Title
Serum albumin promotes stem cell proliferation and neurogenesis in a rodent model of post-traumatic epilepsy

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Publication Date
2015

Peer reviewed|Thesis/dissertation

Serum albumin promotes stem cell proliferation and neurogenesis in a rodent model of post-traumatic epilepsy

By

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A dissertation in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Neuroscience

in the

Graduate Division

of the

University of California, Berkeley

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Summer 2015

Abstract

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Traumatic brain injuries (TBIs) cause the breakdown of the blood-brain barrier (BBB) and the extravasation of serum albumin, and induce epileptiform activity via astrocytic transforming growth factor β (TGF-β) signaling. Intracerebroventricular (ICV) infusion of serum albumin alone is sufficient to induce seizures in rodents and has been associated with increased TGF-β signaling and synaptogenesis, which contribute to epileptogenesis. This study demonstrates that ICV infusion of serum albumin also promotes the proliferation of neural stem cells (NSCs) and aberrant neurogenesis in the dentate gyrus of the adult mouse. Treatment with an angiotensin II type 1 receptor antagonist (Losartan) prevents these changes. Furthermore, we demonstrate that serum albumin alone does not affect the proliferation of these NSCs *in vitro*, and is not sufficient to induce the secretion of astrocytic proliferative signals. This suggests that the increase in proliferation observed in animals exposed to serum albumin may require activity-induced proliferative signals from epileptiform activity, contributions from other cells in the niche in addition to astrocytes, cell-to-cell contact signals, or all three. These observations suggest a mechanism for the increase in neurogenesis observed in the post-injury epileptic brain and offer an opportunity to develop a therapeutic intervention to prevent epileptogenesis after TBIs.

Acknowledgements

I would like to thank my mentor; Dr. Daniela Kaufer for her exceptional leadership, encouragement, and support. I can only hope that our professional relationship continues to grow and develop as I move to the next stage of my academic endeavors. This project was a collaboration with Dr. Alon Friedman and his team of researchers at the Ben Gurion University in Beer Sheva, Israel. I would like to thank Dr. Friedman and his team including Itai Weissberg, Dan Z. Milikovsky, and Guy Bar-Klein for their teachings, support, guidance, and cooperation.

During the developmental and execution of this work, I had the honor and privilege of working with an incredibly bright and talented group of undergraduate students who provided great technical support. They include Maribel Patiño, Charmi Balsara, Louis Tan, Christian Felix, Carmen Maria Conroy, Luis Rangel, Ana Ibarra, Leslie Pedraza, Maxine Nanthavong, and Victoria Servin. Aaron Friedman and Drs. Luisa Cacheaux, Lydia Wood, and Soo Kim also provided a great amount of technical support, and I would like to thank them for their relentless drive supporting this project. I am also grateful to the rest of the Kaufer laboratory: Drs. David Covarrubias, Anna Geraghty, Kereshmeh Taravosh-Lahn, Noopur Amin, Samuel Sakhai, Andrea Nicholas, Elizabeth Kirby, Alana Wong, and Laurel Barchas, Vlad Senatorov, and Shawn Shirazi. Thank you for creating a supportive work environment and for extending your sincere friendship. In addition, I would like to thank my committee members, Drs. David Schaffer, Sanjay Kumar, and Scott Baraban for their time, advice, and material support for my experiments. I would also like to thank Drs. John Ngai, Russell Fletcher, Michael Shapira, Egle Cekanaviciute, Marion Buckwalter, and Mr. Jeremy Hamilton for having directly contributed to my education, technical proficiency in surgical procedures and protocols used for this project, and logistical support.

Finally, I would like to deeply thank my family. Throughout the completion of this work, my family has been an unlimited source of encouragement, support, and motivation for which I am eternally grateful. I am very fortunate to have their supportive and endless love, patience, and confidence in my success. Thank you.

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Chapter 1 – INTRODUCTION

Glial cells in neurological disorders

The nervous tissues that comprise the central nervous system contain glial cells, epithelial cells, stem cells, and neurons. The relative amounts of these cells and the intricate ways in which they connect to one other give rise to the rich repertoire of behaviors exhibited by animals. Accordingly, neurological disorders are the result of errors within the assortment of molecular interactions that these cells undertake. Astrocytes, oligodendrocytes, and Schwann cells together form a group of glial cells referred to as macroglia. Combined with microglia, immune system cells that mobilize to the brain to perform a protective role, these glial cells support the development and function of neurons and their networks. Glial cells and neurons share an embryonic precursor cell in their lineage but are morphologically and functionally distinct from neurons. There are many types of glial cells, and mounting evidence suggests that glial cells may be as heterogeneous as neurons. Research on glia has enjoyed a resurgence in recent years, thanks in part to an ever-increasing number of studies highlighting the critical roles played by glia in the development of the nervous system and in neurological disorders. Glia have been shown to regulate the blood-brain barrier and act as gatekeepers of the vasculature of the brain, thereby modulating the development of many neuropathological processes, from cerebral ischemia (Nedergaard & Dirnagl, 2005) to migraines (Bartley, 2009; Thalakoti et al., 2007). Furthermore, glial cells are important for the repair of nervous tissue after injury and also contribute to neuropathology in neurodegenerative diseases.

Glial cells play important roles in several hereditary neurodegenerative diseases, including amyotrophic lateral sclerosis, spinocerebellar ataxia, Parkinson's disease, and Huntington's disease, in which mutated proteins associated with these neurological disorders act via glial cells to reduce the efficiency of their supportive roles and facilitate the release of neurotoxic compounds (Lobsiger & Cleveland, 2007). For example, mutated SOD1 has been shown to act via astrocytes and microglia to accelerate neurodegeneration in amyotrophic lateral sclerosis (Brites & Vaz, 2014; Papadeas, Kraig, O'Banion, Lepore, & Maragakis, 2011). Bergmann glia have been shown to express a polyglutamine-expanded ataxin-7 that directly induces the neurodegeneration of Purkinje cells by impairing glutamate transport (Custer et al., 2006).

One important function of glia in the brain is the regulation of the microvasculature by astrocytes (Iadecola & Nedergaard, 2007). The regulation of blood flow in the brain is necessary to meet the demands for oxygen and glucose caused by neuronal activity. Astrocytes regulate the vascular tone of arterioles with which they come into contact in response to changes in intracellular calcium (Filosa & Iddings, 2013; Filosa, Bonev, & Nelson, 2004). This important role of astrocytes also allows them to participate in the development of cerebrovascular disease. Astrocytes have been directly implicated in brain ischemia (Chen & Swanson, 2003; Rossi, Brady, & Mohr, 2007; Takano, Oberheim, Cotrina, & Nedergaard, 2009), a type of cerebrovascular disorder that is often caused by strokes. Astrocytes are known to both protect and damage neurons affected by an interruption in blood supply. In their protective role, astrocytes are known to use their stores of glycogen to provide energy to neurons deprived of glucose (A. M. Brown & Ransom, 2007; Dringen, Gebhardt, & Hamprecht, 1993; Rossi et al., 2007; Stobart & Anderson, 2013; S. W. Suh et al., 2007b). On the other hand, astrocytes can also

damage neurons via lactic acidosis and the release of neurotransmitters, such as glutamate, which is thought to produce a neurotoxic effect after ischemic brain damage(Chen & Swanson, 2003; Rossi et al., 2007). Another reason why astrocytes have been implicated in the protection of neurons is their ability to express voltage-gated ion channels and neurotransmitter receptors that are co-activated at synapses and participate in the removal of potentially toxic excitatory amino acids (Auld & Robitaille, 2003). Astrocytes also help support synaptic homeostasis by providing trophic factors that support the survival and proliferation of adult-generated neurons (Song, Stevens, & Gage, 2002), defend against oxidative stress (Dringen, Pfeiffer, & Hamprecht, 1999; Peuchen et al., 1997), maintain glutamate, ion, and water balances, participate in scar formation and tissue repair, and facilitate synapse formation and remodeling(Bélanger & Magistretti, 2009; Bélanger, Allaman, & Magistretti, 2011; Volterra & Meldolesi, 2005). However, impairments in any of these physiological processes can lead to the pathogenesis of multiple developmental, genetic, idiopathic and acquired neurological disorders(Nedergaard, Ransom, & Goldman, 2003; Takano et al., 2009). Because astrocytes are also connected, via gap junctions (Anders et al., 2014; Bennett, Contreras, Bukauskas, & Sáez, 2003; Dermietzel, Hertberg, Kessler, & Spray, 1991; Giaume et al., 1991; Sáez, Contreras, Bukauskas, Retamal, & Bennett, 2003), in large astrocytic networks, they are believed to be important in the propagation of stroke-induced damage to neurons surrounding a site of vascular injury (Rossi et al., 2007).

Astrocytes are generally subdivided into two major groups: protoplasmic astrocytes and fibrous astrocytes. Protoplasmic astrocytes populate the gray matter and have numerous processes that end in sheet-like appendages that make contact with and encircle capillaries and blood vessels and envelope cell bodies and synapses. Fibrous astrocytes populate regions of white matter tracts and have small, elongated processes that make contact with and encircle capillaries, blood vessels, and axons at the nodes of Ranvier. Combined, astrocytes greatly outnumber neurons and thus have the ability to create separation within neuronal networks and between adjacent synapses. This spatial separation serves as insulation that prevents the unintended propagation of action potentials between neighboring circuits. For example, astrocytes express K^+ inward-rectifier channels that make them highly permeable to K^+. This permeability helps maintain constant K^+ concentrations in the extracellular space surrounding neurons. Concentrations of extracellular K^+ change after neuronal firing, because repolarization requires neurons to open voltage gated K^+ channels that allow the flow of K^+ into extracellular space. Astrocytes are charged with the task of buffering these changes to maintain an ionic balance conducive to this repolarization process and to proper neuronal communication. This astrocytic function is especially important during episodes of hyperactivity because, as neurons generate and propagate more action potentials, excess amounts of K^+ can build up in the extracellular space and prevent neuronal repolarization, thereby reducing the efficiency of signaling between neurons. Astrocytes also perform other important functions associated with efficient signaling, such as neurotransmitter re-uptake and metabolic processing to terminate signaling. In a model of traumatic brain injury, we demonstrated that K^+ buffering and neurotransmitter re-uptake is affected by serum albumin that escapes from the vasculature after injury, and that this process leads to epileptogenesis (Cacheaux et al., 2009; David et al., 2009; Friedman, Kaufer, & Heinemann, 2009; Weissberg, Reichert, Heinemann, & Friedman, 2011).
All of these examples of neurological disorders demonstrate how, working together, glial cells and neurons establish specific networks of interconnected neurons that mediate behaviors and

allow for cognition in humans. Deleterious genetic variations or injuries can negatively affect these harmonious co-dependent interactions and produce neurological disorders.

Adult neurogenesis and glial cells

After embryonic development, the brain retains the ability to proliferate and generate more neurons, albeit in restricted niches (Ming & Song, 2011). The process of generating functionally mature neurons from stem cells residing in the adult brain is called adult neurogenesis. Altman and Das provided the first anatomical evidence that the postnatal brain continues to generate new granule cells in the dentate gyrus of the hippocampi of rats (Altman & Das, 1965). Twenty years after this pioneering discovery, another group demonstrated that songbirds possess the ability to recruit and functionally integrate newly born neurons into existing networks of the CNS (Paton & Nottebohm, 1984). These observations promoted a race to reveal the locations, molecular mechanisms, and biological significance of adult neurogenesis. The study of adult neurogenesis moved in leaps and bounds after the introduction of 5-bromo-2'deoxyuridine (BrdU), a thymidine analog that allowed a cell's lineage to be traced back to a temporally specific period of proliferation in which dividing cells incorporated this exogenous molecule into their deoxyribonucleic acid (DNA) during the DNA replication phase of the cell cycle (Kuhn, Dickinson-Anson, & Gage, 1996). This technique was later enhanced with the introduction of 5-ethynyl-2'-deoxyuridine (Salic & Mitchison, 2008; SB et al., 2008; Zeng et al., 2010) (EdU), another thymidine analog that, when combined with click chemistry, circumvents many of the limitations of cell cycle analysis and lineage tracing using BrdU incorporation (Cappella, Gasparri, Pulici, & Moll, 2008).

Adult neurogenesis is restricted to the subgranular zone (SGZ) in the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles, under typical conditions. After injury, neurogenesis can be induced in other regions of the CNS (Gould, 2007), but the mechanisms through which this phenomenon occurs remain elusive.

Neural stem cells (NSCs) drive the neurogenic activity of the nervous system. A stem cell is a type of undifferentiated cell that 1) exhibits the ability to self-renew through cell division and 2) has the capacity to generate specialized cell types through the differentiation of its progeny (Gage, 2000). Although the debate regarding the specific lineage-relationship of putative NSCs in the adult brain remains an open discussion (Alvarez-Buylla & Lim, 2004; Lugert et al., 2010; Ming & Song, 2011; H. Suh et al., 2007a), it is commonly accepted that, in the SGZ, proliferating radial and nonradial precursors generate intermediate progenitors that sequentially generate neuroblasts (Ming & Song, 2005; 2011). These neuroblasts can differentiate into motile immature neurons that migrate to the inner granule cell layer and further differentiate into mature dentate granule cells. These cells quickly develop dendrites that reach into the molecular layer and axons that establish synapses with neurons in the CA3 region through the mossy fiber pathway of the hilus (Zhao, Teng, Summers, Ming, & Gage, 2006). These morphological changes allow the granule cells to fully integrate into the hippocampal circuitry (Ge, Sailor, Ming, & Song, 2008). Throughout their maturation process, these newly developed granule cells exhibit changes in their responsiveness to the neurotransmitters to which they are exposed. Newly developed granule cells are first activated by gamma-aminobutyric acid (GABA) (Bhattacharyya et al., 2008; Ge et al., 2006) and then by glutamatergic synaptic inputs (Espósito

et al., 2005; Overstreet-Wadiche, Bromberg, Bensen, & Westbrook, 2006). Newborn neurons are hyperexcitable and exhibit greater plasticity than mature granule cells (Ge et al., 2008; Schmidt-Hieber, Jonas, & Bischofberger, 2004).

There are various intracellular and extracellular molecular mechanisms that regulate different phases of adult neurogenesis (Faigle & Song, 2013; Leone et al., 2014; Ma et al., 2010; Ming & Song, 2011; Mu, Lee, & Gage, 2010; Ninkovic & Götz, 2007; Wakabayashi, Hidaka, Fujimaki, Asashima, & Kuwabara, 2014; Zhao, Deng, & Gage, 2008). Additionally, adult neurogenesis is regulated by environmental factors, such as physiological stimuli in the case of exercise (Garrett, Lie, Hrabé de Angelis, Wurst, & Hölter, 2012; van Praag, Kempermann, & Gage, 1999), enrichment (Kempermann, Kuhn, & Gage, 1997; 1998), stress (Danzer, 2012; Grégoire, Bonenfant, Le Nguyen, Aumont, & Fernandes, 2014; Kirby et al., 2013; Mirescu & Gould, 2006; Naninck et al., 2015; Saaltink & Vreugdenhil, 2014; Schoenfeld & Gould, 2012; 2013), and disease (Felling & Levison, 2003; Stranahan et al., 2008; Winner, Kohl, & Gage, 2011). Seizures also have a potent regulatory effect on adult neurogenesis, increasing the proliferation of some stem cells in the SGZ and SVZ (Jessberger & Parent, 2011) and depleting the stem cell pool via differentiation in regions where epileptic foci are established (Sierra et al., 2015).

Some of the extracellular molecular mechanisms involved in the regulation of adult neurogenesis are products of the interplay between neural stem cells, neurons, and glia. Most classes of glial cells are directly or indirectly associated with the process of adult neurogenesis. To begin, it is noteworthy to highlight the morphological (radial processes) and molecular (expression of Glial fibrillary acidic protein [GFAP]) similarities between the glia-like neural stem cells, in both neurogenic zones (SVZ and SGZ), and astrocytes (D. D. Wang & Bordey, 2008). The similarities between these populations of cells have led some to propose that these neural stem cells form another subcategory of astrocytes (Morrens, Van Den Broeck, & Kempermann, 2012). These cells produce new neurons, create an environment that is permissive of neurogenesis, and are themselves generated in a parallel and independently regulated process(Morrens et al., 2012). As cells in the niche, astrocytes position themselves adjacent to progenitor cells and newborn neurons and seem to have an intimate cell-to-cell contact relationship with these cells, suggesting that they may play a key role in regulating adult neurogenesis (Plümpe et al., 2006; Shapiro, Korn, Shan, & Ribak, 2005). Moreover, ephrin-B2 presented by hippocampal astrocytes has been found to regulate neurogenesis *in vivo*, through this proposed juxtacrine mechanism(Ashton et al., 2012). Nonetheless, astrocytes are also known to be involved in several other paracrine signaling pathways, such as Notch (Givogri et al., 2006; Magnusson et al., 2014; Wilhelmsson et al., 2012), Sonic Hedgehog (Garcia, Petrova, Eng, & Joyner, 2010; Y. Wang et al., 2014; H. Yang et al., 2012b), and Wingless (Lie et al., 2005; C. Yang et al., 2012a), which are all implicated in the regulation of adult neurogenesis. Astrocytes are also known to produce several growth factors and neurotrophic factors that regulate the process of adult neurogenesis, such as fibroblast growth factor-2 (FGF2), vascular endothelial growth factor (VEGF), and ciliary neurotrophic factor (CNTF) (Morrens et al., 2012). More on the astrocytic regulation of adult neurogenesis is presented in chapters 2 and 3.

Transforming growth factor β superfamily and neurogenesis

The transforming growth factor β (TGF-β) superfamily, and its associated signaling pathways, are just one example of the many molecular mechanisms involved in the regulation of neurogenesis in naïve and injured animals (Y. He et al., 2014; Villapol, Logan, & Symes, 2013; Yousef, Morgenthaler, et al., 2015b). Some of the members of this family that have been directly implicated in the regulation of neurogenesis include the bone morphogenetic proteins (BMPs) (Colak et al., 2008; D. A. Lim et al., 2000; Maćkowiak, Chocyk, Markowicz-Kula, & Wedzony, 2004; Mercier & Douet, 2014; Mira et al., 2010; Yousef, Morgenthaler, et al., 2015b), Activins (Abdipranoto-Cowley et al., 2009; Rodríguez-Martínez & Velasco, 2012; Rodríguez-Martínez, Molina-Hernández, & Velasco, 2012), and TGF-β1, TGF-β2, and TGF-β3 (Aigner & Bogdahn, 2008; Buckwalter et al., 2006; Graciarena, Roca, Mathieu, Depino, & Pitossi, 2013; Wachs et al., 2006).

The complete superfamily of TGF-β cytokines is composed of nearly 40 proteins, including BMPs, growth differentiation factors (GDFs), activins, inhibins, nodal, lefty, mullerian inhibiting substances (MIS), and the three types of TGF-β proteins mentioned above (de Caestecker, 2004; J, M, & C, 2011; Massague, 1992; Massague, Cheifetz, Ignotz, & Boyd, 1987; Romano et al., 2012). This family is divided in two main categories, based on the type of receptor to which the proteins bind. The first group binds to the ALK4, ALK5, and ALK 7 types of TGF-β type I receptors (TGF-βRI) and is known as the TGF-β/Activin group. The second group binds to the ALK1, ALK2, ALK3, and ALK6 types of TGF-βRI and is known as the BMP/GDF. Both of these groups use a similar process of signal transduction that starts with binding of one of these cytokines to specific type I and II transmembrane serine threonine/kinase receptors that phosphorylate intracellular Smad proteins, which in turn translocate to the nucleus to regulate gene transcription. Because these cytokines primarily bind to specific types of TGF-βRI, the specificity in the type of Smad protein that is activated, and thus the signaling cascade that is used, is provided by the identity of the TGF-βRI. For example, the ligands TGF-β, nodal, and activin bind to the TGF-βRII first, and this receptor recruits and phosphorylates the ALK4, ALK5, or ALK 7 TGF-βRIs. Once phosphorylated, these TGF-βRIs are activated and only phosphorylate Smad 2 and Smad 3 proteins. This process transduces a signal to the interior of the cell, and the type of Smad protein involved in the signaling cascade will dictate the transcriptional changes driven by the stimulus. For a complete review of this signaling pathway, please refer to the following (de Caestecker, 2004; Heldin & Moustakas, 2012).

Neurons and astrocytes from the CNS produce all three isoforms of TGF-β (Bar-Klein et al., 2014; Vivien & Ali, 2006). In the adult brain in particular, receptors for TGF-β are found in the cortex, hippocampus, striatum, brainstem, and cerebellum (Blottner, Wolf, Lachmund, Flanders, & Unsicker, 1996; Flanders, Ren, & Lippa, 1998; Villapol et al., 2013). Furthermore, neurons, astrocytes, microglia, and endothelial cells in the cortical gray matter express TGF-βRI and TGF-βRII (Bar-Klein et al., 2014; De Groot, Montagne, Barten, Sminia, & Van Der Valk, 1999). This suggests that most cells in the CNS could potentially respond to cytokines of this superfamily and activate TGF-β signaling, the downstream effects of which are known to control the proliferation and differentiation of stem cells in neurogenic niches and the maturation and survival of their progeny in the adult brain (Buckwalter et al., 2006; Y. He et al., 2014; Moustakas & Heldin, 2009; Villapol et al., 2013). In the adult human brain, TGF-β1 is expressed

in the hippocampus and is known to be unregulated by aging (Werry, Enjeti, Halliday, Sachdev, & Double, 2010). Supporting a role for TGF-β signaling in the regulation of neurogenesis, studies have found prominent activation of TGF-β signals in the adult dentate gyrus and the expression of downstream Smad proteins in this neurogenic zone (Y. He et al., 2014). Additionally, studies characterizing the neurogenesis that occurs after traumatic brain injury have demonstrated that the dentate gyrus undergoes significant alterations to the mRNA expression levels of components and targets of the TGF-β signaling pathway, suggesting a regulatory role for TGF-β in post-injury neurogenesis (Logan, Villapol, & Symes, 2013).

Recently, we showed that serum albumin also has the ability to bind to the TGF-βRII (Cacheaux et al., 2009) and preferentially activate the ALK5 pathway in astrocytes (Bar-Klein et al., 2014). This activation results in the increased phosphorylation of Smad 2 proteins in cultured astrocytes (Bar-Klein et al., 2014) and cortical lysates from animals exposed to serum albumin (Cacheaux et al., 2009). This novel mechanism for activating the TGF-β signaling pathway results in similar transcriptional changes to those observed in animals exposed to TGF-β1, and this response can be blocked with antibodies for TGF-βRII and SB431542, a selective inhibitor of the ALK5, ALK4, and ALK7 types of TGF-βRI. We have also shown that this albumin-dependent activation of the TGF-β signal is blocked in the brain after treatment with the angiotensin II type 1 receptor antagonist, losartan (Bar-Klein et al., 2014), which is also known to block TGF-β signals (Campistol et al., 1999; Cohn et al., 2007; Khalil et al., 2000; Lavoie et al., 2005; D. S. Lim et al., 2001). Because serum albumin demonstrates this unique ability to activate the TGF-β signaling pathway via the ALK5 type I TGF-βR, a known regulator of the late stages of adult hippocampal neurogenesis (Y. He et al., 2014), its role in the regulation of neurogenesis after breakdown of the blood-brain-barrier needed to be ascertained to better understand the physiological effects of the extravasation of albumin after brain injury.

Seizures and Epilepsy

Seizures are another potent regulator of neurogenesis in the brain. Seizures are transient and abnormal changes in brain function caused by hyperactivity and hypersynchrony of neuronal networks (Kandel, 2013). Epilepsy is a brain disorder that causes spontaneous recurrent seizures that lead to abnormal behavior and/or sensations (Jessberger & Parent, 2011). Epidemiological studies have demonstrated that, in the United States, approximately 3% of those who live to the age of 80 years are diagnosed with epilepsy (Kandel, 2013). The signs and symptoms of seizures that cause epilepsy depend on the location of the affected brain regions. These can include positive and negative effects, such as the gain of a sensory experience (e.g., seeing flashing lights or olfactory hallucinations) or the loss of consciousness or a particular sensory capability (e.g., temporary blindness).

Seizures are classified as focal seizures or generalized seizures. Abnormal firing in a small group of neurons causes focal seizures, and their manifestation depends on the brain region where those neurons are located. These types of seizures may or may not affect consciousness, depending on the foci that act as the origin for the seizure, and may or may not extend to other brain regions. If a focal seizure extends to other brain regions, the complexity of its symptomology increases, and if it causes a loss of consciousness, the seizure may then be considered a secondarily generalized seizure.

Generalized seizures affect both brain hemispheres from their onset. They can be subcategorized into convulsive and non-convulsive generalized seizures, depending on whether the seizures are associated with the stiffening of all muscles at the same time (tonic movements) or the jerking of all extremities (clonic movements). An example of a nonconvulsive generalized seizure is the typical absence seizure. In this subcategory of generalized seizures, individuals suffering the seizure exhibit cessation of all motor activity, loss of consciousness, and retained postures. They may appear to be in a trance for a brief period of time that usually lasts less than 10 seconds. The most commonly observed type of convulsive seizure is the tonic-clonic generalized seizure. These seizures begin without warning, and are often characterized by an initial involuntary cry made by air that is quickly expelled from the lungs through the tonic contraction of the diaphragm and thorax. This tonic phase is also characterized by a rigid posture caused by the simultaneous stiffening of all muscles, and cyanosis. The tonic phase usually lasts 30 seconds in humans, and quickly transitions to the clonic phase, in which the extremities will jerk for up to 2 minutes. The clonic phase is followed by the postictal phase, in which neuronal activity slowly returns to normal functioning, and the individual who just suffered the seizure may express physical exhaustion and sleepiness and complain of muscle soreness.

Epilepsy, as defined by the International League Against Epilepsy's official report of 2014, is defined as a disease of the brain that is characterized by any of the following conditions: 1) At least 2 two unprovoked seizures occurring more than 24 hours apart; 2) one unprovoked seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years; 3) diagnosis of an epilepsy syndrome (Fisher et al., 2014). The last part of this definition is important for the field of epilepsy research because it allows, for the first time, the identification of epilepsy in patients in which only an epilepsy syndrome has been diagnosed, including patients in whom only one seizure was observed but a preponderance of genetic and environmental information is known and indicative of the presence of the disease. Previously, observations used in a clinical setting to diagnose epilepsy depended on behavioral observations collected during or after the occurrence of seizures. This paradigm shift arose in the wake of scientific discoveries that revealed important information about the cellular, molecular, and genetic mechanisms involved in the development of seizures and epilepsy.

The ability to draw observations from the cellular mechanisms involved in the development of seizures is advantageous for scientists and clinicians intending to generalize conclusions and design therapeutic approaches. Despite myriad clinically-defined seizures, a good understanding of the process of seizure generation can be drawn from studying the electrophysiological behavior of cells causing focal seizures. The abnormal hyperactive and hypersynchronous activity of a focal seizure originates from a seizure focus. This focus must have the ability to generate hyperactive signaling and spread that signaling to neighboring regions that will synchronize with the focus to cause the seizure.

The hyperactivity of the focus may be caused by different cellular factors or changes in synaptic connections caused by injury, blood clots, and tumors (Kandel, 2013). Much of the knowledge gathered about this type of hyperactive and hypersynchronous neuronal activity comes from work performed using animal models of focal seizures. In most of these studies, seizures were

induced via focal electrical stimulation, focal lesion, or by acute injection of convulsing agents, such as pilocarpine or kainic acid. After the induction of seizures, neuronal behavior is observed in most cases via electroencephalogram (EEG), electrocorticoencephalogram (ECog), or directly in cells using *in vitro* brain slice preparations. These approaches have offered vast amounts of information but have 1) centered on neuronal behavior, and 2) not provided an opportunity to study the epileptogenic period because they depend on the artificial and acute induction of status epilepticus (SE). A model that more closely resembles the natural process of epileptogenesis will offer a unique opportunity to understand the relationships between neurons and glial cells that lead to hyperactivity and hypersynchrony at the seizure focus and to develop prophylactic treatments that prevent seizures and epilepsy in at-risk individuals.

To design such a model, it is necessary to decide which epileptogenic conditions are most suitable for replication in the laboratory. The causes of epilepsy vary, and the temporal frameworks in which these causes operate remain poorly understood or unknown in most cases. The uncertainty of when a seizure will occur is precisely why epilepsy is such a debilitating condition. One environmental factor that is known to have the ability to give rise to seizures and epilepsy is a traumatic brain injury (TBI) (Annegers, Hauser, Coan, & Rocca, 1998; Herman, 2002; Shlosberg, Benifla, Kaufer, & Friedman, 2010). The epileptogenic window in post-traumatic epilepsy could be as short as 24 hours (H.-C. Wang et al., 2008) or a week in humans (Herman, 2002), and 2-3 days in rodents (Bar-Klein et al., 2014), which makes TBI a suitable insult to model when studying the natural processes of seizure development and epileptogenesis.

Traumatic Brain Injury and Epilepsy

Traumatic brain injury (TBI) is the leading cause of death in young adults and children in developed countries (Shlosberg et al., 2010). In the US, an estimated 1.7 million people sustain TBIs annually (Faul, Xu, Wald, & Coronado, 2010), and approximately 53,000 die from these injuries (Coronado et al., 2011), making TBI-related deaths responsible for approximately one third of all injury-related deaths (Coronado et al., 2011). Armed conflict increases this proportion, and during the Iraq campaigns nearly two-thirds of injured US troops evacuated for treatment at Walter Reed Army Medical Center suffered TBIs (Warden, 2006). Survivors of TBI are confronted with lifelong disabilities that force them into dependency on clinical care. In the US alone, over 2% of the population is estimated to experience TBI-associated disabilities (Thurman, Alverson, Dunn, Guerrero, & Sniezek, 1999) at a cost of $60 billion each year in medical services and loss-of-productivity costs (Shlosberg et al., 2010).

Epilepsy is one of the disabilities suffered by most survivors of TBIs. The relationship between TBIs and epilepsy has been well-documented (Annegers et al., 1998; Herman, 2002; Shlosberg et al., 2010), but although several studies have highlighted a role for the blood brain barrier in epileptogenesis (Oby & Janigro, 2006), the pathophysiological mechanism that catalyzes post-traumatic epilepsy (PTE) remains poorly understood. Studies in rodents and humans have offered important observations that form the basis for this thesis. One such observation is that the epileptic brain is characterized by numerous alterations to hippocampal architecture (McAuliffe et al., 2011; Murphy & Danzer, 2011; Murphy et al., 2011; Parent, Elliott, Pleasure, Barbaro, & Lowenstein, 2006; Sierra et al., 2015). Additionally, in several models of epilepsy and in humans

suffering from epilepsy, seizures have been shown to increase neurogenesis (Parent et al., 2006; 1997).

As described above, neurogenesis is the process of generating and integrating functionally mature neurons derived from a progenitor cell. Work with various mammalian species has contributed to the understanding that this process begins during embryonic development and progresses through adulthood in the subgranular zone (SGZ) of the hippocampus (Ming & Song, 2005). Adult hippocampal neurogenesis is tightly regulated by internal and external cues including aging, hormones, physical activity, and seizures (Ming & Song, 2005). Of these processes, the dramatic increase in hippocampal neurogenesis associated with seizures requires further study, as the association between seizures and the molecular pathways of the proliferation of NSCs remains poorly understood. This relationship has been difficult to investigate because the methods available for evoking seizures *in vivo* do not offer the time resolution required to assess the effects of seizures as neurogenesis progresses. The recent discovery that exposure to albumin both *in vivo* and *ex vivo* (brain slices) results in delayed and prolonged seizure activity (Ivens et al., 2007) represents an opportunity to assess the relationship between seizures and neurogenesis. Seizures are associated with the uptake of albumin by astrocytes. This uptake is mediated by transforming growth factor β receptors (Ivens et al., 2007) (TGF-βR) and causes a transcriptional change that severely affects the ability of astrocytes to regulate the ionic balance at the synapse (David et al., 2009), thereby facilitating the development of seizures (David et al., 2009; Ivens et al., 2007). Even though this effective model of epileptogenesis is able to evoke electrophysiological neuronal responses similar to those seen in epileptic seizures, its relationships to changes in neurogenesis and changes in hippocampal architecture remain untested in this model of PTE (which I will refer to as the albumin exposure model [AEM]).

In these experiments, we addressed the relationship between the development of seizure formation, neurogenesis, and hippocampal architecture by testing the hypothesis that 1) the AEM exhibits increased neurogenesis and 2) these newly born cells develop aberrantly, facilitating the establishment of a recurrent circuit.

Chapter 2 – Exposure to serum albumin promotes cell proliferation in the adult hippocampus

HYPOTHESIS:

Upon exposure to serum albumin, adult mice exhibit an increase in neurogenesis in the dentate gyrus of the hippocampus.

INTRODUCTION

Studies of rodent models of epilepsy and acute seizures have demonstrated increased neurogenesis in the SGZ (Parent, 2003) after seizures and suggest that seizure-induced neurogenesis may involve aberrant neuroblast migration to produce ectopic granule cells (Parent et al., 2006) and altered cell integration that is facilitated by mossy fibre sprouting, neuronal hypertrophy and persistence of hilar basal dendrites on adult-generated granule neurons (McAuliffe et al., 2011; Murphy et al., 2011). These processes are believed to contribute to the persistent hippocampal hyperexcitability that is characteristic of epilepsy. Accordingly, when adult neurogenesis is genetically suppress before acute pilocarpine-induced seizures, there is a reduction in the frequency of chronic seizures (Cho et al., 2015). This reduction is did not completely impede epileptogenesis, suggesting that adult neurogenesis that is dependent contributes to epilepsy but is not strictly required. However, additional reports have also shown that pharmacological inhibition of adult neurogenesis is sufficient to attenuate spontaneous recurrent seizures (Jung et al., 2004; 2006). These seemingly contradictory observations highlight the necessity to ascertain the functional role of aberrant newborn neurons. Other studies have demonstrated that the pronounced neuronal remodeling that is a hallmark of temporal lobe epilepsy includes the migration of differentiated granule cells (Murphy & Danzer, 2011) that integrate and establish connections with sprouting mossy fibers from the CA3. Such increases in proliferation, faulty migration, and abnormal integration have not been tested in the AEM, and could be responsible for providing the architectural changes necessary to form a recurrent circuit that exhibits epileptic seizures.

Here we demonstrate that exposure to serum albumin promotes an increase in cell proliferation in the adult hippocampus. Furthermore, we demonstrate that this increase in proliferation is dependent in part on the activation of nestin positive glia-like cells (Type 1 NSCs) and/or Type 2a NSCs, and Type 3 doublecortin positive neuroblasts. Given that newly born neurons are hyperexcitable, these findings provide an additional plausible and contributive mechanism for the development of seizures and epilepsy in the AEM (Bar-Klein et al., 2014; Cacheaux et al., 2009; David et al., 2009; Ivens et al., 2007; Seiffert et al., 2004; Weissberg, Wood, Kamintsky, & Vazquez, 2015) and following TBI. Characterizing the electrophysiological characteristics of these newborn cells would provide valuable insights.

RESULTS

Serum albumin increases proliferation in the dentate gyrus of the dorsal hippocampus of adult mice

To test the hypothesis that exposure to serum albumin causes an increase in proliferation in the dentate gyrus of the hippocampi of adult mice, the lateral ventricles of animals were infused with serum albumin at physiological concentration (0.4 mM) in artificial cerebrospinal fluid (aCSF) for 7 days, using micro-osmotic pumps placed subcutaneously between the shoulder blades, and connected through an infusion line to a cannula (Fig. 1A). Pumps were removed seven days after surgical implantation. Twenty-two days after pump removal, animals were given a single intraperitoneal (IP) injection of EdU (50 mg/kg). Twenty-four hours after the EdU injections, the animals were perfused and their brains were fixed, sectioned with a cryostat, and prepared for immunohistochemistry (Fig. 1A). The brain sections were probed with antibodies for proliferating cell nuclear antigen (PCNA), a proliferation marker of cells in the DNA synthesis phase of the cell cycle (Moldovan, Pfander, & Jentsch, 2007); Ki-67, a proliferation marker of cells in all active phases of the cell cycle (Scholzen & Gerdes, 2000); and doublecortin (DCX), a marker of proliferative Type 3 neuroblasts and immature neurons (Ashton et al., 2012; J. P. Brown et al., 2003; Couillard-Després et al., 2005; Horesh et al., 1999; Plümpe et al., 2006) (Fig. 1B). Click-it Chemistry was used to label cells that had incorporated EdU within the previous 24 hours. Quantification of this immunohistological assay demonstrated that animals infused with serum albumin exhibited increased proliferation 30 days post-treatment, as indicated by PCNA positive immunostaining (149% increase, p=0.0090), Ki-67 positive immunostaining (145% increase, p=0.0244), and the number of positively labeled cells after the Click-it reaction (155% increase, p=0.0348; Fig. 1C), when compared to control animals infused with aCSF. Animals infused with serum albumin also exhibited increased neurogenesis 30 days post-treatment, as indicated by DCX positive immunostaining (86% increase, p=0.0428; Fig. 1C), when compared to control animals infused with aCSF (Alb, n=3; aCSF, n=3).

To test weather the exposure to serum albumin causes an increase in proliferation of nestin positive NSCs in the dentate gyrus of the dorsal hippocampus of adult mice, the lateral ventricles of *Nestin-CreER^{T2}; R26-stop$^{loxP/loxP}$-lacZ* mice were infused with serum albumin at physiological concentration (0.4 mM) in aCSF for 7 days, using micro-osmotic pumps placed subcutaneously between the shoulder blades, and connected through an infusion line to a cannula. To induce recombination and label mitotic nestin-positive cells, these mice were administered 150 mg/kg tamoxifen IP dissolved in corn oil for the first 5 days of ICV infusion of the experimental solutions. Pumps were removed seven days after surgical implantation. Twenty-three days after

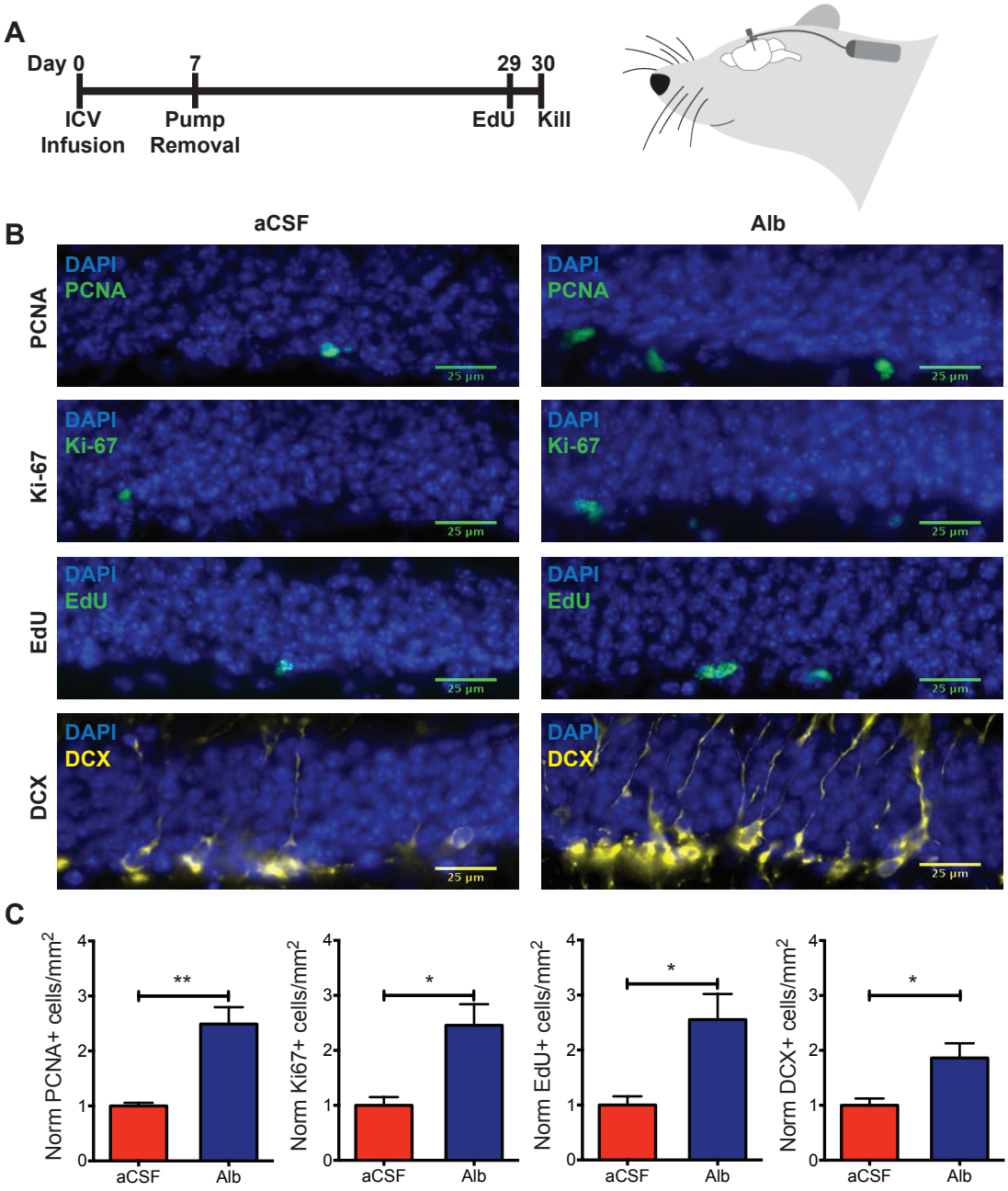

Figure 1. Serum albumin increases proliferation 30 days post-treatment in the dentate gyrus of the hippocampus of adult mice. **A.** Schematic of experimental time course and the surgical implantation. **B.** Representative photomicrographs demonstrating PCNA, Ki-67, and DCX expression, and EdU labeling in the dentate gyri of adult mice treated with artificial cerebrospinal fluid (aCSF) or serum albumin (Alb) for 30 days via intracerebroventricular (ICV) infusion with osmotic pumps, and IP EdU injections 24 hours before perfusion. Scale bar, 25 µm. **C.** Quantification of PCNA, Ki-67, DCX or EdU labeled cells/mm^2 in the dentate gyri of aCSF and Alb treated mice. N values are: aCSF, n=3; Alb, n=3. Six 20 µm sections with 2 dentate gyri per mouse were used for counting labeled cells. Bars indicate mean ± SEM. *$p < 0.05$, **$p< 0.01$.

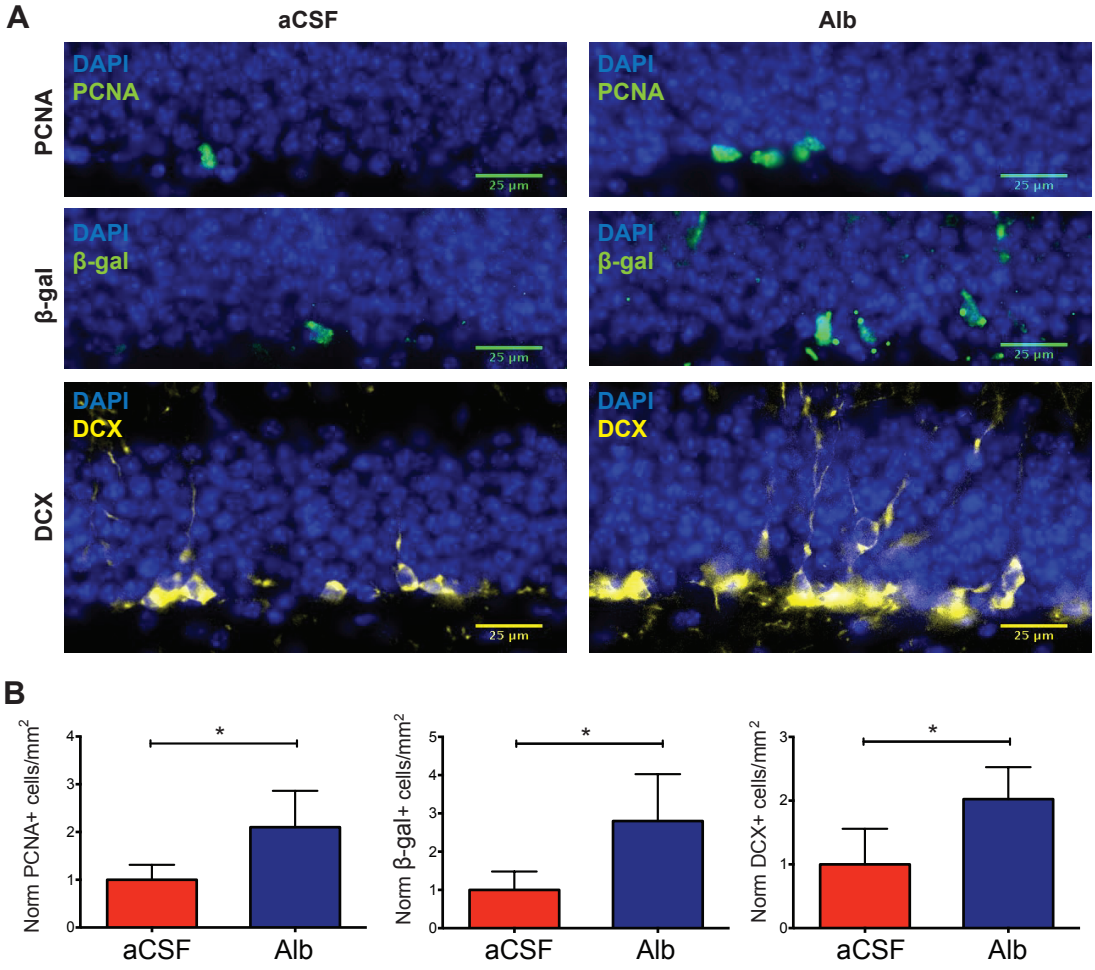

Figure 2. Serum albumin increases proliferation 30 days post-treatment in the dentate gyrus of the hippocampus of adult *Nestin-CreER^{T2}; R26-stop$^{loxP/loxP}$-lacZ* mice. **A.** Schematic of experimental time course. **B.** Representative photomicrographs demonstrating PCNA, β-gal, and DCX expression in the dentate gyri of adult mice treated with aCSF or serum albumin (Alb) for 30 days via ICV infusion with osmotic pumps. Recombination and labeling of mitotic cells was induced with IP injections of tamoxifen (150 mg/kg) for the first 5 days of ICV infusions. Scale bar, 25 μm. **C.** Quantification of PCNA, β-gal, and DCX labeled cells/mm^2 in the dentate gyri of aCSF and Alb treated mice. N values are: aCSF, n=5; Alb, n=6. Six 20 μm sections with 2 dentate gyri per mouse were used for counting labeled cells. Bars indicate mean ± SEM. *$p < 0.05$.

pump removal, animals were perfused and their brains were fixed, sectioned with a cryostat, and prepared for immunohistochemistry (Fig. 2A). The brain sections were probed with antibodies for PCNA, DCX, and β-galactosidase (β-gal), here used as a reporter of recombination that was expressed in nestin-positive glia-like cells (Type 1 NSCs) and/or nestin-positive Nonradial precursor (Type 2a NSCs) (Ashton et al., 2012; Lagace et al., 2007) (Fig. 2B). Quantification of this immunohistological assay demonstrated that animals infused with serum albumin exhibited increased proliferation, as indicated by PCNA positive immunostaining (110% increase, p=0.0148), and increased neurogenesis 30 days post-treatment, as indicated by DCX positive immunostaining (102% increase, p=0.0108) when compared to control animals infused with aCSF (Fig. 2C). Animals infused with serum albumin also exhibited increased proliferation of

nestin-positive glia-like cells (Type 1 NSCs) and/or nestin-positive Nonradial precursor (Type 2a NSCs), as indicated by the expression of β-gal, when compared to control animals infused with aCSF (Fig. 2C; 180% increase, p=0.0134; aCSF, n=5; Alb, n=6).

Serum albumin increases proliferation in the dorsal hippocampus during the recurrent seizure phase of the albumin exposure model of epilepsy

To assess whether the observed albumin-induced changes in proliferation and neurogenesis in the adult hippocampus were also present at earlier time points, we conducted a series of experiments to collect observations during the early stages of the albumin exposure model of epilepsy (7 days post albumin exposure), when recurrent seizures are already measurable with telemetric EEG (Weissberg et al., 2015). We compared these observations with groups observed at 15 and 30 days post-treatment. We hypothesized that the changes in proliferation and neurogenesis observed 30 days post-treatment in animals exposed to serum albumin were associated with the epileptogenic process induced by albumin exposure, and would thus be observed at 7 and 14 days post-treatment. To test this, we infused the lateral ventricles of adult mice with serum albumin (0.4 mM) in aCSF for 7, 14, and 30 days, using micro-osmotic pumps. Pumps were removed seven days after surgical implantation. Animals were perfused 7, 14, and 30 days post-treatment, and brains were fixed, sectioned, and prepared for immunohistochemistry. Brain sections were probed with antibodies for PCNA and DCX (Fig. 3A-L). Quantification of this immunohistological assay demonstrated that animals infused with serum albumin exhibited increased proliferation, as indicated by PCNA positive immunostaining, 7 days post-treatment (84% increase, $p<0.050$), 14 days post-treatment (67% increase, $p<0.050$), and 30 days post-treatment (123% increase, $p<0.001$), when compared to control animals infused with aCSF (Fig. 3M). Quantification of this immunohistological assay also demonstrated that animals infused with serum albumin exhibited increased neurogenesis, as indicated by DCX positive immunostaining at 14 days post-treatment (39% increase, $p<0.050$), and 30 days post-treatment (45% increase, $p<0.050$), but not 7 days post-treatment, when compared to control animals infused with aCSF (Fig. 3N). N values were: (7 days; Alb, n=4; aCSF, n=3), (14 days; Alb, n=5; aCSF, n=4), and (30 days; Alb, n=5; aCSF, n=4).

To test whether this proliferative response was homogeneous throughout the hippocampus, we compartmentalized the dentate gyrus into anatomical regions known to exhibit proliferative potential and further quantified PCNA labeled cells in the hilus (Fig. 4A). We found that the increase in proliferation observed in animals exposed to serum albumin was restricted to the granule cell layer of the dentate gyrus, and there were no changes in proliferation in the hilus at 7, 14, and 30 days post-treatment (Fig. 4B). These observations suggest that cells in the stem cell niche of the granule cell layer drive the proliferative effect of serum albumin, and proliferative cells in the hilus do not respond via proliferation at this stage after the initial insult.

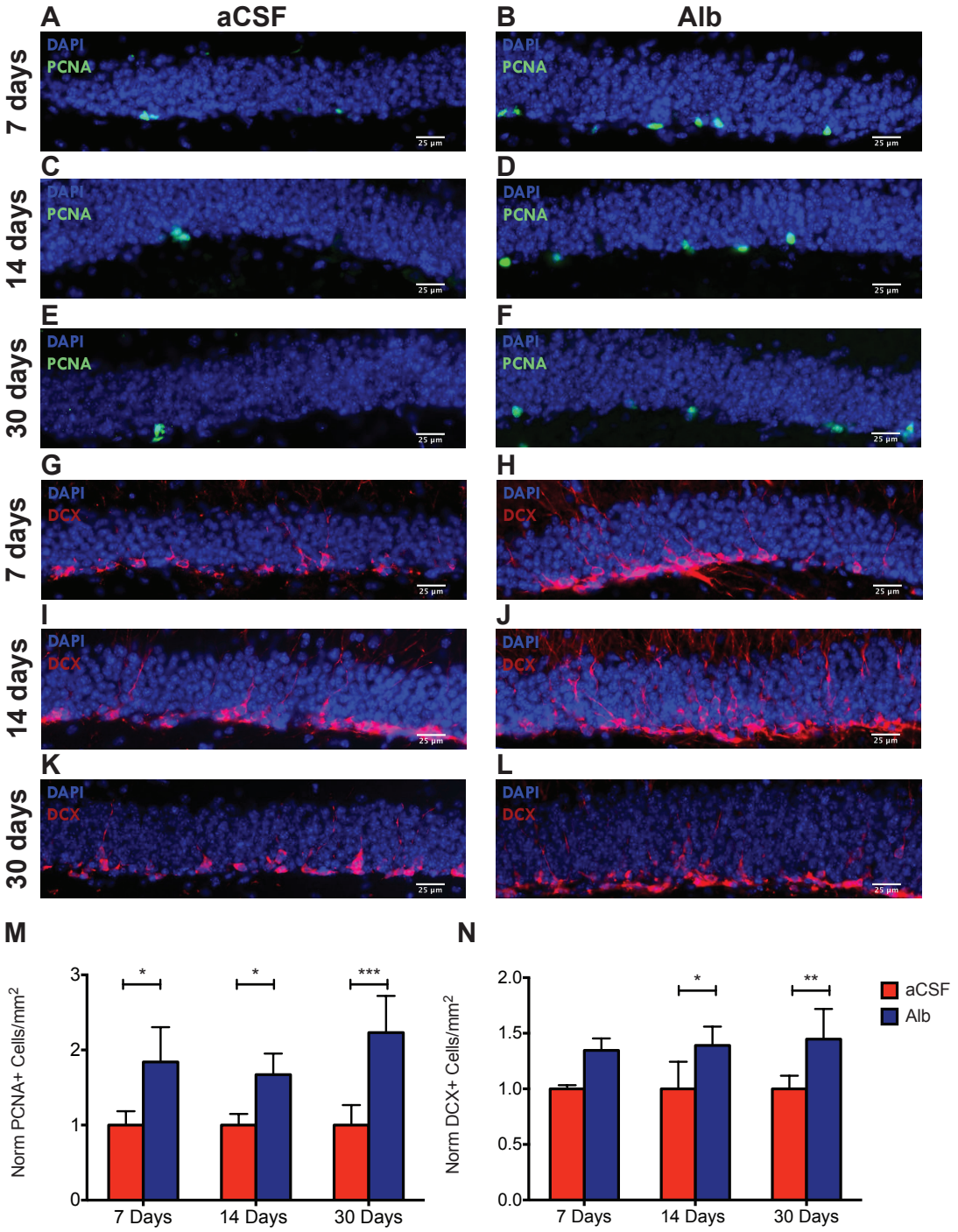

Figure 3. Serum albumin increases proliferation 7, 15, and 30 days post-treatment and neurogenesis 15 and 30 days post-treatment in the dentate gyrus of the hippocampus of adult mice. **A-F.** Representative photomicrographs demonstrating Proliferating Cell Nuclear Antigen (PCNA) expression in the dentate gyri of adult mice treated with artificial cerebrospinal fluid (aCSF) (**A, C, E**) or serum albumin (Alb) (**B, D, F**) for 7, 14, or 30 days via intracerebroventricular infusion with an osmotic pump. Scale bar, 25 µm. **G-L.** Representative photomicrographs demonstrating doublecortin (DCX) expression in the dentate gyri of adult mice treated with aCSF (**G, I, K**) or Alb (**H, J, L**) for 7, 14, or 30 days via intracerebroventricular infusion with an osmotic pump. **M.** Quantification of cells/mm^2 expressing PCNA in the dentate gyri of aCSF and Alb treated mice. **N.** Quantification of cells/mm^2 expressing DCX in the dentate gyri of aCSF and Alb treated mice. N values were: 7 days aCSF, n=3; 7 days Alb, n=4; 14 days aCSF, n= 4; 14 days Alb, n=5; 30 days aCSF, n=4; 30 days Alb, n=5. Six 20 µm sections with 2

dentate gyri per mouse were used for counting labeled cells. Bars indicate mean ± SEM. *p < 0.05, **p<0.01, ***p<0.001. Two way ANOVA with Bonferroni posttest used for comparison between treatments.

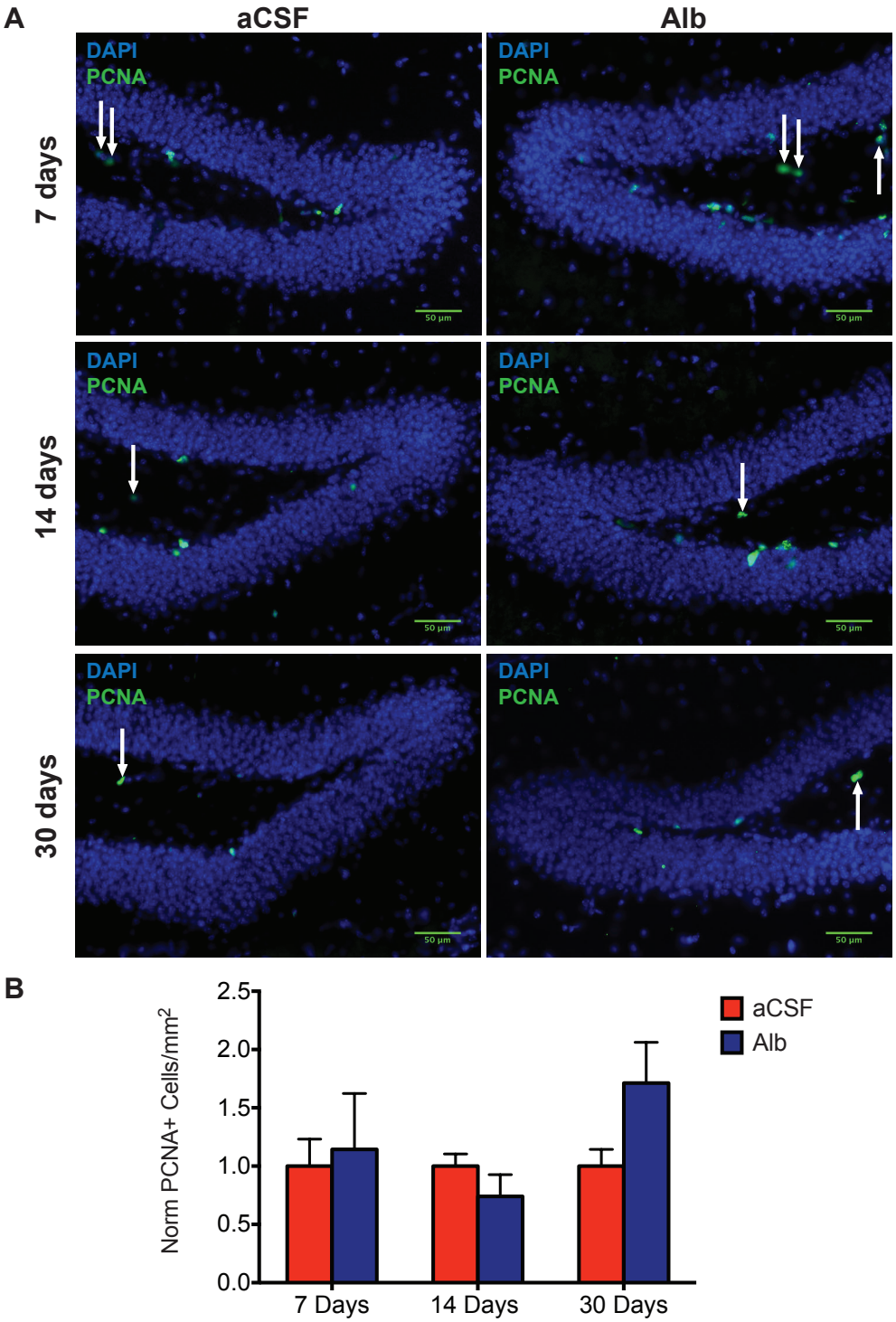

Figure 4. Serum albumin does not change proliferation in the hilus 7, 15, and 30 days post-treatment. **A.** Representative photomicrographs demonstrating Proliferating Cell Nuclear Antigen (PCNA) expression in the hilus of adult mice treated with artificial cerebrospinal fluid (aCSF) or serum albumin (Alb) for 7, 14, or 30 days via intracerebroventricular infusion with an osmotic pump. Scale bar, 50 µm. **B.** Quantification of cells/mm^2 expressing PCNA in the hilus of aCSF and Alb treated mice. N values were: 7 days aCSF, n=3; 7 days Alb, n=4; 14 days aCSF,

n= 4; 14 days Alb, n=5; 30 days aCSF, n=4; 30 days Alb, n=5. Six 20 µm sections with 2 dentate gyri per mouse were used for counting labeled cells. Bars indicate mean ± SEM. Significance is $p \leq 0.05$. Two way ANOVA with Bonferroni post-test used for comparison between treatments.

DISCUSSION

We proposed the AEM as a model to study post-traumatic epilepsy because it simulates the consequence of BBB disruption – the exposure of the brain secluded environment to the serum protein albumin, that we previously demonstrated to set the cascade of events leading to the generation of epilepsy such as the activation of inflammatory TGF-β signaling, reactive astrogliosis, and the electrophysiological traits of post-injury epileptiform activity (Bar-Klein et al., 2014; Cacheaux et al., 2009; Campistol et al., 1999; David et al., 2009; Weissberg et al., 2011). We now report, for the first time significant increase in the proliferation of cells in the dorsal SGZ of the dentate gyrus in the AEM within the period of epiletogenesis (5-7 days post albumin exposure), and for up to 30 days after exposure to serum albumin. This increased proliferation is consistent with the increases in proliferation observed in the pilocarpine and kainic models of epilepsy in rodents (Gray & Sundstrom, 1998; Parent et al., 1997; 2006; Sierra et al., 2015) and the increases in the mRNA expression of Ki-67 that we reported, in animals exposed to serum albumin, 24, and 48 hours after exposure (Cacheaux et al., 2009). Some of the NSCs involved in this albumin-dependent increase in proliferation are nestin-positive, as also reported with similar increases in proliferation in the controlled cortical impact (CCI) injury model of TBI (Logan et al., 2013) and the kainic acid model of epilepsy (Sierra et al., 2015).

We also report for the first time that exposure to serum albumin increased the number of neuronal fate committed DCX-positive cells.. Previous observations demonstrated that, once astrocytes are activated by serum albumin via the ALK5 type I receptor of the TGF-β signaling pathway, they secrete TGF-β1 (Bar-Klein et al., 2014), which is the canonical ligand of ALK5. Because ALK5 has been shown to positively regulate late stages of adult neurogenesis (Y. He et al., 2014), this feedback loop of TGF-β signaling suggests a mechanism through which two drivers of neurogenesis may influence the development of this phenotype in the AEM; seizures induce 1) a transient increase in the proliferation of neural stem cells and progenitors and 2) an increase in the secretion of astrocytic TGF-β1 that promotes the survival and maturation of DCX-positive immature neurons. To test the hypothesis, a fate-mapping study is necessary to assess whether nestin-positive NSCs that proliferate during albumin exposure during the pre-seizure phase produce neurons that contribute to the establishment of a hyperexcitable and recurrent circuit. Some of the morphological markers that could help distinguish this type of aberrantly developed adult-generated granule cells include hilar ectopic granule cells (Parent et al., 2006), granule cells with basal dendrites (Jessberger et al., 2007; Walter, Murphy, Pun, Spieles-Engemann, & Danzer, 2007), and granule cells that are integrated with sprouted mossy fibers (Jessberger et al., 2007; Kron, Zhang, & Parent, 2010; Parent et al., 1997). Using β-gal as a lineage tracer, induced via recombination in nestin-positive cells, should achieve this objective. In accordance with a recent report, two hypotheses explain the possible lineage fates for cells born from nestin-positve NSCs after exposure to serum albumin. First, if albumin-induced seizures meet the criteria described for the model of mesial temporal lobe epilepsy described by Sierra et al., (Sierra et al., 2015) (i.e., animals develop seizures [repetitive-spike and slow-wave discharges lasting 10 seconds or more] within six hours of insult that are synchronized with behavioral stage 4-5 generalized seizures according to the Racine scale, and last for up to 50

days; animals must also exhibit interictal discharges lasting up to 200 ms), then most β-gal-positive cells should develop into astrocytes. Second, if albumin-induced seizures are closer to those described in the reference's model of epileptiform activity (animals do not develop repetitive-spike and slow-wave discharges lasting 10 seconds or more but instead exhibit epileptiform discharges, as early as 6 hours after insult, that continue intermittently for up to 50 days), then animals will exhibit increased numbers of β-gal-positive cells when compared to controls, and most of these β-gal-positive cells will develop into neurons. In the AEM, animals exposed to serum albumin exhibit a latent period of 4.9 ±1.3 days with no seizure activity. This makes hypothesis 1 very unlikely. Therefore, we expect that this milder model of seizure onset and epileptogenesis will more closely resemble hypothesis 2, with animals exposed to serum albumin exhibiting increased numbers of β-gal-positive cells with most of these cells developing into neurons. Increased ALK5 signaling, first activated by infused serum-albumin and later induced by increased TGF-β1 levels caused by epileptiform activity in the neurogenic zone, can up-regulate the proliferation of nestin-positive glia-like cells (Type 1 NSCs) (Logan et al., 2013), without depleting the pool of stem cells (Sierra et al., 2015). Simultaneously, this increase in ALK5 signaling can help support the survival and maturation of DCX-positive immature neurons (Y. He et al., 2014), thus creating an environment in which TGF-β-dependent increases in the proliferation of nestin-positive glia-like cells (Type 1 NSCs) and the maturation of newborn neurons drive the development and sustainment of a hyperactive and hypersynchronous network. In contrast with this hypothesis, high levels of soluble TGF-β1 overproduced by astrocytes in transgenic mice (Buckwalter et al., 2006), high levels of TGF-β1 in aged mice (Yousef, Conboy, et al., 2015a), and infusion of high levels of recombinant TGF-β1 in the SVZ and SGZ of rats (Wachs et al., 2006), lead to the strong inhibition of neurogenesis. These apparently contradictory results are likely explained by the highly context-dependent effects of activating TGF-β signaling pathways in general, the different cell types involved in a response to an epileptogenic insult, and the still poorly understood mechanism of action of albumin as an agonist of the TGF-βRI ALK5.

I address this knowledge gap by evaluating the roles involved in the cell-autonomous activation of TGF-β signaling in the neurogenic niche in response to albumin exposure in the next chapter. Furthermore, because ALK5 can transduce signals from all three isoforms of TGF-β and some growth and differentiation factors (GDFs) (Mazerbourg & Hsueh, 2006), it remains undetermined whether any of these molecules also contribute to the albumin-induced activation of ALK5 *in vivo*. These interactions seem unlikely, as we reported no expression of TGF-β2, TGF-β3, and GDF9 (a known ligand for ALK5) after exposure to serum albumin (Cacheaux et al., 2009). Nevertheless, the possibility that additive contributions to ALK5 signaling could play a role in albumin-induced proliferation and neurogenesis ought to be considered because expression of these factors may vary in the neurogenic niche. Mechanisms that are independent of TGF-signaling could also play a role in the increased proliferation and neurogenesis described above. We previously reported that albumin exposure causes an increase in the expression of Gadd45b (Cacheaux et al., 2009). This gene is a neural activity-induced immediate early gene that is expressed in mature hippocampal neurons and is required for the activity-induced DNA demethylation of specific promoters that facilitate the expression of genes necessary for adult neurogenesis (Ma et al., 2009). For example, one gene expressed as a result of epigenetic modifications performed by Gadd45b is brain-derived neurotrophic factor (BDNF). This proliferative signal promotes the proliferation of neural precursors and dendritic growth in

newborn neurons in the adult hippocampus. Correspondingly, BDNF expression is increased in animals exposed to serum albumin (Cacheaux et al., 2009).

These findings demonstrate that serum albumin is a novel and potent regulator of NSC proliferation and adult neurogenesis and may be a key driver of changes in network architecture that occur after traumatic brain injuries. Because these mechanisms are involved in the development of epilepsy and may play important roles in the prevention and regulation of neurological injury and illness, the AEM offers a unique opportunity to investigate the roles of newborn neurons in the diseased brain.

Chapter 3 – Culture media conditioned by astrocytes exposed to serum albumin is not sufficient to increase the proliferation of adult neural stem cells *in vitro*

HYPOTHESIS:

Upon exposure to serum albumin, astrocytes promote neural stem cell proliferation via a secreted factor.

INTRODUCTION

Astrocytes, microglia, mature neurons, endothelial cells, and adult NSCs and their progeny comprise the cellular components of the adult neurogenic niche (Ming & Song, 2011). Astrocytes from the neurogenic hippocampus and the SVZ have been demonstrated to play very active regulatory roles in adult neural stem cell proliferation and neurogenesis *in vitro* (D. A. Lim & Alvarez-Buylla, 1999; Song et al., 2002). Studies using hippocampal astrocytes have found that these cells express a number of secreted and membrane-bound factors, such as interleukin 1β (IL-1β), interleukin 6 (IL-6) (Barkho et al., 2006), and ephrin-B2 (Ashton et al., 2012), that are known to regulate proliferation and neuronal differentiation, as well as neuronal migration, maturation, and synaptogenesis (Ming & Song, 2011). To test whether astrocytes also play a role in the albumin-dependent increase in proliferation and neurogenesis reported above, we isolated hippocampal astrocytes from P1 rat pups, grew them on 10 cm plates for 7 days, and exposed them to serum albumin (0.5 μM). After 24 hours incubation, the serum albumin was washed off the cultures, and fresh media was added to feed and collect any molecules secreted by astrocytes activated by serum albumin. This astrocyte-conditioned media (ACM) was collected 24 hours later and used to treat nestin-positive/Sox-2-positive/GFAP-negative adult hippocampal NSCs (nonradial precursors [Type 2a cells]). These NSCs were incubated with serum albumin (0.5 μM), astrocyte-conditioned media, astrocyte-conditioned media from astrocytes treated with serum albumin (0.5 μM), or control media for 24 hours and exposed to EdU (30 μM) during the last 6 hours of treatment to label proliferating cells. To measure the effect of albumin on proliferation, we probed the cultured adult NSCs with antibodies for PCNA and Ki-67 and used Click-it Chemistry reveal cells that had incorporated EdU.

We found that neither direct exposure to serum albumin nor albumin-treated ACM elicit a change in the proliferation of NSCs *in vitro*. These results suggest that the increase in proliferation observed in animals exposed to serum albumin may involve the contributions of other cells in addition to astrocytes, or is dependent on neural activity.

RESULTS

Exposure to serum albumin does not change the proliferation of neural stem cells *in vitro*

To test whether albumin can lead to NSCs proliferation in a cell autonomous way, we grew nestin-positive/Sox-2-positive/GFAP-negative adult rat hippocampal NSCs in media formulated to maintain populations of proliferative but non-differentiating neural progenitor cells. This effect is in part due to fibroblast growth factor-2 (FGF-2), which was used to supplement the

media. This media will be referred to as neural stem cells' media (NSCm). After we grew adult rat hippocampal NSCs in NSCm for 48 hours, we exposed some cultures to serum albumin (0.5 μM) for 24 hours with or without SJN, a selective inhibitor of the TGF-β type I receptor ALK5. After 18 hours of exposure, we introduced EdU to these cultures of NSCs. Six hours later, we fixed and prepared the cultures for immunocytochemistry (Fig. 5A). We labeled cells that expressed PCNA and Ki-67, or those that incorporated EdU (Fig. 5B), and quantified the percentages of cells that were labeled with each of these markers of proliferation. Exposure to serum albumin, with or without SJN, did not change the proliferation of NSCs (PCNA, $p=0.2914$, $n=8$; Ki-67, $p=0.1644$, $n=8$; EdU, $p=0.3587$, $n=8$) under these culturing conditions (Fig. 5C). A Tukey's multiple comparisons test after the ANOVA confirmed that there were no changes in proliferation among groups (Fig. 5C).

Exposure of Neural stem cells to albumin in low-proliferation conditions decreases proliferation

To conduct experiments using astrocyte conditioned media, we used media formulated to support NSCs and astrocytes simultaneously (Song et al., 2002). This media notably lacks FGF and fetal bovine serum, key ingredients of NSCm and astrocyte media, respectively. This media preparation will be referred to as co-culture media (CCm). To test whether CCm affected the proliferation of stem cells, we grew nestin-positive/Sox-2-positive/GFAP-negative adult rat hippocampal NSCs in CCm for 24 hours with or without serum albumin. After the first 18 hours of growth, we added EdU to the cultures of NSCs. Six hours later, we fixed and prepared these cultures for immunocytochemistry (Fig. 6A). We labeled cells that expressed PCNA and Ki-67, or that incorporated EdU (Fig. 6B), and quantified the percentages of cells that were labeled with each of these markers of proliferation. Using an ANOVA, we found that CCm, and CCm with serum albumin did not change the proliferation of NSCs as indicated by the percentage of cells labeled with PCNA and Ki-67 (PCNA, $p=0.0631$, $n=8$; Ki-67, $p=0.5367$, $n=8$; Fig. 6C). A Tukey's multiple comparisons test after the ANOVA confirmed that there were no changes in the expression levels of PCNA and Ki-67 among groups (Fig. 6C). However, we also found that CCm with serum albumin caused the decreased incorporation of EdU (Fig. 6C). A Tukey's multiple comparisons test after the ANOVA demonstrated that the group treated with serum albumin in CCm exhibited 21% decreased EdU uptake when compared to the group treated with CCM alone ($p<0.05$) and a 32% when compared to the group treated with NSCm ($p<0.001$; CCm, $n=8$; CCm+Alb, $n=8$; Fig. 6C).

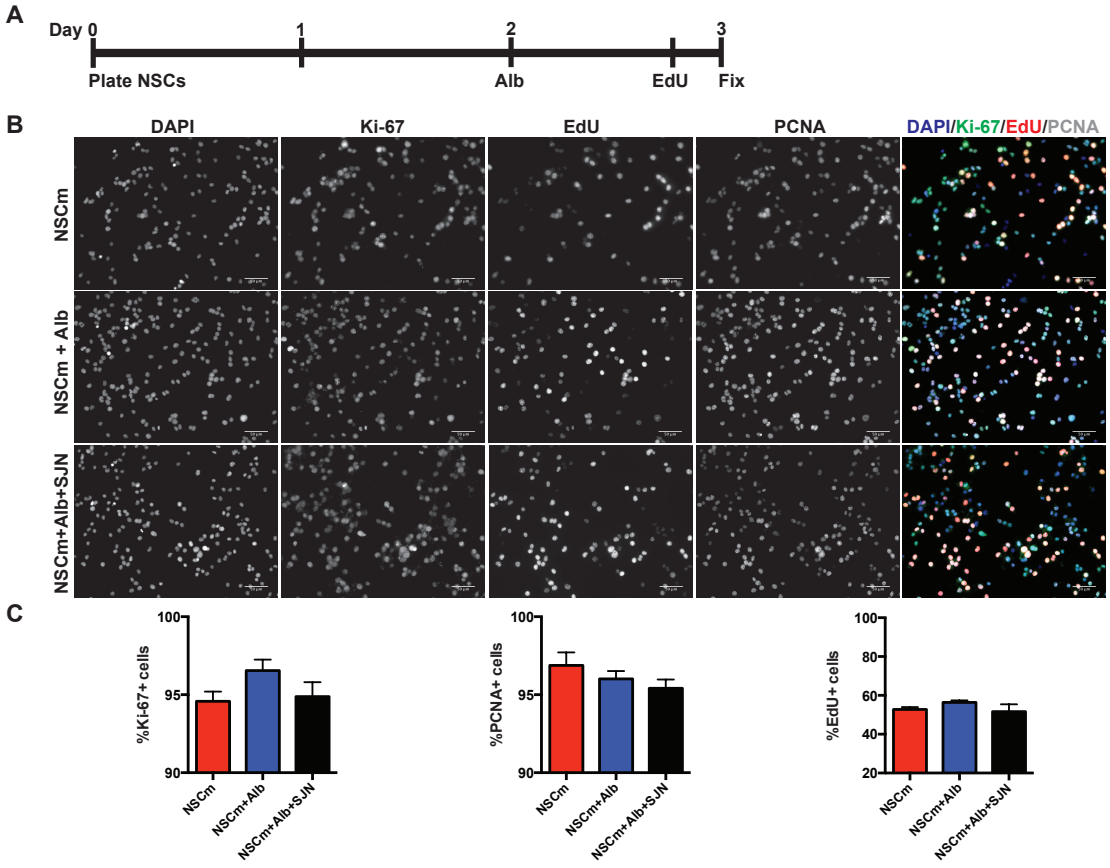

Figure 5. Exposure to serum albumin does not change the proliferation of neural stem cells *in vitro*. **A.** Schematic of experimental time course. **B.** Representative photomicrographs demonstrating Ki-67 and PCNA expression, and EdU labeling in adult neural stem cells (NSC). Cells were grown in neural stem cell media (NSCm) for 48 hours and then exposed to serum albumin (Alb) or Alb with the SJN, a selective inhibitor of the TGF-β type I receptor ALK5. After 18 hours of exposure to Alb or Alb+SJN, all cells were treated with EdU for six hours. Scale bar, 50 µm. **C.** Quantification of the percentage of cells that were labeled with Ki-67, PCNA or EdU. N values are: NSCm, n=8; NSCm+Alb, n=8; NSCm+Alb+SJN, n=8. Bars indicate mean ± SEM. One-way ANOVAs with Tukey's multiple comparisons test were used for comparison between treatments.

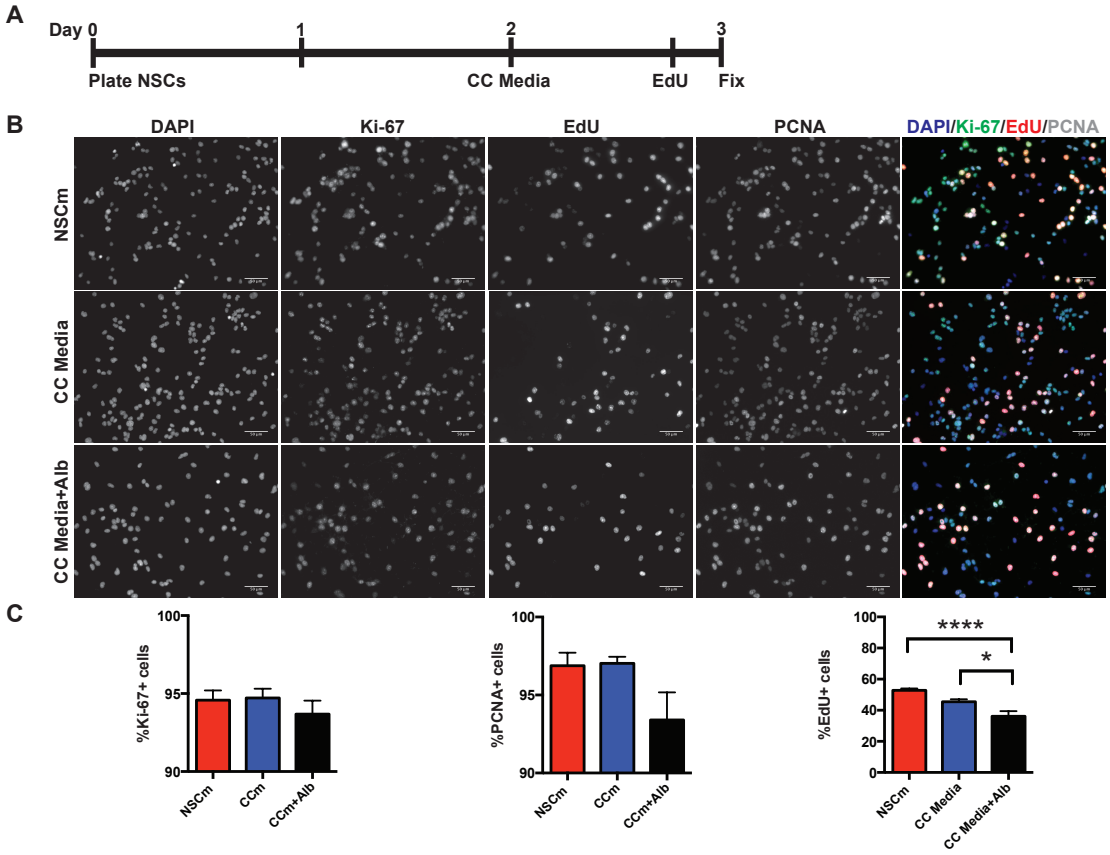

Figure 6. Neural stem cells deprived of FGF and exposed to serum albumin decrease DNA replication *in vitro*. **A.** Schematic of experimental time course. **B.** Representative photomicrographs demonstrating Ki-67 and PCNA expression, and EdU labeling in adult neural stem cells (NSC). Cells were grown in neural stem cell media (NSCm) for 48 hours and then changed to co-culture media (CCm). Some cultures were also exposed to serum albumin (CCm+Alb). After 18 hours of growth in CCm, all cells were treated with EdU for six hours. Scale bar, 50 µm. **C.** Quantification of the percentage of cells that were labeled with Ki-67, PCNA or EdU. N values are: NSCm, n=8; CCm, n=8; CCm+Alb, n=8. Bars indicate mean ± SEM. *p < 0.05, ****p < 0.0001. One-way ANOVAs with Tukey's multiple comparisons test were used for comparison between treatments.

Media conditioned by astrocytes exposed to serum albumin is not sufficient to increase NSC proliferation *in vitro*

To test whether astrocytes mediated the changes in the proliferation of NSCs observed in animals exposed to serum albumin, we treated primary post-natal hippocampal astrocytes with serum albumin for 24 hours. After this incubation, we washed off the serum albumin, provided astrocytes with fresh CCm, and allowed these cells to condition the media for 24 hours. This media will be referred to as astrocyte-conditioned media (ACM). We used ACM to treat nestin-positive/Sox-2-positive/GFAP-negative adult rat hippocampal NSCs for 24 hours. After 18 hours of exposure to ACM, we introduced EdU to the cultures of NSCs. Six hours later, we fixed and prepared these cultures for immunocytochemistry (Fig. 7A). We labeled cells that expressed PCNA and Ki-67, or that incorporated EdU, and quantified the percentages of cells that were labeled with each of these markers of proliferation. Using an ANOVA, we found that treating

NSCs with ACM from astrocytes grown in CCm (ACM), astrocytes grown in CCm with serum albumin (ACM+Alb), and astrocytes grown in CCm with serum albumin and SJN (ACM+Alb+SJN) did not change the proliferation of NSCs, as indicated by the percentages of cells labeled with PCNA and Ki-67 (PCNA, p=0.3987; Ki-67, p=0.0620, n=8; ACM, n=8; ACM+Alb, n=8; ACM+Alb+SJN; Fig. 7B). A Tukey's multiple comparisons test after the ANOVA confirmed that there were no changes in the expression levels of PCNA and Ki-67 among groups (Fig. 7B). However, when we used an ANOVA to analyze the percentages of cells that incorporated EdU, as marker of proliferation within the last six hours of exposure, we found that there were significant differences among the means for the groups (p=0.0028; Fig. 7B). A Tukey's multiple comparisons test after the ANOVA demonstrated that the group treated with serum albumin and SJN exhibited a 31% decreased proliferation when compared to the group treated with serum albumin alone (p<0.01; ACM, n=8; ACM+Alb, n=8; ACM+Alb+SJN, n=8; Fig. 7B).

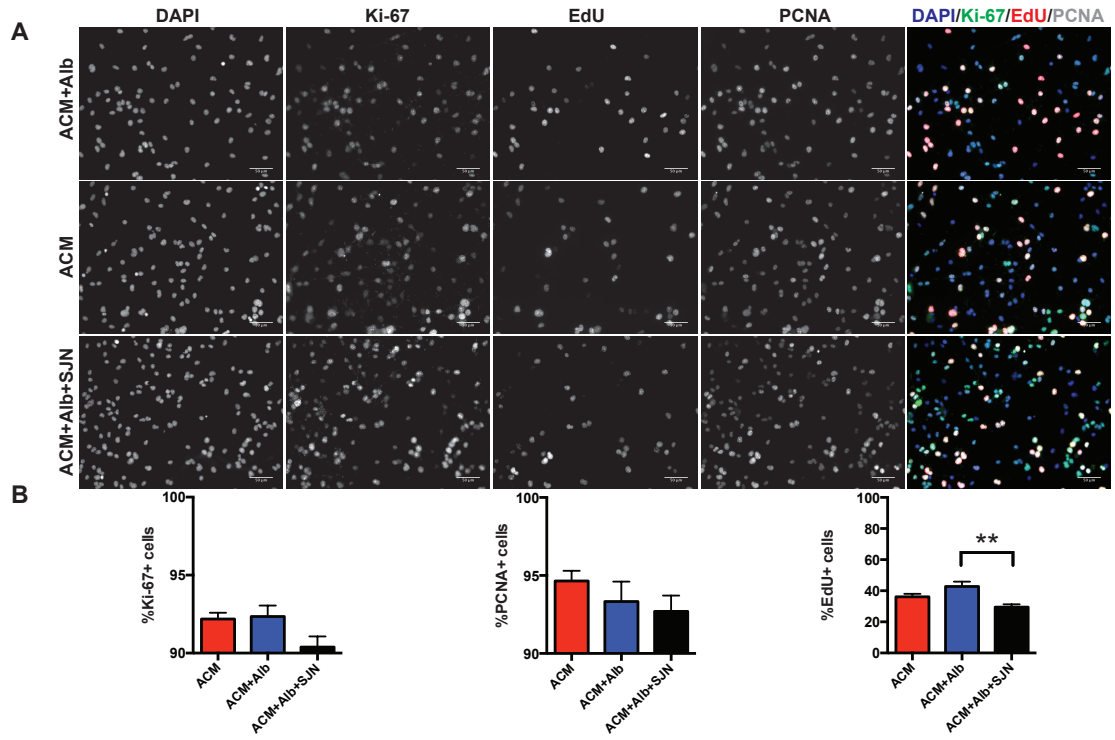

Figure 7. Media conditioned by astrocytes exposed to serum albumin is not sufficient to increase NSC proliferation *in vitro*. **A.** Representative photomicrographs demonstrating Ki-67 and PCNA expression, and EdU labeling in adult neural stem cells (NSC). Cells were grown in neural stem cell media for 48 hours and then changed to astrocyte-conditioned media for 24 hours. The ACM was conditioned by primary post-natal hippocampal astrocytes grown in co-culture media (ACM), co-culture media with serum albumin (ACM+Alb), or co-culture media with serum albumin and SJN (ACM+Alb+SJN). After 18 hours of growth in their respective ACM, all NSCs were treated with EdU for six hours. Scale bar, 50 µm. **B.** Quantification of the percentage of cells that were labeled with Ki-67, PCNA or EdU. N values are: ACM, n=8; ACM+Alb, n=8; ACM+Alb+SJN, n=8. Bars indicate mean ± SEM. *p < 0.05, **p < 0.01. One-way ANOVAs with Tukey's multiple comparisons test were used for comparison between treatments.

DISCUSSION

The astrocyte-dependent epileptogenic process that follows albumin exposure in animals led us to hypothesize that the increase in proliferation and neurogenesis observed after albumin exposure was also mediated by astrocytes. This hypothesis was designed after considering that serum albumin binds to the TGF-β receptor complex (Cacheaux et al., 2009) and preferentially induces activation of the activin receptor-like kinase 5 (ALK5) pathway of TGF-β receptor I in astrocytes (Bar-Klein et al., 2014) and that this process is involved in epileptogenesis in animals (David et al., 2009; Friedman et al., 2009; Ivens et al., 2007; Seiffert et al., 2004; Shlosberg et al., 2010; Weissberg et al., 2011). The proliferation of NSCs in the epileptic brain has been well documented (Gray & Sundstrom, 1998; Parent et al., 1997; 2006; Sierra et al., 2015) and a close relationship between ALK5 TGF-β signaling and increases in late-stage adult neurogenesis has been found (Y. He et al., 2014). Drawing from these observations, and measurements showing that, after epileptogenic injuries, hippocampal concentrations of serum albumin reach 0.76 ± 0.21 μM (Frigerio et al., 2012), we exposed cultured astrocytes and NSCs to 0.5 μM serum albumin to assess its effect on the proliferation of NSCs. This attempt to recreate the environmental conditions found in the hippocampus after the breakdown of the blood brain barrier demonstrated that serum albumin at these low concentrations (the physiological concentration of serum albumin is 0.4 mM) is not sufficient to affect the proliferation of NSCs grown in NSCm. This result may be driven in part by the presence of FGF-2 in NSCm, which is known to promote continuous self-renewal to maintain the stem cell state of these cells (Kirby et al., 2013; Liu et al., 2006; Ming & Song, 2005; 2011; Taupin et al., 2000). This observation suggests that there may be a difference in the potency of the TGF-β signal that is induced by serum albumin, when compared to TGF-β1. Yousef et al., reported that, in Sox2 positive neural progenitor cells treated with TGF-β1 (100 ng/mL) for 24 hours, TGF-β signaling led to decreased proliferation in these cells (Yousef, Conboy, et al., 2015a). However, it is noteworthy to highlight that differences in the cell maintenance protocols used for these experiments may account for this difference. First, we used higher concentrations of FGF-2 to culture NSCs [20 ng/mL as opposed to 10 ng/mL used by the Yousef et al., group (Yousef, Conboy, et al., 2015a)]. This difference in FGF-2 concentrations could have kept the NSCs in our experiments proliferative for a longer duration and at a higher rate. Second, we seeded approximately one third of the number of NSCs (25,000 cells) used by the Yousef et al. group (80,000 cells) in the same amount of culturing area, and we allowed the cells to attach and grow small processes (48 hours post-seeding) before beginning the albumin exposure experiments (Yousef, Conboy, et al., 2015a). Under these conditions, we believe that contact-dependent signaling, or the lack thereof, and even differences in the rates at which nutrients and signals in the environment were used, could have created the differences observed.

When NSCs were grown in CCm, we deprived them of FGF-2 and thus subtracted a signal that maintained their stem cell states, thereby prolonging proliferation. However, this change alone was not sufficient to negatively affect the proliferation of NSCs for 24 hours, and we thus observed no changes in proliferation between cells cultured in NPCm and CCm. This result demonstrated that CCm could be used to study the interactions between heterogeneous populations of cells from the CNS with little or no effect on the proliferation of NSCs. However, this change in environmental conditions appears to have increased the sensitivity of NPCs to signals evoked by exposure to serum albumin. After 24 hours of incubation in CCm, NPCs that

were further treated with serum albumin (0.5 μM) in CCm exhibited decreased proliferation, as revealed by a lower percentage of cells that incorporated EdU. Having validated the efficacy of using CCm to conduct our experiments with astrocyte conditioned media (ACM), we tested whether astrocytes were able to affect the proliferation of NSCs via secreted molecules after exposure to serum albumin. When we compared the proliferation of NSCs treated with ACM to that of NSCs treated with ACM from astrocytes exposed to serum albumin, we found no differences. In contrast, NSCs treated with ACM from astrocytes exposed to serum albumin and SJN exhibited decreased proliferation when compared to NSCs treated with ACM from astrocytes exposed to serum albumin. These observations suggest that blocking ALK5 signaling in astrocytes helps to create an environment that is less conducive to proliferation. This effect could have been driven by a decrease in the concentration of secreted proliferative signals, or by an increase in the concentration of secreted differentiating signals produced by astrocytes.

In summary, these experiments demonstrate that direct serum albumin exposure does not affect the proliferation of NSCs. Furthermore, serum albumin alone was also not sufficient to induce astrocytes to secrete proliferative signals that could drive an increase in proliferation such as that observed in animals exposed to serum albumin. These observations do not discount the role that astrocytes could play in the phenomenon observed *in vivo*, as this model did not investigate the role of juxtacrine (i.e., cell-to-cell contact dependent) astrocytic signaling that could regulate proliferation and neurogenesis (Ashton et al., 2012; Ming & Song, 2011). Furthermore, this model concentrated on the effects on proliferation of a specific subset of NSCs (nestin-positive/Sox-2-positive/GFAP-negative nonradial precursors [Type 2a cells]), while the effect of an astrocyte-secreted molecule could act upon one of at least three additional stem cells found in the neurogenic niche of the SGZ (radial glia-like cells [Type I cells], intermediate progenitor cells, and neuroblasts). Logan et al. demonstrated in a controlled cortical impact model of TBI that brain injuries induce significant alterations in TGF-β, BMP, and activin signaling (Logan et al., 2013). In particular, Runt-related transcription factor-1 (Runx1), which can interact with intracellular TGF-β Smad signaling pathways, is expressed in Nestin-positive/GFAP-positive radial glia-like cells, and these cells are primarily responsible for the increase in proliferation observed after TBI.

Chapter 4 – Exposure to serum albumin promotes aberrant neurogenesis in the adult hippocampus

HYPOTHESIS:

Neurons that develop after exposure to serum albumin develop aberrantly, thus facilitating the establishment of a recurrent circuit.

INTRODUCTION

Adult neurogenesis in the dentate gyrus of the hippocampus shares various similarities with neurogenesis during embryonic and post-natal development (Mathews et al., 2010). Hippocampal granule cells follow a predictable developmental pattern in which immature neurons grow characteristic dendritic and axonal projections before maturing and integrating into

the canonical three-synaptic circuit of the hippocampus (Ambrogini et al., 2004; Laplagne et al., 2006; Markakis & Gage, 1999; Overstreet et al., 2004; Toni et al., 2008; 2007). After epilepsy, this developmental pattern for newly born neurons in the adult hippocampus is aberrantly changed (Parent, 2007). The primary changes include abnormal migration patterns of immature granule cells (Jessberger et al., 2007; McCloskey, Hintz, Pierce, & Scharfman, 2006; Murphy & Danzer, 2011; Parent et al., 2006; Scharfman, Goodman, & Sollas, 2000; Scharfman, Goodman, & McCloskey, 2007; Walter et al., 2007), the development of aberrant basal dendrites on newly born neurons (Jessberger et al., 2007; Murphy et al., 2011; Ribak, Tran, Spigelman, Okazaki, & Nadler, 2000; Shapiro & Ribak, 2006; Walter et al., 2007), altered apical dendritic structures (Murphy et al., 2011), and synaptic organization that contributes to mossy fiber sprouting (Danzer, Kotloski, Walter, Hughes, & McNamara, 2008; Kron et al., 2010; Murphy et al., 2011; Parent et al., 1997). Altered apical dendritic structures occur in cells that are born just before pilocarpine-induced status epilepticus. Cells with these structures fail to develop typical fanlike apical dendrites and include cells with apical dendrites that project obliquely rather than directly into the molecular layer (Murphy et al., 2011). Similar characteristics were described and illustrated in pathologies found in hippocampal samples from patients with epilepsy (M. E. Scheibel & Scheibel, 1973). To quantify this observation, Murphy et al. measured the angle in which these apical dendrites developed relative to the granule cell layer and found that these angles were significantly greater in epileptic animals (Murphy et al., 2011). Drawing from these observations, we measured the angles of apical dendrites in newborn neurons from animals exposed to serum albumin to assess whether the AEM shares this characteristic with epileptic brains.

We found that recently born immature neurons from animals exposed to serum albumin exhibit significant lateralization of their apical dendrites when compared to recently born immature neurons in control animals. The lengths of apical dendrites in animals exposed to serum albumin were not different from the lengths of apical dendrites in control animals. The lateralization of apical dendrites demonstrates that animals exposed to serum albumin experience aberrant neurogenesis and suggest that, as shown elsewhere, these cells may exhibit additional abnormalities in morphology, integration, and function (Jessberger et al., 2007; McAuliffe et al., 2011; Murphy et al., 2011; Murphy & Danzer, 2011; Parent, 2007; Parent et al., 1997; 2006; Walter et al., 2007).

RESULTS

Neurons born during the epileptogenic period of the AEM exhibit lateralized apical dendrites 7, 14, and 30 days post-treatment

To determine whether the AEM involved aberrant neurogenesis, we infused the lateral ventricles of adult mice with serum albumin (0.4 mM) in aCSF for 7, 14, and 30 days using micro-osmotic pumps. Pumps were removed seven days after surgical implantation. Animals were perfused 7, 14, and 30 days post-treatment, and brains were fixed, sectioned and prepared for immunohistochemistry (Fig. 8A). Brain sections were probed with antibodies for DCX (Fig. 8B), and measurements of the angles of apical dendrites in newborn neurons were collected from all treatments groups. The quantification of this immunohistological assay demonstrated that animals exposed to serum albumin exhibit lateralization of primary apical dendrites in newborn

neurons 7 ($p<0.001$), 14 ($p<0.001$), and 30 days ($p<0.001$) post-treatment, when compared to aCSF-treated animals (Fig. 8C). This effect was most notable in animals from the 7-day exposure group and decreased as a function of time, suggesting that neurons born just before or during the epileptogenic period of the AEM may be more susceptible to aberrant development after a traumatic brain injury.

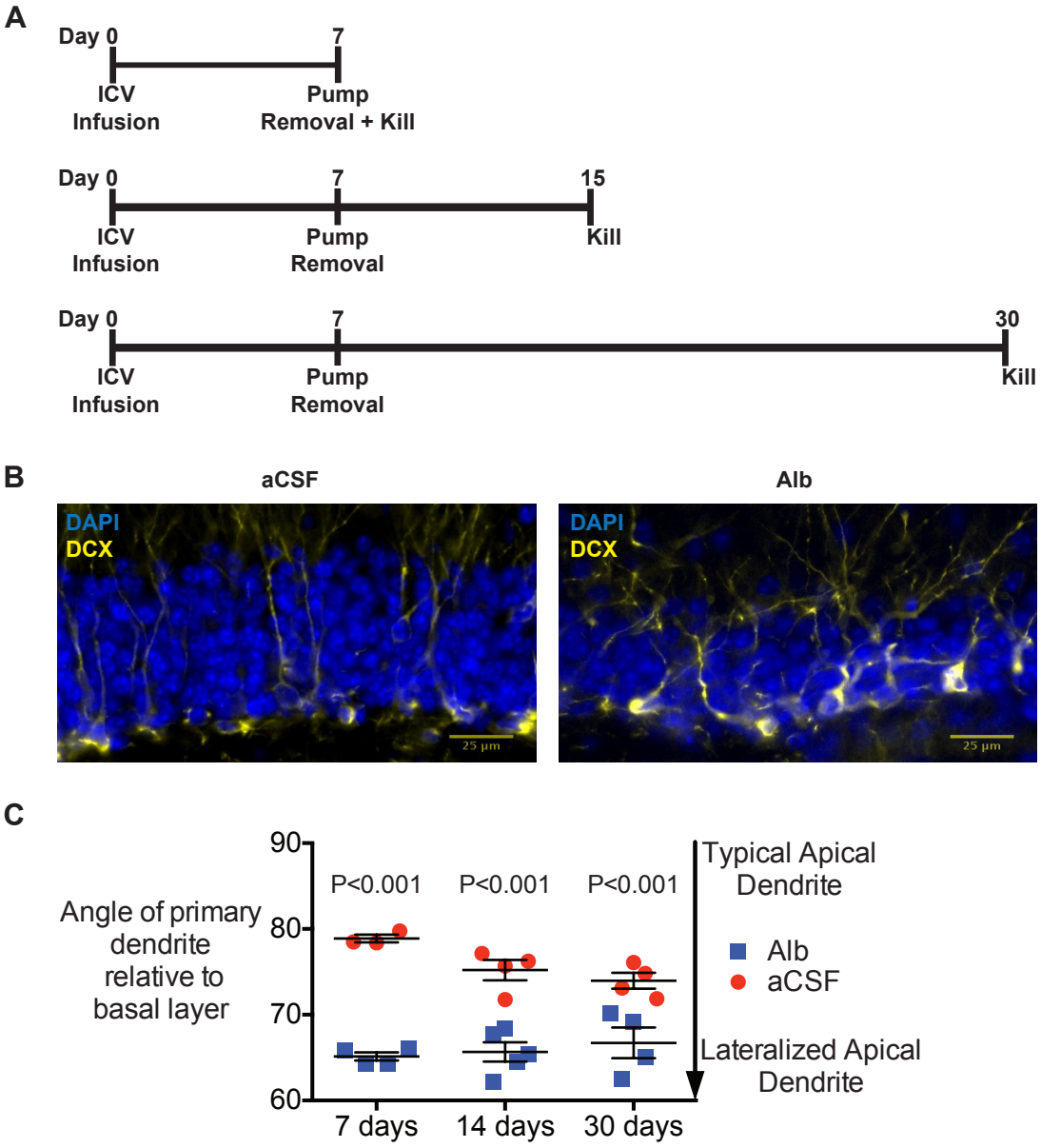

Figure 8. Serum albumin increases aberrant neurogenesis in the dentate gyrus. **A.** Schematic of experimental time course. **B.** Representative photomicrographs demonstrating Doublecortin (DCX) expression in the dentate gyri of adult mice treated with artificial cerebrospinal fluid (aCSF) or serum albumin for 30 days via intracerebroventricular infusion with an osmotic pump. **C.** Quantification of angles of primary apical dendrites relative to basal layer of dentate gyri of mice treated with aCSF and serum albumin. Blue squares represent the mean of the angles measured for one animal treated with serum albumin, and red circles represent the mean of the angles measured for one animal treated with aCSF. Black lines represent the mean ± SEM for a treatment group. N values are: 7 days aCSF, n=3; 7 days Alb, n=4; 14 days aCSF, n=4; 14 days Alb, n=5; 30 days aCSF, n=4; 30 days Alb, n=4. Six 20 µm

sections with 2 dentate gyri per mouse were used for measuring angles of primary apical dendrites. Two way ANOVA with Bonferroni posttest used for comparison between treatments.

Exposure to serum albumin does not change the lengths of apical primary dendrites of immature neurons 7, 14, or 30 days post-treatment

To determine whether newly born neurons exposed to serum albumin exhibited additional morphological changes, we measured the lengths of the apical primary dendrites of immature DCX-positive neurons in the dentate gyrus of the hippocampi of adult mice. To do this, we infused the lateral ventricles of adult mice with serum albumin (0.4 mM) in aCSF for 7, 14, and 30 days using micro-osmotic pumps. Pumps were removed seven days after surgical implantation. Animals were perfused 7, 14, and 30 days post-treatment, and brains were fixed, sectioned and prepared for immunohistochemistry (Fig. 8A). Brain sections were probed with antibodies for DCX (Fig. 8B). The quantification of this immunohistological assay demonstrated that exposure to serum albumin does not change the lengths of apical primary dendrites of immature neurons 7, 14, or 30 days post-treatment (Table 1). We further analyzed the lengths of these dendrites in the dentate granule cell layer and the molecular layer individually, and observed no changes (Table 1). These observations suggest that, at these stages of post-traumatic recovery, newborn neurons exposed to serum albumin grow apical dendrites at a rate similar to that of newborn neurons exposed to aCSF, but these dendrites bud from the cell body at a more lateralized site.

Table 1. Structure of AD of immature neurons exposed to serum albumin versus controls

			AD length breakdown (μm)	
	No. of cells	AD length (μm)	DGC-L	ML
Newborn aCSF control (7 days)	60	65.61	37.58	27.93
Newborn Alb (7 days)	80	75.74	40.93	34.81
Newborn aCSF control (14 days)	80	95.98	50.53	45.45
Newborn Alb (14 days)	100	86.93	47.19	39.74
Newborn aCSF control (30 days)	80	84.44	50.76	33.7
Newborn Alb (30 days)	80	99.55	59.65	39.9
Newborn Alb+Los (30 days)	100	91.06	52.1	38.96

AD, Apical primary dendrite. DGC-L, DCG layer, ML, molecular layer.

Values are means. No significant differences were found between groups.

DISCUSSION

It is hypothesized that the aberrant integration of newly born granule cells into the adult brain contributes to epileptogenesis. However, this hypothesis has not been completely accepted because some evidence suggests that newborn neurons in the epileptic adult brain may protect the brain by reducing excitability (Jakubs et al., 2006). A newer and more inclusive view of this phenomenon is that, in the epileptic brain, both of these processes develop simultaneously, thus allowing some newborn neurons to integrate aberrantly and lead to hyperexcitability while others attempt to balance this effect to protect the brain. In this new heterogeneous model of the process of neuronal integration in the epileptogeneic brain, cells that are born shortly before or after status epilepticus and exhibit aberrant morphologies (Arisi & Garcia-Cairasco, 2007; Heinrich et al., 2006; Murphy et al., 2011; Overstreet-Wadiche et al., 2006; Shapiro & Ribak, 2006) have been implicated in the epileptogenic process. Some of the aberrant morphologies associated with significantly higher spine densities on the dendrites of newborn granule cells include long basal dendrites, large somas, or both (Murphy et al., 2011). Granule cells with long basal dendrites have also been shown to have more contact with sprouted mossy fibers, and it is thus hypothesized that they are the recipients of more recurrent signals. However, it is noteworthy that these aberrantly developed newborn neurons represent ~10% of newborn granule cells in the pilocarpine model of epilepsy. Considering these observations, we hypothesize that these abnormal granule cells in the pilocarpine model of epilepsy are similar to those characterized in human epileptic brains (Campe, Spencer, & De Lanerolle, 1997; Franck, Pokorny, Kunkel, & Schwartzkroin, 1995; M. E. Scheibel & Scheibel, 1973) and in the AEM. Furthermore, in characterizing the altered dendritic trajectories of newborn cells in the AEM, we found that, similar to the pilocarpine model, animals exposed to serum albumin exhibit aberrant neurogenesis during and 30 days after the epileptogenic period.

The morphological observations described above suggest that these aberrant newborn granule cells possess higher excitability and thus promote the onset of seizures and epilepsy. Having demonstrated that the AEM includes aberrant newborn cells, we must investigate whether these aberrant cells contribute to the epileptogenic process or develop as a result of the epileptogenic process itself. This model lends itself to this type of investigation because, as we have demonstrated (Weissberg et al., 2015), this model has an epileptogenic window of up to three days in which TGF-β signaling is active but no seizures occur. Traumatic brain injuries are known to increase TGF-β signaling in the hippocampus (Logan et al., 2013; Villapol et al., 2013), and this increase is associated with the proliferation of radial glia-like cells (Type I stem cells) in the SGZ and the increased survival and dendritic development of immature neurons (Y. He et al., 2014). Thus, it is possible that TGF-β signaling aids the survival and development of dendritic processes in newborn neurons exposed to serum albumin and TGF-β1 secreted by astrocytes.

To investigate the process of integration in the AEM, we focused on the dendritic trajectories of the apical dendrites of newly born granule cells and the lengths of these dendrites. We found that, similar to the pilocarpine model of epilepsy, the AEM includes newborn granule cells with more lateralized apical dendrites. These observations suggest that the AEM has a similar heterogeneity of integration of adult-generated granule cells, and these newborn cells that develop lateralized apical dendrites may be involved in the development of basal dendrites in the

AEM. If so, these cells may develop to receive robust afferent and recurrent input that could underlie the development of post-traumatic epilepsy. This type of recurrent excitatory input is hypothesized to support epileptogenesis (Sutula & Dudek, 2007; Sutula, He, Cavazos, & Scott, 1988) because cells that receive more recurrent input are thought to contribute more significantly to the development of seizures. The possibility that newborn cells with more lateralized apical dendrites continue to develop into basal dendrites, with increased synaptic inputs, should be investigated, as this finding could reveal a marker for the population of cells that directly lead to the development of seizures after TBI. Specifically targeting these cells would be advantageous for most proposed therapeutic interventions because attempts to prevent epileptogenesis by reducing neurogenesis have produced conflicting results, with groups showing partial (Jung et al., 2004; 2006; Sugaya, Maru, Kudo, Shibasaki, & Kato, 2010) or no efficacy (Pekcec, Fuest, Mühlenhoff, Gerardy-Schahn, & Potschka, 2008; Pekcec, Lüpke, Baumann, Seifert, & Potschka, 2011; Pekcec, Mühlenhoff, Gerardy-Schahn, & Potschka, 2007).

In the AEM, the epileptogenic process is preceded by astrocytic activation and an increase in TGF-β signaling. Here, we demonstrate for the first time that, when these changes occur in the neurogenic niche, they result in increased proliferation and aberrant neurogenesis. These newborn aberrant neurons share morphological abnormalities with aberrant cells implicated in epileptogenesis and, because our model is uniquely positioned to allow interventions into the epileptogenic process, we can exploit our ability to regulate TGF-β signaling to prevent these changes in the population of cells that inhabit the hippocampus.

Chapter 5 – Losartan prevents albumin exposure-dependent proliferation and aberrant neurogenesis in the adult hippocampus

HYPOTHESIS:

Upon exposure to albumin, the TGF-β signaling pathway mediates an increase in proliferation and aberrant neurogenesis in the dentate gyrus of the adult hippocampus.

INTRODUCTION

When our group developed the AEM, we demonstrated that 1) exposure to serum albumin initiated a sequence of events that generate focal paroxysmal hypersynchronous episodes (Ivens et al., 2007; Seiffert et al., 2004), similar to those seen in epileptic seizures, and 2) that this process is mediated by astrocytic TGF-β signaling. One unique characteristic of the AEM is that it exhibits delayed and prolonged epileptiform activity (Ivens et al., 2007) and thus provides an opportunity to study the epileptogenic period.

During the epileptogenic period, astrocytes bind and uptake serum albumin via the TGF-βR (Ivens et al., 2007). This binding activates the TGF-β signaling pathway and induces a transcriptional change in astrocytes that leads to ionic imbalances at the synapse and the development of seizures. Albumin uptake and seizures can be prevented by the administration of TGF-βR blockers (Cacheaux et al., 2009) and inhibitors of the TGF-β signaling pathway (Bar-Klein et al., 2014). However, the relationship between the activation of the TGF-β signaling pathway by serum albumin and neurogenesis remained untested, along with the role that this neurogenic episode could have in remodeling the architecture of the hippocampus.

To test the hypothesis that, upon exposure to albumin, the TGF-β signaling pathway mediates an increase in proliferation and neurogenesis in the dentate gyrus of the adult hippocampus, we treated animals with serum albumin and Losartan, an FDA-approved inhibitor of the TFG-β signaling pathway. We found that, by blocking the TFG-β signaling pathway, we prevented the increases in proliferation and neurogenesis observed in animals exposed to serum albumin.

RESULTS

Losartan prevents the albumin-induced increase in proliferation and neurogenesis observed 30 days post-treatment in the dentate gyrus of the hippocampi of adult mice

To test the hypothesis that the TGF-β signaling pathway mediates an albumin-induced increase in proliferation in the dentate gyrus of the hippocampi of adult mice, we infused the lateral ventricles of animals with serum albumin at physiological concentration (0.4 mM) in artificial cerebrospinal fluid (aCSF), serum albumin (0.4 mM) and Losartan (10 μM) in aCSF, or aCSF for 7 days using micro-osmotic pumps placed subcutaneously between the shoulder blades and connected through an infusion line to a cannula. Pumps were removed seven days after surgical implantation. Twenty-three days after pump removal, animals were perfused and their brains were fixed, sectioned with a cryostat, and prepared for immunohistochemistry. The brain

sections were probed with antibodies for PCNA and DCX (Fig. 9A). The quantification of this immunohistological assay demonstrated that animals infused with serum albumin exhibited increased proliferation 30 days post-treatment, as indicated by PCNA positive immunostaining (123% increase, $p<0.001$; Fig. 9B), when compared to control animals infused with aCSF, and that the addition of Losartan to the infusion prevented this increase (Fig. 9B). Animals infused with serum albumin also exhibited increased neurogenesis 30 days post-treatment, as indicated by DCX positive immunostaining (45% increase, $p<0.01$; Fig. 9B), when compared to control animals infused with aCSF, and this change was also prevented by the addition of Losartan to the infusion.

Losartan prevents the albumin-induced lateralization of apical dendrites observed in aberrant neurons born during the epileptogenic period of the AEM 30 days post-treatment

To test the hypothesis that the TGF-β signaling pathway mediates the albumin-induced lateralization of apical dendrites observed in aberrant neurons born in the dentate gyrus of the hippocampi of adult mice, we infused the lateral ventricles of animals with serum albumin at physiological concentration (0.4 mM) in artificial cerebrospinal fluid (aCSF), serum albumin (0.4 mM) and Losartan (10 μM) in aCSF, or aCSF for 7 days using micro-osmotic pumps placed subcutaneously between the shoulder blades and connected through an infusion line to a cannula. Pumps were removed seven days after surgical implantation. Twenty-three days after pump removal, animals were perfused and their brains were fixed, sectioned with a cryostat, and prepared for immunohistochemistry. The brain sections were probed with antibodies for DCX (Fig. 9A) and measurements of the angles of apical dendrites in newborn neurons were collected from all treatment groups. The quantification of this immunohistological assay demonstrated that animals exposed to serum albumin exhibit lateralization of primary apical dendrites in newborn neurons 30 days post-treatment ($p<0.001$) when compared to aCSF-treated animals, and the addition of Losartan to the infusion prevented these changes (Fig. 9C).

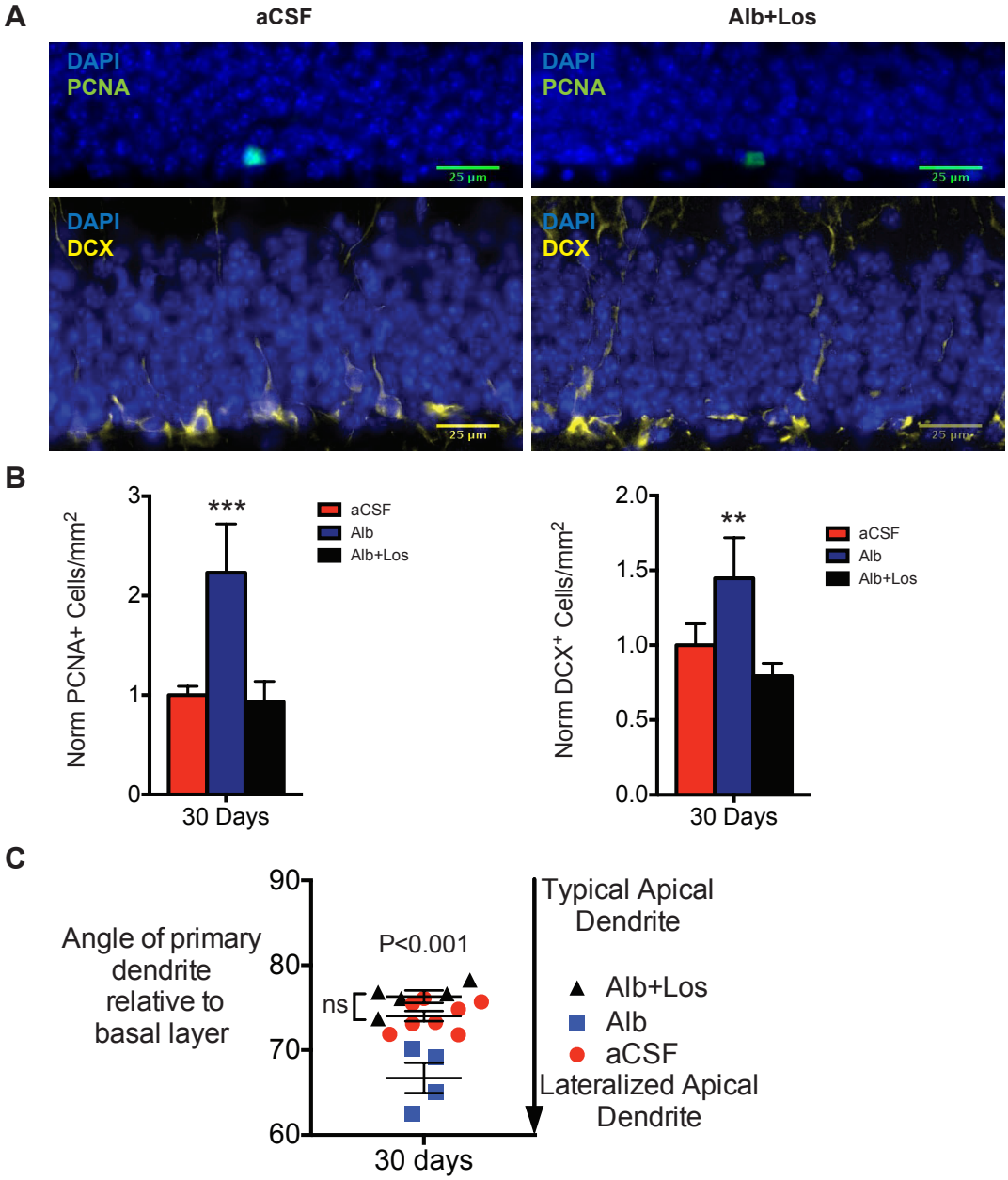

Figure 9. Losartan ICV infusion prevents the albumin-induced increase in proliferation and neurogenesis in the dentate gyrus of the hippocampus. **A.** Representative photomicrographs demonstrating Proliferating Cell Nuclear Antigen (PCNA) and Doublecortin (DCX) expression in the dentate gyri of adult mice treated with artificial cerebrospinal fluid (aCSF) or serum albumin and Losartan (Alb+Los) for 30 days via intracerebroventricular infusion with an osmotic pump. **B.** Quantification of cells/mm^2 expressing PCNA or DCX in the dentate gyri of aCSF and Alb+Los treated mice. **C**. Quantification of angles of primary apical dendrites relative to basal layer of dentate gyri of mice treated with aCSF, serum albumin (Alb), and Alb+Los. Blue squares represent the mean of the angles measured for one animal treated with Alb, red circles represent the mean of the angles measured for one animal treated with aCSF, and black triangles represent the mean of the angles measured for one animal treated with Alb+Los. Black lines represent the mean ± SEM for a treatment group. N values are: aCSF, n=4; Alb, n=5; Alb+Los, n=5. Six 20 µm sections with 2 dentate gyri per mouse were used for counting labeled cells and measuring angles of primary apical dendrites. Bars indicate mean ± SEM. *p < 0.05, **p<0.01, ***p<0.001. One-way

ANOVA with Dunnett's multiple comparison tests were used for comparing the number of cells/mm^2 expressing PCNA or DCX and the angles of primary apical dendrites between treatments.

DISCUSSION

Accumulating observations have demonstrated that the breakdown of the blood-brain-barrier and TGF-β signaling play a role in the development of post-traumatic epilepsy (Bar-Klein et al., 2014; Cacheaux et al., 2009; David et al., 2009; Ivens et al., 2007; Oby & Janigro, 2006; Seiffert et al., 2004; Shlosberg et al., 2010; Tomkins et al., 2007; 2008; Weissberg et al., 2011). We have also shown that local exposure of the cortex and the hippocampus to serum albumin is sufficient to induce seizures, and that the pathway used for this epileptogenic process is cell-specific and mediated by astrocytic TGF-β signaling (Bar-Klein et al., 2014; Cacheaux et al., 2009; Ivens et al., 2007; Weissberg et al., 2015). Furthermore, we have demonstrated that epileptogenesis in animals exposed to serum albumin and TGF-β1 can be prevented by the co-infusion of antibodies for TGF-βRII; SB431542, an inhibitor of the TGF-βRI ALK5 and its relatives ALK4 and ALK7; SJN, a selective inhibitor of the TGF-βRI ALK5; and Losartan, a FDA-approved angiotensin II receptor, type 1 (AT1) antagonist drug (Bar-Klein et al., 2014; Cacheaux et al., 2009; Weissberg et al., 2015).

In these experiments, we showed for the first time that the increases in proliferation and aberrant neurogenesis induced by exposure to serum albumin are dependent on TGF-β signaling at the site of injury. This observation demonstrated that, in the AEM, TGF-β signaling is also implicated in the activation of NSCs and the development of aberrant newborn granule cells. These aberrant neurons may underlie the hyperexcitability that is characteristic of the epileptic brain, and finding a mechanism to prevent their development offers the promise of the development of an anti-epileptic regimen to be used after TBIs. To test weather albumin-induced increased proliferation and neurogenesis are dependent on TGF-β signaling , we used Losartan, a common anti-hypertensive AT1 antagonist that was previously shown to antagonize TGF-βR signaling in animal models of chronic renal insufficiency, cardiomyopathy, and Marfan syndrome (Cohn et al., 2007; Lavoie et al., 2005; D. S. Lim et al., 2001). Furthermore, this compound has also been shown to have a neuro-protective effect in an *in vitro* model of the O_2-glucose depletion that occurs after ischemic injury (Wu et al., 2010). Losartan infusion blocked the albumin-increased proliferation and neurogenesis. The prevention of increased proliferation and neurogenesis can be attributed to one of several possible explanations, or a combination thereof: the blocking of TGF-β signaling, AT1 signaling, or the prevention of seizures, all of which have been associated with intracellular Smad2/3 signaling and the proliferation, differentiation, and survival of newborn neurons (Bar-Klein et al., 2014; Cohn et al., 2007; Y. He et al., 2014; Jessberger, Römer, Babu, & Kempermann, 2005; Lavoie et al., 2005; D. S. Lim et al., 2001; Logan et al., 2013; Parent et al., 2006; Villapol et al., 2013).

One reason why we cannot rule out the possibility that these effects are mediated by AT1 signaling is that angiotensin II has the ability to increase TGF-β1 levels in the CNS (Lanz et al., 2010). However, reports demonstrating that losartan decreases the severity of seizures in the WAR rat model of epilepsy (Pereira et al., 2010), enhances the efficacy of antiepileptic drugs in mice (Łukawski, Janowska, Jakubus, Tochman-Gawda, & Czuczwar, 2010), and reduces the albumin-induced increase in p-Smad2/3 levels (Bar-Klein et al., 2014), offer a convincing

argument that its effect on epileptogenesis may be driven by the signaling promiscuity of AT1 and the ability of AT1 to phosphorylate Smad3 despite insufficient TGF-βR activation (Rodríguez-Vita et al., 2005; W. Wang et al., 2006).

These findings support our hypothesis that the TGF-β signaling pathway mediates an albumin-dependent increase in proliferation and aberrant neurogenesis in the dentate gyrus of the adult hippocampus and suggest that losartan might become one of the first prophylactic treatments available to prevent epilepsy after TBIs. Future experiments using losartan in the pilocarpine and kainic acid modes of epilepsy to test whether losartan has the ability to prevent the increases in proliferation and neurogenesis reported in these models (Gray & Sundstrom, 1998; Jessberger et al., 2005; 2007; Parent et al., 1997; 2006; Radley & Jacobs, 2003; Shapiro & Ribak, 2006) would be useful. If so, these observations would suggest a stronger role for TGF-β signaling in driving the changes in proliferation and the development of aberrant newly born granule cells that are reported in these models. This could imply that, more than status epilepticus or seizures, which are sporadic and can deplete the stem cell pool (Sierra et al., 2015), persistent TGF-β signaling in the neurogenic zone supports neurogenesis (Y. He et al., 2014) and thus increases the number of aberrant adult-generated granule cells that may contribute to the establishment of recurrent circuitry.

CONCLUSION

Increased proliferation and neurogenesis are found in rodent models of traumatic brain injury (Logan et al., 2013; Villapol et al., 2013) and in epilepsy in rodents and humans(Gray & Sundstrom, 1998; Jessberger et al., 2005; 2007; Parent et al., 1997; 2006; Radley & Jacobs, 2003; Shapiro & Ribak, 2006). Breakdown of the blood brain barrier (BBB) after vascular injury and traumatic brain injury leads to the extravasation of serum albumin into the brain parenchyma, and this process in turn activates astrocytic TGF-β signaling. Traumatic brain injuries are also known to increase TGF-β signaling in the neurogenic niche of the adult hippocampus, and this type of signaling has been implicated in the proliferation of NSCs. Additionally, increased TGF-β signaling after albumin exposure and the opening of the BBB has been associated with epileptiform activity that develops after a latent period and persists for up to 32 days in rodents. This type of epileptiform activity has been associated with the increased proliferation of NSCs and increased neurogenesis in the adult hippocampus. Furthermore, ALK5 TGF-β signaling in the hippocampus has been shown to support the survival and maturation of newly born immature neurons in the adult brain.

In this thesis, I first demonstrate that exposure to serum albumin induces an increase in the proliferation of NSCs in the adult hippocampus. I demonstrated that this increase in proliferation is driven in part by nestin-positive NSCs, and that it is significant shortly after the beginning of the phase of recurrent seizures in the AEM. This increase in proliferation is restricted to the granule cell layer, as I did not find changes in the number of proliferating cells in the hilus of the dentate gyrus of the hippocampus. I then investigated the relationship between this increase in proliferation and the number of newly born neurons and found that animals exposed to serum albumin also exhibit increased neurogenesis, characterized by an accumulation of neuronal fate-committed DCX-positive cells in the granule cell layer. I then investigated the morphology of

albumin-induced newborn neurons and revealed that neurons born after albumin exposure develop aberrantly, exhibit morphological characteristics associated with higher recurrent inputs, and thus may play a greater role in epileptogenesis. To prevent these changes in network architecture, I used an FDA-approved inhibitor of TGF-β signaling that prevented the observed increases in proliferation and aberrant neurogenesis. Finally, I examined whether the increase in proliferation found in animals exposed to serum albumin involved secreted signaling molecules from astrocytes and nestin-positive/Sox2-positive/GFAP-negative nonradial progenitor cells (Type 2a cells). I found that direct exposure to serum albumin does not affect the proliferation of these NSCs *in vitro*, and does not induce the secretion of astrocytic proliferative signals. This observation led me to conclude that the increase in proliferation observed in animals exposed to serum albumin may require activity-induced proliferative signals from epileptiform activity and contributions from other cells in the niche, in addition to astrocytes.

Even with the rapid increase in the availability of antiepileptics observed over the last 20 years, a community-based study in southern France estimated that as many as 22.5% of patients with epilepsy suffer from drug-resistant epilepsies that do not respond to pharmacological interventions. This work has identified a novel function of serum albumin in driving network remodeling after breakdown of the BBB, and these changes might play an important role in the development of post-traumatic epilepsy. Furthermore, the efficacy of losartan at preventing these changes revealed a novel mechanism through which this compound might intervene with the epileptogenic process. Additional work is necessary to determine whether aberrant neurons generated after albumin exposure contribute to epileptiform activity in the AEM and whether blocking this effect with losartan affects the healing process that is activated after brain injuries. Finally, identification of the cell type specific pathways that drive these increases in proliferation and neurogenesis will aid in the development of targeted antiepileptic drugs.

MATERIALS AND METHODS

Animals

The Animal Care and Use Committee at the University of California, Berkeley, approved all animal procedures conducted as part of this project. I used adult male C57BL/6J mice (Jackson Laboratory). Animals were housed (5 mice/cage) on a 12 hour light dark cycle with lights on at 07:00 hours. Mice were allowed to acclimate to the animal facility for 1 week before experiments began.

In vivo fate mapping

Nestin-CreER^{T2}; R26-stop$^{loxP/loxP}$-lacZ (a kind gift from D. Schaffer, University of California, Berkeley) was used to label the lineage of cells born during the epileptogenic period. Genotyping was performed using the following primers:

Cre-F (5′-ACCAGCCAGCTATCAACTCG-3′)
Cre-R(5′- TTACATTGGTCCAGCCACC-3′), 200bp.

lacZ-F (5′-GTCAATCCGCCGTTTGTTCCCACG-3′)
lacZ-R (5′-CCAGTACAGCGCGGCTGAAATCAT-3′), 400 bp.

wtRosa-F (5′-GGAGCGGGAGAAATGGATATG-3′)
wtRosa-R (5′- AAAGTCGCTCTGAGTTGTTAT-3′), 600bp.

Adult *Nestin-CreER^{T2}; R26-stop$^{loxP/loxP}$-lacZ* mice received a daily intraperitoneal injection of tamoxifen (150 mg/kg; Sigma-Aldrich, St. Louis, MO) dissolved in corn oil for five days after pump implantation to induce recombination and label mitotic cells.

Intracerebroventricular pump implantation

Osmotic pumps were implanted as previously described (Weissberg et al., 2011). Briefly, surgery was performed on adult male mice under isofluorane anesthesia (2%). Using a sterotaxic frame, a 0.7 mm diameter hole was drilled through the skull over the somatosensory cortex (0.5 mm caudal, 1 mm lateral to bregma) and anterior to the hippocampus. Micro-osmotic pumps (ALZET, Cupertino, CA) were filled with 200 µL of either 0.4 mM bovine serum albumin (Alb; Sigma) solution or 0.4 mM bovine serum albumin and 10 µM Losartan in artificial cerebrospinal fluid (aCSF) as previously described (Seiffert et al., 2004), and placed subcutaneously between the shoulder blades. In a subset of animals, 10% of the BSA was replaced with Alexa Fluor 488 conjugated BSA (Life Technologies, San Francisco CA) (2.68gr/l). Sham controls were implanted with pumps containing aCSF. Pumps delivered their contents into the right lateral cerebral ventricle via a brain infusion kit (ALZET, 0008851). Pumps were extracted under isoflurane anesthesia 7 days after implantation.

EdU injections

5-ethynyl-2'-deoxyuridine (EdU, Life Technologies) was dissolved in physiological saline. Mice were injected with EdU intraperitoneally (50 mg/kg) 22 days after pump removal (24 hours before perfusions).

Immunohistochemical staining

Mice were anesthetized with Euthasol euthanasia solution and transcardially perfused with ice cold heparinized physiological saline (10 units heparin/mL physiological saline) followed by 4% paraformaldehyde (PFA, Sigma-Aldrich) in 0.1 M phosphate buffered saline (PBS). Brains were dissected, post-fixed in 4% PFA for 24 hours at 4 ºC, and equilibrated in 30% sucrose in PBS. After the equilibration step, brains were embedded in Tissue-Tek O.C.T. compound (Sakura, Torrance, CA) and stored at -80 ºC until sectioned with a cryostat. Frozen 20 µm sections were mounted on Fisherbrand Superfrost/Pus microscope slides (Thermo Fisher Scientific, Waltham, MA). Immunostaining was performed on six 20-µm sections with 2 dentate gyri per mouse.

Double immunohistochemical staining for proliferating cell nuclear antigen (PCNA) and Ki-67 was performed concomitantly with click chemistry to identify proliferating cells expressing PCNA and Ki-67 and cells that incorporated EdU. Staining was conducted after antigen-retrieval with 10 mM Sodium Citrate, pH 6.0 at 95 ºC for 30 minutes and blocking with normal donkey

serum. A mouse anti-PCNA primary antibody (1:500; Abcam, Cambridge, MA) and a rabbit anti-Ki67 primary antibody (1:500; Abcam) were used. The secondary antibodies used were AlexaFluor 488 donkey anti-mouse (1:500; Invitrogen, Carlsbad, CA) and Cy3 donkey anti-rabbit (1:200; Jackson ImmunoResearch, West Grove, PA) Secondary antibodies were incubated for 4 hours at room temperature in blocking buffer. A Click-iT EdU Alexa Fluor 647 Imaging Kit (Life Technologies) was used to label cells that incorporated EdU. Nuclear staining was performed with Vectashield hard-set mounting medium with DAPI (Vector Laboratories, Burlingame, CA).

Double immunohistochemical staining for PCNA and Doublecortin (DCX) was performed to label proliferating cells, neuroblasts, and immature neurons. The staining was completed after antigen-retrieval with 10 mM Sodium Citrate, pH 6.0 at 95 ºC for 30 minutes and blocking with normal donkey serum. A mouse anti-PCNA primary antibody (1:500; Abcam) and a guinea-pig anti-DCX primary antibody (1:500; Millipore, Billerica, MA) were used. Secondary antibodies used were AlexaFluor 488 donkey anti-mouse (1:500; Invitrogen) and Cy3 donkey anti-guinea-pig (1:500; Jackson ImmunoResearch). Secondary antibodies were incubated for 4 hours at room temperature in blocking buffer. Nuclear staining was performed with Vectashield hard-set mounting medium with DAPI (Vector Laboratories).

Triple immunohistochemical staining for PCNA, β-gal, and DCX was performed after antigen-retrieval with 10 mM Sodium Citrate, pH 6.0 at 95 ºC for 30 minutes and blocking with normal donkey serum. A mouse anti-PCNA primary antibody (1:500; Abcam), chicken anti- β-gal primary antibody (1:200; Abcam), and a guinea-pig anti-DCX primary antibody (1:500; Millipore) were used. Secondary antibodies used were AlexaFluor 488 donkey anti-chicken (1:500; Jackson ImmunoResearch), Cy3 donkey anti-mouse (1:500; Jackson ImmunoResearch), and a Cy5 donkey anti-guinea-pig (1:500; Jackson ImmunoResearch). Secondary antibodies were incubated for 4 hours at room temperature in blocking buffer. Nuclear staining was performed with Vectashield hard-set mounting medium with DAPI (Vector Laboratories).

Culturing adult hippocampal neural stem cells

The isolation of adult neural stem cells from rodents is described in greater detail previously (Gage, 2000). Adult rat hippocampal neural stem cells from Millipore were used. Cells were cultured at 37 ºC, 5% CO_2 in plates and Nunc Lab-Tek chamber slides (Thermo Fisher) coated with poly-ornithine (Sigma-Aldrich) and laminin (Invitrogen). Dulbecco's modified Eagle medium (DMEM)/F-12 (1:1) (Invitrogen) supplemented with N2 (Invitrogen) and 20 ng/ml recombinant human FGF-2 (PeproTech, Rocky Hill, NJ) was used. Cells were fed growth media every other day until they reach the desired confluency, at which point the media was swapped for co-culturing media, as described in (Song et al., 2002), astrocyte conditioned media (co-culture media conditioned by astrocytes for 24 hours) or the intended experimental conditions. Six hours before fixing, stem cells were treated with 30 μM EdU.

Culturing primary hippocampal astrocytes

The method described by McCarthy and Vellis was used to harvest primary astrocytic cultures from P1-2 Sprague Dawley rat pup hippocampi (McCarthy & de Vellis, 1980). In accordance

with this method, hippocampi were dissected in ice-cold media and cut into small pieces with a blade. The pieces were then digested using papain from papaya latex extract (Sigma-Aldrich) in HBSS (Invitrogen) for 20 minutes at 37 ºC. Papain was inactivated using 10% horse serum, and the separated cells were centrifuged for 1 minutes at 350xg. The resulting pellet was re-suspended in HBSS and the cells were then triturated with serological pipettes, micropipettes, and flame-polished pipettes of progressively smaller circumferences. Cells were then plated in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (Anexia Biologix, Dixon, CA) and 1% Penicillin/Streptomycin (Invitrogen) at a density of 3 million cells per T75 flask. Once cells reached confluency, they were shaken on an orbital shaker at 225 rpm for 120 minutes at 37 ºC to mechanically detach microglia. Detached and floating microglia were washed with warm PBS 5 times. Astrocytes were then chemically detached from the flasks with trypsin and re-plated in 100 mm plastic plates. When these pure astrocyte cultures reached 90% confluency, they were washed 3 times with warm PBS, and the media was changed to serum free co-culture media, described in (Song et al., 2002). Cells were incubated for 24 hours at 37 ºC, 5% CO_2 and then treated with co-culture media with 0.5 µM bovine serum albumin (Alb; Sigma-Aldrich), 0.5 µM Alb and 30 µM SJN 2511 (Tocris, Minneapolis, MN), or received fresh serum-free media and were incubated for 24 hours at 37 ºC, 5% CO_2. Cells were washed with warm PBS 3 times, and new serum-free media was given to all of the experimental cultures to begin the conditioning of this media. This astrocyte-conditioned media was collected 24 hours later and used to treat cultures of adult neural stem cells.

Immunocytochemistry

For the *in vitro* proliferation assay, cells were fixed with 4% PFA for 15 minutes at room temperature and washed with ice cold TBS. Cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature. Blocking was conducted with 5% normal donkey serum in TBS with 0.1% Triton X-100 for 2 hours at room temperature. Primary antibodies were incubated overnight at 4 ºC in blocking buffer. Proliferating stem cells were labeled with a mouse anti-PCNA primary antibody (1 µg/mL; Abcam) and a rabbit anti-Ki67 primary antibody (5 µg/mL; Abcam). Secondary antibodies used were AlexaFluor 488 donkey anti-rabbit (1:500; Jackson ImmunoResearch) and AlexaFluor 647 donkey anti-mouse (1:500; Jackson ImmunoResearch). Secondary antibodies were incubated for 4 hours at room temperature in blocking buffer. Proliferating cells that incorporated EdU were also labeled with click chemistry using a Click-iT EdU Alexa Fluor 594 Imaging Kit (Life Technologies). Nuclear staining was performed with Vectashield hard-set mounting medium with DAPI (Vector Laboratories). Fluorescent images were captured with an inverted epifluorescence microscope (Zeiss Axio observer Z1) using the Metamorph imaging system.

Quantification of immunofluorescence assays

Cells labeled by immunohistochemical assays were counted in the dorsal dentate gyri and subgranular zones under a 20x air objective (Zeiss, Oberkochen, Germany). The area sampled was calculated using Metamorph software (Molecular Devices, Sunnyvale, CA) and used to calculate the number of positive cells per millimeter squared. Six 20 µm sections per animal, spanning the hippocampus, were sampled for quantification. Researchers were blinded to the group allocation of samples, specifically when doing stereology. For the immoctochemical

assays, 80 random fields of each condition were collected using with a 20x air objective and an automated imager. Fluorescently labeled nuclei were counted with an automated cell counter application from the Metamorph software (Molecular Devices). Researchers were blinded to the group allocation of samples when doing using this application to count the labeled cells.

Statistics

When only two groups were compared, we used unpaired t-tests and considered $p<0.05$ as significant. To study the effect of Losartan on proliferation, neurogenesis, and dendritic structures, we used one-way ANOVAs followed by Dunnett's multiple comparison tests with the appropriate control groups as references. To study the effects of albumin exposure on proliferation, neurogenesis, and dendritic structures at three different time points, we used two-way ANOVAs with Bonferroni's multiple comparison tests. One-way ANOVAs with Tukey's multiple comparisons test were used for comparison between treatments of the *in vitro* experiments.

References:

Abdipranoto-Cowley, A., Park, J. S., Croucher, D., Daniel, J., Henshall, S., Galbraith, S., et al. (2009). Activin A is essential for neurogenesis following neurodegeneration. *Stem Cells (Dayton, Ohio)*, *27*(6), 1330–1346. http://doi.org/10.1002/stem.80

Aigner, L., & Bogdahn, U. (2008). TGF-beta in neural stem cells and in tumors of the central nervous system. *Cell and Tissue Research*, *331*(1), 225–241. http://doi.org/10.1007/s00441-007-0466-7

Altman, J., & Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *The Journal of Comparative Neurology*, *124*(3), 319–335.

Alvarez-Buylla, A., & Lim, D. A. (2004). For the long run: maintaining germinal niches in the adult brain. *Neuron*, *41*(5), 683–686.

Ambrogini, P., Lattanzi, D., Ciuffoli, S., Agostini, D., Bertini, L., Stocchi, V., et al. (2004). Morpho-functional characterization of neuronal cells at different stages of maturation in granule cell layer of adult rat dentate gyrus. *Brain Research*, *1017*(1-2), 21–31. http://doi.org/10.1016/j.brainres.2004.05.039

Anders, S., Minge, D., Griemsmann, S., Herde, M. K., Steinhäuser, C., & Henneberger, C. (2014). Spatial properties of astrocyte gap junction coupling in the rat hippocampus. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1654), 20130600–20130600. http://doi.org/10.1098/rstb.2013.0600

Annegers, J. F., Hauser, W. A., Coan, S. P., & Rocca, W. A. (1998). A population-based study of seizures after traumatic brain injuries. *The New England Journal of Medicine*, *338*(1), 20–24. http://doi.org/10.1056/NEJM199801013380104

Arisi, G. M., & Garcia-Cairasco, N. (2007). Doublecortin-positive newly born granule cells of hippocampus have abnormal apical dendritic morphology in the pilocarpine model of temporal lobe epilepsy. *Brain Research*, *1165*, 126–134. http://doi.org/10.1016/j.brainres.2007.06.037

Ashton, R. S., Conway, A., Pangarkar, C., Bergen, J., Lim, K.-I., Shah, P., et al. (2012). Astrocytes regulate adult hippocampal neurogenesis through ephrin-B signaling. *Nature Neuroscience*, *15*(10), 1399–1406. http://doi.org/10.1038/nn.3212

Auld, D. S., & Robitaille, R. (2003). Glial Cells and Neurotransmission. *Neuron*, *40*(2), 389–400. http://doi.org/10.1016/S0896-6273(03)00607-X

Bar-Klein, G., Cacheaux, L. P., Kamintsky, L., Prager, O., Weissberg, I., Schoknecht, K., et al. (2014). Losartan prevents acquired epilepsy via TGF-β signaling suppression. *Annals of Neurology*, *75*(6), 864–875. http://doi.org/10.1002/ana.24147

Barkho, B. Z., Song, H., Aimone, J. B., Smrt, R. D., Kuwabara, T., Nakashima, K., et al. (2006). Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. *Stem Cells and Development*, *15*(3), 407–421. http://doi.org/10.1089/scd.2006.15.407

Bartley, J. (2009). Could glial activation be a factor in migraine? *Medical Hypotheses*, *72*(3), 255–257. http://doi.org/10.1016/j.mehy.2008.09.048

Bennett, M. V. L., Contreras, J. E., Bukauskas, F. F., & Sáez, J. C. (2003). New roles for astrocytes: gap junction hemichannels have something to communicate. *Trends in Neurosciences*, *26*(11), 610–617. http://doi.org/10.1016/j.tins.2003.09.008

Bélanger, M., & Magistretti, P. J. (2009). The role of astroglia in neuroprotection. *Dialogues in*

Clinical Neuroscience, *11*(3), 281–295.
Bélanger, M., Allaman, I., & Magistretti, P. J. (2011). Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metabolism*, *14*(6), 724–738. http://doi.org/10.1016/j.cmet.2011.08.016
Bhattacharyya, B. J., Banisadr, G., Jung, H., Ren, D., Cronshaw, D. G., Zou, Y., & Miller, R. J. (2008). The chemokine stromal cell-derived factor-1 regulates GABAergic inputs to neural progenitors in the postnatal dentate gyrus. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *28*(26), 6720–6730. http://doi.org/10.1523/JNEUROSCI.1677-08.2008
Blottner, D., Wolf, N., Lachmund, A., Flanders, K. C., & Unsicker, K. (1996). TGF-beta rescues target-deprived preganglionic sympathetic neurons in the spinal cord. *The European Journal of Neuroscience*, *8*(1), 202–210.
Brites, D., & Vaz, A. R. (2014). Microglia centered pathogenesis in ALS: insights in cell interconnectivity. *Frontiers in Cellular Neuroscience*, *8*, 117. http://doi.org/10.3389/fncel.2014.00117
Brown, A. M., & Ransom, B. R. (2007). Astrocyte glycogen and brain energy metabolism. *Glia*, *55*(12), 1263–1271. http://doi.org/10.1002/glia.20557
Brown, J. P., Couillard-Després, S., Cooper-Kuhn, C. M., Winkler, J., Aigner, L., & Kuhn, H. G. (2003). Transient expression of doublecortin during adult neurogenesis. *The Journal of Comparative Neurology*, *467*(1), 1–10. http://doi.org/10.1002/cne.10874
Buckwalter, M. S., Yamane, M., Coleman, B. S., Ormerod, B. K., Chin, J. T., Palmer, T., & Wyss-Coray, T. (2006). Chronically increased transforming growth factor-beta1 strongly inhibits hippocampal neurogenesis in aged mice. *The American Journal of Pathology*, *169*(1), 154–164.
Cacheaux, L. P., Ivens, S., David, Y., Lakhter, A. J., Bar-Klein, G., Shapira, M., et al. (2009). Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *29*(28), 8927–8935. http://doi.org/10.1523/JNEUROSCI.0430-09.2009
Campe, von, G., Spencer, D. D., & De Lanerolle, N. C. (1997). Morphology of dentate granule cells in the human epileptogenic hippocampus. *Hippocampus*, *7*(5), 472–488. http://doi.org/10.1002/(SICI)1098-1063(1997)7:5<472::AID-HIPO4>3.0.CO;2-J
Campistol, J. M., Iñigo, P., Jimenez, W., Lario, S., Clesca, P. H., Oppenheimer, F., & Rivera, F. (1999). Losartan decreases plasma levels of TGF-beta1 in transplant patients with chronic allograft nephropathy. *Kidney International*, *56*(2), 714–719. http://doi.org/10.1046/j.1523-1755.1999.00597.x
Cappella, P., Gasparri, F., Pulici, M., & Moll, J. (2008). A novel method based on click chemistry, which overcomes limitations of cell cycle analysis by classical determination of BrdU incorporation, allowing multiplex antibody staining. *Cytometry. Part a : the Journal of the International Society for Analytical Cytology*, *73*(7), 626–636. http://doi.org/10.1002/cyto.a.20582
Chen, Y., & Swanson, R. A. (2003). Astrocytes and brain injury. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *23*(2), 137–149.
Cho, K.-O., Lybrand, Z. R., Ito, N., Brulet, R., Tafacory, F., Zhang, L., et al. (2015). Aberrant hippocampal neurogenesis contributes to epilepsy and associated cognitive decline. *Nature Communications*, *6*, 6606. http://doi.org/10.1038/ncomms7606

Cohn, R. D., van Erp, C., Habashi, J. P., Soleimani, A. A., Klein, E. C., Lisi, M. T., et al. (2007). Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. *Nature Medicine*, *13*(2), 204–210. http://doi.org/10.1038/nm1536

Colak, D., Mori, T., Brill, M. S., Pfeifer, A., Falk, S., Deng, C., et al. (2008). Adult Neurogenesis Requires Smad4-Mediated Bone Morphogenic Protein Signaling in Stem Cells. *The Journal of Neuroscience*, *28*(2), 434–446. http://doi.org/10.1523/JNEUROSCI.4374-07.2008

Coronado, V. G., Xu, L., Basavaraju, S. V., McGuire, L. C., Wald, M. M., Faul, M. D., et al. (2011). Surveillance for traumatic brain injury-related deaths--United States, 1997-2007. *MMWR. Surveillance Summaries : Morbidity and Mortality Weekly Report. Surveillance Summaries / CDC*, *60*(5), 1–32. Retrieved from http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=21544045&retmode=ref&cmd=prlinks

Couillard-Després, S., Winner, B., Schaubeck, S., Aigner, R., Vroemen, M., Weidner, N., et al. (2005). Doublecortin expression levels in adult brain reflect neurogenesis. *The European Journal of Neuroscience*, *21*(1), 1–14. http://doi.org/10.1111/j.1460-9568.2004.03813.x

Custer, S. K., Garden, G. A., Gill, N., Rueb, U., Libby, R. T., Schultz, C., et al. (2006). Bergmann glia expression of polyglutamine-expanded ataxin-7 produces neurodegeneration by impairing glutamate transport. *Nature Neuroscience*, *9*(10), 1302–1311. http://doi.org/10.1038/nn1750

Danzer, S. C. (2012). Depression, stress, epilepsy and adult neurogenesis. *Experimental Neurology*, *233*(1), 22–32. http://doi.org/10.1016/j.expneurol.2011.05.023

Danzer, S. C., Kotloski, R. J., Walter, C., Hughes, M., & McNamara, J. O. (2008). Altered morphology of hippocampal dentate granule cell presynaptic and postsynaptic terminals following conditional deletion of TrkB. *Hippocampus*, *18*(7), 668–678. http://doi.org/10.1002/hipo.20426

David, Y., Cacheaux, L. P., Ivens, S., Lapilover, E., Heinemann, U., Kaufer, D., & Friedman, A. (2009). Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *29*(34), 10588–10599. http://doi.org/10.1523/JNEUROSCI.2323-09.2009

de Caestecker, M. (2004). The transforming growth factor-β superfamily of receptors. *Cytokine & Growth Factor Reviews*, *15*(1), 1–11. http://doi.org/10.1016/j.cytogfr.2003.10.004

De Groot, C. J., Montagne, L., Barten, A. D., Sminia, P., & Van Der Valk, P. (1999). Expression of transforming growth factor (TGF)-beta1, -beta2, and -beta3 isoforms and TGF-beta type I and type II receptors in multiple sclerosis lesions and human adult astrocyte cultures. *Journal of Neuropathology and Experimental Neurology*, *58*(2), 174–187.

Dermietzel, R., Hertberg, E. L., Kessler, J. A., & Spray, D. C. (1991). Gap junctions between cultured astrocytes: immunocytochemical, molecular, and electrophysiological analysis. *The Journal of Neuroscience*, *11*(5), 1421–1432.

Dringen, R., Gebhardt, R., & Hamprecht, B. (1993). Glycogen in astrocytes: possible function as lactate supply for neighboring cells. *Brain Research*, *623*(2), 208–214.

Dringen, R., Pfeiffer, B., & Hamprecht, B. (1999). Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. *The Journal of Neuroscience*, *19*(2), 562–569.

Espósito, M. S., Piatti, V. C., Laplagne, D. A., Morgenstern, N. A., Ferrari, C. C., Pitossi, F. J., & Schinder, A. F. (2005). Neuronal differentiation in the adult hippocampus recapitulates

embryonic development. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *25*(44), 10074–10086. http://doi.org/10.1523/JNEUROSCI.3114-05.2005
Faigle, R., & Song, H. (2013). Signaling mechanisms regulating adult neural stem cells and neurogenesis. *Biochimica Et Biophysica Acta*, *1830*(2), 2435–2448. http://doi.org/10.1016/j.bbagen.2012.09.002
Faul, M., Xu, L., Wald, M. M., & Coronado, V. G. (2010). Traumatic Brain Injury in the United States: Emergency
Department Visits, Hospitalizations and Deaths 2002–2006
. Retrieved from http://www.cdc.gov/traumaticbraininjury/pdf/blue_book.pdf
Felling, R. J., & Levison, S. W. (2003). Enhanced neurogenesis following stroke. *Journal of Neuroscience Research*, *73*(3), 277–283. http://doi.org/10.1002/jnr.10670
Filosa, J. A., & Iddings, J. A. (2013). Astrocyte regulation of cerebral vascular tone. *American Journal of Physiology. Heart and Circulatory Physiology*, *305*(5), H609–19. http://doi.org/10.1152/ajpheart.00359.2013
Filosa, J. A., Bonev, A. D., & Nelson, M. T. (2004). Calcium dynamics in cortical astrocytes and arterioles during neurovascular coupling. *Circulation Research*, *95*(10), e73–81. http://doi.org/10.1161/01.RES.0000148636.60732.2e
Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., et al. (2014). ILAE official report: a practical clinical definition of epilepsy. *Epilepsia*, *55*(4), 475–482. http://doi.org/10.1111/epi.12550
Flanders, K. C., Ren, R. F., & Lippa, C. F. (1998). Transforming growth factor-betas in neurodegenerative disease. *Progress in Neurobiology*, *54*(1), 71–85.
Franck, J. E., Pokorny, J., Kunkel, D. D., & Schwartzkroin, P. A. (1995). Physiologic and Morphologic Characteristics of Granule Cell Circuitry in Human Epileptic Hippocampus. *Epilepsia*, *36*(6), 543–558. http://doi.org/10.1111/j.1528-1157.1995.tb02566.x
Friedman, A., Kaufer, D., & Heinemann, U. (2009). Blood–brain barrier breakdown-inducing astrocytic transformation: Novel targets for the prevention of epilepsy. *Epilepsy Research*, *85*(2-3), 142–149. http://doi.org/10.1016/j.eplepsyres.2009.03.005
Frigerio, F., Frasca, A., Weissberg, I., Parrella, S., Friedman, A., Vezzani, A., & Noé, F. M. (2012). Long-lasting pro-ictogenic effects induced in vivo by rat brain exposure to serum albumin in the absence of concomitant pathology. *Epilepsia*, *53*(11), 1887–1897. http://doi.org/10.1111/j.1528-1167.2012.03666.x
Gage, F. H. (2000). Mammalian neural stem cells. *Science (New York, N.Y.)*, *287*(5457), 1433–1438.
Garcia, A. D. R., Petrova, R., Eng, L., & Joyner, A. L. (2010). Sonic hedgehog regulates discrete populations of astrocytes in the adult mouse forebrain. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *30*(41), 13597–13608. http://doi.org/10.1523/JNEUROSCI.0830-10.2010
Garrett, L., Lie, D. C., Hrabé de Angelis, M., Wurst, W., & Hölter, S. M. (2012). Voluntary wheel running in mice increases the rate of neurogenesis without affecting anxiety-related behaviour in single tests. *BMC Neuroscience*, *13*, 61. http://doi.org/10.1186/1471-2202-13-61
Ge, S., Goh, E. L. K., Sailor, K. A., Kitabatake, Y., Ming, G.-L., & Song, H. (2006). GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature*, *439*(7076), 589–593. http://doi.org/10.1038/nature04404
Ge, S., Sailor, K. A., Ming, G.-L., & Song, H. (2008). Synaptic integration and plasticity of new

neurons in the adult hippocampus. *The Journal of Physiology*, *586*(16), 3759–3765. http://doi.org/10.1113/jphysiol.2008.155655

Giaume, C., Fromaget, C., Aoumari, el, A., Cordier, J., Glowinski, J., & Gros, D. (1991). Gap junctions in cultured astrocytes: single-channel currents and characterization of channel-forming protein. *Neuron*, *6*(1), 133–143.

Givogri, M. I., de Planell, M., Galbiati, F., Superchi, D., Gritti, A., Vescovi, A., et al. (2006). Notch signaling in astrocytes and neuroblasts of the adult subventricular zone in health and after cortical injury. *Developmental Neuroscience*, *28*(1-2), 81–91. http://doi.org/10.1159/000090755

Gould, E. (2007). How widespread is adult neurogenesis in mammals? *Nat Rev Neurosci*, *8*(6), 481–488. http://doi.org/10.1038/nrn2147

Graciarena, M., Roca, V., Mathieu, P., Depino, A. M., & Pitossi, F. J. (2013). Differential vulnerability of adult neurogenesis by adult and prenatal inflammation: role of TGF-β1. *Brain, Behavior, and Immunity*, *34*, 17–28. http://doi.org/10.1016/j.bbi.2013.05.007

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

Grégoire, C.-A., Bonenfant, D., Le Nguyen, A., Aumont, A., & Fernandes, K. J. L. (2014). Untangling the influences of voluntary running, environmental complexity, social housing and stress on adult hippocampal neurogenesis. *PloS One*, *9*(1), e86237. http://doi.org/10.1371/journal.pone.0086237

He, Y., Zhang, H., Yung, A., Villeda, S. A., Jaeger, P. A., Olayiwola, O., et al. (2014). ALK5-dependent TGF-β signaling is a major determinant of late-stage adult neurogenesis. *Nature Neuroscience*, *17*(7), 943–952. http://doi.org/10.1038/nn.3732

Heinrich, C., Nitta, N., Flubacher, A., Müller, M., Fahrner, A., Kirsch, M., et al. (2006). Reelin deficiency and displacement of mature neurons, but not neurogenesis, underlie the formation of granule cell dispersion in the epileptic hippocampus. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *26*(17), 4701–4713. http://doi.org/10.1523/JNEUROSCI.5516-05.2006

Heldin, C.-H., & Moustakas, A. (2012). Role of Smads in TGFβ signaling. *Cell and Tissue Research*, *347*(1), 21–36. http://doi.org/10.1007/s00441-011-1190-x

Herman, S. T. (2002). Epilepsy after brain insult: targeting epileptogenesis. *Neurology*, *59*(9 Suppl 5), S21–6. Retrieved from http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=12428028&retmode=ref&cmd=prlinks

Horesh, D., Sapir, T., Francis, F., Wolf, S. G., Caspi, M., Elbaum, M., et al. (1999). Doublecortin, a stabilizer of microtubules. *Human Molecular Genetics*, *8*(9), 1599–1610.

Iadecola, C., & Nedergaard, M. (2007). Glial regulation of the cerebral microvasculature. *Nature Neuroscience*, *10*(11), 1369–1376. http://doi.org/10.1038/nn2003

Ivens, S., Kaufer, D., Flores, L. P., Bechmann, I., Zumsteg, D., Tomkins, O., et al. (2007). TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. *Brain : a Journal of Neurology*, *130*(Pt 2), 535–547. http://doi.org/10.1093/brain/awl317

Jakubs, K., Nanobashvili, A., Bonde, S., Ekdahl, C. T., Kokaia, Z., Kokaia, M., & Lindvall, O. (2006). Environment matters: synaptic properties of neurons born in the epileptic adult brain develop to reduce excitability. *Neuron*, *52*(6), 1047–1059. http://doi.org/10.1016/j.neuron.2006.11.004

Jessberger, S., & Parent, J. M. (2011). Adult Neurogenesis in Epilepsy. In *Neurogenesis in the Adult Brain II* (pp. 37–52). Springer Japan. http://doi.org/10.1007/978-4-431-53945-2_3

Jessberger, S., Römer, B., Babu, H., & Kempermann, G. (2005). Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. *Experimental Neurology*, *196*(2), 342–351. http://doi.org/10.1016/j.expneurol.2005.08.010

Jessberger, S., Zhao, C., Toni, N., Clemenson, G. D., Li, Y., & Gage, F. H. (2007). Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling. *The Journal of Neuroscience*, *27*(35), 9400–9407. http://doi.org/10.1523/JNEUROSCI.2002-07.2007

Jung, K.-H., Chu, K., Kim, M., Jeong, S.-W., Song, Y.-M., Lee, S.-T., et al. (2004). Continuous cytosine-b-D-arabinofuranoside infusion reduces ectopic granule cells in adult rat hippocampus with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. *The European Journal of Neuroscience*, *19*(12), 3219–3226. http://doi.org/10.1111/j.0953-816X.2004.03412.x

Jung, K.-H., Chu, K., Lee, S.-T., Kim, J., Sinn, D.-I., Kim, J.-M., et al. (2006). Cyclooxygenase-2 inhibitor, celecoxib, inhibits the altered hippocampal neurogenesis with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. *Neurobiology of Disease*, *23*(2), 237–246. http://doi.org/10.1016/j.nbd.2006.02.016

Kandel, E. R. (2013). Principles of neural science. New York : McGraw-Hill.

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature*, *386*(6624), 493–495. http://doi.org/10.1038/386493a0

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1998). Experience-induced neurogenesis in the senescent dentate gyrus. *The Journal of Neuroscience*, *18*(9), 3206–3212.

Khalil, A., Tullus, K., Bakhiet, M., Burman, L. G., Jaremko, G., & Brauner, A. (2000). Angiotensin II type 1 receptor antagonist (losartan) down-regulates transforming growth factor-beta in experimental acute pyelonephritis. *The Journal of Urology*, *164*(1), 186–191.

Kirby, E. D., Muroy, S. E., Sun, W. G., Covarrubias, D., Leong, M. J., Barchas, L. A., & Kaufer, D. (2013). Acute stress enhances adult rat hippocampal neurogenesis and activation of newborn neurons via secreted astrocytic FGF2. *eLife*, *2*, e00362. http://doi.org/10.7554/eLife.00362

Kron, M. M., Zhang, H., & Parent, J. M. (2010). The developmental stage of dentate granule cells dictates their contribution to seizure-induced plasticity. *The Journal of Neuroscience*, *30*(6), 2051–2059. http://doi.org/10.1523/JNEUROSCI.5655-09.2010

Kuhn, H. G., Dickinson-Anson, H., & Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *The Journal of Neuroscience*, *16*(6), 2027–2033.

Lagace, D. C., Whitman, M. C., Noonan, M. A., Ables, J. L., DeCarolis, N. A., Arguello, A. A., et al. (2007). Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *27*(46), 12623–12629. http://doi.org/10.1523/JNEUROSCI.3812-07.2007

Lanz, T. V., Ding, Z., Ho, P. P., Luo, J., Agrawal, A. N., Srinagesh, H., et al. (2010). Angiotensin II sustains brain inflammation in mice via TGF-beta. *The Journal of Clinical Investigation*, *120*(8), 2782–2794. http://doi.org/10.1172/JCI41709

Laplagne, D. A., Espósito, M. S., Piatti, V. C., Morgenstern, N. A., Zhao, C., van Praag, H., et al. (2006). Functional convergence of neurons generated in the developing and adult

hippocampus. *PLoS Biology*, *4*(12), e409–e409. http://doi.org/10.1371/journal.pbio.0040409
Lavoie, P., Robitaille, G., Agharazii, M., Ledbetter, S., Lebel, M., & Larivière, R. (2005). Neutralization of transforming growth factor-beta attenuates hypertension and prevents renal injury in uremic rats. *Journal of Hypertension*, *23*(10), 1895–1903.
Leone, L., Fusco, S., Mastrodonato, A., Piacentini, R., Barbati, S. A., Zaffina, S., et al. (2014). Epigenetic modulation of adult hippocampal neurogenesis by extremely low-frequency electromagnetic fields. *Molecular Neurobiology*, *49*(3), 1472–1486. http://doi.org/10.1007/s12035-014-8650-8
Lie, D. C., Colamarino, S. A., Song, H.-J., Désiré, L., Mira, H., Consiglio, A., et al. (2005). Wnt signalling regulates adult hippocampal neurogenesis. *Nature*, *437*(7063), 1370–1375. http://doi.org/10.1038/nature04108
Lim, D. A., & Alvarez-Buylla, A. (1999). Interaction between astrocytes and adult subventricular zone precursors stimulates neurogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(13), 7526–7531.
Lim, D. A., Tramontin, A. D., Trevejo, J. M., Herrera, D. G., García-Verdugo, J. M., & Alvarez-Buylla, A. (2000). Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron*, *28*(3), 713–726.
Lim, D. S., Lutucuta, S., Bachireddy, P., Youker, K., Evans, A., Entman, M., et al. (2001). Angiotensin II blockade reverses myocardial fibrosis in a transgenic mouse model of human hypertrophic cardiomyopathy. *Circulation*, *103*(6), 789–791.
Liu, Y., Song, Z., Zhao, Y., Qin, H., Cai, J., Zhang, H., et al. (2006). A novel chemical-defined medium with bFGF and N2B27 supplements supports undifferentiated growth in human embryonic stem cells. *Biochemical and Biophysical Research Communications*, *346*(1), 131–139. http://doi.org/10.1016/j.bbrc.2006.05.086
Lobsiger, C. S., & Cleveland, D. W. (2007). Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. *Nature Neuroscience*, *10*(11), 1355–1360. http://doi.org/10.1038/nn1988
Logan, T. T., Villapol, S., & Symes, A. J. (2013). TGF-β superfamily gene expression and induction of the Runx1 transcription factor in adult neurogenic regions after brain injury. *PloS One*, *8*(3), e59250. http://doi.org/10.1371/journal.pone.0059250
Lugert, S., Basak, O., Knuckles, P., Häussler, U., Fabel, K., Götz, M., et al. (2010). Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. *Cell Stem Cell*, *6*(5), 445–456. http://doi.org/10.1016/j.stem.2010.03.017
Ma, D. K., Jang, M.-H., Guo, J. U., Kitabatake, Y., Chang, M.-L., Pow-Anpongkul, N., et al. (2009). Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science (New York, N.Y.)*, *323*(5917), 1074–1077. http://doi.org/10.1126/science.1166859
Ma, D. K., Marchetto, M. C., Guo, J. U., Ming, G.-L., Gage, F. H., & Song, H. (2010). Epigenetic choreographers of neurogenesis in the adult mammalian brain. *Nature Neuroscience*, *13*(11), 1338–1344. http://doi.org/10.1038/nn.2672
Maćkowiak, M., Chocyk, A., Markowicz-Kula, K., & Wedzony, K. (2004). Neurogenesis in the adult brain. *Polish Journal of Pharmacology*, *56*(6), 673–687.
Magnusson, J. P., Göritz, C., Tatarishvili, J., Dias, D. O., Smith, E. M. K., Lindvall, O., et al. (2014). A latent neurogenic program in astrocytes regulated by Notch signaling in the mouse. *Science (New York, N.Y.)*, *346*(6206), 237–241.

http://doi.org/10.1126/science.346.6206.237
Markakis, E. A., & Gage, F. H. (1999). Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. *The Journal of Comparative Neurology*, *406*(4), 449–460. http://doi.org/10.1002/(SICI)1096-9861(19990419)406:4<449::AID-CNE3>3.0.CO;2-I
Massague, J. (1992). Receptors for the TGF-beta family. *Cell*, *69*(7), 1067–1070.
Massague, J., Cheifetz, S., Ignotz, R. A., & Boyd, F. T. (1987). Multiple type-beta transforming growth factors and their receptors. *Journal of Cellular Physiology. Supplement*, *Suppl 5*, 43–47.
Mathews, E. A., Morgenstern, N. A., Piatti, V. C., Zhao, C., Jessberger, S., Schinder, A. F., & Gage, F. H. (2010). A distinctive layering pattern of mouse dentate granule cells is generated by developmental and adult neurogenesis. *The Journal of Comparative Neurology*, *518*(22), 4479–4490. http://doi.org/10.1002/cne.22489
Mazerbourg, S., & Hsueh, A. J. W. (2006). Genomic analyses facilitate identification of receptors and signalling pathways for growth differentiation factor 9 and related orphan bone morphogenetic protein/growth differentiation factor ligands. *Human Reproduction Update*, *12*(4), 373–383. http://doi.org/10.1093/humupd/dml014
McAuliffe, J. J., Bronson, S. L., Hester, M. S., Murphy, B. L., Dahlquist-Topalá, R., Richards, D. A., & Danzer, S. C. (2011). Altered patterning of dentate granule cell mossy fiber inputs onto CA3 pyramidal cells in limbic epilepsy. *Hippocampus*, *21*(1), 93–107. http://doi.org/10.1002/hipo.20726
McCarthy, K. D., & de Vellis, J. (1980). Preparation of Separate Astroglial and Oligodendroglial Cell Cultures from Rat Cerebral Tissue. *The Journal of Cell Biology*, *85*(3), 890–902. http://doi.org/10.2307/1609060?ref=no-x-route:e106c71eb59156b523460f1bac063cbb
McCloskey, D. P., Hintz, T. M., Pierce, J. P., & Scharfman, H. E. (2006). Stereological methods reveal the robust size and stability of ectopic hilar granule cells after pilocarpine-induced status epilepticus in the adult rat. *The European Journal of Neuroscience*, *24*(8), 2203–2210. http://doi.org/10.1111/j.1460-9568.2006.05101.x
Mercier, F., & Douet, V. (2014). Bone morphogenetic protein-4 inhibits adult neurogenesis and is regulated by fractone-associated heparan sulfates in the subventricular zone. *Journal of Chemical Neuroanatomy*, *57-58*, 54–61. http://doi.org/10.1016/j.jchemneu.2014.03.005
Ming, G.-L., & Song, H. (2005). Adult neurogenesis in the mammalian central nervous system. *Annual Review of Neuroscience*, *28*, 223–250. http://doi.org/10.1146/annurev.neuro.28.051804.101459
Ming, G.-L., & Song, H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron*, *70*(4), 687–702. http://doi.org/10.1016/j.neuron.2011.05.001
Mira, H., Andreu, Z., Suh, H., Lie, D. C., Jessberger, S., Consiglio, A., et al. (2010). Signaling through BMPR-IA regulates quiescence and long-term activity of neural stem cells in the adult hippocampus. *Cell Stem Cell*, *7*(1), 78–89. http://doi.org/10.1016/j.stem.2010.04.016
Mirescu, C., & Gould, E. (2006). Stress and adult neurogenesis. *Hippocampus*, *16*(3), 233–238. http://doi.org/10.1002/hipo.20155
Moldovan, G.-L., Pfander, B., & Jentsch, S. (2007). PCNA, the maestro of the replication fork. *Cell*, *129*(4), 665–679. http://doi.org/10.1016/j.cell.2007.05.003
Morrens, J., Van Den Broeck, W., & Kempermann, G. (2012). Glial cells in adult neurogenesis. *Glia*, *60*(2), 159–174. http://doi.org/10.1002/glia.21247

Moustakas, A., & Heldin, C.-H. (2009). The regulation of TGFbeta signal transduction. *Development (Cambridge, England)*, *136*(22), 3699–3714. http://doi.org/10.1242/dev.030338

Mu, Y., Lee, S. W., & Gage, F. H. (2010). Signaling in adult neurogenesis. *Current Opinion in Neurobiology*, *20*(4), 416–423. http://doi.org/10.1016/j.conb.2010.04.010

Murphy, B. L., & Danzer, S. C. (2011). Somatic translocation: a novel mechanism of granule cell dendritic dysmorphogenesis and dispersion. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *31*(8), 2959–2964. http://doi.org/10.1523/JNEUROSCI.3381-10.2011

Murphy, B. L., Pun, R. Y. K., Yin, H., Faulkner, C. R., Loepke, A. W., & Danzer, S. C. (2011). Heterogeneous integration of adult-generated granule cells into the epileptic brain. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *31*(1), 105–117. http://doi.org/10.1523/JNEUROSCI.2728-10.2011

Naninck, E. F. G., Hoeijmakers, L., Kakava-Georgiadou, N., Meesters, A., Lazic, S. E., Lucassen, P. J., & Korosi, A. (2015). Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice. *Hippocampus*, *25*(3), 309–328. http://doi.org/10.1002/hipo.22374

Nedergaard, M., & Dirnagl, U. (2005). Role of glial cells in cerebral ischemia. *Glia*, *50*(4), 281–286. http://doi.org/10.1002/glia.20205

Nedergaard, M., Ransom, B., & Goldman, S. A. (2003). New roles for astrocytes: redefining the functional architecture of the brain. *Trends in Neurosciences*, *26*(10), 523–530. http://doi.org/10.1016/j.tins.2003.08.008

Ninkovic, J., & Götz, M. (2007). Signaling in adult neurogenesis: from stem cell niche to neuronal networks. *Current Opinion in Neurobiology*, *17*(3), 338–344. http://doi.org/10.1016/j.conb.2007.04.006

Oby, E., & Janigro, D. (2006). The Blood?Brain Barrier and Epilepsy. *Epilepsia*, *47*(11), 1761–1774. http://doi.org/10.1111/j.1528-1167.2006.00817.x

Overstreet, L. S., Hentges, S. T., Bumaschny, V. F., de Souza, F. S. J., Smart, J. L., Santangelo, A. M., et al. (2004). A transgenic marker for newly born granule cells in dentate gyrus. *The Journal of Neuroscience*, *24*(13), 3251–3259. http://doi.org/10.1523/JNEUROSCI.5173-03.2004

Overstreet-Wadiche, L. S., Bromberg, D. A., Bensen, A. L., & Westbrook, G. L. (2006). Seizures accelerate functional integration of adult-generated granule cells. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *26*(15), 4095–4103. http://doi.org/10.1523/JNEUROSCI.5508-05.2006

Papadeas, S. T., Kraig, S. E., O'Banion, C., Lepore, A. C., & Maragakis, N. J. (2011). Astrocytes carrying the superoxide dismutase 1 (SOD1G93A) mutation induce wild-type motor neuron degeneration in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(43), 17803–17808. http://doi.org/10.1073/pnas.1103141108

Parent, J. M. (2003). Injury-Induced Neurogenesis in the Adult Mammalian Brain. *The Neuroscientist*, *9*(4), 261–272. http://doi.org/10.1177/1073858403252680

Parent, J. M. (2007). Adult neurogenesis in the intact and epileptic dentate gyrus. *Progress in Brain Research*, *163*, 529–540. http://doi.org/10.1016/S0079-6123(07)63028-3

Parent, J. M., Elliott, R. C., Pleasure, S. J., Barbaro, N. M., & Lowenstein, D. H. (2006). Aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. *Annals of Neurology*, *59*(1), 81–91. http://doi.org/10.1002/ana.20699

Parent, J. M., Yu, T. W., Leibowitz, R. T., Geschwind, D. H., Sloviter, R. S., & Lowenstein, D. H. (1997). Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *17*(10), 3727–3738.

Paton, J. A., & Nottebohm, F. N. (1984). Neurons generated in the adult brain are recruited into functional circuits. *Science (New York, N.Y.)*, *225*(4666), 1046–1048.

Pekcec, A., Fuest, C., Mühlenhoff, M., Gerardy-Schahn, R., & Potschka, H. (2008). Targeting epileptogenesis-associated induction of neurogenesis by enzymatic depolysialylation of NCAM counteracts spatial learning dysfunction but fails to impact epilepsy development. *Journal of Neurochemistry*, *105*(2), 389–400. http://doi.org/10.1111/j.1471-4159.2007.05172.x

Pekcec, A., Lüpke, M., Baumann, R., Seifert, H., & Potschka, H. (2011). Modulation of neurogenesis by targeted hippocampal irradiation fails to affect kindling progression. *Hippocampus*, *21*(8), 866–876. http://doi.org/10.1002/hipo.20802

Pekcec, A., Mühlenhoff, M., Gerardy-Schahn, R., & Potschka, H. (2007). Impact of the PSA-NCAM system on pathophysiology in a chronic rodent model of temporal lobe epilepsy. *Neurobiology of Disease*, *27*(1), 54–66. http://doi.org/10.1016/j.nbd.2007.04.002

Pereira, M. G. A. G., Becari, C., Oliveira, J. A. C., Salgado, M. C. O., Garcia-Cairasco, N., & Costa-Neto, C. M. (2010). Inhibition of the renin-angiotensin system prevents seizures in a rat model of epilepsy. *Clinical Science*, *119*(11), 477–482. http://doi.org/10.1042/CS20100053

Peuchen, S., Bolaños, J. P., Heales, S. J., Almeida, A., Duchen, M. R., & Clark, J. B. (1997). Interrelationships between astrocyte function, oxidative stress and antioxidant status within the central nervous system. *Progress in Neurobiology*, *52*(4), 261–281.

Plümpe, T., Ehninger, D., Steiner, B., Klempin, F., Jessberger, S., Brandt, M., et al. (2006). Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neuroscience*, *7*(1), 77. http://doi.org/10.1186/1471-2202-7-77

Radley, J. J., & Jacobs, B. L. (2003). Pilocarpine-induced status epilepticus increases cell proliferation in the dentate gyrus of adult rats via a 5-HT1A receptor-dependent mechanism. *Brain Research*, *966*(1), 1–12.

Ribak, C. E., Tran, P. H., Spigelman, I., Okazaki, M. M., & Nadler, J. V. (2000). Status epilepticus-induced hilar basal dendrites on rodent granule cells contribute to recurrent excitatory circuitry. *The Journal of Comparative Neurology*, *428*(2), 240–253. http://doi.org/10.1002/1096-9861(20001211)428:2<240::AID-CNE4>3.0.CO;2-Q

Rodríguez-Martínez, G., & Velasco, I. (2012). Activin and TGF-β Effects on Brain Development and Neural Stem Cells. *CNS & Neurological …*. http://doi.org/10.2174/187152712804546306

Rodríguez-Martínez, G., Molina-Hernández, A., & Velasco, I. (2012). Activin A promotes neuronal differentiation of cerebrocortical neural progenitor cells. *PloS One*, *7*(8), e43797–e43797. http://doi.org/10.1371/journal.pone.0043797

Rodríguez-Vita, J., Sánchez-López, E., Esteban, V., Rupérez, M., Egido, J., & Ruiz-Ortega, M. (2005). Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a transforming growth factor-beta-independent mechanism. *Circulation*, *111*(19), 2509–2517. http://doi.org/10.1161/01.CIR.0000165133.84978.E2

Romano, V., Raimondo, D., Calvanese, L., D'Auria, G., Tramontano, A., & Falcigno, L. (2012).

Toward a better understanding of the interaction between TGF-β family members and their ALK receptors. *Journal of Molecular Modeling*, *18*(8), 3617–3625. http://doi.org/10.1007/s00894-012-1370-y

Rossi, D. J., Brady, J. D., & Mohr, C. (2007). Astrocyte metabolism and signaling during brain ischemia. *Nature Neuroscience*, *10*(11), 1377–1386. http://doi.org/10.1038/nn2004

Saaltink, D.-J., & Vreugdenhil, E. (2014). Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition? *Cellular and Molecular Life Sciences : CMLS*, *71*(13), 2499–2515. http://doi.org/10.1007/s00018-014-1568-5

Salic, A., & Mitchison, T. J. (2008). A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(7), 2415–2420. http://doi.org/10.1073/pnas.0712168105

Sáez, J. C., Contreras, J. E., Bukauskas, F. F., Retamal, M. A., & Bennett, M. V. L. (2003). Gap junction hemichannels in astrocytes of the CNS. *Acta Physiologica Scandinavica*, *179*(1), 9–22. http://doi.org/10.1046/j.1365-201X.2003.01196.x

SB, B., J, B., KR, G., BJ, A., ST, C., & A, S. (2008). Detection of S-phase cell cycle progression using 5-ethynyl-2“-deoxyuridine incorporation with click chemistry, an alternative to using 5-bromo-2-”deoxyuridine antibodies. *BioTechniques*, *44*(7), 927–929.

Scharfman, H. E., Goodman, J. H., & Sollas, A. L. (2000). Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. *The Journal of Neuroscience*, *20*(16), 6144–6158.

Scharfman, H., Goodman, J., & McCloskey, D. (2007). Ectopic granule cells of the rat dentate gyrus. *Developmental Neuroscience*, *29*(1-2), 14–27. http://doi.org/10.1159/000096208

Scheibel, M. E., & Scheibel, A. B. (1973). Scheibel: Hippocampal pathology in temporal lobe... - Google Scholar. Epilepsy: Its Phenomena in ….

Schmidt-Hieber, C., Jonas, P., & Bischofberger, J. (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature*, *429*(6988), 184–187. http://doi.org/10.1038/nature02553

Schoenfeld, T. J., & Gould, E. (2012). Stress, stress hormones, and adult neurogenesis. *Experimental Neurology*, *233*(1), 12–21. http://doi.org/10.1016/j.expneurol.2011.01.008

Schoenfeld, T. J., & Gould, E. (2013). Differential effects of stress and glucocorticoids on adult neurogenesis. *Current Topics in Behavioral Neurosciences*, *15*, 139–164. http://doi.org/10.1007/7854_2012_233

Scholzen, T., & Gerdes, J. (2000). The Ki-67 protein: from the known and the unknown. *Journal of Cellular Physiology*, *182*(3), 311–322. http://doi.org/10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9

Seiffert, E., Dreier, J. P., Ivens, S., Bechmann, I., Tomkins, O., Heinemann, U., & Friedman, A. (2004). Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *24*(36), 7829–7836. http://doi.org/10.1523/JNEUROSCI.1751-04.2004

Shapiro, L. A., & Ribak, C. E. (2006). Newly born dentate granule neurons after pilocarpine-induced epilepsy have hilar basal dendrites with immature synapses. *Epilepsy Research*, *69*(1), 53–66. http://doi.org/10.1016/j.eplepsyres.2005.12.003

Shapiro, L. A., Korn, M. J., Shan, Z., & Ribak, C. E. (2005). GFAP-expressing radial glia-like cell bodies are involved in a one-to-one relationship with doublecortin-immunolabeled newborn neurons in the adult dentate gyrus. *Brain Research*, *1040*(1-2), 81–91.

http://doi.org/10.1016/j.brainres.2005.01.098
Shlosberg, D., Benifla, M., Kaufer, D., & Friedman, A. (2010). Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nature Reviews. Neurology*, *6*(7), 393–403. http://doi.org/10.1038/nrneurol.2010.74
Sierra, A., Martín-Suárez, S., Valcárcel-Martín, R., Pascual-Brazo, J., Aelvoet, S.-A., Abiega, O., et al. (2015). Neuronal hyperactivity accelerates depletion of neural stem cells and impairs hippocampal neurogenesis. *Cell Stem Cell*, *16*(5), 488–503. http://doi.org/10.1016/j.stem.2015.04.003
Song, H., Stevens, C. F., & Gage, F. H. (2002). Astroglia induce neurogenesis from adult neural stem cells. *Nature*, *417*(6884), 39–44. http://doi.org/10.1038/417039a
Stobart, J. L., & Anderson, C. M. (2013). Multifunctional role of astrocytes as gatekeepers of neuronal energy supply. *Frontiers in Cellular Neuroscience*, *7*, 38. http://doi.org/10.3389/fncel.2013.00038
Stranahan, A. M., Arumugam, T. V., Cutler, R. G., Lee, K., Egan, J. M., & Mattson, M. P. (2008). Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nature Neuroscience*, *11*(3), 309–317. http://doi.org/10.1038/nn2055
Sugaya, Y., Maru, E., Kudo, K., Shibasaki, T., & Kato, N. (2010). Levetiracetam suppresses development of spontaneous EEG seizures and aberrant neurogenesis following kainate-induced status epilepticus. *Brain Research*, *1352*, 187–199. http://doi.org/10.1016/j.brainres.2010.06.061
Suh, H., Consiglio, A., Ray, J., Sawai, T., D'Amour, K. A., & Gage, F. H. (2007a). In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. *Cell Stem Cell*, *1*(5), 515–528. http://doi.org/10.1016/j.stem.2007.09.002
Suh, S. W., Bergher, J. P., Anderson, C. M., Treadway, J. L., Fosgerau, K., & Swanson, R. A. (2007b). Astrocyte glycogen sustains neuronal activity during hypoglycemia: studies with the glycogen phosphorylase inhibitor CP-316,819 ([R-R*,S*]-5-chloro-N-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmethyl)propyl]-1H-indole-2-carboxamide). *The Journal of Pharmacology and Experimental Therapeutics*, *321*(1), 45–50. http://doi.org/10.1124/jpet.106.115550
Sutula, T. P., & Dudek, F. E. (2007). Unmasking recurrent excitation generated by mossy fiber sprouting in the epileptic dentate gyrus: an emergent property of a complex system. *Progress in Brain Research*, *163*, 541–563. http://doi.org/10.1016/S0079-6123(07)63029-5
Sutula, T., He, X. X., Cavazos, J., & Scott, G. (1988). Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science (New York, N.Y.)*, *239*(4844), 1147–1150.
Takano, T., Oberheim, N., Cotrina, M. L., & Nedergaard, M. (2009). Astrocytes and ischemic injury. *Stroke; a Journal of Cerebral Circulation*, *40*(3 Suppl), S8–12. http://doi.org/10.1161/STROKEAHA.108.533166
Taupin, P., Ray, J., Fischer, W. H., Suhr, S. T., Hakansson, K., Grubb, A., & Gage, F. H. (2000). FGF-2-responsive neural stem cell proliferation requires CCg, a novel autocrine/paracrine cofactor. *Neuron*, *28*(2), 385–397.
Thalakoti, S., Patil, V. V., Damodaram, S., Vause, C. V., Langford, L. E., Freeman, S. E., & Durham, P. L. (2007). Neuron-glia signaling in trigeminal ganglion: implications for migraine pathology. *Headache*, *47*(7), 1008–23– discussion 24–5. http://doi.org/10.1111/j.1526-4610.2007.00854.x

Thurman, D. J., Alverson, C., Dunn, K. A., Guerrero, J., & Sniezek, J. E. (1999). Traumatic brain injury in the United States: A public health perspective. *The Journal of Head Trauma Rehabilitation*, *14*(6), 602–615. Retrieved from http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10671706&retmode=ref&cmd=prlinks

Tomkins, O., Friedman, O., Ivens, S., Reiffurth, C., Major, S., Dreier, J. P., et al. (2007). Blood-brain barrier disruption results in delayed functional and structural alterations in the rat neocortex. *Neurobiology of Disease*, *25*(2), 367–377. http://doi.org/10.1016/j.nbd.2006.10.006

Tomkins, O., Shelef, I., Kaizerman, I., Eliushin, A., Afawi, Z., Misk, A., et al. (2008). Blood-brain barrier disruption in post-traumatic epilepsy. *Journal of Neurology, Neurosurgery, and Psychiatry*, *79*(7), 774–777. http://doi.org/10.1136/jnnp.2007.126425

Toni, N., Laplagne, D. A., Zhao, C., Lombardi, G., Ribak, C. E., Gage, F. H., & Schinder, A. F. (2008). Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nature Neuroscience*, *11*(8), 901–907. http://doi.org/10.1038/nn.2156

Toni, N., Teng, E. M., Bushong, E. A., Aimone, J. B., Zhao, C., Consiglio, A., et al. (2007). Synapse formation on neurons born in the adult hippocampus. *Nature Neuroscience*, *10*(6), 727–734. http://doi.org/10.1038/nn1908

van Praag, H., Kempermann, G., & Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience*, *2*(3), 266–270. http://doi.org/10.1038/6368

Villapol, S., Logan, T. T., & Symes, A. J. (2013). Role of TGF-β Signaling in Neurogenic Regions After Brain Injury. *Trends in Cell …*.

Vivien, D., & Ali, C. (2006). Transforming growth factor-beta signalling in brain disorders. *Cytokine & Growth Factor Reviews*, *17*(1-2), 121–128. http://doi.org/10.1016/j.cytogfr.2005.09.011

Volterra, A., & Meldolesi, J. (2005). Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci*, *6*(8), 626–640. http://doi.org/10.1038/nrn1722

Wachs, F.-P., Winner, B., Couillard-Després, S., Schiller, T., Aigner, R., Winkler, J., et al. (2006). Transforming growth factor-beta1 is a negative modulator of adult neurogenesis. *Journal of Neuropathology and Experimental Neurology*, *65*(4), 358–370. http://doi.org/10.1097/01.jnen.0000218444.53405.f0

Wakabayashi, T., Hidaka, R., Fujimaki, S., Asashima, M., & Kuwabara, T. (2014). MicroRNAs and epigenetics in adult neurogenesis. *Advances in Genetics*, *86*, 27–44. http://doi.org/10.1016/B978-0-12-800222-3.00002-4

Walter, C., Murphy, B. L., Pun, R. Y. K., Spieles-Engemann, A. L., & Danzer, S. C. (2007). Pilocarpine-induced seizures cause selective time-dependent changes to adult-generated hippocampal dentate granule cells. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *27*(28), 7541–7552. http://doi.org/10.1523/JNEUROSCI.0431-07.2007

Wang, D. D., & Bordey, A. (2008). The astrocyte odyssey. *Progress in Neurobiology*, *86*(4), 342–367. http://doi.org/10.1016/j.pneurobio.2008.09.015

Wang, H.-C., Chang, W.-N., Chang, H.-W., Ho, J.-T., Yang, T.-M., Lin, W.-C., et al. (2008). Factors predictive of outcome in posttraumatic seizures. *The Journal of Trauma*, *64*(4), 883–888. http://doi.org/10.1097/TA.0b013e31804a7fa4

Wang, W., Huang, X. R., Canlas, E., Oka, K., Truong, L. D., Deng, C., et al. (2006). Essential

role of Smad3 in angiotensin II-induced vascular fibrosis. *Circulation Research*, *98*(8), 1032–1039. http://doi.org/10.1161/01.RES.0000218782.52610.dc

Wang, Y., Jin, S., Sonobe, Y., Cheng, Y., Horiuchi, H., Parajuli, B., et al. (2014). Interleukin-1β induces blood-brain barrier disruption by downregulating Sonic hedgehog in astrocytes. *PloS One*, *9*(10), e110024. http://doi.org/10.1371/journal.pone.0110024

Warden, D. (2006). Military TBI during the Iraq and Afghanistan wars. *Journal of Head Trauma Rehabilitation*, *21*(5), 398–402.

Weissberg, I., Reichert, A., Heinemann, U., & Friedman, A. (2011). Blood-Brain Barrier Dysfunction in Epileptogenesis of the Temporal Lobe. *Epilepsy Research and Treatment*, *2011*, 1–10. http://doi.org/10.1155/2011/143908

Weissberg, I., Wood, L., Kamintsky, L., & Vazquez, O. (2015). Albumin induces excitatory synaptogenesis through astrocytic TGF-β/ALK5 signaling in a model of acquired epilepsy following blood–brain barrier dysfunction. *Neurobiology of …*. http://doi.org/10.1523/JNEUROSCI

Werry, E. L., Enjeti, S., Halliday, G. M., Sachdev, P. S., & Double, K. L. (2010). Effect of age on proliferation-regulating factors in human adult neurogenic regions. *Journal of Neurochemistry*, *115*(4), 956–964. http://doi.org/10.1111/j.1471-4159.2010.06992.x

Wilhelmsson, U., Faiz, M., de Pablo, Y., Sjöqvist, M., Andersson, D., Widestrand, A., et al. (2012). Astrocytes negatively regulate neurogenesis through the Jagged1-mediated Notch pathway. *Stem Cells (Dayton, Ohio)*, *30*(10), 2320–2329. http://doi.org/10.1002/stem.1196

Winner, B., Kohl, Z., & Gage, F. H. (2011). Neurodegenerative disease and adult neurogenesis. *The European Journal of Neuroscience*, *33*(6), 1139–1151. http://doi.org/10.1111/j.1460-9568.2011.07613.x

Wu, X., Kihara, T., Hongo, H., Akaike, A., Niidome, T., & Sugimoto, H. (2010). Angiotensin receptor type 1 antagonists protect against neuronal injury induced by oxygen-glucose depletion. *British Journal of Pharmacology*, *161*(1), 33–50. http://doi.org/10.1111/j.1476-5381.2010.00840.x

Yang, C., Iyer, R. R., Yu, A. C. H., Yong, R. L., Park, D. M., Weil, R. J., et al. (2012a). β-Catenin signaling initiates the activation of astrocytes and its dysregulation contributes to the pathogenesis of astrocytomas. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(18), 6963–6968. http://doi.org/10.1073/pnas.1118754109

Yang, H., Feng, G.-D., Olivera, C., Jiao, X.-Y., Vitale, A., Gong, J., & You, S.-W. (2012b). Sonic hedgehog released from scratch-injured astrocytes is a key signal necessary but not sufficient for the astrocyte de-differentiation. *Stem Cell Research*, *9*(2), 156–166. http://doi.org/10.1016/j.scr.2012.06.002

Yousef, H., Conboy, M. J., Morgenthaler, A., Schlesinger, C., Bugaj, L., Paliwal, P., et al. (2015a). Systemic attenuation of the TGF-β pathway by a single drug simultaneously rejuvenates hippocampal neurogenesis and myogenesis in the same old mammal. *Oncotarget*, *6*(14), 11959–11978.

Yousef, H., Morgenthaler, A., Schlesinger, C., Bugaj, L., Conboy, I. M., & Schaffer, D. V. (2015b). Age-Associated Increase in BMP Signaling Inhibits Hippocampal Neurogenesis. *Stem Cells (Dayton, Ohio)*, *33*(5), 1577–1588. http://doi.org/10.1002/stem.1943

Zeng, C., Pan, F., Jones, L. A., Lim, M. M., Griffin, E. A., Sheline, Y. I., et al. (2010). Evaluation of 5-ethynyl-2′-deoxyuridine staining as a sensitive and reliable method for studying cell proliferation in the adult nervous system. *Brain Research*, *1319*, 21–32. http://doi.org/10.1016/j.brainres.2009.12.092

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell*, *132*(4), 645–660. http://doi.org/10.1016/j.cell.2008.01.033

Zhao, C., Teng, E. M., Summers, R. G., Ming, G.-L., & Gage, F. H. (2006). Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience*, *26*(1), 3–11. http://doi.org/10.1523/JNEUROSCI.3648-05.2006

Łukawski, K., Janowska, A., Jakubus, T., Tochman-Gawda, A., & Czuczwar, S. J. (2010). Angiotensin AT1 receptor antagonists enhance the anticonvulsant action of valproate in the mouse model of maximal electroshock. *European Journal of Pharmacology*, *640*(1-3), 172–177. http://doi.org/10.1016/j.ejphar.2010.04.053