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases." J Neurosci **23**(25): 8692-8700.

Wähämaa, H., H. Schierbeck, H. S. Hreggvidsdottir, K. Palmblad, A.-C. Aveberger, U. Andersson and H. E. Harris (2011). "High mobility group box protein 1 in complex with lipopolysaccharide or IL-1 promotes an increased inflammatory phenotype in synovial fibroblasts." Arthritis Research & Therapy **13**(4): R136.

Wang, J., Z. J. Lin, L. Liu, H. Q. Xu, Y. W. Shi, Y. H. Yi, N. He and W. P. Liao (2017). "Epilepsy-associated genes." Seizure **44**: 11-20.

Wang, Q., P. Hong, H. Gao, Y. Chen, Q. Yang, M. Jiang and H. Li (2016). "An interneuron progenitor maintains neurogenic potential in vivo and differentiates into GABAergic interneurons after transplantation in the postnatal rat brain." Sci Rep **6**: 19003.

Wartenberg, H. C. and B. W. Urban (2004). "Single sodium channels from human skeletal muscle in planar lipid bilayers: characterization and response to pentobarbital." J Anesth **18**(2): 100-106.

Watt, J. A. and N. K. Hobbs (2000). "Interleukin-1beta immunoreactivity in identified neurons of the rat magnocellular neurosecretory system: evidence for activity-dependent release." J Neurosci Res **60**(4): 478-489.

Webster, K. M., M. Sun, P. Crack, T. J. O'Brien, S. R. Shultz and B. D. Semple (2017). "Inflammation in epileptogenesis after traumatic brain injury." J Neuroinflammation **14**(1): 10.

Wiebe, S., W. T. Blume, J. P. Girvin, M. Eliasziw and T. Effectiveness Efficiency Surgery (2001). "A randomized, controlled trial of surgery for temporal-lobe epilepsy." New England Journal of Medicine **345**(5): 311-318.

Wonders, C. P. and S. A. Anderson (2006). "The origin and specification of cortical interneurons." Nat Rev Neurosci **7**(9): 687-696.

Wu, X., Y. Fu, G. Knott, J. Lu, G. Di Cristo and Z. J. Huang (2012). "GABA signaling promotes synapse elimination and axon pruning in developing cortical inhibitory interneurons." J Neurosci **32**(1): 331-343.

Xanthos, D. N. and J. Sandkuhler (2014). "Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity." Nat Rev Neurosci **15**(1): 43-53.

Yalçın, Ö. (2012). "Genes and molecular mechanisms involved in the epileptogenesis of idiopathic absence epilepsies." Seizure - European Journal of Epilepsy **21**(2): 79-86.

Yang, S., Z. W. Liu, L. Wen, H. F. Qiao, W. X. Zhou and Y. X. Zhang (2005). "Interleukin-1beta enhances NMDA receptor-mediated current but inhibits excitatory synaptic transmission." Brain Res **1034**(1-2): 172-179.

Yu, D. X., F. P. Di Giorgio, J. Yao, M. C. Marchetto, K. Brennand, R. Wright, A. Mei, L. McHenry, D. Lisuk, J. M. Grasmick, P. Silberman, G. Silberman, R. Jappelli and F. H. Gage (2014). "Modeling hippocampal neurogenesis using human pluripotent stem cells." Stem Cell Reports **2**(3): 295-310.

Yu, F. H., M. Mantegazza, R. E. Westenbroek, C. A. Robbins, F. Kalume, K. A. Burton, W. J. Spain, G. S. McKnight, T. Scheuer and W. A. Catterall (2006). "Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy." Nat Neurosci **9**(9): 1142-1149.

Zeng, L. H., N. R. Rensing and M. Wong (2009). "The Mammalian Target of Rapamycin Signaling Pathway Mediates Epileptogenesis in a Model of Temporal Lobe Epilepsy." Journal of Neuroscience **29**(21): 6964-6972.

Zhang, K., H. Xu, L. Cao, K. Li and Q. Huang (2013). "Interleukin-1beta inhibits the differentiation of hippocampal neural precursor cells into serotonergic neurons." Brain Res **1490**: 193-201.

Zhang, W., R. Yamawaki, X. Wen, J. Uhl, J. Diaz, D. A. Prince and P. S. Buckmaster (2009). "Surviving hilar somatostatin interneurons enlarge, sprout axons, and form new synapses with granule cells in a mouse model of temporal lobe epilepsy." J Neurosci 29(45): 14247-142

Zhou, C., C. Qi, J. Zhao, F. Wang, W. Zhang, C. Li, J. Jing, X. Kang and Z. Chai (2011). "Interleukin-1beta inhibits voltage-gated sodium currents in a time- and dose-dependent manner in cortical neurons." Neurochem Res **36**(6): 1116-1123.

Zunszain, P. A., C. Anacker, A. Cattaneo, S. Choudhury, K. Musaelyan, A. M. Myint, S. Thuret, J. Price and C. M. Pariante (2012). "Interleukin-1beta: a new regulator of the kynurenine pathway affecting human hippocampal neurogenesis." Neuropsychopharmacology **37**(4): 939-949.