



2018

INSULIN-LIKE GROWTH FACTOR-1 OVEREXPRESSION MEDIATES HIPPOCAMPAL REMODELING AND PLASTICITY FOLLOWING TBI

Erica Latrice Littlejohn

University of Kentucky, ericalittlejohn2016@gmail.com

Digital Object Identifier: <https://doi.org/10.13023/etd.2018.292>

[Right click to open a feedback form in a new tab to let us know how this document benefits you.](#)

Recommended Citation

Littlejohn, Erica Latrice, "INSULIN-LIKE GROWTH FACTOR-1 OVEREXPRESSION MEDIATES HIPPOCAMPAL REMODELING AND PLASTICITY FOLLOWING TBI" (2018). *Theses and Dissertations--Physiology*. 39. https://uknowledge.uky.edu/physiology_etds/39

This Doctoral Dissertation is brought to you for free and open access by the Physiology at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Physiology by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Erica Latrice Littlejohn, Student

Dr. Kathryn E. Saatman, Major Professor

Dr. Kenneth Campbell, Director of Graduate Studies

INSULIN-LIKE GROWTH FACTOR-1 OVEREXPRESSION MEDIATES
HIPPOCAMPAL REMODELING AND PLASTICITY FOLLOWING TBI

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Medicine
at the University of Kentucky

By
Erica Latrice Littlejohn

Lexington, Kentucky

Director: Dr. Kathryn E. Saatman, Professor of Physiology

Lexington, Kentucky

2018

Copyright © Erica Latrice Littlejohn 2018

ABSTRACT OF DISSERTATION

INSULIN-LIKE GROWTH FACTOR-1 OVEREXPRESSION MEDIATES HIPPOCAMPAL REMODELING AND PLASTICITY FOLLOWING TBI

Every year over 2.5 million traumatic brain injuries (TBI) occur and are the leading cause of death and disability among adolescents. There are no approved treatments for TBI. Survivors suffer from persistent cognitive impairment due to posttraumatic tissue damage and disruption of neural networks which significantly detract from their quality of life. Posttraumatic cognitive impairment depends in part on the brain's limited ability to repair or replace damaged cells. Immature neurons in the hippocampus dentate gyrus, a brain region required for learning and memory, are particularly vulnerable to TBI. Insulin-like growth factor-1 (IGF1) is a potential therapeutic for TBI because it is a potent neurotrophic factor capable of mediating neuroprotection, neuro-repair, and neurogenesis. We hypothesized that conditional IGF1 overexpression in the mouse hippocampus following experimental controlled cortical impact injury (CCI) would enhance posttraumatic neurogenesis chronically. To this end, conditional astrocyte-specific IGF1 overexpressing mice (IGFtg) and wild-type (WT) mice received CCI or sham injury. The proliferation marker BrdU was used to label

neurons born the first week after injury. Six weeks after injury, when surviving posttrauma-born neurons would be fully developed, we counted proliferated cells (BrdU+) and the subset expressing a mature neuronal marker (NeuN+/BrdU+) in the hippocampus. We also assessed cognitive performance during radial arm water-maze reversal (RAWM-R) testing, a neurogenesis-sensitive assay. IGF1 promoted end-stage maturity and decreased mis-migration of neurons born after trauma. These effects coincide with IGF1 induced improvements in performance on neurogenesis sensitive cognition following TBI.

Mammalian target of rapamycin (mTOR), an early signaling molecule downstream of IGF1, has been identified as a potential target for TBI interventions because of its regulatory role in neuronal plasticity and neurogenesis. However, recent studies have also reported maladaptive plasticity and recovery associated with posttraumatic mTOR activation. It is imperative to elucidate the mechanism of action of IGF1 during pre-clinical evaluations. We hypothesized that IGF1 mediates posttraumatic neurogenic effects through IGF1 induction of mTOR activation. We injured cohorts of IGFtg and WT mice and harvested their brains for immunohistochemistry to assess IGF1 overexpression effects on posttraumatic mTOR activation at 1, 3, and 10 days post-injury (dpi). We found that IGF1 upregulated mTOR activation following TBI in a region-specific manner at 1 and 3dpi. To determine if IGF1 regulated differentiation and arborization through the mTOR pathway, injured WT and IGFtg mice received daily i.p. injections of rapamycin (10mg/kg), the inhibitor of mTOR, or its vehicle for 7 days. Vehicle and rapamycin administration began 3dpi, after the cells dividing at the peak of

posttraumatic proliferation were labeled with BrdU. IGF1 enhancement of posttraumatic neurogenesis was not dependent on mTOR activation.

In summary, IGF1 directs newborn neuron localization, promotes end-stage maturation, and chronically improves cognition. IGF1 can stimulate posttraumatic neurogenesis and plasticity independent of mTOR activation. These data suggest that IGF1 can stimulate neuron replacement following trauma-induced hippocampal neuron loss and cognitive improvement. Further studies should investigate IGF1 and mTOR inhibition as a combination therapy for neurorehabilitation.

KEYWORDS: Neurogenesis, Insulin-like Growth Factor-1, mTOR, Traumatic Brain Injury, Cognitive Recovery, Dentate Gyrus

Erica Latrice Littlejohn_____

July 1, 2018_____
Date

INSULIN-LIKE GROWTH FACTOR-1 OVEREXPRESSION MEDIATES
HIPPOCAMPAL REMODELING AND PLASTICITY FOLLOWING TBI

By

Erica Latrice Littlejohn

Kathryn E. Saatman, Ph.D.

Director of Dissertation

Kenneth Campbell, Ph.D.

Director of Graduate Studies

July 1, 2018

*For my mother and grandmother, Linda Littlejohn and Fannie Jacobs-Littlejohn, whose
unconditional love and support will never be forgotten.*



Acknowledgements

I am very grateful for the journey to my Doctorate in Physiology. In addition to receiving world class training from leaders in neuroscience and brain trauma, I have learned many insightful lessons. I would like to warmly express my gratitude to the members of the community that supported me along the way.

I want to thank the Center for Graduate and Professional Diversity Initiatives (CGPDI) and Black Graduate and Professional Student Association (BGPSA) were instrumental to my success on UK's campus. I want to especially thank Dr. Chassity Holliman-Douglas, Dr. Cleo Price, and members of BGPSA.

Thank you to the entire department of Physiology (PGY) and the Spinal Cord and Brain Injury Research Center (SCoBIRC). The faculty, administrators, and students of have been so warm and supportive. SCoBIRC is truly a family. Zel, thank you for knowing everything and making every last minute need a possibility! Thank you Linda for allowing me to live in the scope room with you! Thank you to all the faculty with open doors who easily give advice and encouragement, with special thanks to Bret Smith, Ed Hall, John Gensel and Kimberly Nixon.

Many thanks to my dear lab mates, my work would literally be impossible without all of you! Special thanks to Diana Sama, you and I were in the trenches my dear friend! Amanda Bolton-Hall, you have been such a reliable source of encouragement. Isabel, thank you for being my partner in my previous ephys life! Carrie Boychuk and Kati Halmos, you are everything! My dear Danielle Scott, there is no challenge you don't

rise up to meet. Your presence ensures my daily dose of work-fun and a loving audience for my comedy and complaints! Special thanks to the amazing Anna Juras, Binoy, Jen, Sindhu, Shaun, Anthony. All my nearly 25 lab mates that have rotated through Kathy's lab for different seasons and reasons have been amazing!

I have made amazing lifelong friends in graduate school though PGY and BGPSA that kept me going. Drs. Jenna, Stacy, Katie, and Laura, I love you ladies! Drs. Ese and Courtney, you are my sisters forever. Mike, you are an incredible friend. So many memories with all of you!!

Thank you so much Dr. Lang and Dr. Soltani from the University of Chicago. You are a major part of the reason I applied to graduate school. I will never forget how much you believed in me and my potential.

Dr. Kathryn Saatman, my mentor and advisor, I hope that I am a reflection of every one of your strengths and gifts as a woman in science. You taught me to pursue excellence in every aspect of biomedical research and to do so without compromise. I hope to imitate your unfailing ethics, integrity, and professionalism throughout my career. It has truly been a privilege to train with you, thank you so much.

Thank you to my extended family at Bethel Harvest Church, I love you. Your support in this journey has been life giving. I am thankful for my friends and relatives for your love and encouragement over the last 20 plus years!

I extend my deepest thank you to my dad, Joan, Quianna, Brandon, Candace, and Untie Renee. Family, you are my breath of fresh air. You moved to Kentucky with me and without your support, compassion, and faithfulness I could not be the woman that I

am today. Beronica Littlejohn and Bridget Reyes, I could write one million love letters to honor what your support has meant to me and it wouldn't be enough. Thank you for making it ok for me to be me. To my mommy (Linda Littlejohn) and granny (Fannie Jacobs), my highest honor as a woman is to be the best parts of the both of you. You are the integrity, grit, passion, resilience, intelligence, creativity, curiosity and optimism that mark my character.

Thank you to my I am. Jesus, no greater love has any man shown than to lay down his life for his friends. Thank you for being the power and light that sustains me. Thank you for this journey, PhD, and future.

TABLE OF CONTENTS

Acknowledgements.....	iii
List of figures.....	x
CHAPTER 1: Introduction	1
Traumatic Brain Injury.....	1
Prevalence and etiology.....	1
Clinical manifestations of TBI	2
Experimental models	5
Trauma disrupts learning and memory.....	9
CCI: A preclinical model of learning and memory impairment.....	9
Primary and secondary injury to the hippocampus	12
Trauma alters hippocampal neurogenesis	14
Adult neurogenesis	14
Adult neurogenesis following TBI	17
IGF1 in development and injury	20
IGF1 introduction	20
IGF1 and mTOR signaling in neural development	22
IGF1 and mTOR signaling in trauma	23
Summary	26
CHAPTER 2: IGF1 increases long-term survival of posttrauma-born neurons while inhibiting ectopic localization in the hippocampus	28
Introduction.....	28

Materials and Methods	30
Animal Care.....	30
Controlled Cortical Impact Injury	31
BrdU Administration	32
Histology and Immunohistochemistry	32
Image Acquisition.....	33
Radial Arm Water Maze.....	35
Statistical Analyses.....	37
Results	38
Dentate gyrus cells that proliferate acutely after trauma survive 6 weeks	38
IGF1 selectively promotes end-stage fate maturation of immature neurons resulting in stable neurogenesis 6wks post-injury.....	38
IGF1 enhances outward migration of posttrauma-born neurons in the GCL	40
IGF1 attenuates ectopic localization of posttrauma-born neurons in the dentate gyrus.....	41
IGF1 improves reversal learning in RAWM 6 weeks after CCI.....	41
Discussion	43
Summary.....	43
Survival.....	44
Migration	46

IGF1 and cognitive flexibility	49
CHAPTER 3: IGF1 posttraumatic hippocampal remodeling is independent of mTOR ..	62
Introduction	62
Materials and Methods	65
Animals.....	65
Controlled Cortical Impact Injury	66
Rapamycin administration.....	66
BrdU Administration	67
Immunohistochemistry and histology	67
Microscopy image analysis	68
Statistical Analyses.....	71
Results	71
IGF1 overexpression potentiates injury-induced mTOR activation in predominantly neuronal sub-regions of DG	71
Potentiation of mTOR activation by IGF1 overexpression does not enhance early posttraumatic proliferation	73
Rapamycin administration increases posttraumatic neural differentiation in IGF1 overexpressing mice.....	74
Rapamycin treatment does not alter posttraumatic positioning of immature neurons.....	75

IGF1 overexpression confers resistance to rapamycin sensitive posttraumatic arbor development in immature neurons	77
Discussion	78
Summary of findings	78
mTOR activation	79
Proliferation and early survival	80
Differentiation	82
Migration	83
Dendritic arbor development	85
Considerations	87
CHAPTER 4: Discussion and Conclusions	101
Review of major findings	101
Posttraumatic neuronal differentiation	102
Impact of posttraumatic IGF1 overexpression on SVZ neurogenesis.....	103
Mature posttrauma-born neuron integration	104
IGF1 and posttraumatic hippocampal remodeling	106
A role for adult-born neurons in emotion-related behavior	108
IGF1 and mTOR: posttraumatic signaling relationship in neurogenesis	110
Considerations	114
References	119
VITA	145

List of Figures

Figure 2.1: Hippocampal cells that proliferate acutely after injury persist long-term in the dentate gyrus.	51
Figure 2.2: End-stage fate maturation of cells proliferated during the first week after injury results in long-term neurogenesis and gliogenesis.	52
Figure 2.3: IGF1 selectively enhances long-term neurogenesis but not gliogenesis.	54
Figure 2.4: IGF1 overexpressing mice had increased glial marker immunolabeling in the ML 6weeks post-CCI.	56
Figure 2.5: IGF1 increases the proportion of new mature neurons that migrate deep within the granule cell layer but decreases the proportion that localizes ectopically to the hilus and molecular layer after injury.	57
Figure 2.6: IGF1 overexpression increased the density of outward migrating NeuN+BrdU+ in the GCL, but not the density of ectopically localized neurons.	59
Figure 2.7: Injured mice extinguish memory of old goal arm earlier following IGF1 overexpression.	60
Figure 3.1: Experimental design.	88
Figure 3.2: IGF1 selectively potentiates injury-induced mTOR activity in the dentate gyrus GCL and HL after moderate injury.	89
Figure 3.3: IGF1 selectively potentiates injury-induced mTOR activity in the dentate gyrus GCL and HL after severe injury.	91
Figure 3.4: Following injury pS6 is activated in astrocytes and progenitors in the DG... ..	93
Figure 3.5: Moderate injury is sufficient to isolate IGF1 overexpression induced mTOR activation in neuronal layers of contralateral hemisphere.	94
Figure 3.6: Daily administration of rapamycin inhibits posttraumatic mTOR activation 72hr following injury.	95
Figure 3.7: The density of proliferated cells in the DG is not altered by IGF1 overexpression or rapamycin treatment at 10 days after injury.	96
Figure 3.8: Rapamycin administration increases posttraumatic neuronal differentiation in IGF1 overexpressing mice.	97
Figure 3.9: Rapamycin does not influence radial migration within the GCL or ectopic hilar migration or following IGF1 overexpression.	98

Figure 3.10: IGF1 overexpression increases dendritic arbor length and complexity and provides resistance to rapamycin induced dendritic pruning following moderate injury. 99

CHAPTER 1: Introduction

Traumatic Brain Injury

Prevalence and etiology

Traumatic brain injury (TBI) is the disruption of brain function caused by the application of force to the brain. Pathological force can result from intentional and unintentional impact on the head. Nearly 2.8 million TBI are reported annually (Coronado et al. 2012, Taylor et al. 2017), which is still an underestimate since many mild injuries go unreported (Meehan et al. 2013). Nearly 9 billion US dollars are spent annually on hospitalization and rehabilitative care for TBI survivors, representing an alarming economic burden to society (Faul et al. 2015).

Common mechanisms of TBI include being struck by an object, motor-vehicle accident, intentional self-harm, assault, and falls. Injuries can range from mild to severe. Different populations are disproportionately affected by different mechanisms of injury. The elderly and toddlers have a higher frequency of falls, while people 15-44 years of age are disproportionately receiving TBI from motor-vehicle incidents (Taylor et al. 2017). Blast-related head trauma disproportionately affects military personnel (Reneer et al. 2011). While legislation has mandated safer driving precautions, improving TBI surveillance and evidence-based guidelines of care have been shown to lessen TBI prevalence and improve patient outcomes (Jagger et al. 1987, Coronado et al. 2012). However, there are still no FDA approved pharmacological treatment options for TBI.

Lack of treatment options is due in part to the neurological complexity of the brain pathology (Marklund et al. 2011).

There is a high mortality rate associated with hospitalization for severe TBI. More than 25% of patients suffering from severe TBI die (Taylor et al. 2017). Multiple discrete and often overlapping neuroanatomical pathologies present as severe TBI in the clinical setting (Saatman et al. 2008). Injuries can encompass any combination of pathologies not limited to skull fractures, epidural hemorrhage, subdural hemorrhage, subarachnoid hemorrhage, intraventricular hemorrhage, axonal injury and brain contusion. The heterogeneity of severe injury makes it difficult to classify and well as challenging to manage treatment (Morales et al. 2005, Hawryluk et al. 2015).

Clinical manifestations of TBI

Annually, there are approximately 5.3 million people receiving rehabilitative care for head trauma that suffer from persistent cognitive impairments and disabilities (Langlois et al. 2006). Early evaluations used during emergency room visits can inform clinicians of potential motor, cognitive, and behavioral impairments associated with TBI. Three common indices that can predict outcomes are the Glasgow Coma Scale (GCS), duration of loss of consciousness, and duration of posttraumatic amnesia. The Glasgow Coma Scale (GCS) is a crude physical and cognitive evaluation that predicts disability following TBI (Husson et al. 2010). Patients receiving very low GCS scores within 24 hours of injury are predictive of poor outcomes (Hart et al. 2014). Survivors of TBI can enter the hospital in a coma, a deep state of unconsciousness and are unresponsive to the environment. The duration of an individual's coma can also be reliably used to predict future impairments (Sherer et al. 2008). A coma duration of fewer than two weeks is

rarely associated with severe long-term disabilities (Hart et al. 2014). The length of posttraumatic amnesia is a powerful predictor of outcomes and disability following trauma. Amnesia is most commonly experienced as a transient cognitive impairment following injury (Nakase-Richardson et al. 2009, Friedland et al. 2016). Posttraumatic amnesia is the inability to retrieve memories stored prior to the injury (Whiting et al. 2006). Posttraumatic amnesia that resolves within a month of injury correlates with a low likelihood of disability (Nakase-Richardson et al. 2009).

While all evaluations have limitations to their power of predicting outcomes, the GCS, duration of loss of consciousness, and posttraumatic amnesia are all strong predictors of future disabilities. No one method is more predictive than the other (Konigs et al. 2016). The multifactorial nature and complexity of TBI create difficulty in predicting cognitive disability since many measures rely on the neuro-responsiveness of the patient. Many studies measure the extent of disability by measuring the time to return to work. Time to return to work is correlated with widely used measures like GSC, duration of loss of consciousness, and duration of posttraumatic amnesia (Cifu et al. 1997). The interpretation of these types of outcomes can be confounded by factors like an injury to other organ systems, access to rehabilitative care, and socioeconomic factors (Holland et al. 2003, Forslund et al. 2014). The ability to predict outcomes is further complicated in the case of mild to moderate TBI injuries. Patients with mild to moderate injuries rarely have poor GCS, loss of consciousness, or amnesia upon admission to the emergency room (Konigs et al. 2016).

Although controversy surrounds the best measures to predict and rate cognitive disability, there is a consensus that brain trauma can initiate, contribute to, and exacerbate

cognitive impairment. Over 65% of patients suffering from moderate to severe TBI self-report long-term cognitive impairments (Whiteneck et al. 2004). Common cognitive disabilities at all severity levels include memory and executive function deficits (Rabinowitz et al. 2014). Memory deficit is an important factor underlying the inability to perform activities of daily living. Impairment of short recall interferes with planning, decision making, and executing decisions and is often a precursor to dementia (Shively et al. 2012). Damage to structures including but not limited to cortex, brainstem, and hippocampus interrupt neuronal conduction, contributing to impairment of new memory formation (Watson et al. 1995, Cantu 2001). Damage to the hippocampus producing hippocampal atrophy is associated with chronic memory impairment following TBI (Ariza et al. 2006). Over 43% of all moderate to severely injured patients have posttraumatic executive function disorders that last for 6 months or longer (Selassie et al. 2008). Executive function impairment describes limited control and direction of lower level cognitive function like learning and memory, planning, decision making, motivation, self-control and emotion-related behavior. Memory disruption affecting executive function is primarily due to the limited ability to organize information effectively for encoding and retrieval (Dikmen et al. 2009). These impairments affect daily activities ranging from making a grocery list, carrying out plans with friends, to performance at work. Two primary types of injury to the frontal cortex result in executive function deficits: 1) shearing of axons (diffuse axonal injury) from acceleration and then rapid deceleration and 2) focal cortical contusion from contact with the head. Both types of injury result in deep brain damage that not only affects the frontal cortex but also its areas of projection like the hippocampus (Cicerone et al. 2006). Aging studies suggest

that prefrontal cortex atrophy can reduce hippocampus-prefrontal cortex connectivity resulting in impaired long-term episodic memory formation (Mander et al. 2013). Damage to multiple brain regions together contributes to cognitive impairment following TBI.

The National Institute of Health's National Institutes for Neurological Disorders and Stroke has impacted policy and supplied funding to centralize clinical study design and protocols for posttraumatic assessment of long-term cognitive disabilities and reporting (Cicerone et al. 2006, Maas et al. 2007). The International Mission on Prognosis and Clinical Trial Design in TBI (IMPACT) study was initiated in 2003 to create a database for all TBI clinical trial datasets from Trauma Centers all over the western world. Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) is a multicenter clinical trial that is currently enrolling patients from all over the US. Through these databases, scientific investigators and clinicians will have access to shared patient tomography, cognitive assessments, and samples, increasing the power of the scientific study and promoting collaboration. These initiatives will provide organization and centralization of TBI clinical data that can inform TBI neurocritical care management, provide evidence-based treatment recommendations and guide experimental models of TBI.

Experimental models

Introduction

Due to the heterogeneity of brain damage observed in TBI (Saatman et al. 2008), it is unlikely that a single target treatment will be able to attenuate cognitive deficits

caused by a combination of TBI pathologies that could include any combination of diffuse axonal injury, epidural/sub-epidural hematoma, intraventricular hematoma, and focal contusion. Because of the heterogeneous nature of human TBI, researchers primarily use models that replicate combined trauma pathologies. The most common injury models are controlled cortical impact injury (CCI), fluid percussion injury (FPI), acceleration injury (weight-drop), blast injury, and closed head injury (CHI). Less common models include those that combine hematoma and tissue penetration. Animal models of TBI give the power to isolate specific pathophysiology and develop targeted treatments. Rodents are the most widely used animal model because of their cost, standardized outcome measures and small size. As preclinical treatments move closer to clinical trial phase, drugs are sometimes screened in larger animals. The development of animal models has been essential to understanding the mechanisms underlying neural network disruption and cognitive impairment. Each injury model targets specific aspects of pathology and its associated cognitive impairment parameters of human TBI.

Focal contusion models

In humans, focal contusion injuries occur as a result of blunt force trauma from motor vehicle incidents, falls, assaults, and combat. Contusion injuries are amongst the more severe brain injuries and are present in the majority of TBI-related hospitalizations that result in death and in long-term cognitive disability (Kurland et al. 2012). Lateral FPI and CCI are the most common models used to produce moderate to severe TBI. These injury models have been adapted to rodents, ferrets, swine, and monkeys (Johnson et al. 2015). In addition to contusion, these models can be scaled to concurrently produce

additional aspects of TBI including subdural hematoma, axonal injury, and intracerebral hematoma (Xiong et al. 2013).

In the lateral FPI model, a craniotomy is generated midway between bregma and lambda suture, at the intersection of the parietal and temporal bones. The dura is left intact, and water pressure is used to displace the exposed brain tissue. This model was designed to incorporate the contrecoup effects of TBI that are often seen in human TBI (Eakin et al. 2015). Lateral FPI predominantly models moderate to severe injury and generates cortical cell death, intracerebral hematoma and edema. Within a week after injury, distant structures like the hippocampus and the thalamus undergo neuronal damage in rats (Cortez et al. 1989). Lateral FPI results in progressive cortical damage that continues beyond 6 months after injury (Pierce et al. 1998). Lateral FPI produces acute motor deficits and chronic long-term deficits in memory. This model has several limitations including limited control of parameters like pressure and dwell time. Water pressure levels are typically set by adjusting the drop height of the pendulum that hits the fluid reservoir. Additionally, while the lateral FPI severity is scalable, small fluctuations in craniotomy size can produce vast differences in pathology (Vink et al. 2001). The skull is rarely left without fracture following severe TBI in humans (Yellinek et al. 2016). The lateral FPI model is widely used in rats, its application to mice is technically challenging and has limited its use in this species. This is unfortunate because the majority of transgenic animal models are generated in mice.

CCI can be used to model moderate to severe injury very reliably. CCI is a TBI model in which a portion of the brain is exposed, and a pneumatically or electromagnetically driven piston impacts the intact dura. Parameters such as speed,

depth of tissue displacement and dwell time can be adjusted increasing the flexibility of this model to study specific parameters of injury. The injuries are controlled with precision, and there is no opportunity for rebound injury since control of the impact is computerized. CCI lesions are generated to typically target the pre-frontal cortex, midline, or parietal cortex. Parasagittal CCI is associated with decreased bleeding from sagittal sutures as compared to other impact locations (Osier et al. 2016), making it an optimal location for surgery. This model produces aspects of human injury including cortical tissue cell death, subdural hematoma, axonal injury, the decline in cerebral blood flow and blood-brain barrier disruptions (Osier et al. 2016). CCI can produce acute motor impairment and long-term memory and learning deficits beyond 1 year after injury (Dixon et al. 1999).

Studies have shown that adjusting the measures of impact depth and speed of impact can each independently be used to cause the graded severity of cognitive dysfunction (Washington et al. 2012). Because the piston makes direct contact with the exposed tissue, the piston shape can be adjusted to produce distinctly different injury pathologies (Pleasant et al. 2011). Severe CCI generated with a flat impactor tip produces more cortical tissue strain and a faster rate of neurodegeneration compared to CCI injury produced with a rounded tip (Pleasant et al. 2011). Compared to a rounded tip, the flat impactor tip produced more neocortical hemorrhage and hippocampal cell death acutely after injury (Pleasant et al. 2011). The geometry of the tip may play a role in the development of posttraumatic seizures. Studies that have reported that 9% to 15% of rodents develop injury-induced seizures following severe CCI injury have used a flat tip

was used (Hunt et al. 2010, Bolkvadze et al. 2012). Researchers developing studies to investigate posttraumatic epilepsy should consider tip selection carefully.

The CCI injury model has been essential to studying the pathophysiology that underlies acute and chronic posttraumatic behavior impairment and cognitive disability. Rodents are most often used for CCI, primarily due to low cost, ease of surgery, and standardization of outcomes across research labs. Behavior outcomes are sensitive to the location of the craniotomy and impact. Injury to the frontal cortex following CCI has been associated with impairments in emotional behaviors, executive function, and reversal learning (Morales et al. 2005, Chou et al. 2016, Osier et al. 2016). CCI injury over the parietal cortex has been associated with impaired spatial learning, reversal learning, novel object recognition, cognitive flexibility, and anxiety (Washington et al. 2012, Zhao et al. 2012, Bondi et al. 2014). Grading injury severity of CCI is associated with a graded degree of cognitive dysfunction (Yu et al. 2009, Rabinowitz et al. 2014).

Trauma disrupts learning and memory

CCI: A preclinical model of learning and memory impairment

The sequelae of cognitive dysfunction resulting from TBI depends on the specific pathology and location of TBI. Trauma to the brain that causes degeneration of the hippocampus has the potential to impact a wide range of functions, behaviors, and cognitive abilities. Hippocampi receive and send information to the cingulate cortex, entorhinal cortex, septal nuclei, ventral striatum, hypothalamus, and thalamus (Shah et al. 2012, Lovblad et al. 2014). Damage to the temporal lobe producing atrophy of the hippocampus and its major aspect of axonal output, the fornix, contribute to learning and

memory impairment (Tate et al. 2000, Ariza et al. 2006). A majority of severe TBI survivors suffer from chronic learning and memory impairments that dramatically inhibit the daily activities of living (Rabinowitz et al. 2014). Damage to the hippocampus can produce hippocampus-dependent impairments in spatial learning and memory in both human TBI and animal models (Miller et al. 2013, Vorhees et al. 2014). A study showed that caudate putamen and hippocampus interact in a compensatory manner to update spatial recognition memory when subjects are navigating an environment (Voermans et al. 2004). TBI leads to progressive damage to axons of neurons that project to the thalamus and hippocampus, orbital-frontal cortex damage and temporal pole degeneration (Warner et al. 2010, Leunissen et al. 2014). It is well established that the hippocampus plays an important role in retrieving spatial memories that encode space, place, time, and context. Place cells in the human hippocampus activate when people navigate an environment. These place cells reactivate when a person must recall and retrieve memories related to the environmental context to complete a task (Miller et al. 2013). TBI patients have limited ability to ignore old misleading cues, update current information and adjust responses according to new cue rules. The ability to adapt one's behavior in response to changes in the environment is called cognitive flexibility (Dajani et al. 2015). Cognitive flexibility impairment has been correlated with caudate putamen volume loss and change of shape after trauma (Leunissen et al. 2014).

Experimental CCI has been used to study motor deficits, alterations in anxiety, aggression, depression, and learning and memory (Lyeth et al. 1990, Dixon et al. 1999, Washington et al. 2012). Lesion to the hippocampus following CCI causes deficits in learning and memory (Dixon et al. 1999). There is widespread cell death throughout the

hippocampus. Many treatments that enhance hippocampal cell survival also attenuate learning and memory deficits following trauma (Patel et al. 2010, Barha et al. 2011, Madathil et al. 2013). The Morris Water Maze (MWM) is a standardized test that is reliably used to assess learning and memory following severe CCI injury. The MWM test is designed to test an animal's spatial working memory and reference memory and requires episodic memory function to form memories about space and distance in relationship to its training (Vorhees et al. 2014). The rodents are trained to perform the MWM task in a circular pool. Aversion to water motivates the animals to use context cues to learn to locate the escape platform that is hidden beneath the surface of the water. underwater in a circular pool. When investigators ran batteries of test to determine which assay was most sensitive to CCI injury severity, MWM consistently revealed a TBI induced learning and memory deficits that persisted from 7 to 90 days after injury (Scheff et al. 1997, Washington et al. 2012, Zhao et al. 2012). Like path learning in humans, the MWM is a hippocampus-dependent task (Vorhees et al. 2014).

The hippocampus is also required to for generating declared episodic memory; retrievable memories developed from a lived event that represent a past place and time. Modification of the traditional MWM gives the ability to test different aspects of learning. After an animal learns the platform location, the escape platform can be moved to a novel location to test reversal learning. During reversal learning, the animal must extinguish the old memory and use working memory to adopt a new strategy to find the platform. The flexibility to replace a previously learned spatial with a new spatial strategy is impaired by hippocampal volume loss (Kleinknecht et al. 2012). Experimental TBI

produces deficits in reversal learning and cognitive flexibility that are detectable using MWM assessments (Yu et al. 2009, Vorhees et al. 2014, Taib et al. 2017).

Primary and secondary injury to the hippocampus

CCI causes pronounced acute and chronic hippocampal neurodegeneration beneath the site of injury as well as damage in regions the hippocampus projects to (Hall et al. 2005, Osier et al. 2016). It is well established that the integrity of the hippocampal circuit is required for appropriate learning and memory function (Dixon et al. 1999, Xavier et al. 1999, Ariza et al. 2006). The hippocampus can be segmented into three major regions, the CA1, CA3, and dentate gyrus (DG) (Dillon et al. 2017). Place cells, pyramidal cells of the CA1, CA3, and DG, are used to make a map of the environment. The DG is a sub-region of the hippocampus and is required for aspects of spatial learning in rodents (Garthe et al. 2009, Aimone et al. 2010). The DG is essential to the ability of the hippocampus to create spatial-temporal context by which it can retrieve stored memories and apply the information to a new situation. Studies have shown that the DG is required to separate similar patterns by creating distinct representations of information (Knierim 2015). Following trauma, the hippocampus undergoes edema, decreased blood vessel density, free-radical damage, deafferentation and glutamate toxicity resulting in neuron loss and hippocampal atrophy (Bareyre et al. 1997, Ariza et al. 2006, Greve et al. 2009, Atkins 2011, Hayward et al. 2011). A major source of input to the DG is from axons of the entorhinal cortex and hippocampal nuclei that synapse onto dendrites of neurons residing in the granule cell layer (GCL). Axons of granule cells travel through the polymorphic layer (hilus) and terminate at divergent locations (Amaral et al. 2007).

In rodents, hippocampal neurons begin expressing markers for cell-mediated apoptosis within hours after injury in the CA1, CA3, GCL, and hilus (HL) and continue apoptosis throughout the first week after injury (Schoch et al. 2012). Inhibiting posttraumatic apoptosis has been shown to improve behavior 7d after CCI and increase cell survival in the DG (Yang et al. 2016). The DG houses the HL and GCL and is the only hippocampal site where new neurons are produced throughout adulthood. Dentate hilar neurons and newborn neurons in the GCL are the most vulnerable populations of hippocampal neurons following TBI (Witgen et al. 2005). The population of hilar GABAergic interneurons and mossy cells undergo cell death following injury (Lowenstein et al. 1992). These two inhibitory neuronal subtypes relay and filter information from the GCL by mediating excitation of distant granule cells and inhibiting GCL excitation (Amaral et al. 2007). Within the GCL there are three populations of neurons altered by TBI; basket neurons, granule neurons and immature neurons. The basket cell is a local GABAergic interneuron at the polymorphic border of the GCL that regulates granule cell excitation. GABAergic interneurons have a similar susceptibility to injury as the other inhibitory cells in the DG (Jinde et al. 2013). The principal cell of the GCL is the granule cell; its presence in the GCL is 100:1 compared to basket cells. Granule cells are organized to form a cell layer 60-100 μ m thick which is bordered by the HL and ML. The majority of the granule cells in the adult GCL neurons were generated during development; they express mature neuron marker (NeuN) and generate glutamatergic output (Amaral et al. 2007). Studies have shown that while there are dramatic apoptosis and degeneration in the GCL after injury, only a small proportion the degenerating cells are NeuN positive following injury (Zhou et al. 2012). Rather, most of

the degenerating cells in the GCL express markers for newly born neurons (Gao et al. 2008). Preferential immature neuron death is a conserved phenotype in multiple experimental models of TBI irrespective of TBI severity.

Trauma alters hippocampal neurogenesis

Adult neurogenesis

It is widely known that CNS is comprised primarily of post-mitotic cells and that neurons do not undergo cell division. For many years, it was thought that neurons are generated during development and that mammals lose the capacity to generate new neurons once they enter the postnatal period of life. Joseph Altman and colleagues discovered proliferation and neuron generation in the brain (Altman et al. 1967). The discovery that humans have adult hippocampal neurogenesis (Spalding et al. 2013) localized to a region of the brain essential for recovery from learning and memory impairment has generated a high volume of research regarding this form of plasticity (Kempermann et al. 2015). Neurogenesis is still a relatively new research field, and the functional role of human adult neurogenesis is not well understood.

Adult neurogenesis is a multistep process that includes neuron generation and end-stage maturation. During early survival, neural stem cells (NSC) in the adult neurogenic niche proliferate and differentiate into neurons. Depending on environmental stimuli and context, newborn neurons can proceed through development to end-stage maturation and integrate into the environment (Kempermann et al. 2015). There are two primary locations where mammals generate new neurons throughout adulthood: along the lateral ventricles and in the dentate gyrus. New neurons generated in the subventricular

niche migrate along the rostral migratory stream to the olfactory bulb. The olfactory bulb has a high rate of neuron turnover and this population of new neurons continuously repopulates the olfactory bulb (Conover et al. 2017). The second well-characterized location resides within the dentate gyrus. The subgranular zone (SGZ) is the microregion of the GCL where it borders the polymorphic layer. This neurogenesis permissive region contains neural progenitors and their progeny, as well as endothelial cells, astrocytes, interneurons, and axon terminals from the ventral tegmental area, the septum and local interneurons (Palmer et al. 2000, Kempermann et al. 2015). The glia and interneurons present in the SGZ play an important role in maintaining the neurogenic niche. The SGZ is heavily vascularized to meet the metabolic needs of neurodevelopment and deliver humoral and endothelial cell-derived growth factors including FGF, VEGF and IGF1 (Palmer et al. 2000, Ryu et al. 2016). Local and humoral growth factors are required to mediate proliferation, differentiation, and survival of progenitor cells and immature neurons (Anderson 2002; Pickel 2016; Ryu). The astrocytes in the SGZ have a dual role in regulating neurogenesis. SGZ astrocytes serve as niche regulating cells by delivering factors that mediate neuronal differentiation through cell to cell contact (Song et al. 2002, Alvarez-Buylla et al. 2004, Ashton et al. 2012). Specialized astrocytes in the SGZ, Type-1 radial glia, serve as NSCs that can proliferate and become neural precursors. These progenitors can transition into immature neurons or astrocytes and exit the SGZ. The interneurons in the SGZ are responsible for maintaining the quiescence of the stem cell population. Without local GABA tone, radial glial stem cells exit quiescence and divide (Song et al. 2012).

NSCs are primarily a quiescent population in the SGZ that express the stem cell marker nestin. The morphology of Type-1 radial glia is distinct from other progenitors. They have apical processes that extend through the GCL and have GABA and AMPA neurotransmitter receptors to sense external stimuli prompting division. These cells have division programs to divide asymmetrically (keep stem-ness) and symmetrically (both cells differentiate). These NSCs divide and produce Type-2 a/b precursors which have a high capacity to divide, but a completely different morphology. Type-2a cells still express some astrocyte markers, but they are horizontal cells and do not possess apical processes that extend through the GCL. These cells still express the stem cell marker nestin, but they also express the Eomes transcription factor (Tbr2). These are cells capable of transitioning between proliferation and cell fate selection. Type-2b cells are Type-1 progeny that develop from Type-2a and select a neuronal fate. At this stage, they no longer express astrocyte markers, and they begin to express the immature neuron markers Prox1 and Dcx. When nestin is down-regulated in Dcx positive cells (Type-3) they have exited the cell cycle and typically have little to no capacity to proliferate (Jessberger et al. 2005). These cells begin neuronal maturation, a process that lasts 3 weeks. During neuronal maturation, Type-3 neurons primarily receive synaptic GABAergic somatic and dendritic input, which is excitatory because of their high internal chloride gradient (Ge et al. 2008). Between 2-3 weeks of age, the Type-3 immature neurons undergo GABA receptor synapse dependent and NMDA receptor-dependent competitive survival to proceed to end-stage maturity (Tashiro et al. 2006, Ge et al. 2007, Ge et al. 2008). Only 30-70% of Dcx positive neurons survive to the end-stage development, due in part to programmed cell death. Postnatally generated neurons

only account for 30% of the mature granule cells in the GCL (Kempermann et al. 2015, Ryu et al. 2016). The adult GCL maintains a relatively constant number of granule cells (Amaral et al. 2007), revealing a role for programmed cell death. During development, type-3 neurons that fail to integrate synaptically undergo Bax-mediated cell death (Oppenheim (Sun et al. 2004). During the Type-3 stage, immature neurons migrate to positions within the GCL where they will localize permanently. Reelin released by interneurons of the niche and HL acts as a guidance cue which directs the new granule cells away from the HL and further into the GCL (Gong et al. 2007). As new neurons exit the Type-3 phase, they begin to express the mature neuron marker NeuN. These mature neurons are morphologically and functionally similar to developmentally born granule cells.

Adult neurogenesis following TBI

Trauma dramatically disrupts the process of neurogenesis. Several methods are used to trace the lineage and fate of a cell in vivo. Assessment of endogenous protein expression is a means to study neurons at the different stages of neurogenesis following injury. However, the majority of the classical NSC markers are also markers of reactive astrocytes (Gotz et al. 2005, Robel et al. 2011). Ki67 is a well characterized marker of cell division that labels cells that have exited the cell cycle within 2-3 days of assessment. Reactive astrocyte proliferation occurs in the hippocampus after injury, presenting difficulty in interpretation when using markers of cell division to target SGZ neurogenesis following injury (Borges et al. 2006). PSA-NCAM and POMC are neuronal proteins that are expressed during the first 3 weeks of neuron birth (Kempermann et al. 2015). These markers label a heterogeneous population of neurons. To accurately

birthdate a cell, cells can be labeled at the time of their division using Bromodeoxyuridine (BrdU) or retroviral labeling. BrdU is a thymidine analog that replaces the thymidine nucleoside during DNA replication (s-phase) of a cell undergoing division. Retroviruses are used to label cells undergoing cell division because the virus can integrate into the DNA only when the nuclear envelope dissolves during late prophase. BrdU labeling is much more widely used because of the cost, ease of delivery, and reliability of the labeling. However, BrdU labeling of dividing precursors can be diluted as precursors continue to divide. This should be taken into consideration when quantifying BrdU positivity using immunoreactivity. There are also methods of genetic labeling that harness the cell's upregulation of neurogenesis markers to drive fluorescent protein production during the developmental stage of interest. Of course, the use of these tools is dependent on the scientific question being asked.

Type-3 immature neurons are extremely vulnerable to TBI. Within the first week after injury, markers for cell degeneration predominantly localize to immature neurons compared with other cell types in the DG (Zhou et al. 2012). However, TBI transiently increases proliferation of Type-1 and Type-2a cells in rodents (Yu et al. 2008, Gao et al. 2013). Trauma findings are consistent with studies that show that stressors like ischemia and seizure stimulate hippocampal stem cell proliferation (Yagita et al. 2001, Jessberger et al. 2005). TBI causes a transient increase in the number of Type-3 immature neurons within the first 2-3 weeks following injury (Dash et al. 2001, Rola et al. 2006). Reactive neurogenesis is not a robust means to replace the lost neurons. Many of these new cells do not survive the final stages of development to become mature neurons (Kernie et al. 2001, Gao et al. 2013, Wang et al. 2016). Additionally, the type-3 neurons present after

TBI have severely stunted dendritic arbors compared to the same neuron population in uninjured animals (Carlson et al. 2004, Villasana et al. 2015).

Injury causes the mislocalization of a subset of Type-3 neurons into the hilus (Villasana et al. 2015, Ibrahim et al. 2016, Shapiro 2017). New neurons may mislocalize before their transition into Type-3 neurons (Yu et al. 2008). The time frame of mislocalization coincides with the trauma-induced loss of HL interneurons. HL interneurons express migratory factors that prevent neuroblasts from migrating into the HL (Lowenstein et al. 1992, Butler et al. 2016). Developing adult-born neurons typically migrate radially away from the SGZ and localize a short distance away in the inner third of the GCL (Cameron et al. 2001). Injury potentiates radial migration of neuroblasts, resulting in their localization toward the outer edge of the GCL where much older granule cells localize (Villasana et al. 2015). Nonetheless, posttrauma-born neurons can survive to end-stage maturity and may functionally integrate wherever they are localized (Scharfman et al. 2003, Myers et al. 2013, Villasana et al. 2015).

While the functional consequence of altered neurogenesis is not fully understood, an increase in the number of neurons that stably integrate into the circuit may be beneficial in attenuating cognitive impairment. The specific role of immature neurons following injury is controversial. Several studies have noted that increases in immature neuron density are associated with reduced learning deficits (Griesbach et al. 2004, Scharfman et al. 2005, Bekinschtein et al. 2013, Bekinschtein et al. 2014, Meng et al. 2014, Jacotte-Simancas et al. 2015). Studies have shown that Type-3 neurons are important for distinguishing between two similar patterns (pattern separation), even though they are not synaptically mature (Ge et al. 2008, Clelland et al. 2009). Adult-born

neurons that reach end-stage maturity have also been shown to contribute to cognitive improvement. Increasing the number of postnatal-born end-stage neurons by inhibiting BAX-mediated apoptosis of NSCs and neuroblasts is sufficient to improve the ability to discriminate between similar contexts in rodents (Sahay et al. 2011). Decreasing the number of mature neurons impairs cognitive flexibility, the ability to ignore a previously learned strategy and replace it with a new strategy as needed (Garthe et al. 2009, Anacker et al. 2017). Decreasing the number of posttrauma-born neurons that reach end-stage maturity using a genetic ablation model impairs recovery of DG dependent spatial learning following CCI (Blaiss et al. 2011). Further studies are required to elucidate the role of posttraumatic neurogenesis in pattern separation and cognitive flexibility following injury.

IGF1 in development and injury

IGF1 introduction

Growth factors act on different tissue systems of the body to promote growth, development, and survival. They are upregulated during prenatal development and dramatically down-regulated postnatally as mammals age. Several growth factors are essential to mammalian development, including insulin-like growth factor 1 (IGF1). Studies have shown that IGF1 is required for normotypic brain growth across species, including humans (Anlar et al. 1999). The majority of IGF1 is produced by the liver under control of growth hormone. IGF1 is also locally produced in organs like the brain and can be expressed independently of growth hormone regulation (Nieto-Estevez et al. 2016). Because of its ability to cross the blood-brain barrier, both locally produced IGF1 and humoral IGF1 regulate brain growth. Global brain expression of IGF1 in the adult

brain is only 4% of its developmental concentration with production limited to select regions (Olfactory bulb, cortex, hippocampus) (Ye et al. 1997). While the IGF1 receptor is ubiquitously expressed on all neural cell types, it is enriched in neuron dense regions like the hippocampus (Bondy et al. 1992). IGF1 can act in an endocrine, paracrine or autocrine fashion to mediate growth signaling. Both IGF1 and insulin bind to both the IGF1 receptor. However, IGF1 binds to the IGF1 receptor with 100x greater affinity than does insulin (Ocran et al. 1988, Pomerance et al. 1988, Schumacher et al. 1991). The two growth factors activate nearly identical signaling mechanisms for metabolism and plasticity (Pomerance et al. 1988, Zheng et al. 2004, Muhic et al. 2015, Nieto-Estevez et al. 2016). Insulin signaling is well characterized in fat, muscle and liver tissue and we have learned much about the function of IGF1 through IGF1/insulin signaling studies.

IGF1 is a member of the insulin family of peptides and peptide hormones. Its binding is regulated by IGF1 binding proteins which prevent its degradation while in circulation and mediate IGF1 availability to its receptor. Neurotrophic factor stimulation of the receptor tyrosine kinase (RTK) activates the PI3K/AKT pathway in CNS tissue. Free IGF1 binds to the membrane-bound IGF1 RTK to initiate a trophic response cascade. Upon activation the IGF1 RTK dimerizes and auto-phosphorylates internal tyrosine residues that serve as a docking site for insulin response substrate (IRS) and Src homology and Collagen (SHC) adapter kinase (Thirone et al. 1998, Zheng et al. 2004). IRS and SHC interact in a competitive manner for pathway selection (Sasaoka et al. 2001). IRS activates the phosphatidylinositide 3-kinases (PI3K) and protein kinase B (AKT), PI3K-AKT signaling pathway. SHC activates the mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) kinase signaling

pathway, MAPK-ERK. These pathways regulate different aspects of proliferation, growth, differentiation, and survival (D'Ercole et al. 2008), but the PI3K-AKT pathway predominates in mediating IGF1 actions in CNS.

IGF1 and mTOR signaling in neural development

IGF1 is upregulated during development in regions undergoing neurogenesis and is dramatically downregulated days after birth. IGF1 has a distinct role in neurogenesis during development. Studies have shown that during development IGF1 stimulates proliferation of neuroblasts. The IGF1 receptor is required for maintenance of the DG stem cell (Type-1 and Type-2a) population during development. Developmental IGF1 receptor deletion in nestin-expressing NSCs causes them to undergo caspase-3 mediated apoptosis, resulting in progressive postnatal brain atrophy (Liu et al. 2009).

Multiple key effectors mediating the role of IGF1 in neural development have been identified. One of the most well-studied effectors of IGF1 actions in the brain is mTOR, the mammalian target of rapamycin. In 1972 the antibiotic rapamycin was discovered and, in addition to antifungal and antibacterial properties, it was found to inhibit antigen-induced proliferation of T-cells and B-cells (Abraham et al. 1996). It was later found to inhibit cancer cell proliferation, which led to the discovery of its target mTOR (Seto 2012). In addition to mTOR being a downstream target of IGF1 receptor signaling, many cell growth, cancer, and development studies have shown that IGF1 receptor signaling and mTOR activation produce similar physiological outcomes, suggesting that IGF1 mediates these functions through mTOR signaling. For example, chronic treatment with rapamycin increases lifespan in rodents (Garza-Lombo et al. 2016), as does developmental inhibition of the IGF1 receptor (Kappeler et al. 2008).

However, developmental inhibition of IGF1 or mTOR can cause small birth weights, brain atrophy, and retardation (Liu et al. 2009, Lee 2015). During brain development both IGF1 signaling and mTOR signaling mediate neuronal proliferation and differentiation. Hyperactivation of mTOR through developmental deletion of its suppressor TSC1 causes CNS tumors due to unchecked expansion of NSCs and their premature differentiation (Magri et al. 2013). Postnatal deletion of PTEN, an inhibitor of IGF1/PI3K signaling, in nestin-expressing cells resulted in NSC expansion and premature neuronal differentiation (Zhu et al. 2012). Drugs that inhibit mTOR activation inhibit NSC proliferation (Park et al. 2012). This often makes it difficult for researchers to address whether IGF1 signaling mediates the induction of neuronal differentiation through mTOR activation since no new neurons are left to analyze. Both IGF1 signaling and mTOR activation promote dendritic and axonal process outgrowth. When effectors downstream of IGF1 receptor signaling (PI3K and AKT) are constitutively active in neuronal cultures, dendritic arbor length and complexity increase in a mTOR dependent manner (Jaworski et al. 2005).

IGF1 and mTOR signaling in trauma

Growth factors may play an essential role in regulating neuronal survival, oxidative stress, neural plasticity, and cognitive recovery after TBI (Zhou et al. 2003, DeKosky et al. 2004, Griesbach et al. 2004, Mahmood et al. 2007, Chiaretti et al. 2008, Ning et al. 2011). Following brain injury in humans, a transient increase in VEGF, nerve growth factor (NGF), and IGF1 are detectable in the plasma or cerebral spinal fluid of TBI patients (Wildburger et al. 2001, Chiaretti et al. 2008, Matsuo et al. 2013, Larphaveesarp et al. 2015, Feeney et al. 2017). This is consistent with experimental injury models that show transient upregulation of several growth factors, not limited to

erythropoietin, VEGF, NGF, and IGF1, in the hippocampus of injured rodents (DeKosky et al. 2004, Lee et al. 2010, Madathil et al. 2010, Schober et al. 2010). Increased serum levels in human TBI and increased levels of exogenous IGF1 in experimental TBI are associated with improved memory (Saatman et al. 1997, Feeney et al. 2017). When rats were treated with IGF1 following fluid percussion injury, IGF1 attenuated the injury-induced spatial memory deficit (Saatman et al. 1997). Increasing brain levels of IGF1 following injury using a transgenic overexpression model attenuated memory impairment acutely after injury (Madathil et al. 2013).

IGF1 protein upregulation is detectable ipsilateral to injury within 1hr after injury (Madathil et al. 2010), most noticeably in the peri-contusion area. Protein detection is limited by the rapid turnover of IGF1. However, increases in posttraumatic IGF1 receptor activation are still detectable in the hippocampus 24h after injury (Rubovitch et al. 2010). IGF1 has also been shown to promote survival and decrease the number of degenerating hippocampal cells in the CA3 and DG of the dentate gyrus (Madathil et al. 2013). Increasing brain levels of IGF1 following injury by transgenic overexpression or central infusion enhances posttraumatic neurogenesis in the DG (Carlson et al. 2014, Carlson et al. 2018). IGF1 restored the dendritic arbor complexity and length of new neurons following injury (Carlson et al. 2014). It is important to investigate IGF1 as a potential therapeutic target for brain injury.

How IGF1 regulates neuroprotection and neuroplasticity following injury is less well understood. After brain injury, PI3K/AKT activity is transiently upregulated within hours after injury in the cortex and hippocampus (Jenkins et al. 2002, Park et al. 2012). Additionally, AKT activity has been shown to be potentiated in response to posttraumatic

increases of IGF1 in the cortex and hippocampus (Madathil et al. 2013). Within 48h of brain injury mTOR, a factor that mediates the upregulation of protein translation downstream of PI3K/AKT activity in the injured cortex and hippocampus is transiently activated (Chen et al. 2007, Park et al. 2012, Guo et al. 2013). S6K and S6, substrates directly activated by mTOR signaling, have been shown to be upregulated for the first 3 days after injury in the cortex and hippocampus (Park et al. 2012).

The effect of mTOR activation on posttraumatic cognitive recovery has been incongruent. Chronic mTOR inhibition by central infusion of rapamycin impairs long-term spatial memory storage in naïve rats (Dash et al. 2006). Pretreatment with centrally infused mTOR inhibitors worsened spatial memory and learning after mild closed head injury (Zhu et al. 2014). A single injection of rapamycin delivered to the ipsilateral ventricle immediately before moderate CCI reduced activation of S6 in the hippocampus, but did not alter posttraumatic spatial learning (Park et al. 2012). Intraperitoneal injection of rapamycin at 1hr after moderate CCI prevented injury-induced mTOR activation of S6 in the hippocampus and attenuated acute spatial learning deficits (Nikolaeva et al. 2016). Inhibition of mTOR with an AMPK-dependent mTOR inhibitor, metformin, after moderate CCI also attenuated TBI induced memory deficits in rodents (Hill et al. 2016). In experimental models of posttraumatic epilepsy, mTOR inhibition by rapamycin reduced the development of posttraumatic seizures (Guo et al. 2013, Butler et al. 2015). Posttraumatic induction of mTOR activation may have to reach a threshold level to impair cognition. Mice that are haploinsufficient for the endogenous mTOR inhibitor, TCS1/2, have impaired learning only after mild closed head injury (Rozas et al. 2015). It is clear that dysregulation of mTOR activity may impair posttraumatic recovery.

Like IGF1 signaling, mTOR activation can regulate cell survival following trauma. mTOR regulates apoptosis and survival during CNS development (Magri et al. 2013, Lee 2015). The basic function of mTOR activity is to regulate homeostasis. Cells undergoing stress can save energy by downregulating mTOR activity to prolong survival. However, when mTOR activation levels are reduced too low, programmed cell death can be induced (Feng et al. 2010, Hung et al. 2012). Following injury, mTOR is activated in NSCs, neurons, and reactive glia in the cortex and hippocampus after injury (Chen et al. 2007, Park et al. 2012, Wang et al. 2016). Inhibiting mTOR activation following weight drop injury reduced trauma induced caspase activation and apoptosis markers (Wang et al. 2017). Inhibiting posttraumatic mTOR activation also inhibits injury-induced NSC proliferation and neurogenesis in the hippocampus (Butler et al. 2015, Wang et al. 2016). Posttraumatic rapamycin treatment inhibits axonal process outgrowth, inhibiting injury-induced aberrant sprouting after severe TBI (Guo et al. 2013, Butler et al. 2015). It is unclear whether and to what extent IGF1 alters mTOR activation and its regulation of posttraumatic neuronal survival and neurogenesis in the hippocampus.

Summary

TBI is a devastating disease associated with a billion-dollar economic burden and the potential for long-term disability. There are currently no approved pharmacological treatments for TBI. IGF1 attenuates injury-induced deficits in hippocampus-dependent learning and memory tasks. Following injury, IGF1 also confers hippocampal neuroprotection and stimulates neurogenesis and dendritic arbor recovery. However, the long-term fate of posttrauma-born neurons following IGF1 intervention and their

contribution to neurogenesis dependent behaviors after injury is not known. This study elucidates the long-term effects of IGF1 on posttraumatic neurogenesis and behavior.

The IGF1 signaling pathway confers neuroprotection and stimulates neuroplasticity following trauma. This study tests the hypothesis that mTOR activation is critical to the enhancement of hippocampal neurogenesis by IGF1 after injury. This dissertation provides new insights into the mechanism of post-traumatic IGF1 signaling in plasticity.

CHAPTER 2: IGF1 increases long-term survival of posttrauma-born neurons
while inhibiting ectopic localization in the hippocampus

Introduction

TBI is the leading cause of death among people under 40 and is predicted to be the leading cause of death in the world by 2020 (Fleminger et al. 2005, Popescu et al. 2015). Survivors of mild to severe cases of TBI can suffer from a progressive decline in cognition and from long-term disability (Watson et al. 1995, Ratcliff et al. 2005, Flanagan 2015, Schachar et al. 2015). Cognitive impairment is due in part to the brain's limited capacity to repair and replace lost neurons.

Throughout adulthood, neural stem cells in the subgranular zone (SGZ) of the hippocampal dentate gyrus can proliferate and differentiate into new principal granule neurons of the granule cell layer (GCL) (Amaral et al. 2007). During the normal progression of adult neurogenesis, nearly 80% of newly born neurons undergo apoptosis within four weeks of proliferating (Cameron et al. 2001, Dayer et al. 2003, Kempermann et al. 2003, Ryu et al. 2016). Surviving neurons form synapses and are functionally integrated into the local hippocampal circuitry (van Praag et al. 2002, Jessberger et al. 2008). Although the function of surviving adult-born neurons is not completely understood, they have been shown to contribute to contextual fear memory and extinction (Snyder et al. 2009, Ishikawa et al. 2016), spatial memory (Farioli-Vecchioli et al. 2008) (Lu et al. 2007), pattern separation (Sahay et al. 2011), and cognitive flexibility) (Burghardt et al. 2012, Sahay et al. 2011). Increasing the number of new adult-born neurons that survive to maturity has been shown to be sufficient to increase cognitive flexibility in naïve mice (Sahay et al. 2011).

Trauma has a pronounced impact on immature hippocampal neurons and their development following injury. Contusive brain injury results in the selective death of immature neurons in the GCL within the first week of injury (Rola et al. 2006, Gao et al. 2008). While brain insults transiently stimulate a period of proliferation, resulting in recovery of immature neuron numbers (Rola et al. 2006, Carlson et al. 2014), the majority of post-trauma-born immature neurons do not survive to maturity after TBI (Lu et al. 2005, Bye et al. 2011, Ning et al. 2011, Gao et al. 2013, Wang et al. 2016). Injury results in a minimal increase in end-stage mature neurons, diminishing the potential long-term impact of stable posttraumatic neurogenesis. Within the injured hippocampus posttrauma-born neurons develop with atypical and stunted dendritic arbors (Carlson et al. 2014, Villasana et al. 2015, Ibrahim et al. 2016), which may inhibit synapse-dependent maturation and survival (Tashiro et al. 2006, Ge et al. 2008, Deng et al. 2010). Brain injury also increases the number of newly born neurons that migrate ectopically from the GCL into the molecular layer (ML) and hilar layer (HL) (Parent et al. 1997, Scharfman et al. 2000, Niv et al. 2012, Villasana et al. 2015, Shapiro 2017). Mislocalization and aberrant development may interfere with the function and survival of neurons born following TBI.

Insulin-like growth factor-1 (IGF1) is a potent growth factor that is expressed at high levels during neuronal development and at lower levels in the adult CNS (Ocrant et al. 1988, Nieto-Estevez et al. 2016). IGF1 stimulates neurite outgrowth (Kim et al. 1997), is required for appropriate migration of neural progenitors during development (Hurtado-Chong et al. 2009), and promotes newborn neuron survival (Baker et al. 1999). In the context of injury, we have shown that IGF1 increases the number of immature neurons

born after trauma and restores their dendritic arborization to the levels of uninjured controls (Carlson et al. 2014). Additionally, IGF1 has been shown to attenuate acute posttraumatic cognitive dysfunction (Saatman et al. 1997, Rubovitch et al. 2010, Madathil et al. 2013).

Here, we utilized a transgenic mouse model in which conditional overexpression of IGF1 by astrocytes results in increased hippocampal levels of IGF1 acutely after contusive brain injury (Madathil et al. 2013). At six weeks post-injury, a time point beyond the window of maturation-dependent survival of adult-born dentate gyrus granule cells (Dayer et al. 2003), we examined the effects of IGF1 overexpression on the survival and localization of matured granule neurons born within the first week after TBI. IGF1 overexpression increased the number of posttrauma-born neurons that survived to end-stage maturity and reduced ectopic migration following injury. IGF1 overexpression improved radial-arm water maze reversal learning during the 6-week period corresponding to enhanced stable neurogenesis. We show that IGF1 promotes cognitive flexibility six weeks after TBI.

Materials and Methods

Animal Care

Transgenic mice with astrocyte-specific conditional overexpression of IGF1 were described previously (Ye et al. 2004, Madathil et al. 2013). The mice were bred in-house by crossing heterozygous tTAGFAP mice with heterozygous IGF1pTRE mice, yielding double transgenic mice carrying both transgenes (tTAGFAP/IGF1pTRE) (Madathil et al. 2013, Carlson et al. 2014). IGF1 double transgenic mice (IGFtg) and their wildtype (WT)

littermates were fed doxycycline supplemented chow (200mg/kg) to suppress IGF1 expression from birth until 2 weeks prior to surgery, after which they received standard chow. The mice were provided with food and water ad libitum at the University of Kentucky Medical Center animal vivarium. They were housed in temperature controlled rooms ($23 \pm 2^{\circ}\text{C}$) with a 14/10-h light/dark cycle. All procedures involving animals were approved by the University of Kentucky's Institutional Animal Care and Use Committee (approval # 2013-1156).

Controlled Cortical Impact Injury

Controlled cortical impact (CCI) injury was performed as previously described (Madathil et al. 2013, Carlson et al. 2014). Briefly, isoflurane-anesthetized mice received a 5-mm diameter craniotomy over the left parietal cortex. For sham-injured mice, a cranioplasty was placed over the exposed cortex and the incision was closed. In CCI-injured mice, a cortical contusion was produced using a pneumatically driven impactor device (Precision System Instruments) or a stereotaxic electromagnetic impactor (Leica Biosystems). A rounded 3mm diameter impactor tip was used to deliver a 0.8mm (pneumatic) or 1.1mm (electromagnetic) depth impact at a velocity of 3.5m/s. A cohort of male and female mice 8-12 weeks old were randomized for CCI or sham injury groups for GCL immunohistochemical analysis (8-9 CCI and 3 shams per genotype) and a second cohort was used for behavioral analysis (9-11 CCI per genotype).

BrdU Administration

Beginning 24h after CCI or sham injury each animal received two i.p. injections of 5-Bromo-2'-deoxyuridine (BrdU; 50 mg/kg in saline; Fisher Scientific) at an 8h interval each day until 7 days post-injury (dpi). Proliferating cells were labeled from one to seven days after injury to capture the majority of the proliferative phase triggered by TBI (Rola et al. 2006, Carlson et al. 2014).

Histology and Immunohistochemistry

Animals were deeply anesthetized by sodium pentobarbital (Fatal-Plus Solution, Vortech Pharmaceuticals) and transcardially perfused with heparinized saline followed by 10% buffered formalin 42d after injury. Brains were removed 24h after post-fixation in 10% formalin, cryoprotected using 30% sucrose solution, and snap frozen in cold isopentanes ($\leq -25^{\circ}\text{C}$). Frozen brains were cut in a coronal plane at 40 μm thickness. A series of 9-10 sections taken at 400 μm intervals spanning the entire cerebrum were stained with 0.5% Cresyl Violet (Acros Organics).

Immunofluorescence was performed using published protocols for free-floating sections (Madathil et al. 2013, Carlson et al. 2014) on three tissue sections selected at 400 μm intervals spanning the injury epicenter (between -1.5 to -2.5mm bregma). To expose BrdU epitopes, the tissue was incubated in 2N HCl (Fisher Scientific) at room temperature with agitation for 1h, followed by 100mM borate for 10 minutes to neutralize residual HCl. The tissue was rinsed overnight in TBS at 4 $^{\circ}\text{C}$ prior to a 30 min room temperature incubation in TBS with 0.1% Triton-X-100 and 5% Normal Horse Serum (NHS). Primary antibodies were diluted in TBS with 0.1% Triton-X-100 and 5% NHS and tissue was incubated overnight at 4 $^{\circ}\text{C}$ with agitation. Primary antibodies used were

anti-NeuN (rabbit, 1:500, Millipore Sigma), anti-GFAP (rabbit polyclonal, 1:1000, Millipore Sigma), anti-Iba1 (rabbit polyclonal, 1:1000, Wako), and anti-BrdU (rat monoclonal, 1:1000, Abcam). Secondary antibodies were conjugated with Alexa-488, Cy-3 or Alexa-594 (Invitrogen). The omission of primary antibody served as a negative control. Sections damaged during processing were omitted from analysis for one mouse because the tissue was.

Image Acquisition

Images were taken on a confocal epifluorescence microscope (C2 Tie Nikon confocal microscope) capturing the dentate gyrus for each section per animal. BrdU+ and BrdU+NeuN+ cells in the densely packed GCL of the hippocampus ipsilateral to impact were imaged at 100x magnification under oil and at 0.75 μm step intervals through a 25 μm depth. Maximum intensity projection images of glia labeled with BrdU in the ML were generated from confocal images taken at 1.5 μm step intervals through 20 μm of each section. Colocalization of markers was confirmed using 3D reconstructions of the cells of interest to avoid false positives.

Region of Interest Analysis

To control for possible differences in cell counts due to variations in region size as a function of the anatomical location of section counted, injury or genotype, the volume of the region was determined. In a profile image of each section, the region of interest (ROI) was outlined. The GCL area was auto-detected using NIS-Elements' ROI area function. The ML region of interest, which had a sparse distribution of labeled proliferated cells compared to the GCL area of interest, was manually traced. The outlined ML ROI contained at least 225 DAPI-labeled cells per section in the lateral 2/3

aspect above the upper blade (0.044mm² to 0.059mm² sample area range) (Guzowski et al. 1999, Orozco et al. 2001, Nixon et al. 2008). The depth of the region imaged on the confocal microscope was multiplied by the area to determine the volume, expressed as mm³.

Cell Quantification

To evaluate the effect of IGF1 on adult-born neuronal survival in the GCL, the number of BrdU+ and BrdU+NeuN+ cells were manually counted in the GCL, ML, and hilus (HL). Cells were determined to reside in the GCL if they were within one cell distance (0-10µm) of the GCL border and the soma was adjacent to other NeuN+ cells along the border (Villasana et al. 2015). As principal granule cells are the only type of neuron generated by adult neurogenesis in the dentate gyrus, BrdU+NeuN+ neurons located in the HL and ML are considered ectopic (Amaral et al. 2007, Scharfman 2016). Colocalization of markers was confirmed by making 3D reconstructions of the cells of interest to avoid false positives. To determine if IGF1 increased the survival of posttrauma proliferated glial cells, BrdU+, BrdU+Iba1+, and BrdU+GFAP+ cells were counted in the ML. The following criteria were used to identify BrdU+ cells in the ML for automated counts: colocalization of BrdU with DAPI, spherical cell shape, and a nucleus 5-16 µm in diameter (NIS-elements software). To ensure an unbiased approach, BrdU+ cells were counted and annotated before evaluation of co-labeling with either cell type marker (GFAP or Iba1). Cell counts are expressed as density, cells per volume (mm³).

Neuronal Positioning Analysis

To localize posttrauma proliferated neurons (BrdU+NeuN+) in the GCL at 42dpi, the position of the center of the cell in relation to the GCL/HL border was determined as previously described (Han et al. 2016, Ibrahim et al. 2016). The center of the soma of BrdU+NeuN+ cells within the GCL was annotated using NIS-elements, and cells were binned into the inner 1/3 of the GCL (iGCL) or outer 2/3 of the GCL (oGCL).

Radial Arm Water Maze

The water maze behavior assessment describes a spatial learning test in which animals are trained to locate a fixed platform hidden beneath the surface of a pool. The water component serves as an aversive motivator that encourages rodents to escape quickly. Decreased exploration of areas that do not contain the platform over contiguous trials indicates learning. The reversal paradigm introduces a novel platform location after animals have learned to find the original location. How quickly rodents decrease preference for the old location is an indicator of improved cognitive flexibility (Garthe et al. 2009). Cognitive flexibility has been linked to an increase in the number of mature adult-born neurons (Garthe et al. 2009).

To measure spatial reference memory and learning, mice were tested using an established 2-day radial arm water maze (RAWM) protocol (Alamed et al. 2006, Morganti et al. 2015) beginning 39 days after injury by an observer blinded to animal genotype. We utilized a six-arm maze with an arm length of 30 cm and a common circular swim diameter of 40 cm within a 160 cm diameter pool. The pool was filled with 20-21°C water, made opaque using tempura paint (Rich Art Co., Northvale, NJ), to a level approximately 2 cm above a clear (hidden) circular platform. The platform was

placed at the end of an arm approximately 7 cm away from the side and back walls. During visible trials, a cue was placed on the edge of the platform. The pool was enclosed by a black curtain on which four unique geometric extra-maze visual cues were affixed.

On day 1 (acquisition day), mice were trained over four blocks of 3 trials to identify the platform location, alternating between visible and hidden platform trials (Morganti et al. 2015). Block 5 on day 1 consisted of 3 hidden platform trials, yielding a total of 15 trials. On day 1, mice unable to locate the platform within 60 seconds were gently guided to it. On day 2, reference memory was tested using 15 hidden platform trials, conducted in 5 blocks of 3 trials each (blocks 6 to 10). Mice received a third day of testing, during which the platform was moved to a novel location, at least 1 arm away from the original location (Alamed et al. 2006, Selenica et al. 2014). On day 3, mice received 15 hidden platform trials in the novel location performed in five blocks. Mice were tested in groups of 4-5 and never entered the same start arm as the previous trial. A mouse was considered to have entered the arm if its whole body crossed the threshold of the arm. Animals received an error point if they entered the wrong arm or did not make an arm choice for more than 15s. Entries into the goal arm were not counted as errors even if the platform was not located. The error data is presented as the average error of hidden platform trials per block.

Upon completion of RAWM testing visual and swimming deficits were evaluated using an open pool visible platform test (Alamed et al. 2006). The latency to find the visible flagged platform in an open pool divided into 4 equal quadrants was assessed over 5 blocks of 3 trials (15 trials total trials). The start position remained constant throughout the testing, while the goal quadrant varied each trial. Animals were excluded from

analysis for failing the visible platform test (Alamed et al. 2006) by receiving a latency of >20 seconds in the last block of the test and ≥ 45 seconds latency across all 15 open pool trials (1 WT and 1 IGFtg). Two visually competent mice were excluded from analyses for non-participation, failing to reach the platform in every trial during the 3 days of testing (1 WT and 1 IGFtg).

All trials were recorded with a digital camera using EthoVision XT 8.0 video tracking software (Noldus Information Technology). The error and latency to platform data was analyzed offline from video analysis by blinded observers. Activity heat-maps were generated by video analysis using Anymaze software (Stoelting Co.).

Statistical Analyses

Data was assembled by an individual blinded to genotype and injury conditions. Statistical analyses and graph generation was performed using GraphPad Prism software 6.0. Cell counting and localization assays revealed no differences across bregma levels within groups. Therefore data was combined across tissue sections for a given animal. Cell counts and cell positioning data were analyzed using a one-way analysis of variance (ANOVA), followed by post-hoc Bonferroni's multiple comparison t-tests where appropriate. Post-hoc comparisons were limited a priori to four, to test for genotype effects across sham groups and across injured groups, and injury effects (sham versus CCI) for WT mice and for IGFtg mice. RAWM and RAWM reversal data were analyzed for each day using a two-way ANOVA with repeated measures. For all comparisons $p < 0.05$ was considered statistically significant. All data are presented as mean + standard error of mean (SEM).

Results

Dentate gyrus cells that proliferate acutely after trauma survive 6 weeks

To determine the effect of IGF1 on long-term survival of cells that proliferate after TBI, we labelled cells dividing within the first seven days after injury with BrdU and quantified the number of BrdU+ cells persisting until 42dpi after injury. Proliferated cells were distributed throughout the dentate gyrus but were more abundant in mice with TBI (Figure 2.1A). Quantification in the GCL (Figure 2.1B) and the ML (Figure 2.1C) confirmed a significant increase in the density of acutely proliferated cells (BrdU+) that survived six weeks in both injured WT and IGFtg mice compared to sham controls. However, overexpression of IGF-1 did not alter the density of proliferated cells in either region of the hippocampus.

IGF1 selectively promotes end-stage fate maturation of immature neurons resulting in stable neurogenesis 6wks post-injury

We identified three major neural cell types represented among the surviving BrdU+ posttrauma-born cells in the dentate gyrus: neurons, astrocytes, and microglia (Figure 2.2). We previously showed that IGF1 stimulates an increase in numbers of immature neurons in the GCL early after CCI brain injury by promoting neuronal differentiation (Carlson et al. 2014). To determine if overexpression of IGF1 supported long-term survival of neurons and glia born acutely after trauma, we quantified the density of proliferated neurons, astrocytes, and microglia at 42 dpi (Figure 2.3). We observed no significant difference in the number of BrdU+ neurons in the GCL of brain-injured WT mice compared to WT shams, suggesting no notable difference in long-term neurogenesis. In contrast, brain-injured IGFtg mice displayed a marked increase in

BrdU+NeuN+ cell density compared to IGFtg shams (Figure 2.3A). The density of surviving posttrauma-born neurons in brain-injured mice overexpressing IGF1 was also significantly higher than that in injured WT mice. Proliferated astrocytes and microglia were counted in the ML of the dentate gyrus, a region predominantly populated by glial cells. Brain injury resulted in a similar increase in the density of proliferated astrocytes present in the ML for WT and IGFtg mice (Figure 2.3C), suggesting that IGF1 overexpression did not enhance the persistence of proliferated astrocytes in the ML. Injury resulted in an increase in the density of posttrauma proliferated microglia present in the ML at 6 weeks post-injury, but this was statistically significant only in WT mice (Figure 2.3E).

While TBI has been shown to increase the number of posttrauma-born cells in the GCL, the proportion of proliferating cells that become mature neurons declines (Lu et al. 2007, Gao et al. 2013, Chen et al. 2016). Consistent with these reports, we found that over 60% of BrdU+ cells co-label as mature neurons in the GCL of sham controls at 6 weeks after surgery, while mature neurons only account for 30% of BrdU+ cells in brain-injured WT mice (Figure 2.3B). IGF1 overexpression significantly increased the proportion of proliferated cells that exhibited a mature neuronal phenotype following injury (Figure 2.3B), restoring the balance of proliferated neurons to over 60%, a level equivalent to sham controls. In contrast, the proportion of posttrauma proliferated cells in the ML that were co-labelled with either glia marker, GFAP or Iba1, was similar for IGFtg and WT brain-injured mice (Figures 2.3D,F).

Measurement of the area of GFAP and Iba1 immunostaining revealed that IGF1 overexpression promoted an increased area of GFAP and Iba1 labeling in the ML

compared to uninjured controls (Figure 2.4A). Together these data suggest that while IGF1 strongly promoted the long-term survival of posttrauma-born neurons, it did not alter the density or proportion of acutely proliferated astrocytes and microglia in the dentate gyrus after CCI brain injury. Astrocyte-specific IGF1 overexpression, however, may stimulate astrocyte hypertrophy, as reported in our previous study of acute responses to brain trauma (Madathil et al. 2013).

IGF1 enhances outward migration of posttrauma-born neurons in the GCL

Earlier studies that characterized the development and migration of newborn neurons within the GCL identified two different regions, the inner and outer zones (Altman et al. 1990, Martin et al. 2002, Kempermann et al. 2003, Zhao et al. 2006). Neural progenitors proliferate in the inner 1/3 of the GCL (iGCL) and migrate toward the outer 2/3 of the GCL (oGCL) as they mature (Kempermann et al. 2003, Schmidt-Hieber et al. 2004, Heigele et al. 2016). Neurons born during development and older granule neurons are preferentially localized in the outer zones in the adult. Newborn neurons migrate further following brain injury (Villasana et al. 2015). A study showed that injury increases the proportion of posttrauma proliferated neurons that localize to the oGCL (Ibrahim et al. 2016). We examined the relative proportions of posttrauma-born neurons localized to the oGCL to determine if IGF1 altered outward migration within the GCL (Figure 2.5A). The majority of the neurons born the first week after surgery localized to the iGCL in all groups (Figure 2.5D). Injury induced a modest increase in the number of posttrauma-born neurons that localized to the oGCL. The density and proportion of posttrauma-born neurons localized to oGCL was only significantly higher than control levels in injured IGFtg mice (Figure 2.5E, Figure 2.6A).

IGF1 attenuates ectopic localization of posttrauma-born neurons in the dentate gyrus

Granule neurons born after brain injury have an increased probability of mislocalization to the HL or ML (Parent et al. 1997, Scharfman et al. 2000, Jessberger et al. 2007, Villasana et al. 2015, Shapiro 2017). Ectopic localization of granule cells may also be stimulated by neurotrophic factors such as BDNF (Scharfman et al. 2005). Therefore, we sought to determine whether increased hippocampal neurogenesis in IGF1 overexpressing mice with brain injury was associated with heightened risk for ectopic localization of adult-born neurons. Ectopically localized new neurons (Figures 2.5B,C) were observed in both groups of brain-injured mice, but not in sham controls. WT injured animals had a significantly higher proportion of proliferated neurons localized to the ML and HL than WT sham mice (Figure 2.5G). Despite increasing the number of proliferated neurons 3-fold (Figure 2.3A), IGF1 overexpression did not significantly increase the number of ectopic neurons in the ML and HL of brain-injured mice compared to injured WT (Figure 2.6B). Compared to WT injured mice, IGF1 overexpression reduced the proportion of proliferated neurons that were ectopically localized following injury (Figure 2.5G).

IGF1 improves reversal learning in RAWM 6 weeks after CCI

Cognitive flexibility describes the process of distinguishing between two similar contexts and then extinguishing a preference for one and replacing it with a novel preference (Anacker et al. 2017). Water maze platform reversal studies show that adult-born hippocampal neurons contribute to cognitive flexibility (Garthe et al. 2009, Garthe et al. 2013). Increasing the number of mature adult-born neurons in the GCL was sufficient to improve pattern separation and cognitive flexibility in naïve animals (Sahay

et al. 2011). To determine if IGF1 overexpression improved hippocampal dependent learning ability or cognitive flexibility, mice were evaluated in a 3d RAWM test that included two days of task acquisition (learning) followed by one day with platform reversal. On days 1 and 2, the numbers of errors did not differ significantly between injured WT and IGFtg mice, indicating similar ability to learn the initial platform location (Figure 2.7A). During day 3 RAWM reversal testing, the platform was moved to a novel goal arm, requiring mice to change their strategy and identify the new platform location. Injured WT and IGFtg mice initially made similar numbers of errors during the first block of day 3 and both learned to find the new platform by the last block of day 3 (Figure 2.7A). Injured IGFtg mice showed an earlier decrease in errors over blocks 10-11 while WT injured mice were more delayed in learning the new location, but this difference was not statistically significant.

Learning a novel goal location after an initial goal location has been established is neurogenesis dependent (Garthe et al. 2009, Burghardt et al. 2012, Garthe et al. 2013). A decrease in time spent in the old (original) location is reflective of the animals' ability to extinguish prior memory and learn a new strategy (Garthe et al. 2009, Saha et al. 2017). Activity maps from day 3 RAWM trials illustrate that injured WT mice had more activity in the vicinity of the old goal arm in blocks 9, 10 and 11 than did injured IGFtg mice (Figure 2.7B). Therefore, we quantified the time spent in the old goal arm during reversal learning on day 3 of the RAWM. Injured IGFtg mice spent significantly less time in the old arm during day 3 of RAWM testing compared to injured WT mice (Figure 2.7C). Our data provides evidence that IGF1 overexpression enhances posttraumatic cognitive flexibility, enabling injured mice to more quickly extinguish their memory of

the old strategy and replace it with a new one. Both WT and IGFtg mice performed similarly on the visual platform test (Figure 2.7D), indicating that behavioral differences between groups are not due to motor or visual deficits (Alamed et al. 2006, Bromley-Brits et al. 2011).

Discussion

Summary

The fate, localization, and function of posttrauma-born neurons in the dentate gyrus six weeks following brain injury were examined in IGF1 overexpressing and WT mice. Three key findings from the studies are presented here. First, of the hippocampal cell types that proliferate after trauma, the population of posttrauma-born neurons that survive to maturity is selectively increased by elevated brain levels of IGF1. IGF1 significantly increased the number of mature posttrauma-born neurons but not the number of astrocytes or microglia proliferated within the first week of injury. Second, we show for the first time that IGF1 regulates migration of adult-born neurons following trauma. Brain-injured IGF1 mice had significantly more newly born neurons localized deep within the GCL. Although IGF1 promoted migration within the GCL, it did not potentiate injury-induced ectopic granule cell localization to the HL and ML. Third, we present a novel effect of IGF1 on cognitive flexibility following trauma. At six weeks, injured IGF1tg mice had improved performance on the day of RAWMR testing, when the hidden platform was moved to a novel location. This data compels further evaluation of IGF1 for its therapeutic potential to promote hippocampal neurogenesis and cognitive flexibility following trauma.

Survival

We show IGF1 overexpression support survival of posttrauma-born neurons to end-stage maturity. Consistent with previous studies, the proportion of acutely proliferated cells that differentiated into neurons and survived to late-stage maturity in injured WT mice was diminished compared to sham controls (Lu et al. 2007, Gao et al. 2013). Posttraumatic IGF1 overexpression stimulated a 3-fold increase in numbers of mature neurons 6 weeks after injury. IGF1 overexpression following injury restored the proportion of proliferated cells that became end-stage mature neurons to sham levels at 6 weeks post-CCI. Posttraumatic IGF1 overexpression dramatically increases neuronal differentiation of SGZ NSCs within 10d of injury (Carlson et al. 2014). Data from in vitro studies and developmental studies suggest that IGF1 is largely increasing the density of neurons that survive to end-stage maturity by inhibition of programmed cell death and enhanced integration of developing neurons (Baker et al. 1999, Jaworski et al. 2005, Kim et al. 2006, Miltiadous et al. 2011, O'Kusky et al. 2012, Carlson et al. 2014, Huat et al. 2014, Nieto-Estevez et al. 2016, Nieto-Estevez et al. 2016). Injury drives extensive cell death of immature neurons and, to a lesser extent, mature neurons in the GCL (Rola et al. 2006, Gao et al. 2008, Gao et al. 2013). Additional posttrauma-born neurons will die during the process of maturation. A critical step in the process of adult neurogenesis is pruning, which maintains a consistent number of principal granule cells in the GCL as mammals age. The majority of 2-3 week old adult-born neurons do not reach end-stage maturity; as many as 80% of these neurons undergo apoptosis during synapse-dependent competitive survival (Cameron et al. 2001, Dayer et al. 2003, Kempermann et al. 2003, Ryu et al. 2016). IGF1 has been shown to prevent apoptosis in

the hippocampus and increase immature neuron dendritic arborization (Miltiadous et al. 2011, Madathil et al. 2013, Rong et al. 2015). We found no difference in the numbers of proliferated cells in the GCL of injured WT and IGFtg mice. By 1-month post-injury only 10-50% of acutely proliferated cells in the GCL of WT mice are mature neurons (Lu et al. 2005, Wang et al. 2016). Gao and colleagues argue that the reduction in mature neurons is not merely due to slowing of the maturation process after TBI, as BrdU+ cells that are NeuN- do not label with the immature neuron marker doublecortin (Gao et al. 2013). Future long-term studies examining the effect of IGF1 overexpression on the rate of proliferated neuron apoptosis in the GCL are necessary to directly elucidate the mechanism that produces increased mature neurons after trauma. In any event, IGF1 overexpression following injury provides a pool of mature neurons that can potentially be recruited to overcome deficits caused by injury.

Although the IGF1 receptor is ubiquitously expressed on all neural cell types (Ocrant et al. 1988, Muhic et al. 2015), our data suggests that IGF1 is selectively enhancing stable neurogenesis after TBI. Our data corroborates existing studies that show that injury stimulates acute glial proliferation in the ML that persists for months after injury (Kernie et al. 2001). However, IGF1 overexpression did not increase the number of proliferated astrocytes or microglia localized to the ML 6 weeks after TBI. Our data contrasts ischemic brain injury findings, that IGF1 treatment stimulates gliosis, producing an increase in astrocytes, microglia and oligodendrocytes in the white matter tracts of fetal sheep (Cao et al. 2003). Although we examined neurons, astrocytes, and microglia, further studies are needed to examine alterations in proliferated oligodendrocyte densities.

In our model, IGF1 overexpression is driven by GFAP, an astrocyte-enriched structural protein. IGF1 overexpressing mice exhibited a significant increase in GFAP immunoreactive area at 6 weeks after injury in the ML compared to uninjured controls and injured WT mice, even though neither the density nor proportion of proliferated astrocytes was increased. This is consistent with our previous finding, that IGF1 overexpression increases GFAP levels in hippocampal homogenates at 24 and 72hrs after CCI compared to WT injured animals (Madathil et al. 2013). Astrocytes play many potentially important roles in the injured brain, including regulating synaptic strength of DG granule cells, attenuating cognitive impairment through hippocampal S100B growth factor release, reducing glutamate excitotoxicity, and mediating immature neuron migration along their processes (Deller et al. 1997, Chen et al. 2003, Kleindienst et al. 2005, Becker et al. 2013, Robinson et al. 2016, Jinnou et al. 2018). An increase in GFAP immunoreactive area was also observed in the GCL and SGZ of injured IGFtg mice compared to WT and sham controls (data not shown). Increased GFAP in close proximity to the GCL and neurogenic niche suggest that astrocytes and possibly GFAP-positive progenitors in the DG may play a role in mediating IGF1 effects on neurogenesis.

Migration

Mature neurons generated by posttraumatic neurogenesis may play an important role in replacing mature granule neurons lost after injury. For the first time, we show that IGF1 can regulate outward migration of posttrauma-born neurons, consistent with a developmentally born phenotype. Although neurogenesis is an ongoing process, the approximate number of adult-born neurons in the GCL is maintained and does not

increase over time (Cameron et al. 2001, Dayer et al. 2003, Kempermann et al. 2003, Amaral et al. 2007). Developmentally born granule neurons, localized to the oGCL of adults, continue to die over time. Adult neurogenesis provides a pool of new neurons that can replace dying hippocampal granule neurons (Dayer et al. 2003). In naïve animals, a subset of adult-born neurons migrate from the neurogenic niche of the SGZ and into the iGCL and oGCL within the first 4 weeks of their birth (Kempermann et al. 2002, Kempermann et al. 2003).

In addition to immature neurons, injury results in a loss of mature granule cells (Zhou et al. 2012, Wang et al. 2016). Mature granule neurons localized to the oGCL were found to colocalize with Caspase-3 (a marker for apoptosis) after injury (Zhou et al. 2012). Although injury promotes a modest increase in the number of posttrauma-born mature neurons that localize to the oGCL, these neurons maintain their injury-induced aberrant dendritic arbors into maturity at 5 weeks post-trauma (Ibrahim et al. 2016). This suggests that although injury has been shown to increase posttrauma-born neurons localized to the oGCL, they may not productively integrate. At 10d post-injury IGF1 overexpression restores immature neuron dendritic arbor length and complexity to uninjured levels (Carlson et al. 2014). We show here that IGF1 increases the density and proportion of mature posttrauma-born neurons that migrate to the oGCL. Futures studies will examine the architecture and function of these cells.

As immature neurons develop in the postnatal brain, they are rarely found localized to the hilus. During the process of adult neurogenesis, immature neurons that mislocalize the HL undergo Bax-mediated apoptosis before they are able to mature (Stallock et al. 2003, Myers et al. 2013). Several models of brain injury, including TBI,

cause adult-born granule neurons to ectopically localize to the HL (Parent et al. 1997, Scharfman et al. 2007, Myers et al. 2013, Shapiro 2017). Consistent with other studies, we show that injury increases the density of ectopically localized posttrauma-born granule cells. However, we are the first to show that IGF decreases the proportion of new neurons that migrate ectopically into the HL. Large numbers of ectopically localized neurons are generated in the epileptic brain and these mislocalized neurons are implicated in seizure development (Parent et al. 1997, Scharfman et al. 2003). Ectopically localized mature granule cells integrate into the hilus are suggested to lower seizure threshold (Scharfman et al. 2003). It was recently reported that IGF1 treatment decreased seizure severity in a kainic acid model of temporal lobe epilepsy (Miltiadous et al. 2011). The magnitude of brain injury induced ectopic localization of neurons is much lower than that observed in models of seizure (Scharfman et al. 2000, Ibrahim et al. 2016), so the role of ectopic hilar neurons is less clear in TBI. IGF1 may be a potential therapeutic for posttraumatic epilepsy.

IGF1 regulates known factors of neuronal migration including GSK3B, PSA-NCAM, CDK5 and reelin (Wang et al. 1999, Jessberger et al. 2008, Hurtado-Chong et al. 2009). Signaling studies have also revealed that inhibiting the activity of GSK3B, a protein inactivated downstream of IGF1 signaling, is sufficient to increase the number of adult-born neurons localized to the oGCL (Ng et al. 2016). During development, IGF1 regulates reelin expression and distribution, as well as reelin-dependent neuron migration levels in naïve mice (Hurtado-Chong et al. 2009). There is also evidence that IGF1 may regulate immature neuron migration indirectly through its effects on other neural cell types. Reelin is a migratory stop signal for dentate granule cells that is expressed by hilar

basket cells (Myers et al. 2013), preventing granule cells from migrating ectopically into the hilus (Gong et al. 2007). While injury causes degeneration of hilar neurons (Lowenstein et al. 1992, Hall et al. 2005, Hunt et al. 2011), IGF1 mediates survival of hilar neurons following trauma (Madathil et al. 2013).

IGF1 and cognitive flexibility

Increasing the number of mature adult-born neurons was sufficient to improve discrimination between two similar contexts, extinguish a learned strategy, and replace it with a new strategy when a novel location goal arm was introduced (Idris et al. 2010, Sahay et al. 2011). Further reinforcing the contribution of posttrauma-born neurons to cognitive flexibility, ablating adult neurogenesis has been shown to impair pattern recognition and cognitive flexibility (Clelland et al. 2009, Burghardt et al. 2012). Inhibiting adult neurogenesis using the anti-proliferation drug temozolomide caused a strong and lasting preference for the old goal arm during Morris water maze testing when the goal arm was moved to a novel location (reversal testing) (Garthe et al. 2009). This study provides evidence that enhancement of neurogenesis through posttraumatic IGF1 overexpression supports long-term cognitive flexibility. Here, WT and IGFtg mice learned to find a hidden platform similarly on days 1-2. The ability to learn where the constant platform goal arm is in the RAWM is dependent on the prefrontal cortex and striatum function of working memory and reference memory (Hyde et al. 1998, Shukitt-Hale et al. 2004). Functional neurogenesis is recruited for the reversal learning aspect of water maze spatial learning tests (Horster et al. 2017). Injured IGFtg mice had a higher density of mature posttrauma-born neurons and learned a new strategy more quickly than WT mice during RAWM reversal. This is consistent with other neurogenesis studies

(Bekinschtein et al. 2013, Bekinschtein et al. 2014, Horster et al. 2017), in which the modification of adult neurogenesis did not interfere the ability to learn a strategy. However, the ability of mice to choose a new strategy when a novel goal location is introduced has been shown to be hippocampus and dentate gyrus dependent (Day et al. 1999, Xavier et al. 1999). When a novel platform location was introduced on day 3, injured IGFtg mice learned to find the new platform earlier and spent significantly less time in the old goal arm compared to injured WT mice. IGFtg mice have a 3-fold increase in the number of mature posttrauma-born neurons at six weeks after injury, a time which parallels their improved cognitive flexibility. This study provides evidence that post-traumatic IGF1 overexpression enhances neurogenesis-sensitive cognition including cognitive flexibility.

Our novel data show that posttraumatic increases in IGF1 are sufficient to improve neurogenesis-sensitive cognitive flexibility. These data have important implications for long-term posttraumatic cognitive impairment. Although frontal lobes are known to dominate directing these functions in human, a role for the hippocampus in cognitive flexibility has been recently defined (Rubin et al. 2014). Over 65% of moderate to severe TBI patients report long-term cognitive deficits (Rabinowitz et al. 2014). Executive function disabilities include impairments in high-level cognition: the ability to plan, make decisions, social behaviours, goal-setting and cognitive flexibility. Modulation of adult hippocampal neurogenesis is potentially a therapeutic target for TBI recovery.

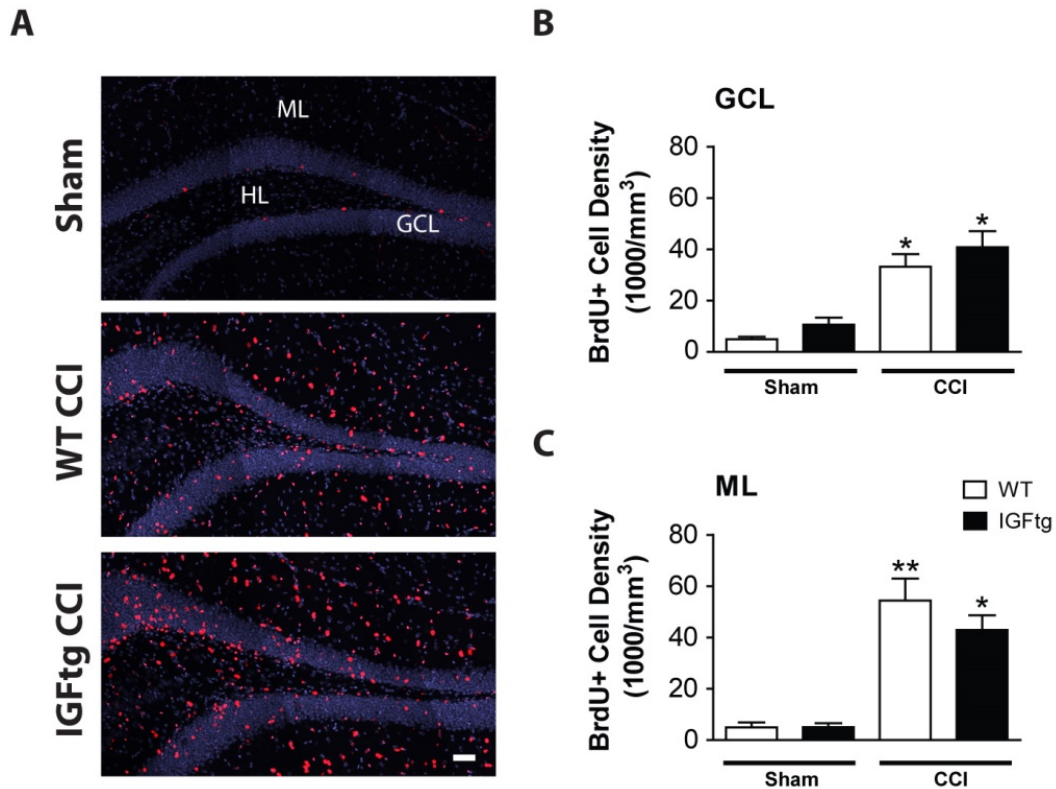


Figure 2.1: Hippocampal cells that proliferate acutely after injury persist long-term in the dentate gyrus.

(A) Cells dividing within seven days following surgery were detected in the dentate gyrus of sham controls, injured wild-type (WT), and injured IGF1-overexpressing (IGFtg) mice at 42d after injury using bromodeoxyuridine (BrdU, red) immunoreactivity. DAPI labeling is shown in blue. Scale bar represents 50 μ m. Controlled cortical impact (CCI) stimulates proliferation in both WT and IGFtg mice. Quantification of BrdU+ cells in (B) the granule cell layer (GCL) and (C) the molecular layer (ML) shows that WT and IGFtg injured mice have increased numbers relative to their respective sham controls. Data presented as mean + SEM (mm³/1000); sham n=3/genotype, CCI n=8-9/genotype; One-way ANOVA, followed by post-hoc Bonferroni's selected comparisons t-tests; *p<0.05 and ** p<0.01, compared to respective sham groups.

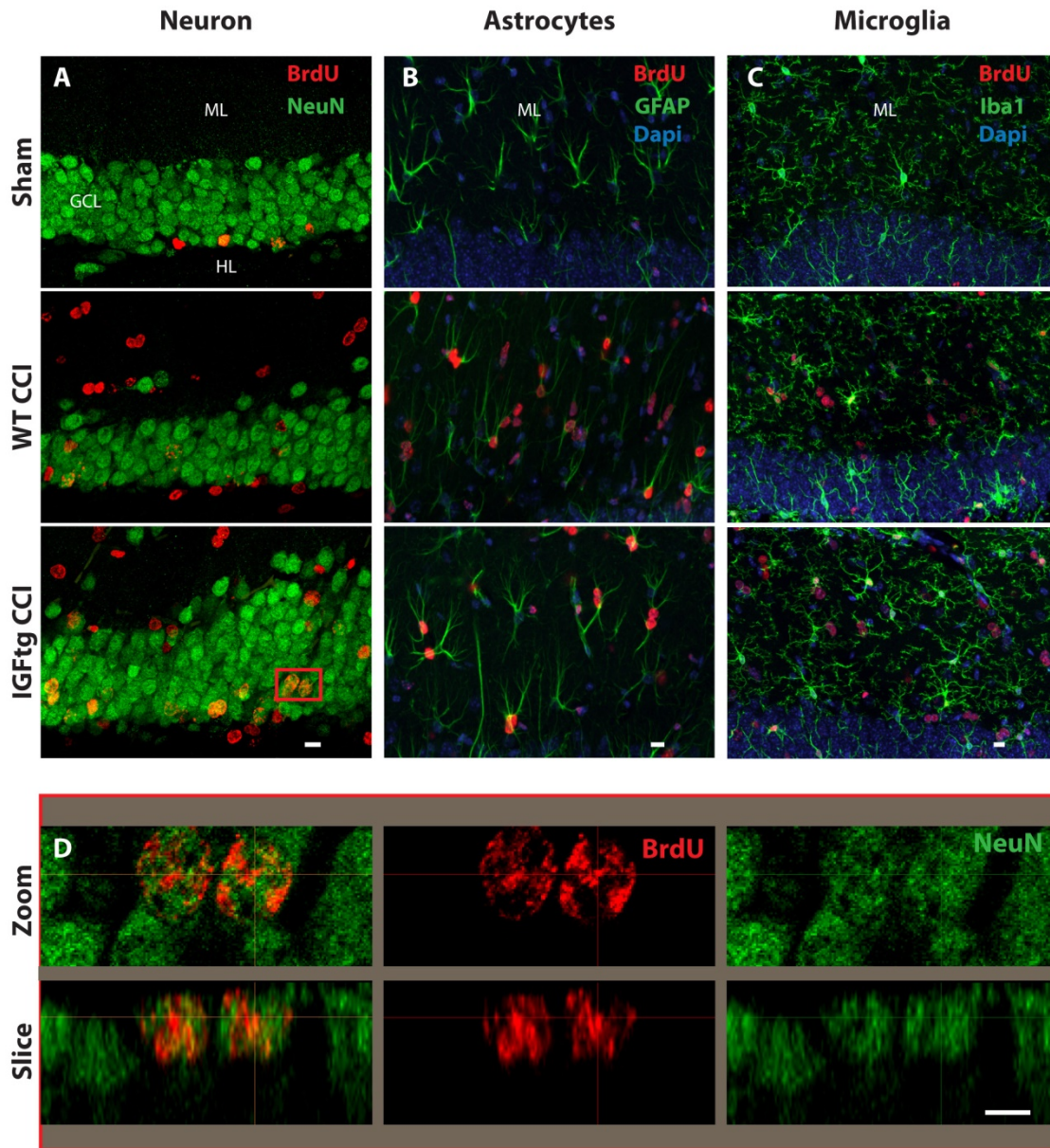


Figure 2.2: End-stage fate maturation of cells proliferated during the first week after injury results in long-term neurogenesis and gliogenesis.

(A) Representative microphotographs from confocal imaging of immunoreactivity of mature neuron-specific protein, Neuronal Nuclei (NeuN, green) with proliferation reporter, bromodeoxyuridine (BrdU, red). Mature posttrauma-born neurons were detected in the granule cell layer (GCL) of sham controls, wild-type (WT) injured, and IGF1-overexpressing (IGFtg) injured mice at 42d after injury. (B) Posttrauma-born astrocytes were co-labeled with glial fibrillary acidic protein (GFAP, green) and BrdU in the molecular layer (ML). (C) Posttrauma-born microglia were co-labeled with ionized calcium binding adaptor molecule 1 (Iba1, green) and BrdU in the ML. The phenotype of posttrauma proliferated cells was confirmed by 3D reconstruction of confocal images. (D) A high-magnification image in the x, y, and z planes of the BrdU+NeuN+ area

indicated by the red box in (A). Figure A-C, scale bar is 10 μ m. Figure D, scale bar is 5 μ m. DAPI is blue; BrdU is red.

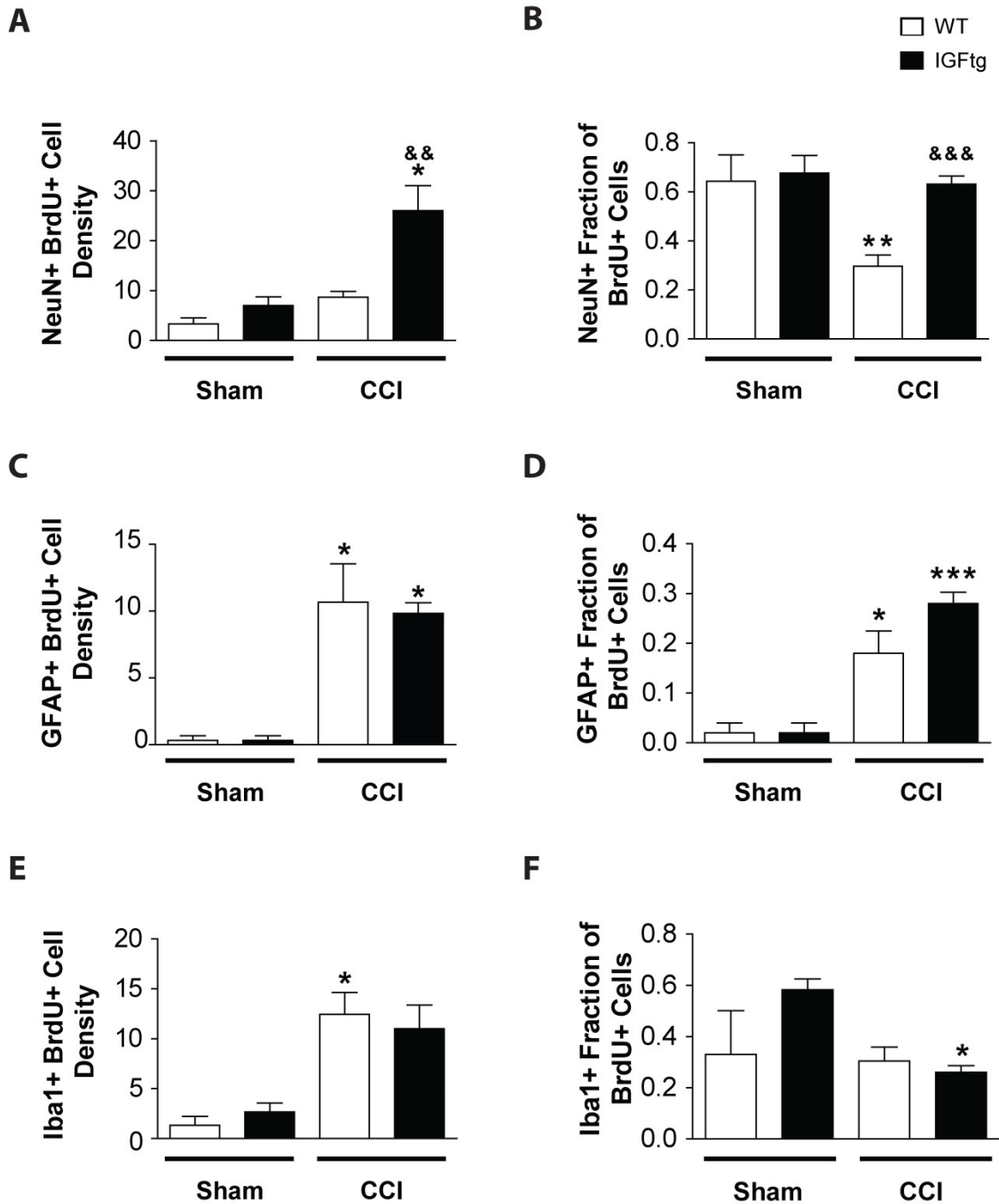


Figure 2.3: IGF1 selectively enhances long-term neurogenesis but not gliogenesis.

Following injury, IGF1 (A) increased the density of surviving posttrauma-born neurons in the granule cell layer (GCL) without increasing the density of posttrauma-born (C) astrocytes or (E) microglia in the molecular layer (ML) compared to injured WT. (B) Injured WT mice exhibited a reduction in the proportion of proliferated progenitors that mature into end-stage neurons, while IGF1 restored this proportion to sham levels. IGF1 overexpression did not increase the proportion of proliferated cells that differentiated into (D) astrocytes or (F) microglia compared to injured WT mice. Data presented as mean +

SEM (1000/mm³). Sham n=3/genotype, CCI n=6-9/genotype; One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc t-tests *p<0.05; **p<0.01; and ***p<0.001 compared to respective sham group. &&p<0.01 and &&&p<0.001 compared to injured WT.

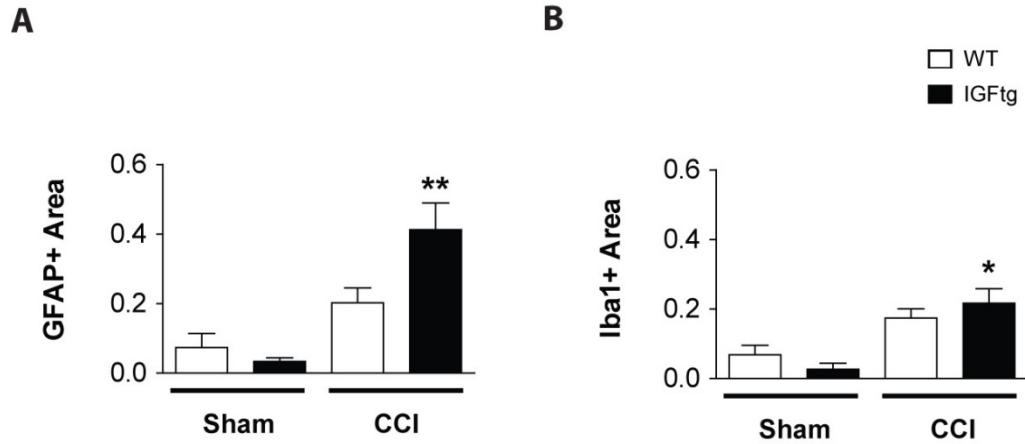


Figure 2.4: IGF1 overexpressing mice had increased glial marker immunolabeling in the ML 6weeks post-CCI.

IGF1 overexpression increased the area of (A) GFAP and (B) Iba1 positive immunoreactivity in the ML following injury compared to IGFtg sham controls. Data presented as mean + SEM (mm²); sham n=3/genotype, CCI n=5-9/genotype; One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc t-tests; *p<0.05 and ** p<0.01, compared to respective sham group.

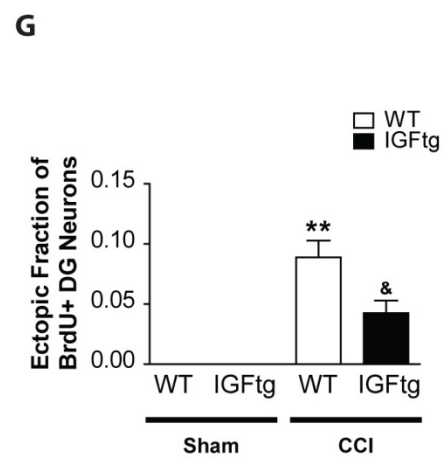
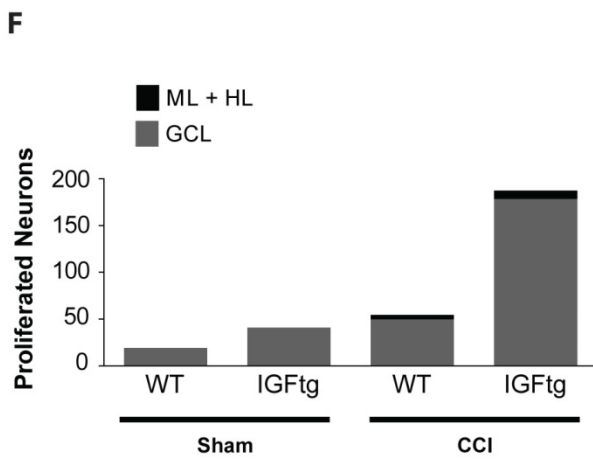
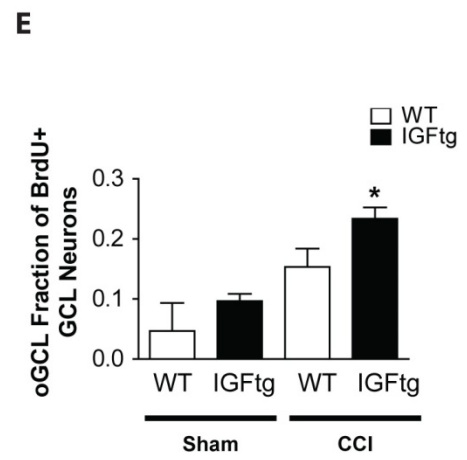
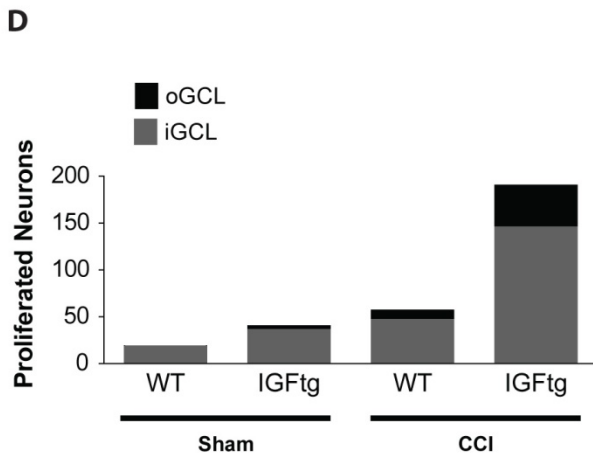
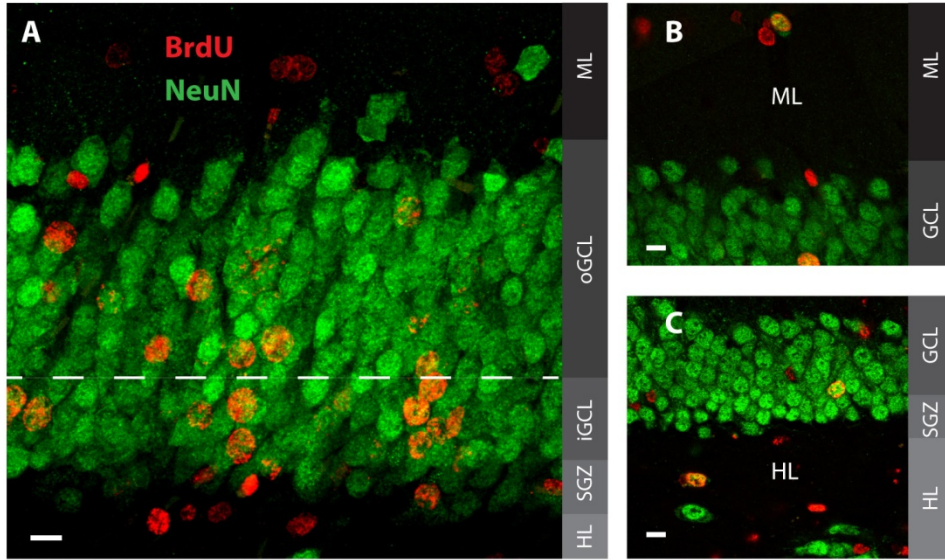


Figure 2.5: IGF1 increases the proportion of new mature neurons that migrate deep within the granule cell layer but decreases the proportion that localizes ectopically to the hilus and molecular layer after injury.

Examples of mature neurons localized to (A) the inner granule cell layer (iGCL) and the outer 2/3rd region of the granule cell layer (oGCL), (B) the molecular layer (ML), and (C) the hilar layer (HL) after controlled cortical impact (CCI) using confocal imaging of Neuronal Nuclei (NeuN, green) and the proliferation reporter, bromodeoxyuridine (BrdU, red). (D) IGF1 overexpression potentiates injury-induced migration of new neurons to the oGCL; (E) IGF1 overexpression increased proportion of mature posttrauma-born GCL neurons localized to the oGCL compared to sham controls. (F) Injury increased ectopic migration of new neurons into the ML and HL. (G) IGF1 overexpression reduced the proportion of recently born mature neurons that ectopically localized to the ML and HL. Figures A-C, scale bar represents 10 μ m. Figures D-G, data presented as mean + SEM. Sham n=3/genotype, CCI n=8-9/genotype; One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc t-tests. *p<0.05; **p<0.01; and ***p<0.001 compared to respective sham group; &p<0.05 compared to injured WT. SGZ, subgranular zone

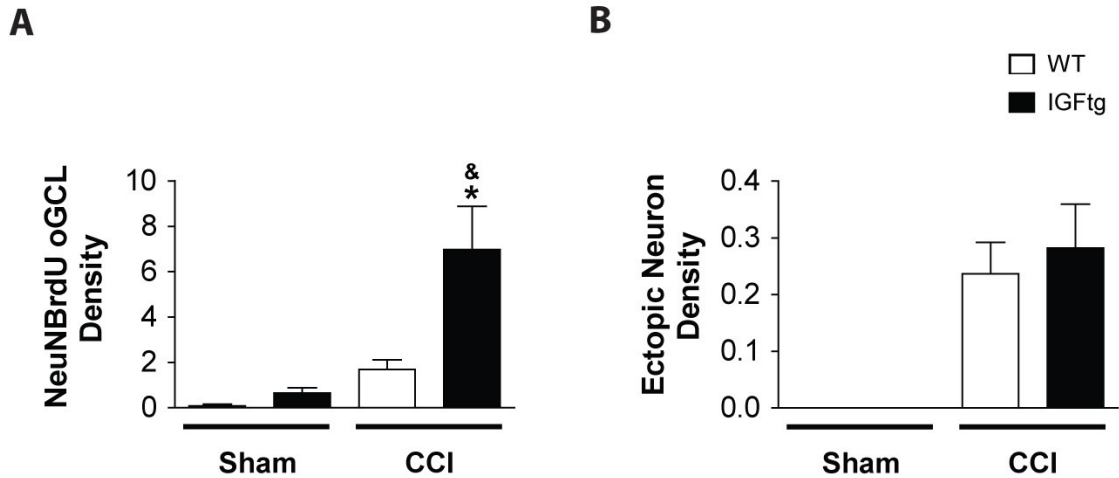
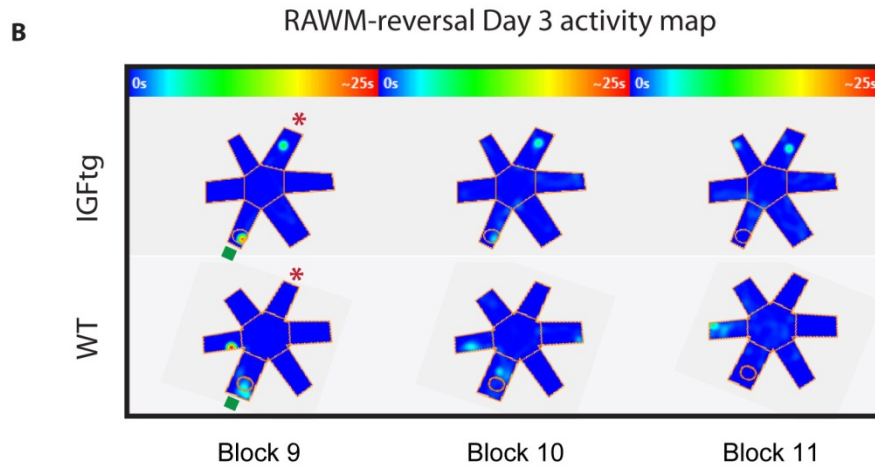
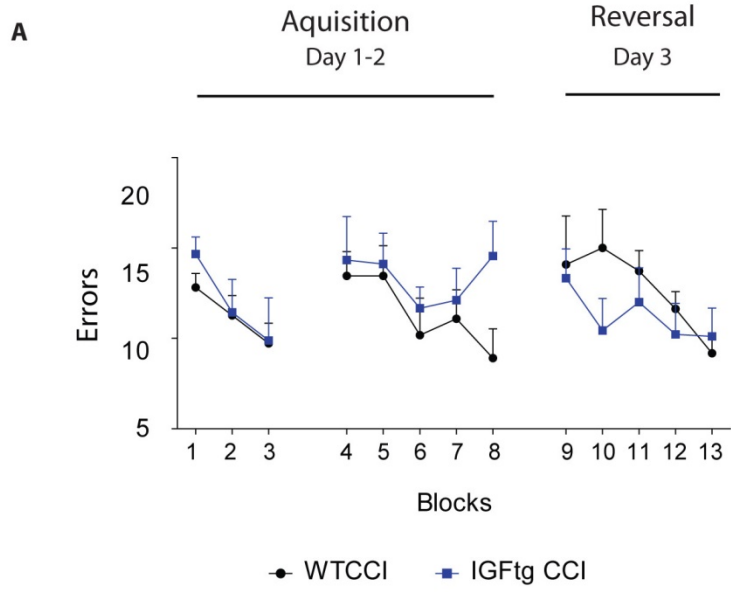
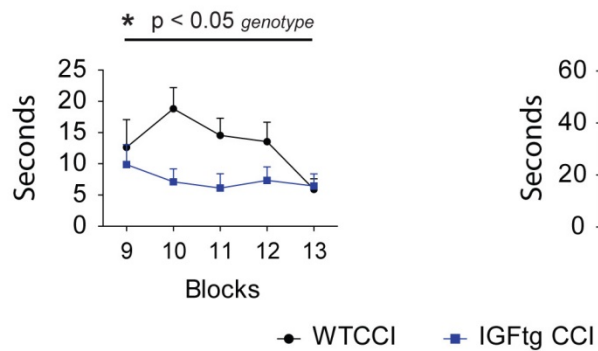


Figure 2.6: IGF1 overexpression increased the density of outward migrating NeuN+BrdU+ in the GCL, but not the density of ectopically localized neurons.

(A) At six weeks after injury, IGF1 overexpression increased the density of proliferated neurons localized to the oGCL compared to sham controls and injured WT. (B) At six weeks after injury, ectopically localized neurons were observed in the ML and HL. Data presented as mean + SEM (1000/mm³). Sham n=3/genotype, CCI n=8-9/genotype; One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc t-tests. *p<0.05 compared to respective sham group. &p<0.05 compared to injured WT.



C Time in Old Goal Arm



D Latency to Visible Platform

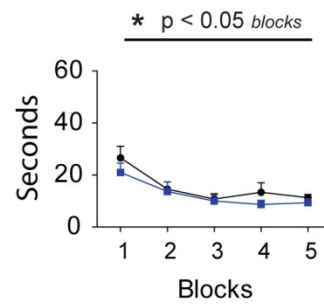


Figure 2.7: Injured mice extinguish memory of old goal arm earlier following IGF1 overexpression.

(A) WT and IGF1 mice learned to find the hidden platform during radial-arm water maze (RAWM) testing and RAWM-reversal. Both WT and IGFtg mice show a decrease in numbers of errors over training days. (B) Maps show activity of representative WT and IGFtg mice during the first three blocks of RAWM reversal testing. IGFtg mice extinguish memory of the old goal arm sooner, after the platform was moved from the original training location (green square) to a novel location (red asterisk) during day 3 RAWM reversal testing. (C) WT mice consistently spent more time in the previous platform location than the IGFtg mice during RAWM reversal testing. (D) Differences between groups were not due to sensory or motor dysfunction. Injured WT and IGFtg mice have similar times to locate the platform at 6 weeks after CCI. Heatmap blue to red coloration indicates increasing amount of time. Data presented as mean + SEM. CCI n=9-11/genotype; Repeated measures ANOVA, p values reported in image.

CHAPTER 3: IGF1 posttraumatic hippocampal remodeling is independent of
mTOR

Introduction

A 2006 study showed that approximately 5 million people who sustained traumatic brain injuries (TBI) are suffering from long-term cognitive impairment and disability (Langlois et al. 2006). The disruption of neural networks resulting from axonal degeneration, neuronal cell death and a limited ability to replace and repair lost cells contributes to cognitive disability (Mander et al. 2013). In response to injury, the brain upregulates signaling that activates developmental plasticity programs which are typically downregulated in adulthood (Anlar et al. 1999). Many forms of adaptive plasticity occur following TBI including cellular reorganization, neural cell-type proliferation, angiogenesis, altered neurotransmitter release, and neurogenesis (Lyeth et al. 1990, Osier et al. 2016)

Hippocampal adult neurogenesis begins with proliferation of neural stem cells (NSCs) in the subgranular zone (SGZ), a unique neurogenic niche in the dentate gyrus that is capable of generating new neurons throughout adulthood (Kempermann et al. 2015). Adult neurogenesis is upregulated in the hippocampal dentate gyrus following TBI in humans (Zheng et al. 2013), primates (Perera et al. 2011) and rodents (Kempermann et al. 2015). The extent and duration of injury-induced neurogenesis is sensitive to injury severity (Wang et al. 2016) and animal model (Snyder et al. 2009). Although upregulation of neurogenesis appears to be a conserved response to brain injury, whether it is beneficial or harmful to recovery remains controversial. Stimulating hippocampal neurogenesis in a healthy brain using growth factors and exercise improves

memory and learning (Deng et al. 2010). Posttraumatic neurogenesis supports spatial learning, reference memory and pattern separation (Sahay et al. 2011, Burghardt et al. 2012, Washington et al. 2012, Bekinschtein et al. 2014). However, it is also a hallmark of posttraumatic seizure activity (Parent et al. 1997, Jessberger et al. 2005, Butler et al. 2015). While the functional role of neurons born following trauma is unclear, ablating posttraumatic neurogenesis can impair cognitive recovery (Blais et al. 2011). Further, the environment of trauma alters immature neuron development. Neurons born after trauma develop with stunted dendritic arbors (Carlson et al. 2014) and maintain this aberrant phenotype as they mature (Villasana et al. 2015, Ibrahim et al. 2016). Trauma alters migration of immature neurons within the granule cell layer (GCL) (Ibrahim et al. 2016) and promotes ectopic migration of new neurons to the hilus (HL) (Shapiro 2017). A subset of posttrauma-born neurons develop into mature neurons capable of synaptic integration with their local environment (Scharfman et al. 2003, Villasana et al. 2015).

Insulin-like Growth factor 1 (IGF1) is an endogenous growth factor that promotes neural plasticity. In the absence of injury, cultured dorsal root ganglia neurons treated with IGF1 had increased process growth (Jones et al. 2003). During development IGF1 stimulates neurogenesis and process outgrowth and mediates immature neuron migration (Hurtado-Chong et al. 2009, Nieto-Estevez et al. 2016). We previously showed that IGF1 overexpression increases immature neuron differentiation and rescues immature neuron dendritic arborization from a stunted injury-induced phenotype (Carlson et al. 2014). Two well described pathways by which IGF1 mediates neuronal growth and survival are the RAS/MAPK/ERK pathway and the PI3K/AKT/mTOR pathway (Kim et al. 1997, Harrington et al. 2004, Zheng et al. 2004, Huat et al. 2014, Nieto-Estevez et al. 2016,

Rhim et al. 2016). In the nervous system PI3K/AKT signaling predominates in mediating many functions of IGF1, including precursor proliferation and differentiation.

AKT activity is transiently induced by injury (Park et al. 2012) and increased brain levels of IGF1 potentiate injury-induced AKT activity in the hippocampus (Madathil et al. 2013). AKT is known activator of the mammalian target of rapamycin (mTOR). A plethora of PI3K/AKT developmental plasticity events are mediated through mTOR signaling including differentiation, migration, process growth and survival of new neurons (Jaworski et al. 2005, Wahane et al. 2014). mTOR activity is transiently increased at 24h to 72h after injury in mature neurons, astrocytes and microglia of the hippocampus (Chen et al. 2007, Park et al. 2012).

It was recently found that injury also activates mTOR activity in NSCs in the neurogenic niche of the hippocampus (Wang et al. 2016). Rapamycin, the specific inhibitor of mTOR, decreases posttraumatic neurogenesis by preventing injury-induced proliferation of NSCs (Butler et al. 2015, Wang et al. 2016). In addition to regulating posttraumatic proliferation, mTOR activation can also mediate maladaptive plasticity. Treatment with rapamycin has been shown to attenuate aberrant sprouting of mossy fibers and seizure activity in models of posttraumatic epilepsy (Butler et al. 2015). It is unclear whether IGF1 overexpression is mediating its effect on posttraumatic neurogenesis through mTOR signaling.

To this end we utilized conditional IGF1 overexpressing animals to determine whether 1) IGF1 overexpression enhanced posttraumatic mTOR activation following moderate or severe controlled cortical impact (CCI) injury and 2) if the neurogenic

effects of IGF1 overexpression in the injured brain are mediated through the mTOR pathway.

Materials and Methods

Animals

We took advantage of injury-induced reactive astrocytosis to conditionally elevate IGF1 levels after injury using a tetracycline inducible mouse model of IGF1 overexpression driven by the glial fibrillary acidic protein (GFAP) promoter. To generate astrocyte-specific IGF1 transgenic mice with conditional overexpression, heterozygous tTAGFAP mice were bred with heterozygous IGF1pTRE mice as previously described (Ye et al. 1997, Madathil et al. 2013). Briefly, by crossing tTAGFAP mice with IGF1pTRE mice, double transgenic mice carrying both the transgenes (tTAGFAP/IGF1pTRE) were generated. In the absence of doxycycline, human IGF1 (hIGF) overexpression expression is astrocyte-specific. IGF1 double transgenic (IGFtg) mice and their wild-type (WT) littermates were fed with doxycycline supplemented mouse chow (200mg/kg) ad libitum until two weeks prior to surgery to block hIGF1 transcription. They received standard mouse chow for at least two weeks to allow transgene expression prior to surgery/injury. The mice were provided with food and water ad libitum at the University of Kentucky Medical Centre animal vivarium where they were housed at a constant temperature ($23 \pm 2^{\circ}\text{C}$) with a 14/10-h light/dark cycle. All procedures involving animals were approved by the University of Kentucky's Institutional Animal Care and Use Committee.

Controlled Cortical Impact Injury

Both male and female littermates were randomly assigned to CCI or sham injury groups once they reached sexual maturity (>8 weeks of age). Surgeons blinded to mouse genotypes and treatments performed moderate or severe CCI on IGFtg (n=47) and WT (n=42) mice. Surgeries were performed as previously described (Madathil et al. 2013). Briefly, anaesthesia was induced using 3% isoflurane. After securing the head of the animal in a stereotaxic frame (David Kopf Instruments, CA), anaesthesia was maintained using 2.5% isoflurane delivered through a nose cone. A midline scalp incision was made, and a 5-mm diameter craniotomy was performed over the left parietal cortex, lateral to the sagittal suture (2.5mm lateral) midway between Bregma and lambda (approximately -1.9 mm). Cortical contusion was produced using a stereotaxic electromagnetic impactor (Leica Biosystems) with a 3mm diameter rounded impactor tip, with a velocity of 3.5m/s to produce moderate (1.0-1.1mm depth) or severe (1.5mm depth) brain injury (Figure 3.1A). IGFtg (n=5) and WT (n=7) sham-injured mice received a craniotomy under isoflurane anaesthesia. After CCI or sham injury, a circular disk made from dental cement was glued over the craniotomy to protect the brain surface, and the scalp was sutured. Mice were placed on a Hova-Bator Incubator (model 1583, Randall Burkey Co., TX) to maintain body temperature until they regained consciousness, after which they were returned to their home cages.

Rapamycin administration

Rapamycin (LC Laboratories, Woburn, MA) was dissolved in 100% ethanol (30mg/ml) and stored at -20°C until use. Immediately before injection, rapamycin was diluted (1:10) into vehicle (5% Tween80, 5% PEG400, 5% EtOH in 1x PBS). Moderately

injured IGFtg and WT mice in a 3-day survival cohort (Figure 3.1B) and in a 10-day survival cohort (Figure 3.1C) were randomly assigned to either vehicle or rapamycin treatment groups for each experiment. The 3-day survival cohort received daily i.p. injections of 10mg/kg rapamycin (n=3/genotype) or vehicle (n= 3/genotype) beginning 1h after CCI. The 10-day survival cohort mice received a daily i.p. injection of 10mg/kg rapamycin (n=8/genotype) or vehicle (n= 6-7/genotype) beginning 4h after the last BrdU injection.

BrdU Administration

5-Bromo-2'-deoxyuridine (BrdU) (Fisher Scientific, Hampton NH) was stored at -20°C until use. The moderate injury 10-day survival cohort of IGFtg and WT mice received 3 intraperitoneal injection (i.p.) injections of BrdU (50 mg/kg in saline) at 4h intervals beginning 72hr after injury (Figure 3.1C). Intraperitoneal BrdU typically incorporates into dividing cells within 4h after injection (Taupin 2007).

Immunohistochemistry and histology

Animals were deeply anesthetised by sodium pentobarbital (FATAL-PLUS SOLUTION V.P.L. 9373 0298-9373-68) and transcardially perfused with heparinised saline followed by 10% buffered formalin. Brains were removed 24h after post-fixation in 10% formalin, then cryoprotected using 30% sucrose solution, and snap frozen in cold isopentanes ($\leq -25^{\circ}\text{C}$). The tissue was cut in a coronal plane at 40 μm thickness. Free-floating immunohistochemistry was performed as previously described on three tissue sections (between -1.5 to -2.5mm bregma) selected at 400 μm intervals spanning the injury epicentre (Madathil et al. 2013, Carlson et al. 2014).

To quantify the extent of mTOR activation, sections were immunolabeled for phosphorylated ribosomal protein S6 (pS6), a well characterized downstream effector of mTOR (Chen et al. 2007). Antigen retrieval was performed using 10mM citric acid pH 6.0 at 60°C. Following three washes in TBS, the sections were incubated in blocking solution (5% normal horse serum in 0.1% Triton X-100 TBS) for 1h. The tissue was then incubated with primary antibody at 4°C overnight. Tissue was immunolabeled for the microtubule protein doublecortin (Dcx), a commonly used immature neuron marker. Posttrauma-born immature neurons were identified by co-immunolabeling the tissue for Dcx and BrdU. To expose BrdU epitopes, the tissue was incubated in 2N HCl (Fisher Scientific) at room temperature with agitation for 1h, followed by 100mM borate for 10 minutes to neutralize residual HCl. The tissue was rinsed overnight in TBS at 4°C prior to a 1h incubation in blocking solution. The tissue was then incubated with primary antibody at 4°C overnight. Primary antibodies used were anti-phospho-S6 (1:100, rabbit, Cell Signalling), anti-Dcx (rabbit monoclonal, 1:2000, Abcam) and anti-BrdU (rat). Secondary antibodies were conjugated with Alexa-488, Cy-3 or Alexa-594 (Invitrogen, CA). Omission of primary antibody served as a negative control.

Microscopy image analysis

All image acquisition and the subsequent analysis was completed by an investigator blinded to the genotype and treatment status of each animal.

Positive immunoreactive area imaging and analysis

For all pS6 analyses, image acquisition software (NIS-Elements software, C2 Tie Nikon confocal microscope) was used to identify the center of the section (tissue depth that contained the maximum area under the curve in the histogram of voxels at each

intensity level). Profile images were then taken at 20x magnification using an epifluorescence microscope (C2 Tie Nikon confocal microscope) capturing the dentate gyrus. All images had identical laser and camera setting, area/pixel (1.23 μ m) and pixel intensity range (0–4095 pixel intensity per 12 bit image). Once all animal sections were imaged, pS6 immunoreactivity was measured using semi-automated image analysis.

The area of pS6 immunoreactivity in the respective region of interest (ROI: GCL, ML, and HL) was identified using thresholding. The binary mask labelled the soma of positively stained cells. For each ROI, the area (mm²) of positive pixels was quantified and normalized to the ROI area. Positive pS6 data was averaged from 3 sections per animal from sham (IGFtg n=5 and WT n=7), moderate CCI (IGFtg n=21 and WT n=18), and severe CCI (IGFtg n=18 and WT n=16) experimental groups.

Cell counting imaging and analysis

For all BrdU+ and BrdU+Dcx+ analyses, images were taken on a confocal epifluorescence microscope (Nikon A1R inverted confocal microscope, Galvano scanner) capturing the epicentre of injury in the dentate gyrus for each animal. The entire ipsilateral DG was imaged at 100x magnification under oil and at 0.75 μ m step intervals through a 20 μ m depth to ensure optical resolution to assess the densely packed BrdU+Dcx+ cells in the GCL. To evaluate the effect of IGF1 on proliferated cells, the number of BrdU+ and BrdU+Dcx+ cells were manually counted in the GCL, HL and ML. Cells were determined to reside in the GCL if they were within one cell distance (0–10 μ m) of the GCL border and the soma was adjacent to other Dcx+ cells along the border (Villasana et al. 2015). Colocalization of markers was confirmed by making 3D reconstructions of the cells of interest to avoid false positives. The volume (mm³) of the

respective ROI (GCL, ML, and HL) was quantified in each section. Cell counts are expressed in density.

Cell localization

Images obtained for BrdU+Dcx+ cell counts were used to assess posttraumatic cell localization. The distance from the hilar border of the SGZ to the center of the cell soma was measured for BrdU+Dcx+ cells in the upper blade of the GCL. The inner GCL (iGCL) layer is defined as the GCL area emanating 0 to 50 μ m from the SGZ (Ibrahim 2016). The outer GCL (oGCL) describes the location of cells residing outward, beyond the iGCL range. Cells localized to the HL, greater than 0-10 μ m from the GCL border were considered ectopic (Villasana et al. 2015). Cell counts and distances were manually quantified.

Sholl analysis

Images obtained for BrdU+Dcx+ cell counts were used to assess dendritic morphology. The Dcx channel immunolabeled hippocampi were acquired as a z-stack (0.75 μ m step size) at 100x magnification using a confocal microscope (Nikon A1R inverted confocal microscope, Melville, NY) from a randomly selected subset of mice (n=4 mice per genotype/treatment). Confocal images were obtained of 8 Dcx-positive immature neurons randomly selected from the DG GCL within a section at the epicenter of injury (-1.9 mm Bregma). BrdU positivity was not assessed during the random selection of neurons. The neuron was not utilized if dendritic processes could not be traced through the confocal z-stack images. Dendritic processes of Dcx-expressing hippocampal granular neurons were semi-manually traced in z-stack images using Image J Simple Neurite Tracer (NIH). The dendritic length was automatically quantified from

trace files by Simple Neurite Tracer. The number of dendritic intersections was quantified for each Dcx-positive neuron by a series of concentric circles (10 μ m intervals) drawn from the center of the cell body using a Sholl analysis plug-in for NIH ImageJ.

Statistical Analyses

Data acquisition was performed by a blinded observer. Graphs were generated and data was analyzed using GraphPad Prism software 5.0. Data presented as mean + standard error of the mean (SEM). Unless otherwise noted, statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Bonferroni selected comparisons post-hoc tests where appropriate. Sholl analysis and cell localization quantified in relationship to distance from the cell soma were analyzed by two-way ANOVA followed by Bonferroni post-hoc tests where appropriate. For all comparisons $p < 0.05$ was considered statistically significant.

Results

IGF1 overexpression potentiates injury-induced mTOR activation in predominantly neuronal sub-regions of DG

To determine if posttraumatic overexpression of IGF1 potentiates injury-induced mTOR activation in the hippocampal DG following injury, we quantified the expression of pS6, a well characterized downstream target of mTOR. The DG of WT (Figure 3.2A-C) and IGFtg (Figure 3.2D-F) brain-injured mice and their sham controls were immunostained for pS6 24h and 72h after moderate (1.0mm) injury. Although pS6 immunoreactivity in WT mice was modestly elevated in the GCL, ML, and HL at 24h after injury, areas within these sub-regions were not significantly different than sham

controls (Figure 3.2G, I, K). At 72h after injury pS6 expression in the DG of injured WT mice was indistinguishable from WT controls (Figure 3.2H, J, L). Injury significantly increased pS6 in the GCL and HL of IGF1 overexpressing mice at 24h after injury in moderately injured mice compared to sham controls (Figure 3.2G, K). The area of pS6 in the GCL of IGFTg mice was also significantly higher than injured WT mice at 24h. Like injured WT mice, pS6 immunoreactivity in the ML of IGFTg mice was not significantly different than sham controls 24h after moderate injury (Figure 3.2I). In IGF1 overexpressing mice, mTOR activity remained elevated in predominantly neuronal regions of the DG at 72h after moderate injury. Both the GCL and HL of IGFTg mice had significantly more pS6 than injured WT and sham controls at 72h after moderate injury (Figure 3.2H, I).

We also examined pS6 immunoreactivity in the DG of WT (Figure 3.3A-C) and IGFTg (Figure 3.3D-F) mice at 24h and 72h after severe injury (1.5mm). Following severe CCI, pS6 immunoreactivity in the GCL of WT mice was indistinguishable from sham controls at 24h and 72h after injury (Figure 3.3G, H). In contrast, severe injury stimulated a robust increase in pS6 expression in the ML of WT mice at 24h and 72h after injury (Figure 3.3I, J) and a transient increase in the HL (Figure 3.3K) compared to sham controls. pS6 in the ML, a predominantly non-neuronal layer, co-labeled with the glial marker for astrocytes (Figure 3.4A). As with moderate injury, IGF1 overexpression resulted in significantly increased pS6 signal in the GCL following severe injury. At 24h after severe injury, pS6 immunoreactivity in IGFTg mice was significantly elevated compared to shams controls only in the GCL (Figure 3.3G), whereas at 72h pS6 was significantly increased in the GCL, ML and HL compared to sham controls (Figure 3.3H,

J, L). In the predominantly neuronal regions of the DG (GCL and HL), pS6 immunoreactivity was also significantly higher than injured WT mice (Figure 3.3H, L).

Taken together, injury-induced mTOR signaling in the DG is sensitive to injury severity. pS6 immunoreactivity is increased at 24h following moderate injury and 72h following severe injury in IGFtg mice. By 10d after injury pS6 expression in the DG of WT and IGFtg mice is indistinguishable from sham controls (data not shown). Interestingly, regardless of severity, injury strongly induces mTOR activation in the GCL of IGF1 overexpressing mice. IGF1 is such a potent activator of mTOR that 72h after moderate injury, even the contralateral GCL and HL displayed increased mTOR activity in IGF1 overexpressing mice compared to injured WT mice and shams (Figure 3.5). Based on these initial findings that 1) IGF1 overexpression sufficiently potentiated mTOR activation after moderate CCI and 2) moderate injury produced selective effects on neuronal regions of the DG of IGFtg mice, the remaining cohorts (Figure 3.1B, C) received moderate injury.

Potentiation of mTOR activation by IGF1 overexpression does not enhance early posttraumatic proliferation

To first validate that the selected and well characterized dose of rapamycin (10mg/kg) (Butler et al. 2015, Butler et al. 2016) was sufficient to inhibit mTOR activity, injured WT and IGFtg mice were treated with daily injections of rapamycin or its vehicle for 3d beginning 1h after CCI (Figure 3.1B). At 72h after moderate injury, S6 activity was effectively silenced in both rapamycin treated WT and IGFtg mice compared to vehicle treated mice (Figure 3.6). This dose was used then to examine the effect of mTOR inhibition on posttraumatic neurogenesis following IGF1 overexpression.

Three days after moderate injury, at the peak of trauma-induced proliferation (Rola et al. 2006), dividing cells in WT (n= 15) and IGFtg (n=14) mice were labeled with BrdU (Figure 3.7A-D). To prevent rapamycin-induced inhibition of posttraumatic NSC proliferation (Paliouras et al. 2012, Butler et al. 2015, Wang et al. 2016), we delayed rapamycin treatment until the labeling of cells proliferated 3dpi had been completed. Although IGF1 overexpression potentiates mTOR activation in the DG within the first 3 days after TBI, both vehicle treated WT (n=7) and IGFtg (n=6) mice had similar densities of proliferated cells in the DG at 10dpi (Figure 3.7E). Similarly, rapamycin did not alter the density of proliferated cells that survived to 10 days in the DG of WT (n=8) and IGFtg (n=8) mice. All four injury groups had similar BrdU+ cell densities throughout the DG (Figure 3.7E) and between DG sub-regions (GCL, ML and HL) (Data not shown). Injured WT mice irrespective of treatment tended to have a higher proportion proliferated DG cells residing in the ML compared to injured IGFtg (1-way ANOVA, p=0.09). Taken together, IGF1 overexpression did not promote increased acute proliferation and the survival of these proliferated cells was not altered by delayed rapamycin treatment. We next examined the role of mTOR signaling in posttraumatic neural differentiation, migration, and growth in these mice.

Rapamycin administration increases posttraumatic neural differentiation in IGF1 overexpressing mice.

The pathway by which IGF1 stimulates neuronal differentiation in the context of injury is unknown. To determine whether mTOR signaling is important for cell fate determination in posttrauma proliferated progenitors following IGF1 overexpression, we quantified the numbers of pretreatment-proliferated (BrdU+) cells that expressed

immature neuron marker (Dcx+) at 10dpi in WT and IGFtg mice following vehicle or rapamycin administration (Figure 3.8A-D). The density of posttrauma proliferated immature neurons in injured WT mice was not altered by rapamycin treatment (Figure 3.8E). IGF1 overexpression did not induce a significant increase in the density of 3dpi proliferated immature neurons (15 ± 1.2 1000/mm³) when compared to WT mice (8 ± 1.3 1000/mm³) following vehicle treatment. However, following rapamycin treatment the density of 3dpi proliferated immature neurons in IGFtg mice (27 ± 3.8 1000/mm³) was 2.5 fold higher than in WT mice (11 ± 2.6 1000/mm³) and nearly 2-fold higher than vehicle-treated IGFtg mice. Rapamycin treatment increased the proportion of BrdU+ cells that commit to a neuronal fate in IGF1 overexpressing mice ($78 \pm 6\%$) compared to WT mice ($42 \pm 8\%$) (Figure 3.8F). The proportion of proliferated cells that had differentiated into immature neurons by 10dpi was similar for both groups of vehicle-treated mice and for WT mice treated with rapamycin. Taken together, rapamycin treatment increased neuronal differentiation in IGF1 overexpressing mice.

Rapamycin treatment does not alter posttraumatic positioning of immature neurons

Injury stimulates migration of SGZ generated immature neurons radially, deeper into the GCL (Villasana et al. 2015, Ibrahim et al. 2016), and ectopically, aberrantly into the hilus (Shapiro 2017). The signaling governing this process is not well understood. Endogenous signaling of both IGF1 and mTOR has been implicated in the regulation of adult-born neurons in the uninjured brain (Garza-Lombo et al. 2016, Nieto-Estevez et al. 2016). At 10dpi the positioning of posttrauma proliferated immature neurons (BrdU+ Dcx+) within the DG of WT and IGFtg mice following mTOR inhibition was examined. The majority of 7-day old neurons in the GCL of both WT and IGFtg mice migrated

within 30 μ m of the SGZ and localized to iGCL irrespective of treatment group (Figure 3.9B). Similar numbers of posttrauma-born immature neurons localized to the iGCL in vehicle-treated WT and IGFtg mice, as well as in rapamycin-treated WT mice (Figure 3.9B). Reflective of the dramatic increase in posttraumatic neural differentiation, the number of 7-day old neurons localized to the iGCL in rapamycin-treated IGF1 overexpressing mice was significantly increased compared to all other groups (Figure 3.9B). Similar numbers and proportion of 7-day old immature neurons migrated deep within the GCL and localized to the oGCL in all injury groups (Figure 3.9C). The population of oGCL-positioned, posttrauma-born immature neurons at 10dpi was not altered by IGF1 overexpression or inhibition of mTOR activation.

To investigate the effect of mTOR activation on injury-induced ectopic migration, the number of 7-day old BrdU+Dcx+ neurons in the HL was quantified. A small number of posttrauma-born neurons localized to the HL in all injury groups (Figure 3.9A). IGF1 overexpressing mice had an increased density of ectopically localized 7-day old neurons (2.4 ± 0.95 1000/mm³) compared WT mice (0.22 ± 0.15 1000/mm³) following vehicle treatment (Figure 3.9D). Rapamycin treatment had no significant effect on injury-induced ectopic localization of 3dpi proliferated neurons in WT (0.62 ± 0.25 1000/mm³) or IGFtg (2.3 ± 0.33 1000/mm³) mice at 10dpi (Figure 3.9D). While IGFtg mice had an increased density of ectopically localized 3dpi proliferated neurons compared to WT mice, ectopic neurons represented a similar proportion of the total number of 3dpi proliferated neurons in the DG for all four groups at 10dpi (Figure 3.9E). Although rapamycin increased the proportion of proliferated cells that became neurons in IGF1 overexpressing animals, it did not alter the proportion of proliferated neurons that localized ectopically.

IGF1 overexpression confers resistance to rapamycin sensitive posttraumatic arbor development in immature neurons

To determine if IGF1 overexpression stimulates dendritic outgrowth in an mTOR dependent manner following injury, we used Sholl analysis to measure length and complexity of dendritic arbors of immature neurons 10dpi in rapamycin and vehicle treated WT and IGFtg mice (Figure 3.10A-D). Consistent with our previous study (Carlson et al. 2014), we found that in vehicle treated mice IGF1 overexpression increased dendritic length of immature neurons compared to WT mice at 10dpi (Figure 3.10E). mTOR inhibition did not significantly alter immature neuron arbor length in either WT or IGFtg mice compared to their respective vehicles groups (Figure 3.10E). Since neither genotype's arbor length was altered by rapamycin treatment, rapamycin-treated IGFtg mice still had significantly longer dendrites than the rapamycin-treated WT mice.

We also averaged the sum of concentric ring intersections per immature neuron for each group to elucidate the effect of mTOR inhibition and IGF1 overexpression on dendritic complexity. In vehicle-treated cohorts, IGF1 overexpression did not significantly increase the total number of arbor crossings of immature neurons compared to WT (Figure 3.10F). mTOR inhibition dramatically diminished arbor complexity in WT mice. Rapamycin reduced total dendritic shell intersections 1.5-fold in WT mice compared to their vehicle-treated controls (5.6 ± 0.5 vs 8.9 ± 0.6 intersections; Figure 3.10F). Immature neurons in IGF1 overexpressing mice were resistant to rapamycin induced impairment of arbor complexity. Total dendritic shell intersections following injury for vehicle (11.4 ± 0.9 intersections) and rapamycin (11.5 ± 0.6 intersections)

treated IGFtg mice were similar (Figure 3.10F). The total arbor crossings of immature neurons in the two rapamycin-treated groups were significantly different from each other, and the reduction in WT arbor complexity made the IGF1 enhancement of arbors even more pronounced (Figure 3.10F).

To understand the relationship between dendritic length and complexity, we analyzed the intersections per shell in relation to the dendritic distance from the soma (two-way ANOVA; significant interaction, $F(51, 2052) = 4.18, p = 0.0001$). With vehicle-treated groups, IGF1 overexpression significantly increased immature neuron dendritic complexity at 60-80 μ m from soma compared to WT mice (Figure 3.10G). mTOR inhibition caused a dramatic downward-shift in dendritic complexity at 50 μ m from the cell in WT mice compared to vehicle treatment (Figure 3.10G). IGF1 overexpressing mice were completely protected from rapamycin-induced reduction of dendritic complexity. At no distance was complexity different between vehicle- and rapamycin-treated IGFtg cohorts. In the presence of rapamycin, IGFtg arbor complexity was significantly higher than WT at 40-90 μ m from soma (Figure 3.10G). IGF1 overexpression relieves posttraumatic dendritic development in immature neurons from mTOR activity dependence.

Discussion

Summary of findings

The key findings of this study determine if IGF1 overexpression modulates posttraumatic neurogenesis through mTOR activation. We first investigated the sub-regional changes in DG mTOR activity and found that while trauma preferentially

induced mTOR activation in the ML layer, IGF1 selectively potentiated acute trauma-induced mTOR activity in the GCL and HL. Next, we found that potentiation of mTOR activation by IGF1 overexpression did not enhance proliferation and that mTOR activation is not required for survival of posttrauma proliferated cells. mTOR activation is not required for posttraumatic neuronal differentiation of NSCs, but its inhibition potentiates IGF1-induced neuronal differentiation. Finally, we found that mTOR activation is required for posttraumatic dendritic arborization. However, the IGF1 overexpression enhances dendritic arborization and prevents rapamycin-induced reduction of dendritic complexity following trauma.

mTOR activation

IGF1 overexpression potentiated injury-induced mTOR activation in the predominantly neuronal regions of the DG. We show that severe injury significantly increases mTOR activation in the ML at 24h and 72h after injury. This is consistent with hippocampal homogenate studies that show that AKT/mTOR/S6K activity is increased between 24h to 72h after injury (Jenkins et al. 2002, Chen et al. 2007, Park et al. 2012). Similar to previous studies, we found that mTOR is predominantly activated in glia of WT mice following injury (Park et al. 2012, Zhu et al. 2014). While the IGF1 receptor is expressed on glia and essentially all neural cell types, neurons and NSCs have very high expression of IGF1 receptor (Ocrant et al. 1988). We found that IGF1 potentiates injury-induced mTOR activation at 24h and 72h after injury in the GCL and HL. IGF1 can signal through the MAPK/ERK pathway, but predominantly signals through the IRS1/PI3K/AKT pathway (Nieto-Estevez et al. 2016) in the CNS. Both the mTOR pathway and the ERK pathway can stimulate aspects of neurogenesis. Injury itself

upregulates IGF1 receptor signaling, as well as AKT/mTOR and MAPK/ERK signaling in the hippocampus (Mori et al. 2002, Chen et al. 2007, Madathil et al. 2010, Rubovitch et al. 2010). We previously showed that IGF1 overexpression potentiates injury-induced AKT activation in the hippocampal homogenates (Madathil et al. 2013). This expands our previous understanding of IGF1 overexpression signaling in the context of injury and reveals preferential IGF1 signaling in predominantly neuronal regions.

Proliferation and early survival

IGF1 overexpression does not enhance early (3d) posttraumatic cell proliferation and inhibiting mTOR does not alter their survival. IGF1 overexpressing and WT mice had a similar densities of 3dpi proliferated cells in the DG and similar densities distributed in the GCL, ML, and HL DG sub-regions cells at 10dpi. Brain injury stimulates proliferation throughout all layers of the DG that peaks 3dpi (Dash et al. 2001, Rola et al. 2006, Gao et al. 2008). Consistent with our previous study, IGF1 overexpression does not potentiate the density of newly divided cells generated by injury-induced proliferation (Carlson et al. 2014).

When quantifying the magnitude of proliferation using BrdU labeling, both cell division and programmed cell death must be taken into consideration. Particular attention is given to the SGZ, a proliferative sub-region of the DG that can generate GCL neurons throughout adulthood. In the uninjured adult brain, Bax-mediated apoptosis plays an important role in eliminating proliferated progenitors in the SGZ, as 29% die within 10d after division (Dayer et al. 2003, Sun et al. 2004). Additionally, expression of pro-apoptotic mediators of programmed cell death is upregulated during the first 14d after injury (Schober et al. 2010). During CNS development and in NSC cultures IGF1 can

increase the density of proliferated neural progenitors through several PI3K dependent mechanisms including regulation of cell division and cell death (Otaegi et al. 2006, Han et al. 2008, Ye et al. 2010, O'Kusky et al. 2012, Zhu et al. 2012, Huat et al. 2014, Wahane et al. 2014). Whether or not IGF1 increases the population of proliferated progenitors and by what mechanism is regulated in part by the neurogenic environment and developmental stage (Cao et al. 2003, Joseph D'Ercole et al. 2008, Liu et al. 2009, Mairet-Coello et al. 2009, Wahane et al. 2014). In the postnatal brain, IGF1 predominantly promotes survival by upregulating expression of anti-apoptotic genes like BCL-2, which suppresses programmed cell death (Baker et al. 1999, Cao et al. 2003, Kim et al. 2006). Taken together, IGF1 is not enhancing the density of proliferated cells directly or indirectly in the context of trauma. It is possible that IGF1 signaling does not synergistically increase proliferation following injury due to ceiling effect caused by decreased NSC availability. The dramatic spike in NSC division caused by trauma reduces the pool of NSC available for future proliferation (Neuberger et al. 2017). Our study suggests that IGF1 does not potentiate injury-induced cell division, which is consistent with our previous report (Carlson et al. 2004).

Rapamycin does not modulate early survival of proliferated cells in the DG. At 10dpi vehicle-treated and rapamycin-treated mice had similar densities cells which proliferated at 3dpi, prior to rapamycin onset. Rapamycin was recently shown to reduce numbers of degenerating neurons in the DG following TBI (Gao et al. 2008, Nikolaeva et al. 2016). Our study suggests that posttrauma-born cells are not a population of DG cells that are protected following rapamycin treatment.

Differentiation

IGF1 signaling has a well-defined role in stimulating NSC neuronal differentiation (Otaegi et al. 2006). Further, insulin-induced neuronal differentiation of cultured rat NSCs was diminished by mTOR inhibition (Han et al. 2008). Our previous study showed that IGF1 overexpression robustly stimulates injury-induced neuronal differentiation in NSCs following severe TBI (Carlson et al. 2014). We hypothesized that IGF1 overexpression would stimulate neuronal differentiation through mTOR activation. However, in our current study, we found that IGF1 overexpression did not significantly enhance neuronal differentiation in vehicle-treated mice following moderate injury. The degree of neurogenesis induced was likely abrogated due to severity; the extent of injury induced neurogenesis has been shown to be injury severity dependent (Wang et al. 2016).

Nonetheless, we found that mTOR activity is not required for neuronal differentiation of proliferated NSCs after injury. This is the first study to investigate the role of posttraumatic mTOR activation in NSC neuronal differentiation in the context of injury. When mTOR activation is genetically inhibited by knocking out its activator Rheb or inhibited by rapamycin in the uninjured brain, neuronal differentiation of NSCs is inhibited (Hartman et al. 2013, Wahane et al. 2014, Garza-Lombo et al. 2016). However, the function of mTOR signaling is sensitive to cell context, available resources, and cellular stress (Gunn et al. 2008, Laplante et al. 2009, Hung et al. 2012). We found that mTOR inhibition by rapamycin does not inhibit neuronal differentiation in the context of injury.

Interestingly, inhibiting mTOR activation following posttraumatic overexpression IGF1 increased NSC neuronal differentiation after injury compared to vehicle-treated

IGFtg and rapamycin-treated WT mice. A plausible explanation is that mTOR negatively feeds-back on IGF1 receptor signaling. Studies have shown that mTORC1 signaling provides negative feedback on IGF1/Insulin signaling by inhibitory phosphorylation of Insulin receptor substrate-1 (IRS-1) (Carlson et al. 2004, Harrington et al. 2004, Um et al. 2004). There is evidence that mTOR inhibition can stimulate hypersensitivity to IGF1/insulin signaling by blocking inhibitory feedback of mTOR on IGF1/Insulin signaling, resulting in increased activation of IRS1/PI3K/AKT signaling (Veilleux et al. 2010). Alternatively, hypersensitivity to IGF1 can potentially redirect signaling through the alternative MAPK pathway. Studies have shown that rapamycin treatment induces both AKT activation and ERK activation (Carracedo et al. 2008, Rastogi et al. 2013). Our study provides evidence that IGF1 overexpression can enhance neuronal differentiation in the presence of mTOR inhibition.

Migration

We found that neither IGF1 overexpression nor mTOR inhibition altered outward migration of posttrauma-born immature neurons into the oGCL or ectopic migration into the HL. Injury increases localization of posttrauma-born neurons to the oGCL, where more mature neurons reside (Villasana et al. 2015, Ibrahim et al. 2016). Consistent with these studies, we found that a small number of immature neurons localized to the outer 2/3 layer of the GCL in all injured animals. We found no increase in the proportion of 7-day old immature neurons that migrated into the oGCL related to treatment or genotype. This was surprising, considering that both IGF1 signaling and mTOR activation can modulate immature neuron migration in an uninjured brain. When the IGF1 receptor is knocked-out during development, SVZ immature neurons fail to migrate to the olfactory

bulb and prematurely accumulate en route (Hurtado-Chong et al. 2009). Conversely, hyperactivation of AKT or mTOR can accelerate newborn neuron dendritic development, integration, and radial migration into the oGCL (Duan et al. 2007, Kim et al. 2009). Both AKT and mTOR increase the proportion of immature neurons that localize to the oGCL in the naïve adult brain, and rapamycin abolishes these effects (Duan et al. 2007, Kim et al. 2009, Zhou et al. 2013). It is likely that the lack of effect of IGF1 overexpression or mTOR inhibition in our study is attributed to the immaturity (7-days old) of the posttrauma proliferated neurons. Duan and colleagues directly hyperactivate mTOR in newborn neurons of the GCL by silencing Disrupted-In-Schizophrenia 1 protein (Disc1), an upstream inhibitor of mTOR (Duan et al. 2007). Compared to controls, when neurons are only 7-days old there is no increase in the proportion that localized to the oGCL (Duan et al. 2007). However, by 2 weeks of age nearly 50% of immature neurons are localized to the oGCL (Duan et al. 2007). We have shown that 6 weeks after proliferation, the proportion of posttrauma-born neurons localized to the oGCL is increased in injured IGF1tg mice compared to controls (Figure 2.6).

Posttraumatic ectopic migration is not dependent on mTOR activation. At 10dpi, 7-day old posttrauma-born neurons were ectopically localized to the HL in all injured groups. Neither IGF1 overexpression nor mTOR inhibition altered the distribution of 7-day old posttrauma-born neurons that ectopically localized to the HL after injury. Compared to WT mice, both IGF1 overexpressing treatment groups had a higher density of 3dpi proliferated neurons in the DG, and both had a significantly higher density of these 7-day old neurons localized to the HL. The increase in ectopically localized immature neurons is consistent with a recent moderate injury study. In the fluid

percussion model of TBI, the density of immature neurons of heterogenous age (1 to 3 weeks old) that ectopically localized to the HL at 7dpi were increased compared to sham controls (Shapiro 2017). The function of these ectopic immature neurons remains unclear, and many of them will not survive to maturity. Adult-born, immature neurons position within 1-2 weeks of their birth (Kuhn et al. 1996, Kempermann et al. 2003, Myers et al. 2013). The majority of ectopically placed immature neurons undergo programmed cell death within this time frame (Sun et al. 2004, Buss et al. 2006, Kim et al. 2007, Ryu et al. 2016). A subset of ectopically localized neurons survive to maturity following injury (Figure 2.6).

It was surprising that mTOR inhibition did not alter ectopic migration of 3dpi proliferated neurons. When the function of mTOR suppressor TSC1 is lost, mTOR activation increases and ectopic migration of adult-born neurons increase (Meikle et al. 2007). Both, mTOR and ectopic neuron localization have been associated with seizure development (Parent et al. 1997, Butler et al. 2015). Rapamycin inhibition of posttraumatic seizure development may not be associated with HL ectopic localization (Guo et al. 2013, Butler et al. 2015). Our study provides evidence that injury does not mediate ectopic localization of immature neurons via the mTOR pathway. The density of posttrauma-born neurons localized to the HL was not altered by rapamycin treatment in either genotype.

Dendritic arbor development

We found that mTOR activity is required for posttraumatic arbor development of immature neurons. The dendritic arbor complexity of immature neurons in rapamycin-treated WT animals was severely stunted compared to that of vehicle-treated WT mice.

Dendritic arbors of immature neurons in the DG are shorter and less complex following severe injury (Carlson et al. 2014, Ibrahim et al. 2016). Many studies examining adult neurogenesis in uninjured animals have shown that mTOR activity regulates dendritic arborization of developing neurons (Jaworski et al. 2005, Tavazoie et al. 2005, Kim et al. 2009, Feliciano et al. 2012). This is the first study to show that posttraumatic dendritic arborization is dependent on mTOR activation.

We show that IGF1 overexpression increases dendritic length and complexity of immature neurons after injury. This is consistent with a previous study that showed that IGF1 overexpression enhanced dendritic length and arbor complexity of immature neurons, restoring them to uninjured levels (Carlson et al. 2014). Interestingly, IGF1 overexpression did not mediate enhancement of immature neuron dendritic arborization through mTOR activation. It was surprising the mTOR inhibition did not alter IGF1-induced arbor enhancement. PI3K activation, a major signaling molecule downstream of IGF1 receptor stimulation, was shown to increase arbor development in neuronal cell cultures in an mTOR-dependent fashion (Jaworski et al. 2005). Similarly, increased activity of AKT, which signals downstream of PI3K, increases dendritic arbor length and complexity in an mTOR dependent manner (Kim et al. 2009). Trauma upregulates mTOR activation in the hippocampus and in DG neural progenitors (Chen et al. 2007, Wang et al. 2016). Although PI3K/AKT signaling predominates in mediating IGF1 signaling in the CNS, the other major arm of IGF1 signaling pathway is still viable. However, very little data exists about the role of RAS/MAPK/ERK in posttraumatic neurogenesis. Both AKT/mTOR and MAPK/ERK can mediate dendritic growth. In neuronal cultures it was shown that MAPK/ERK inhibitors could block exogenous IGF1 induced dendritic arbor

length and complexity (Kim et al. 1997). IGF1 overexpression is likely mediating posttraumatic dendritic enhancement through an alternative pathway. We show here for the first time that posttraumatic IGF1 overexpression mediates outgrowth independent of mTOR activation. The function of enhanced dendritic development may underlie the ability of IGF1 to improve posttraumatic cognitive recovery in DG dependent assessments (Saatman et al. 1997, Madathil et al. 2013). IGF1 can expand the dendritic field, possibly enhancing integration of adult-born neurons. Our study provides evidence that IGF1 can be used in combination with posttraumatic treatments like rapamycin, and prevent seizure development and simultaneously support proper arbor development.

Considerations

Our study provides evidence that enhancement of posttraumatic neurogenesis by IGF1 is independent of mTOR activation. This has far reaching implications. In chemically induced seizure models and trauma induced seizure models, immature neuron numbers increase. However, whether increased neurogenesis participates in the etiology of seizure development or is a byproduct of seizure activity remains controversial. However, there is consensus that treatment with rapamycin reduces seizure development. Rapamycin inhibits neurogenesis, prevents aberrant sprouting of recurrent mossy fibers, and reduces seizure behavior (Buckmaster et al. 2011, Butler et al. 2015, Butler et al. 2016, Butler et al. 2017). This study suggests that IGF1 treatment could be administered with rapamycin as a preclinical therapeutic. This work significantly enhances the body of knowledge regarding the role of mTOR activation and IGF1 in posttraumatic neurogenesis.

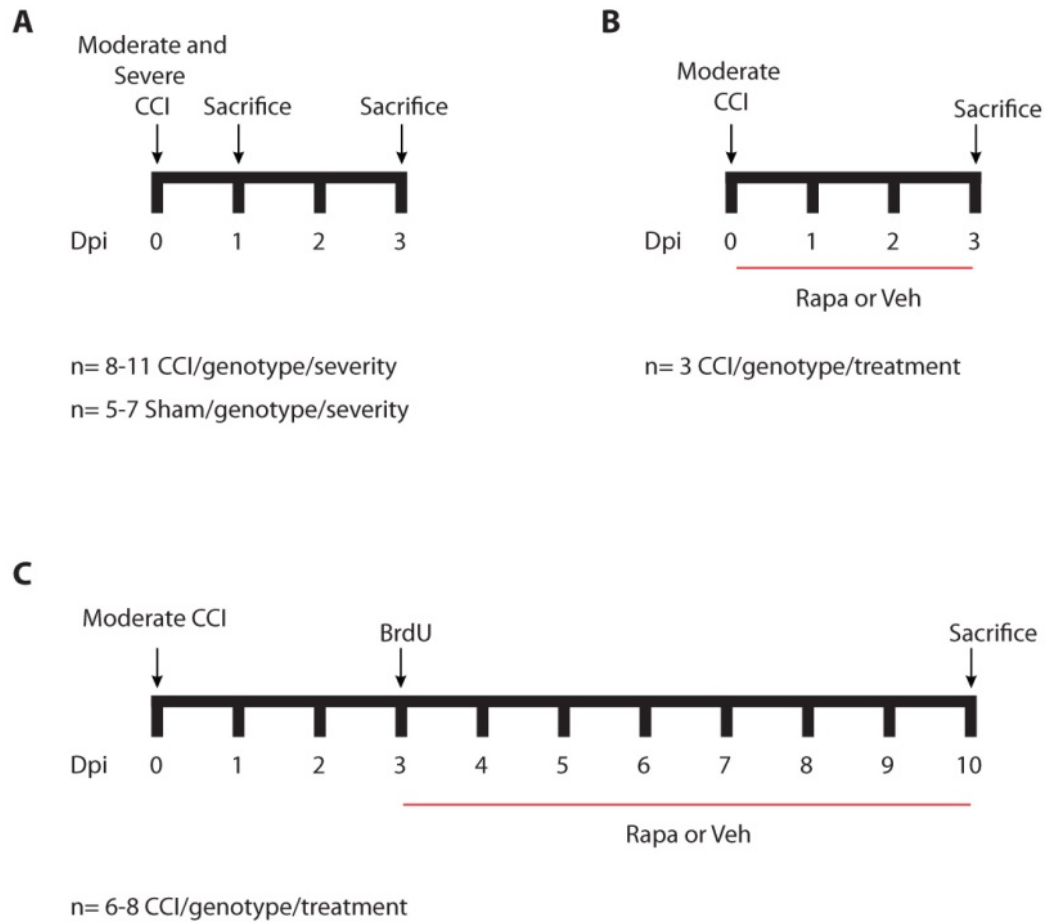
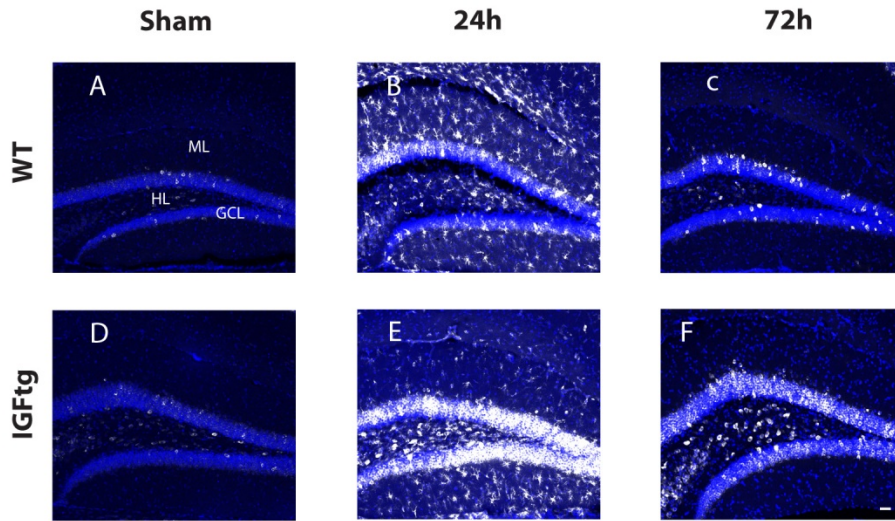


Figure 3.1: Experimental design.

(A) To assess posttraumatic mTOR activity, cohorts of mice were sacrificed 24h or 72h after moderate or severe controlled cortical impact (CCI) injury. Sham mice were sacrificed 72h after receiving a craniotomy. (B) A second cohort of moderately injured mice were treated daily with 10mg/kg rapamycin (Rapa) or vehicle (Veh) beginning 1hr after injury for 3 days to validate effective mTOR inhibition by rapamycin. (C) A third cohort of mice were treated daily with 10mg/kg rapamycin or vehicle beginning 3d after injury and continuing until euthanasia at 10d after injury to examine the contribution of mTOR signaling on posttraumatic neurogenesis. These mice received three injections of BrdU (50mg/kg) 4hrs apart beginning on day 3 post-CCI. Dpi, days post-injury.

Moderate injury



□ WT
 ■ IGFtg

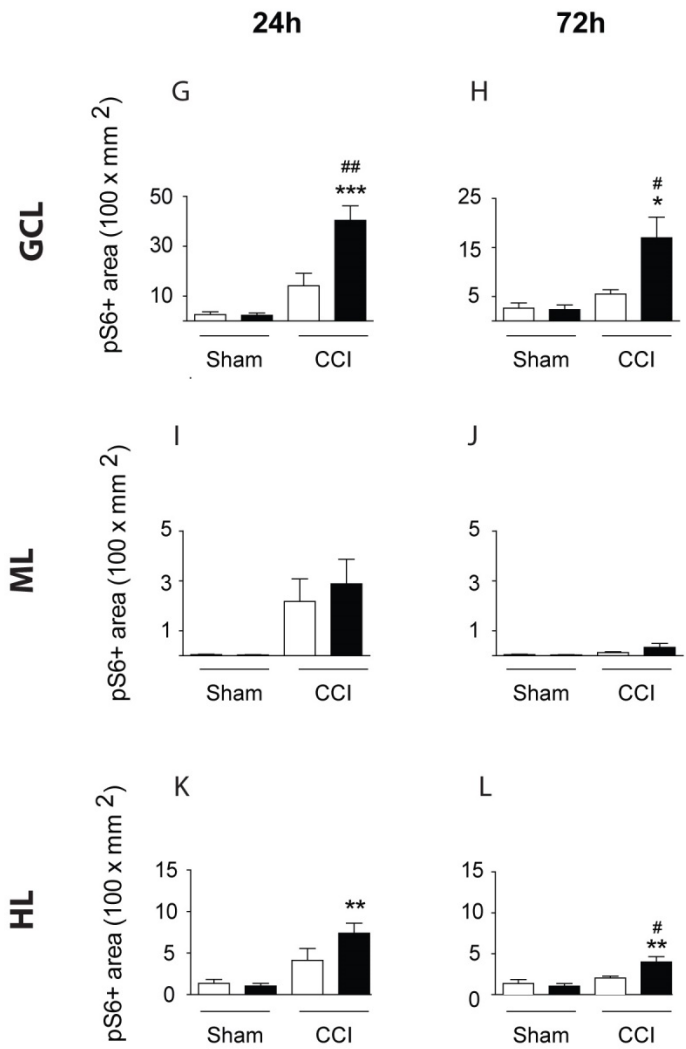
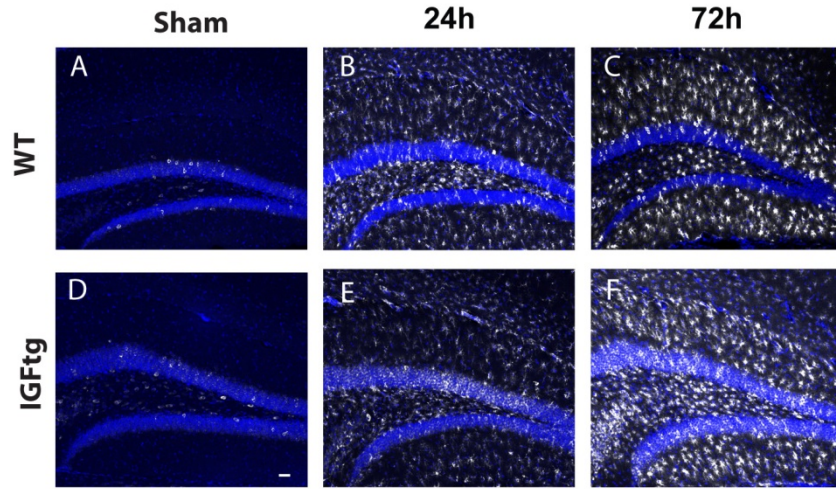


Figure 3.2: IGF1 selectively potentiates injury-induced mTOR activity in the dentate gyrus GCL and HL after moderate injury.

Representative microphotographs from confocal imaging of immunoreactivity of phospho-S6 Ribosomal Protein (pS6, white), a well characterized marker of mTOR activity, in wildtype (WT; A-C) and insulin-like growth factor 1 transgenic (IGFtg; D-F) mice following (A,D) sham injury and (B,E) 24h and (C,F) 72h after moderate controlled cortical impact (CCI) injury. In the granule cell layer (GCL), IGFtg mice exhibit a significantly greater area of pS6 staining at (G) 24h and (H) 72h after CCI when compared to sham IGFtg mice or injured WT mice. In the molecular layer (ML), neither moderate CCI or IGF1 overexpression altered pS6 levels at (I) 24h or (J) 72h post-injury. In the hilus (HL), the area of pS6 labeling was significantly elevated in injured IGFtg mice at (K) 24h and (L) 72h. Data are presented as mean + SEM. ; One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc, * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$ CCI vs Sham. # $p < 0.05$ and ## $p < 0.01$ injured IGFtg vs. injured WT. Scale bar is 100 μ m. DAPI is purple

Severe injury



□ WT
■ IGFtg

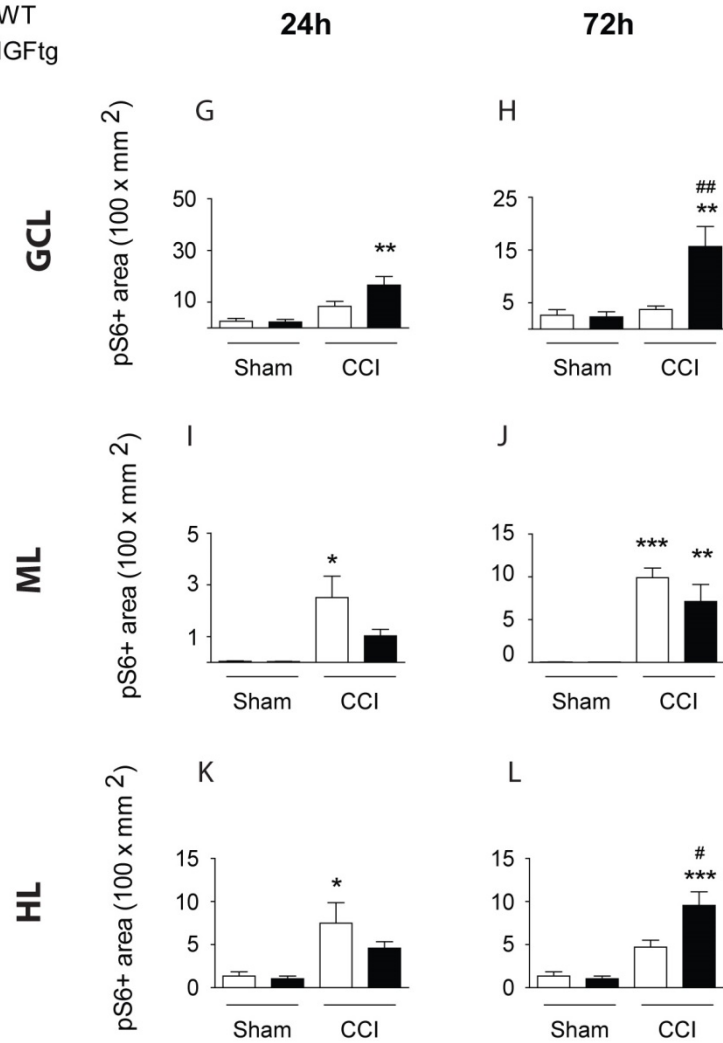
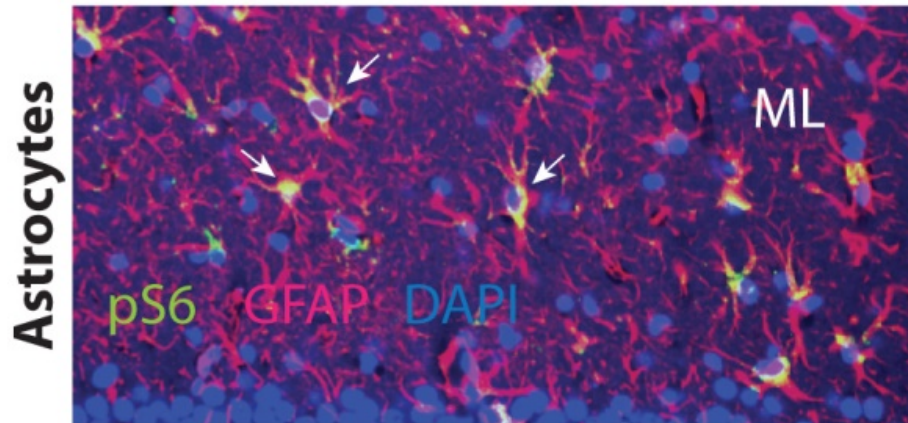


Figure 3.3: IGF1 selectively potentiates injury-induced mTOR activity in the dentate gyrus GCL and HL after severe injury.

Representative microphotographs from confocal imaging of immunoreactivity of phospho-S6 Ribosomal Protein (pS6, white) in wildtype (WT; A-C) and insulin-like growth factor 1 transgenic (IGFtg; D-F) mice following sham (A,D) injury and (B,E) 24h and (C,F) 72h after severe controlled cortical impact (CCI) injury. In the granule cell layer (GCL), IGFtg mice exhibit a significantly greater area of pS6 staining compared to IGFtg sham mice at (G) 24h and (H) 72h after CCI. At 72h after CCI, pS6 levels in the GCL of IGFtg mice are also increased when compared to injured WT mice. In the molecular layer (ML), severe CCI increased pS6 levels at (I) 24h post-injury in injured WT mice, but not injured IGFtg mice compared to their sham controls. (J) Severe injury increased the levels of pS6 staining in the ML of injured WT and injured IGFtg mice compared to their sham controls. In the hilus (HL), severe CCI increased the area of pS6 labeling at (K) 24h after CCI in injured WT mice, but not injured IGFtg mice compared to their sham controls. IGFtg mice exhibit a significantly greater area of pS6 staining at (I) 72h after CCI when compared to sham IGFtg mice or injured WT mice. Data are presented as mean + SEM. One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc, * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$ CCI vs. Sham. # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ injured IGFtg vs. injured WT. Scale bar is 100 μ m. DAPI is purple.

A



B

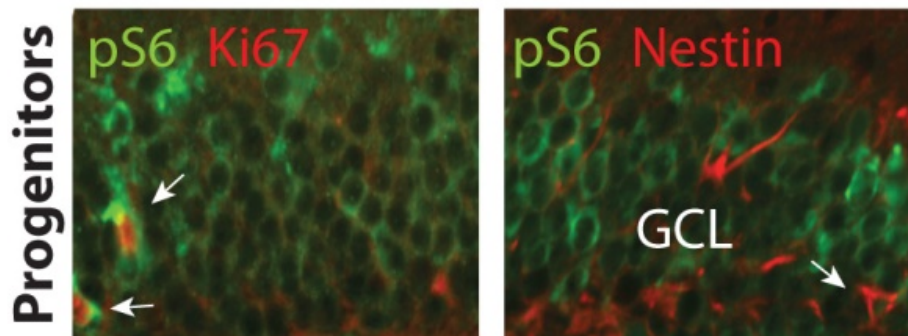


Figure 3.4: Following injury pS6 is activated in astrocytes and progenitors in the DG.

(A) Confocal imaging of astrocyte immunoreactivity for phospho-S6 Ribosomal Protein (pS6, green) and Glial Fibrillary Acidic Protein (GFAP, magenta) in the molecular layer (ML) 72h after severe injury. Dapi is blue (B) Microphotographs depict immunolabeling of pS6 positive cells in the granule cell layer (GCL) and its subgranular zone that are also immunoreactive for the cell division marker Ki67 (red) and the progenitor marker Nestin (red). Arrows are white.

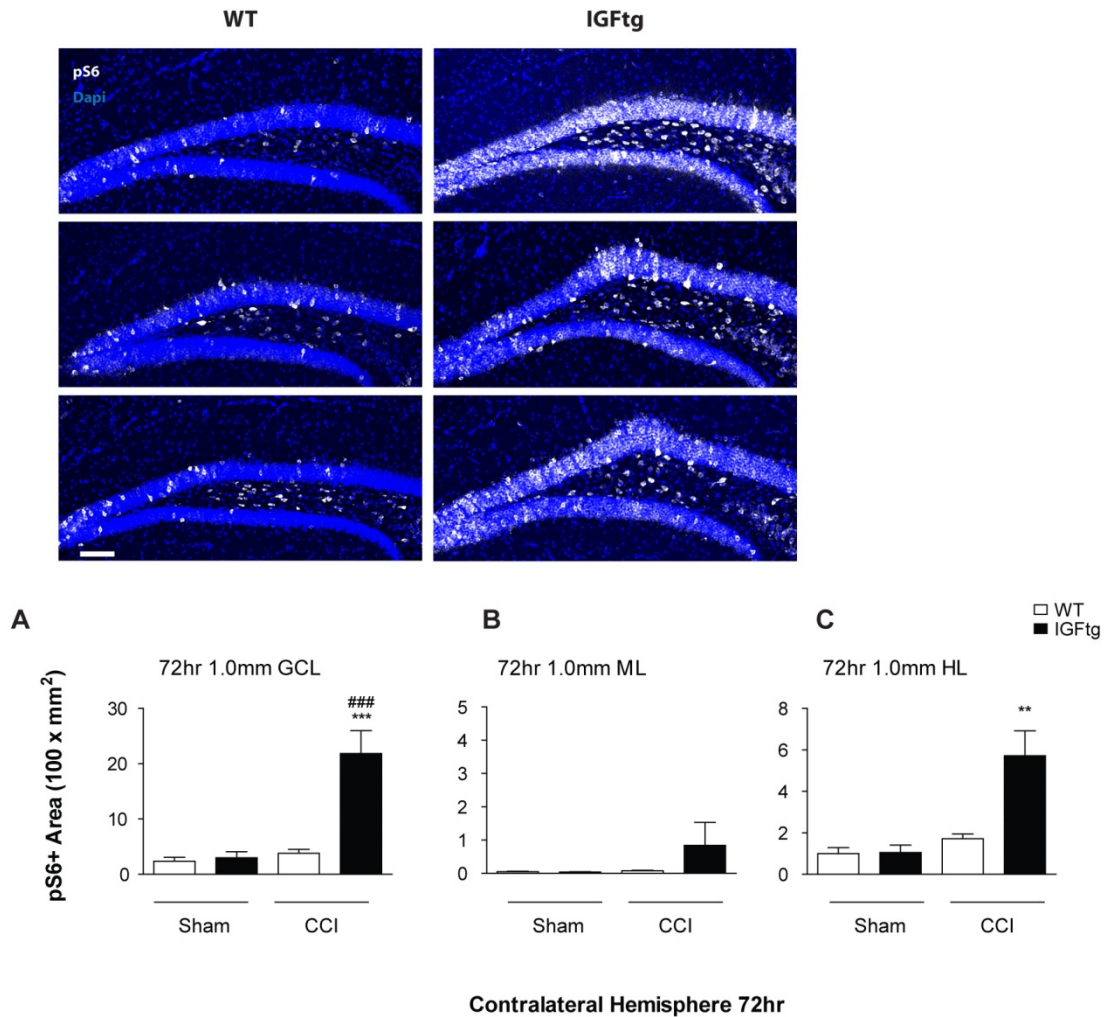


Figure 3.5: Moderate injury is sufficient to isolate IGF1 overexpression induced mTOR activation in neuronal layers of contralateral hemisphere.

Photomicrographs of phospho-S6 Ribosomal Protein (pS6, white) immunoreactivity in the contralateral dentate gyrus (DG) of injured WT (left panels) and injured insulin-like growth factor 1 transgenic (IGFtg; right panels) mice 72h after moderate injury (3 representative animals per group). Posttraumatic upregulation of mTOR activity 72hr after moderate injury, is observed contralateral to injury in the (A) granule cell layer (GCL) and (C) hilus (HL) of injured IGFtg mice compared to IGFtg shams. Moderate injury does not induce mTOR activation in the (B) molecular layer (ML) of the DG contralateral to injury. Data are presented as mean + SEM. One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc, ** $p < 0.01$; and *** $p < 0.001$ CCI vs. Sham. ### $p < 0.001$ injured IGFtg vs. injured WT. DAPI is purple. Scale bar is 100 μ m.

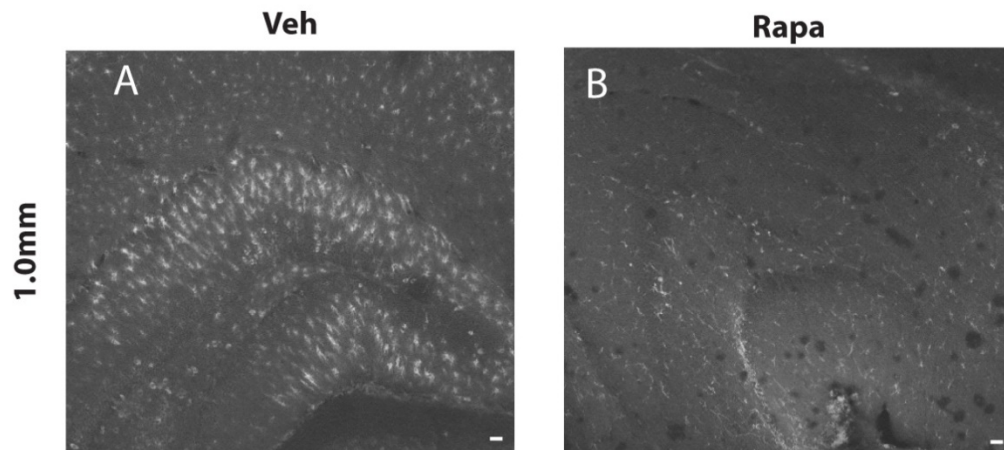


Figure 3.6: Daily administration of rapamycin inhibits posttraumatic mTOR activation 72hr following injury.

Representative images of phospho-S6 Ribosomal Protein (pS6, white) immunoreactivity in the ipsilateral dentate gyrus following daily i.p. injections of (A) vehicle (veh) and (B) 10mg/kg rapamycin (Rapa) for 3 days after moderate injury (3d post-injury survival). Scale bar is 50µm.

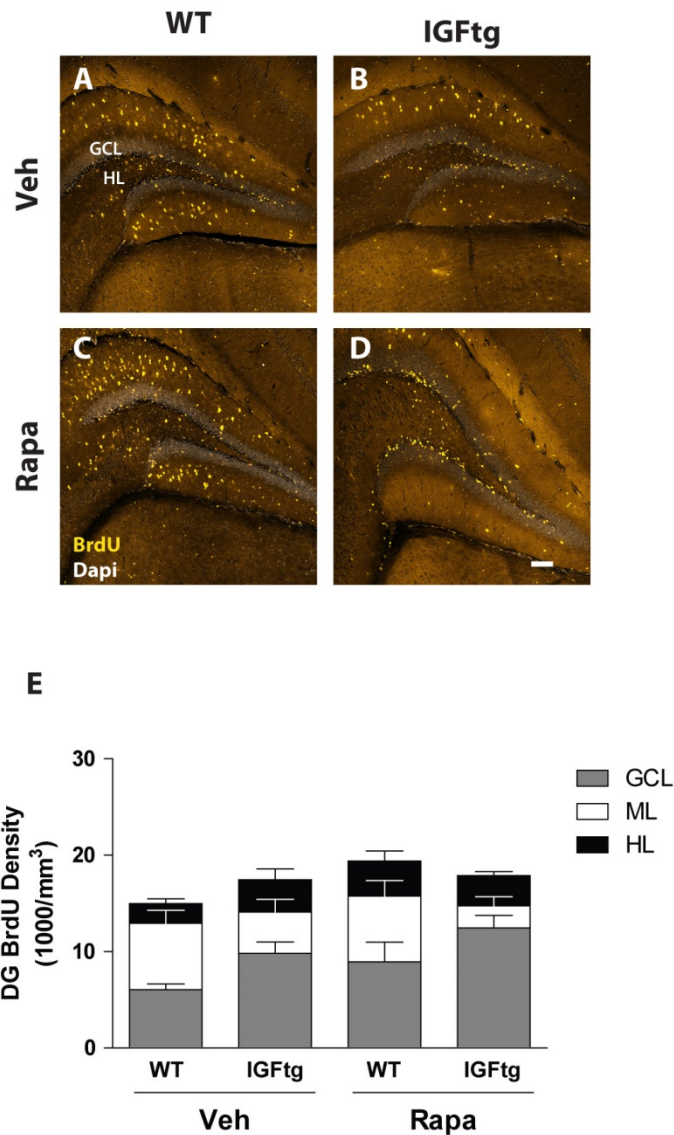


Figure 3.7: The density of proliferated cells in the DG is not altered by IGF1 overexpression or rapamycin treatment at 10 days after injury.

Mice received 3 intraperitoneal injections of bromodeoxyuridine (BrdU) at the peak of trauma-induced proliferation (3 days post-injury) at 4h intervals, ending 4h before the onset of vehicle or 10mg/kg rapamycin administration. Representative images of BrdU (yellow) immunoreactivity in the dentate gyrus (DG) of vehicle-treated (A) Wildtype (WT) and (B) IGF1 transgenic (IGFtg) and 10mg/kg rapamycin-treated (C) WT and (D) IGFtg at 10 days after moderate injury. (E) Injured WT and IGFtg mice had similar densities of proliferated cells in the granule cell layer (GCL), molecular layer (ML) and hilar layer (HL) irrespective of treatment. Data presented as mean + SEM.; One-way ANOVA, $p =$ not significant. Scale bar is 100 μ m. DAPI is white. Rapa, rapamycin. Veh, vehicle.

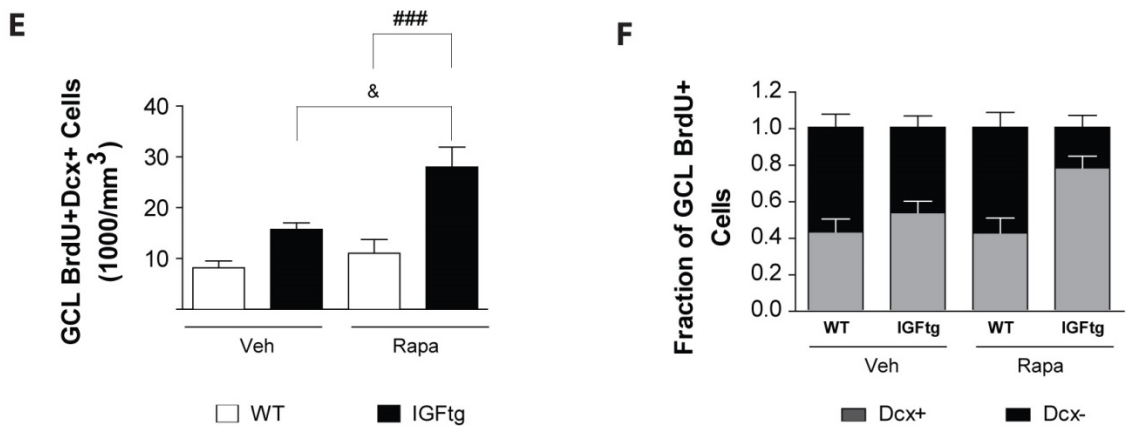
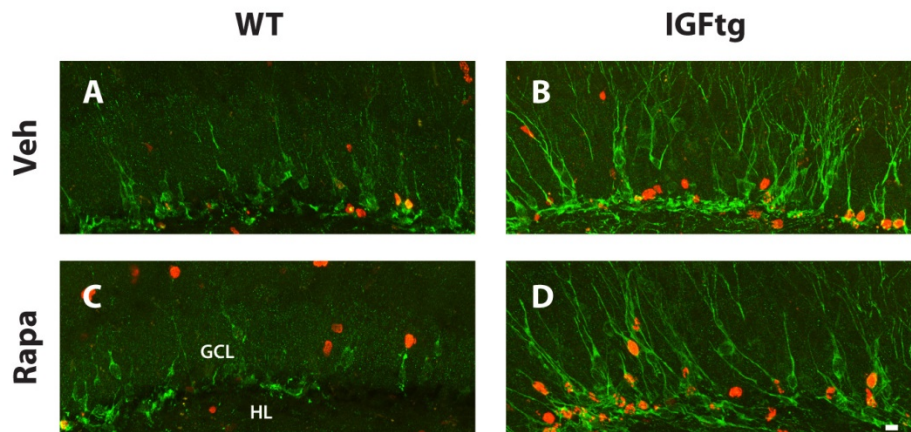


Figure 3.8: Rapamycin administration increases posttraumatic neuronal differentiation in IGF1 overexpressing mice.

Representative images of co-immunoreactivity of doublecortin (Dcx, green), a marker for immature neurons, and the proliferation marker BrdU (red) in the granule cell layer (GCL) of (A,B) vehicle treated and (C,D) rapamycin treated wildtype (WT; A, C) and IGF1 transgenic (IGFTg; B, D) mice 10d after injury. Rapamycin treatment (E) increased the density of BrdU+Dcx+ neurons as well as (F) the proportion of progenitors that differentiated into neurons in IGFTg mice compared to vehicle treatment and compared to rapamycin treatment in WT mice following moderate injury. Data are presented as mean + SEM. One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc t-tests, &p<0.05 IGFTg vehicle vs. IGFTg rapamycin. ###p<0.001 rapamycin WT vs. rapamycin IGFTg. Figure A-D, scale bar is 100µm. Rapa, rapamycin. Veh, vehicle.

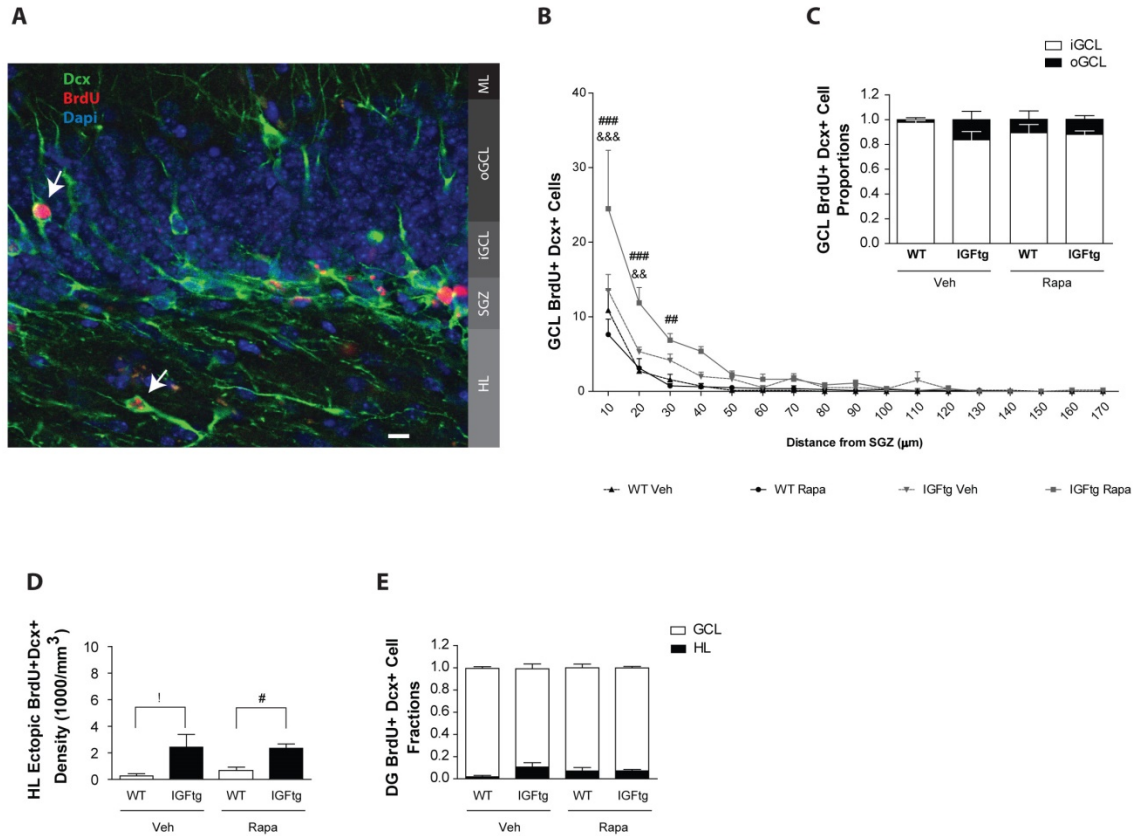


Figure 3.9: Rapamycin does not influence radial migration within the GCL or ectopic hilar migration or following IGF1 overexpression.

(A) Confocal imaging of doublecortin (Dcx, green) with proliferation reporter, bromodeoxyuridine (BrdU, red) in examples of immature neurons localized to the inner granule cell layer (iGCL), the outer 2/3rd region of the granule cell layer (oGCL) and the hilus (HL, white arrow) at 10 days after moderate injury. (B) Quantification of numbers of 3 days post-injury (dpi) proliferated immature neurons as a function of their distance from the subgranular zone (SGZ) as they localize radially toward the oGCL by 10dpi. Rapamycin treated IGFTg mice have significantly more proliferated immature neurons localized to the iGCL compared to vehicle treated controls and rapamycin treated WT mice. (C) However, the proportion of proliferated GCL immature neurons localized to the iGCL or oGCL is consistent across all groups. Rapamycin did not alter outward migration. (D) The density of proliferated immature neurons ectopically localized to the HL is significantly higher in rapamycin-treated IGFTg mice compared to rapamycin-treated WT mice. (E) However, rapamycin does not increase ectopic migration to the HL in IGFTg mice compared WT mice. Data presented as mean + SEM. (B) Two-way ANOVA with Bonferroni post-hoc, $&&p < 0.01$ and $&&&p < 0.001$ IGFTg vehicle vs. IGFTg rapamycin. $##p < 0.01$ and $###p < 0.001$ rapamycin, WT vs. IGFTg. (C,D,E) One-way ANOVA, followed by Bonferroni's selected comparisons post-hoc t-tests, $!p < 0.05$ vehicle, WT vs. IGFTg. $#p < 0.05$ rapamycin, WT vs. IGFTg. Scale bar is $10\mu\text{m}$. Rapa, rapamycin. Veh, vehicle.

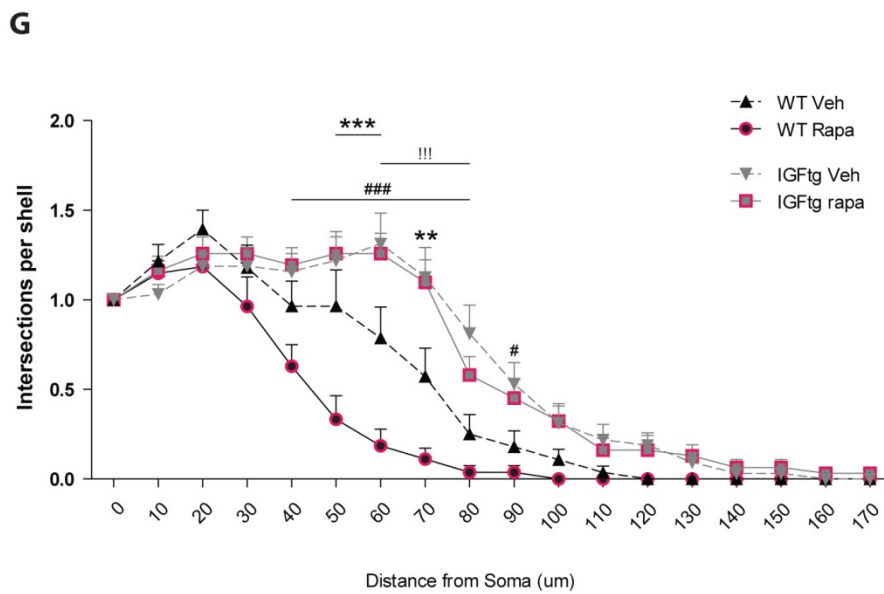
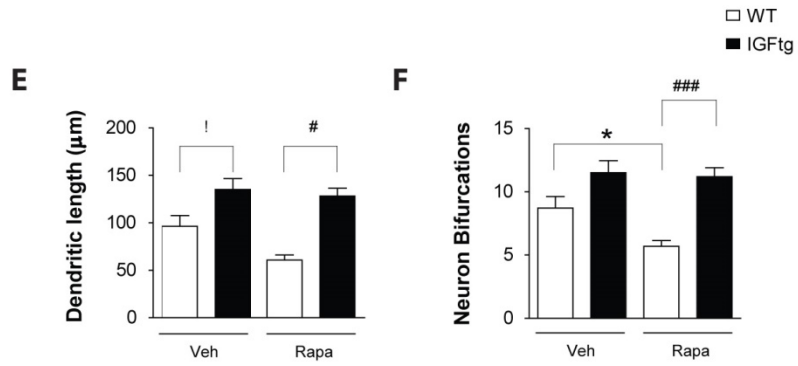
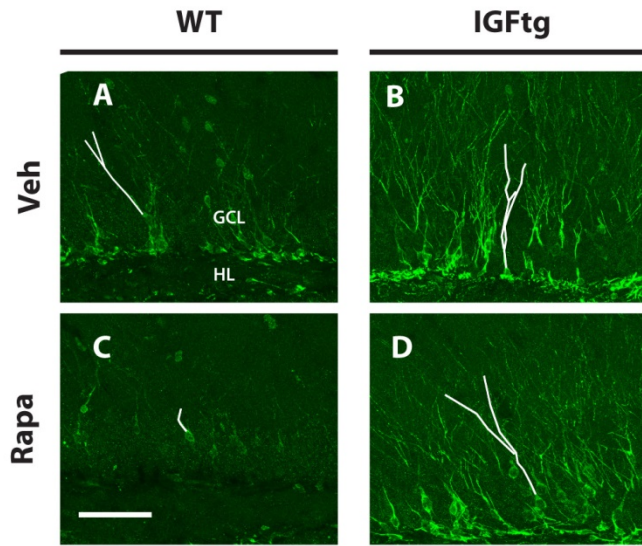


Figure 3.10: IGF1 overexpression increases dendritic arbor length and complexity and provides resistance to rapamycin induced dendritic pruning following moderate injury.

Representative image of doublecortin (Dcx, green) immunoreactive immature neurons following (A,B) vehicle or (C,D) rapamycin treatment in wildtype (WT; A, C) and IGF1 transgenic (IGFtg; B, D) mice 10 days after moderate injury. (E) Irrespective of treatment, IGFtg mice had increased dendritic length compared to WT mice. (F) Vehicle-treated IGFtg mice had more complex arbors than vehicle-treated WT mice. Rapamycin-treated WT mice had a reduction in dendritic complexity compared to vehicle-treated WT controls. Rapamycin-treated IGFtg mice had significantly more dendritic complexity than rapamycin-treated WT mice, while rapamycin had no effect on the dendritic arbor complexity of IGFtg mice compared to their vehicle-treated controls. (G) Sholl analysis displays dendritic complexity over distance from the cell soma, reflecting a rapamycin-induced reduction in complexity in WT mice, and resistance to rapamycin-mediated pruning in IGFtg mice. Data presented as mean + SEM. (E,F) One-way ANOVA with Bonferroni selected pairs post-hoc, * $p < 0.05$ WT vehicle vs. WT rapamycin. # $p < 0.05$ and ### $p < 0.001$ rapamycin, WT vs. IGFtg. ! $p < 0.05$ vehicle, WT vs. IGFtg. (G) Two-way ANOVA with Bonferroni post-hoc, ** $p < 0.01$ and *** $p < 0.001$ WT vehicle vs. WT rapamycin. !!! $p < 0.001$ vehicle, WT vs. IGFtg. # $p < 0.05$ and ### $p < 0.001$ rapamycin, WT vs. IGFtg. Scale bar is 10 μ m. White is overlay of traced Dcx arbor.

CHAPTER 4: Discussion and Conclusions

Review of major findings

IGF1 overexpression drives the fate, localization, and function of posttrauma-born neurons in the dentate gyrus following injury. Conditional overexpression of IGF1 increased the population of posttrauma-born neurons that survive to late state maturity and localize with developmentally born neurons in the GCL. Although IGF1 dramatically increased numbers of posttrauma-born neurons, it did not potentiate injury-induced ectopic migration. Injured IGF1 overexpressing mice had improved performance on the third day of RAWM testing compared to injured WT mice, when the hidden platform was moved to a novel location. When we investigated the signaling mechanism underlying IGF1 enhancement of neurogenesis, we found that IGF1 selectively potentiated trauma induced mTOR activity in the GCL and HL acutely after injury. However, the effects of IGF1 on posttraumatic neurogenesis were not dependent on mTOR activation. Inhibiting mTOR activation did not impair posttraumatic differentiation of neurons after trauma. In fact, only in injured animals overexpressing IGF1 was DG neuronal differentiation sensitive to rapamycin. Treating injured mice with rapamycin in the presence of IGF1 overexpression increased neuronal differentiation 2-fold. In WT mice mTOR activation is required for immature neuron dendritic arborization following trauma. IGF1 overexpression increased dendritic arborization and length and conferred resistance to rapamycin-induced inhibition of arbor complexity and length. This is the first evidence that uncouples IGF regulation of neurogenesis from down-stream mTOR signaling events. This compelling data support that IGF1 should be further evaluated for its therapeutic potential.

Posttraumatic neuronal differentiation

Injury stimulates proliferation in the hippocampal DG of rodents (Rola et al. 2006). IGF1 overexpression enhanced recovery of the immature DGGC population at 10d after injury without increasing proliferation within the GCL compared to injured WT mice (Carlson et al. 2014). In the current work we show that at 6 weeks after injury, the density of acutely proliferated cells in the GCL remained elevated compared to sham controls. Hippocampal DG cells proliferated acutely after trauma have been reported to persist for months after injury (Kernie et al. 2001). Although injured WT and IGFtg mice had similar numbers of acutely proliferated cells localized to the GCL, IGF1 overexpression increased the proportion that colocalized with a mature neuron marker. These data suggest that IGF1 overexpression increases posttraumatic neurogenesis through NSC differentiation and not NSC proliferation. Although IGF1 overexpressing mice have increased neuronal differentiation, IGF1 does not appear to deplete the NSC population after injury. Immunolabeling with the stem cell marker nestin revealed that Type-1 and Type-2a NSCs were present in the SGZ of both WT and IGFtg mice 10d after injury (Carlson et al. 2014).

Only 40% of proliferating cells in the DG SGZ of an uninjured rodent are NSCs (Palmer et al. 2000). Majority of these proliferated cells disappear within four weeks of their division; the remaining proliferated cells are largely neurons (~70%) (Palmer et al. 2000, Kempermann et al. 2003). Experimental TBI potentiates proliferation of the NSC population in the SGZ acutely after injury compared to controls (Kernie et al. 2001, Gao et al. 2013, Wang et al. 2016). It is unclear what proportion of acutely proliferated cells in the injured DG SGZ are NSCs or what proportion of those become neurons. Direct

assessment of the effect of TBI on NSC neuronal differentiation requires continuous sampling of posttrauma-proliferated NSC expression of markers of neuronal fate and degeneration for at least 1 month. Researchers interested in TBI and plasticity must further elucidate the impact and functional consequences of trauma on DG NSC differentiation.

Impact of posttraumatic IGF1 overexpression on SVZ neurogenesis

IGF1 is expressed in abundance in the developing rodent brain and its levels dramatically decline during postnatal development (Ye et al. 1997). IGF1 and the IGF1 receptor are ubiquitously expressed in all CNS cell types (Popken et al. 2005, Joseph D'Ercole et al. 2008, O'Kusky et al. 2012). In the postnatal brain, IGF1 expression is highest in neurons (Bartlett et al. 1991). Subregion examination of the postnatal rodent brain revealed that in the DG, IGF1 and the IGF1 receptor mRNA levels are 100x higher in immature neurons and NSCs than in mature granule cells (Zhang et al. 2007).

In addition to the SGZ, the subventricular zone (SVZ) of the lateral ventricle is highly enriched in NSCs in the adult brain. Newly born neurons from the SVZ migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB) throughout adulthood. The regular supply of new neurons may explain why the OB maintains a relatively high level of IGF1 expression that does not dramatically decline with aging like most regions (Ye et al. 1997, Lee et al. 2018). When IGF1 expression is genetically eliminated from the postnatal brain, immature neuron exit from the SVZ niche is impaired, neuroblast misposition, and the OB number of new neurons is reduced (Hurtado-Chong et al. 2009). IGF1 has an important role in regulating SVZ adult-neurogenesis.

In our studies, we focus on the fate and functional consequence of posttraumatic SGZ DG neurogenesis. Our model is dependent on injury-induced hippocampal astrocyte proliferation and hypertrophy since IGF1 overexpression is coupled to GFAP production. However, following CCI and FPI, an increase in GFAP mRNA and protein levels was observed at the site of the contusion and near the lateral ventricle aspect that is proximal to the site of injury (Dietrich et al. 1999, Madathil et al. 2013). Within the first week after severe CCI, NSC proliferation is stimulated in the SVZ (Radomski et al. 2013). One-month post-CCI, those rodents had reduced numbers of new neurons in the olfactory bulb and impaired olfactory discrimination compared to shams (Radomski et al. 2013). Following aspiration injury to the somatosensory cortex, SVZ neuroblasts exit the RMS and migrate to the injured cortex (Saha et al. 2013). IGF1 overexpression may regulate SVZ derived neuroblast migration after injury. We must take into consideration that IGF1 overexpression may also have a functional effect in progenitor-enriched brain regions and locations undergoing reactive astrogliosis.

Mature posttrauma-born neuron integration

While IGF1 increases the number of posttrauma-born granule cells that survive to late-stage maturity, our design does not allow us to examine their function. The morphology of the limited number of posttrauma-born neurons that survive to late-stage maturity in the absence of IGF1 overexpression is atypical compared to age-matched neurons in sham controls (Villasana et al. 2015). Only one study has investigated the function of mature posttrauma-born DGGCs compared to controls and these data suggest that post-trauma born DGGCs can receive input from the circuit. Mature post-trauma born DGGCs and mature DGGCs in controls had similar frequency and amplitude of

spontaneous and miniature excitatory synaptic events and evoked potentials (Villasana et al. 2015). The study also showed that the mature posttrauma-born neurons had similar paired-pulse facilitation (PPF) responses compared to controls, suggesting similar presynaptic release. IGF1 can increase expression of NMDA receptor subunits, NMDA receptor trafficking and potentiation via PI3K/AKT/GS3Kb signaling (Liu et al. 1995, Skeberdis et al. 2001, Le Greves et al. 2005, Le Greves et al. 2006, Molina et al. 2012, Li et al. 2017). Future studies should employ electrophysiology to investigate the spontaneous and evoked responses in new neurons in mice with post-traumatic IGF1 overexpression to determine if the new neurons are appropriately integrated.

A conditional DcxIGF1-eGFP overexpressing mouse would be extremely useful for accurate and long-term fate mapping and performing cellular recording in the DG. Our studies show that posttraumatic IGF1 has a dramatic effect on Type-3 neurons in the DG after injury (Carlson et al. 2014, Carlson et al. 2018). There is no Dcx or PSA-NCAM conditional tetracycline trans-activator line currently on the market. But with CRISP/CAS9 precision gene editing the line could be developed in C57 mice. Dr. Saatman and colleagues have been using a posttraumatic model of conditional IGF1 overexpression using tetracycline mediated expression for over 5 years (Madathil et al. 2013). A Dcx driven overexpression model would allow researchers to narrow the hypothesis and selectively address IGF1 overexpression effects on immature neurons after injury. This model has advantages over a nestin driven model because one specific stage of neurogenesis can be isolated. Directly examining the role IGF1 overexpression actions on immature neurons will help elucidate the role of IGF1 in posttraumatic neurogenesis.

IGF1 and posttraumatic hippocampal remodeling

The ionotropic glutamate receptor, N-methyl-D-aspartate receptor (NMDAr), has a very important role in facilitating learning and memory. Long-term potentiation (LTP) and long-term depression (LTD) are opposing mechanisms of NMDAr-dependent synaptic plasticity. There is evidence from CA1 experiments in the uninjured hippocampus that IGF1 can regulate LTP and LTD by direct and indirect synapse remodeling. An increase in the ratio of NMDAr subunits NR2B to NR2A increases NMDAr activity, lowers the threshold for LTP in the forebrain, and enhances learning and memory (Bliss et al. 1993, Tang et al. 1999). IGF1 administration has been shown to increase the ratio of NR2B to NR2A mRNA levels in the hippocampus of juvenile and aged rats (Le Greves et al. 2005, Le Greves et al. 2006). In the rodent hippocampus, Glycogen synthase kinase-3 beta (GSK3 β) mediates LTP induced inhibition of LTD in a PI3K/AKT dependent manner (Peineau et al. 2007). When LTP generation is impaired in a mouse model of Autistic Spectrum Disorder, IGF1 treatment rescues LTP generation in hippocampal CA1 pyramidal cells (Bozdagi et al. 2013).

IGF1 overexpression may affect postsynaptic remodeling of hippocampal neurons after injury. This is especially crucial when we considered how glutamate receptor remodeling could affect competitive survival of new neurons. NMDAr activity is required to increase spine density and, thereby, LTP in CA1 neurons in the developing hippocampus (Collin et al. 1997). In the CA1, IGF1 infusion was shown to increase the spine density in aging mice (Shi et al. 2005). The availability and activity of NMDA receptors is critical to the NMDAr-dependent competitive survival that takes place when adult-born DG neurons are 2-3 weeks of age (Tashiro et al. 2006). The effect of IGF1 on

spine density during the development of adult-born immature neurons in the DG is unknown.

Studies have also shown that IGF1 has a role in axonal sprouting and guidance. mTOR activation is heavily implicated in the development of epilepsy and posttraumatic epilepsy (Guo et al. 2013, Butler et al. 2015, Butler et al. 2016, Butler et al. 2017). This was a primary driver for initiating a study design to elucidate the role and requirement for mTOR activation in mediating the effects of IGF1 on posttraumatic hippocampal plasticity. A common phenotype of the epileptic hippocampus is the presence of mossy fiber collaterals aberrantly synapsed onto neighboring granule cells (Hunt et al. 2010). In a model of posttraumatic epilepsy, 8-13 weeks of rapamycin treatment attenuated the presence of these aberrant fibers (Butler et al. 2015). Future experiments should investigate the effect of IGF1 on axonal sprouting after injury.

Studies have shown that astrocytes have important interactions with neuroblasts. Astrocytes release extracellular matrix (ECM) proteins that regulate synaptogenesis (Frischknecht et al. 2009). ECM proteins create the perineural net which is thought to prevent aberrant process growth. During development, restrictive ECM proteins are released after the critical period of neuronal maturation. ECM proteins play a very important role in regulating neurite outgrowth of developing neurons. In the adult hippocampus, aggrecan is selectively expressed in the granule cell layer and the molecular layer of the dentate gyrus and is a potent regulator of process outgrowth (Dauth et al. 2016). When aggrecan is genetically increased in the molecular layer and granule cell layer, adult-born neurons have dramatically reduced dendritic complexity and length compared to controls (Bolos et al. 2018). IGF1 acutely increases reactive

astrocytosis in the molecular after injury (Madathil et al. 2013) and can regulate aggrecan expression in non-neural tissues (Wang et al. 2003). IGF1 may indirectly regulate dendritic arborization through ECM regulation following injury.

A role for adult-born neurons in emotion-related behavior

While learning and memory deficits are of great concern to clinicians and patients, there is a growing need to understand the effect trauma is having on emotion-related behavior. Blast-related injury accounts for over 50% of munition related injuries amongst military personnel (DeWitt et al. 2009). TBI is associated with an increased rate of suicide and expression of posttraumatic stress disorder symptoms including depression and aggression (Nakase-Richardson et al. 2009, Brenner et al. 2011, Brenner et al. 2011, Hart et al. 2011, Walter et al. 2012, Hart et al. 2014). A study showed that over 80% of inmates report incurring a TBI prior to incarceration (Slaughter et al. 2003). A link is beginning to emerge between emotional related behavior and trauma. There may be a role for IGF1 in treating these disorders. Experimental TBI causes chronic atrophy of the hippocampus and fear conditioning impairment at 3 months post-injury in rodents (Titus et al. 2016). Studies have shown that neurogenesis is important for fear conditioning and fear extinction (Snyder et al. 2009, Seo et al. 2015). We show data that IGF1 overexpression increased numbers of posttrauma-born neurons in the dorsal (septal) GCL. We focus on the septal hippocampus because it is the epicenter of injury. Studies have shown that the dorsal hippocampus is more important for spatial memory (Moser et al. 1993) and the ventral (temporal) hippocampus is more important for fear and anxiety related activities (Kjelstrup et al. 2002). In future studies, we could examine the DG and immature neurons in the ventral hippocampus, which sends direct input about fear to the

amygdala (Fanselow et al. 2010). IGF1 may mediate fear associated behavior following trauma by promoting stable neurogenesis.

A clinical study reported that approximately 26% of TBI patients receiving GCS scores ranging from 3-15 within 24h of injury reported that they suffered from major depressive disorder by the 1-year follow up (Hart et al. 2011). Mood disorders affect the ability to socialize, interact at work and at home and perform daily activities. Decreased serum levels of nerve growth factor (NGF) and BDNF are implicated in depression and suicide risk, while increased levels of BDNF and IGF1 are associated with improved mood in depressed patients (Chen et al. 2001, Evans et al. 2004, Kopczak et al. 2015, Wiener et al. 2015, Zhang et al. 2018). Exercise is currently a recommended treatment to reduce depression symptoms and it is associated with increased neurotrophic factors (Kishi et al. 2017, Phillips 2017). Growth factors may play an important role in mood enhancing treatments for depression. There has been difficulty establishing rodent models of human depression because of the nuance and complex features of psychological health diseases (Krishnan et al. 2011). Rodents assays that examine depression-like behaviors including motivation, social interaction, and despair have been developed (Krishnan et al. 2011). Like clinical data, it is well established in rodents that one mechanism by which exercise attenuates depression-like behavior is through BDNF upregulation (Vaynman et al. 2004, Marais et al. 2009, Sleiman et al. 2016). The forced swim test is a measure of despair behavior in rodents. The forced swim test is an assessment of how quickly animals give up trying to escape a pool of water (Can et al. 2012, Tucker et al. 2017). In the context of brain injury, mice show depressive-like behavior during the forced swim behavior test at three weeks after CCI (Washington et

al. 2012). The forced swim assessment could be used to assess if IGF1 overexpression could attenuate injury-induced depression-like behavior.

IGF1 and mTOR: posttraumatic signaling relationship in neurogenesis

mTOR is a common downstream target of numerous neurotrophic factors (Feng et al. 2010, Sengupta et al. 2010, Laplante et al. 2012). Growth factor signaling and mTOR activation support neuroplasticity in the brain. Depending on the context, both have been associated with pathogenesis and recovery in neurodegenerative diseases (Buch 2014, Perluigi et al. 2015). These findings raise concern about the efficacy and safety of targeting neurotrophic factors as potential therapeutics. IGF1 and mTOR signaling are extremely complex and dynamic dependent on the physiological context. Subtle variability between experiments, not limited to species selection, disease state, in vivo versus in vitro, tissue type, and temporal factors, are likely to contribute to paradoxical reports about IGF1/mTOR signaling, fueling controversy among investigators (Wrigley et al. 2017). Although IGF1 stimulation and mTOR activation may produce the same effect, this does not necessarily mean that IGF1 is mediating that effect through its downstream target mTOR. We found that dendritic arbor development of immature neurons in injured IGF1 overexpressing mice was enhanced compared to injured WT mice. mTOR inhibition impaired dendritic outgrowth in recovering WT mice, while the effects of IGF1 on arbor development were insensitive to mTOR inhibition. Rapamycin treatment stimulated neuronal differentiation in IGF1g mice compared to vehicle-treated controls and injured WT mice. Our data suggest that varying pathways of IGF1 signaling mediate distinct aspects of neural development within immature neurons after injury.

Two well-characterized pathways that stimulate growth, differentiation, survival, and neurogenesis downstream of IGF1 signaling include IRS1/PI3K/AKT/mTOR and SHC/RAS/MAPK/MEK (Joseph D'Ercole et al. 2008, Liu et al. 2009). Both pathways have been shown to stimulate dendritic growth in neurons (Jaworski et al. 2005, Kumar et al. 2005, Karelina et al. 2012). Cross talk between the two pathways may also mediate effects of IGF1 signaling in trauma. A role for crosstalk between RAS and PI3K signaling is well described in tumorigenesis (Castellano et al. 2011, Mendoza et al. 2011). Recently, a study showed crosstalk between these pathways in neuronal cultures of dissociated CA1 and CA3 neurons (Kumar et al. 2005). mTOR inhibition blocked RAS/MAPK/MEK dendritic outgrowth but not RAS/PI3K mediated outgrowth (Kumar et al. 2005). Expression of RAS-L16S35 (constitutively active Ras that activates the PI3K pathway) or RAS-L16S40 (constitutively active Ras that activates the MAPK/MEK pathway) in these neurons stimulated dendritic complexity compared to controls. Rapamycin impaired dendritic outgrowth in control neurons and in RAS-MAPK neurons, but not RAS-PI3K expressing neurons (Kumar et al. 2005).

Compensatory signaling between the IRS1/PI3K/AKT/mTOR and SHC/RAS/MEK/ERK pathway has been described in insulin insensitivity studies. When the IRS1 arm is inactivated by negative feedback, the SHC arm can become more sensitive to stimulation. Hyperactivation of the insulin receptor by prolonged growth hormone (GH) administration prior to insulin treatment prevents further activation of IRS1 by insulin in rat hindlimbs (Thirone et al. 1998). Meanwhile, SHC activation sensitivity to insulin treatment was dramatically increased following chronic GH administration (Thirone et al. 1998).

Studies suggest that hyperactivation insensitivity of the IGF1 receptor to IGF1 and the insulin receptor to insulin is mediated by posttranslational negative feedback on IRS1 by mTOR (Um et al. 2004, Shah et al. 2006, Copps et al. 2012). A study showed that IGF1 treatment stimulates migration and enhanced survival of cultured fibroblast (Harrington et al. 2004). TSC1/2 mutation (TSC1/2-) in cultured fibroblasts resulted in constitutively active RHEB, which directly activates the mTORC1 complex (Harrington et al. 2004). The IRS1 substrate was downregulated in TSC1/2- fibroblasts and they were unresponsive to IGF1 treatment. Rapamycin treatment restored sensitivity to IGF1 treatment, enhancing survival in fibroblast from TSC1/2 mutants (Harrington et al. 2004). Activated mTOR/S6 has been shown to directly downregulate IRS1 activity in response in fibroblasts with a mutation in TSC1/2 (Shah et al. 2006).

mTOR can also provide negative feedback on the IGF1/PI3K/AKT signaling through IRS1 independent mechanisms (Wan et al. 2007). mTOR inhibition can potentiate IGF1 signaling when IGF1 effects are not mediated through an mTOR-dependent mechanism. These studies challenge the dogma of insulin and IGF1 signaling. These studies and the data presented here suggest looking at insulin/IGF1 signaling from a holistic view. The overarching purpose of growth factors is to balance homeostasis. Narrowly focusing on isolated effects of individual substrates risks losing sight of compensatory shifts that take place elsewhere and oversimplifying mechanisms.

The data presented here expands the understanding of the mechanisms underlying enhanced plasticity in the posttraumatic DG following IGF1 overexpression. Our goal was to identify the effects of mTOR inhibition on the IGF1-induced enhancement of neurogenesis. IGF1 may enhance posttraumatic differentiation and dendritic outgrowth in

immature neurons following trauma by two different mechanisms. Neuronal differentiation was increased following mTOR inhibition in IGF1 overexpressing mice. Follow-up studies should examine if posttraumatic IGF1-induced neuronal differentiation is dependent on IRS1/PI3K signaling. Studies should investigate the effect of posttraumatic IGF1 overexpression on hippocampal IRS1 substrate phosphorylation and expression and if rapamycin treatment prevents alteration of the substrate. This would suggest that mTOR is a negative regulator of posttraumatic IGF1 action. There is also evidence that the mTOR effector, S6 kinase (pS6K) may downregulate IRS1 activity. pS6K negatively regulates IRS1 activity in response to insulin stimulation. In the absence of S6K insulin sensitivity is increased (Um et al. 2004) and increased S6K activation inhibits IRS1 activation of PI3K (Draznin 2006). S6K hyper-phosphorylates IRS1 inhibitory serine sites and inhibits the ability of IRS1 to bind and activate PI3K. It was recently shown in cultured fibroblasts that constitutively active mTORC1 causes inhibitory phosphorylation and mRNA downregulation of IRS1 (Harrington et al. 2004). Insulin and IGF1 could not activate AKT because constitutively active mTOR/pS6K was negatively feeding back on IRS1 (Harrington et al. 2004). The phenotype was rescued by mTOR inhibition by rapamycin and S6K mRNA silencing.

Dendritic arborization of immature neurons was enhanced by IGF1 overexpression following injury; this effect was insensitive to mTOR inhibition. Our interpretation of the signaling underlying enhanced posttraumatic dendritic arborization following IGF1 overexpression is limited. While mTOR inhibition does not inhibit the arborization effects of IGF1 overexpression on immature neurons, it is unclear if these effects are independent of mTOR activation. mTOR activity may enhance dendritic

arborization in IGFtg mice. However, IGF1 may stimulate dendritic outgrowth through an alternative pathway when IRS1/PI3K/AKT/mTOR signaling is inhibited. Rapamycin diminished dendritic outgrowth in cultured neurons expressing constitutively activated PI3K (Jaworski et al. 2005). However, rapamycin treatment only partially blocked the dendritic outgrowth induced by cultured neurons expressing constitutively activated RAS (Kumar et al. 2005). The inhibition of MAPK or PI3K was able to diminish the enhanced dendritic outgrowth stimulated by Glial Cell-derived Neurotrophic Factor (GDNF) in cultured striatal neurons (Garcia-Martinez et al. 2006). Overactivation of the IGF1 receptor following posttraumatic IGF1 overexpression may lead to downregulation of the IRS1/PI3K/AKT/mTOR and increased activation of the SHC/RAS/MAPK pathway. It is well known that prolonged IGF1 stimulation of the IGF1 receptor can induce PI3K/mTOR dependent downregulation of IRS1/PI3K activation and upregulation of compensatory pathway activation (Rozenfurt et al. 2014). As prolonged IGF1 treatment continues to activate the IGF1 receptor, cultured melanoma cells shift to upregulation of the SHC/RAS/MAPK/ERK when IRS1 activity is lost (Reuveni et al. 2013). The incorporation of IRS1 and SHC inhibitors into future studies could elucidate the signaling underlying IGF1 overexpression effects on dendritic outgrowth following trauma.

Considerations

The function of adult neurogenesis in human diseases of the brain is not well understood. The persistence and extent of hippocampal neurogenesis in the uninjured human brain is a continuing point of controversy (Eriksson et al. 1998, Knoth et al. 2010, Dennis et al. 2016, Boldrini et al. 2018, Sorrells et al. 2018). Gerd Kempermann and colleagues have shown that like rodents, neurogenesis in humans dramatically declines

with age (Knoth et al. 2010). Variation between specimen collection protocols and patient history may contribute to conflicting data regarding the degree of adult neurogenesis between studies. The adult brain tissue from studies reporting nearly no persistent adult neurogenesis was acquired from patients with a variety of causes of death including cancer, epilepsy, brain injury, and chronic illness (Dennis et al. 2016, Sorrells et al. 2018). Gage and colleagues controlled for the cause of death confounders by examining brain specimen from a population of patients with squamous carcinoma of the tongue, larynx, and pharynx (Eriksson et al. 1998). This study also controlled for drugs that may inhibit neurogenesis, and possibly confound results. Proliferation assessments in the DG of the specimens from the Gage study were from patients who did not receive anti-cancer proliferation drugs (Eriksson et al. 1998). When Rene Hen and colleagues designed their experiments to exclude specimen from patients with a history of chronic disease, mental illness, drugs-use for mental illness, and brain injury, they found persistent adult-neurogenesis in the DG (Boldrini et al. 2018). Variations in tissue preservation protocols between studies using human tissue can dramatically influence the interpretation of findings. Studies find that increasing the amount of time between patient death and chemical tissue preservation, postmortem interval (PMI) can decrease brain tissue immunoreactivity for many antigens (Shiurba et al. 1998, Unal-Cevik et al. 2004, Birdsill et al. 2011). Studies that found little to no adult neurogenesis in the DG examined specimen from patients with a PMI of less than 35h in less than 35% of cases, or they did not directly report PMI (Dennis et al. 2016, Sorrells et al. 2018). Studies that support persistent adult neurogenesis collected specimens from patients with a PMI of less than 35h in approximately 78% to 100% of cases (Eriksson et al. 1998, Boldrini et al. 2018).

Future studies elucidating the pattern and extent of human adult neurogenesis in the DG are prerequisites to determining the implications of neurogenesis on recovery from TBI.

The potential clinical value of IGF1 in treating brain injury should not be limited to its effects on adult neurogenesis. IGF1 has been shown to be an effective therapeutic in treating TBI-induced metabolic dysfunction (Hatton et al. 2006). Clinical studies that have shown promising IGF1 treatment outcomes have often administered IGF1 with factors that prolong its systemic circulation and effectiveness. IGF1 was administered with growth hormone (GH) within 72h of moderate to severe brain injury to reduce hypercatabolic metabolism in patients after trauma (Hatton et al. 1997). All patients had improved protein metabolism within 1 week of treatment (Hatton et al. 1997). Patients receiving moderate GCS scores but not poor GSC scores had improved outcomes 6 months following IGF1 treatment (Hatton et al. 1997). A clinical malnutrition study and an efficacy study in TBI patients found that administering growth hormone with IGF1 increased serum levels of IGF1 and the IGF1 binding protein 3 (IGFBP-3) (Rockich et al. 1999, Justova et al. 2001). Circulating IGF1 is often found in complex with IGFBP-3, IGFBP-3 extends IGF1 activity by 20hr (4hrs of action) (Rosenfeld 2006). The combination of IGF1 and GH was sufficient to improve protein metabolism within 24h of treatment onset in TBI patients (Hatton et al. 2006). However, using GH in clinical treatment is controversial, since several studies have reported that GH increases mortality rate long-term (Divall et al. 2013). IGF1 is now commercially available and prescribed under the brand name Mecasermin in combination with IGFBP-3 (Cohen et al. 2014). Future preclinical models elevating effects of IGF1 overexpression should incorporate IGFBPs into the study design, considering their effect on IGF1 half-life. IGF1 needs

further preclinical evaluation to determine the best mode of delivery and what binding protein to accompany it.

The goal of investigating the effects of IGF1 modulation of hippocampal plasticity and understanding the mechanisms underlying those changes is to further evaluate IGF1 as a potential treatment TBI. Drugs that stimulate the IRS1/PI3K/AKT/mTOR pathway are a safety concern because activation of the pathway is contraindicated in diabetes, cancer development, and aspects of seizure development (Mendoza et al. 2011, Tolcher et al. 2015, Wheless 2015, Patnaik et al. 2016). Seizures are a common feature of the human disease Tubular Sclerosis. Tubular sclerosis is a disease caused by an inherited mutation to TSC1/2. The mutation is thought to profoundly affect neurogenesis during development (Rosset et al. 2017). More than 50% of children with TSC mutations die from brain tumors while those that survivor develop seizures, symptoms of autism, and suffer from a low IQ (Joinson et al. 2003, Feliciano et al. 2012, Kotulska et al. 2014). Patients with a TSC mutation frequently have seizures; many have not been responsive to treatment. Recent clinical trials have shown that mTOR inhibition is effective in reducing seizures in children and juveniles with TSC mutations (Wheless 2015, Samuelli et al. 2016, Kilincaslan et al. 2017).

Patients with TSC may benefit from IGF1 driven plasticity combined with inhibition of mTOR induced maladaptive plasticity. A recent study has shown that the hippocampi of patients suffering from temporal lobe epilepsy have significantly high mTOR activity compared to controls (Talos et al. 2018). Seizure development has also been reported in TBI patients (Ferguson et al. 2010, Bramlett et al. 2015). It is unclear whether mTOR contributes to the etiology of these forms of epilepsy or if its inhibition

would reduce seizure activity. The data presented here is essential to the preclinical evaluation of IGF1 for TBI because it introduces the impact of combining IGF1 with mTOR inhibitor. Further studies should be done to evaluate the effect of combining IGF1 and mTOR to treat human diseases of the brain.

References

- Abraham, RT and Wiederrecht, GJ (1996). "Immunopharmacology of rapamycin." Annu Rev Immunol **14**: 483-510.
- Aimone, JB, Deng, W and Gage, FH (2010). "Adult neurogenesis: integrating theories and separating functions." Trends Cogn Sci **14**(7): 325-337.
- Alamed, J, Wilcock, DM, Diamond, DM, Gordon, MN and Morgan, D (2006). "Two-day radial-arm water maze learning and memory task; robust resolution of amyloid-related memory deficits in transgenic mice." Nat Protoc **1**(4): 1671-1679.
- Altman, J and Bayer, SA (1990). "Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods." J Comp Neurol **301**(3): 365-381.
- Altman, J and Das, GD (1967). "Postnatal neurogenesis in the guinea-pig." Nature **214**(5093): 1098-1101.
- Alvarez-Buylla, A and Lim, DA (2004). "For the long run: maintaining germinal niches in the adult brain." Neuron **41**(5): 683-686.
- Amaral, DG, Scharfman, HE and Lavenex, P (2007). "The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies)." Prog Brain Res **163**: 3-22.
- Anacker, C and Hen, R (2017). "Adult hippocampal neurogenesis and cognitive flexibility - linking memory and mood." Nat Rev Neurosci **18**(6): 335-346.
- Anlar, B, Sullivan, KA and Feldman, EL (1999). "Insulin-like growth factor-I and central nervous system development." Horm Metab Res **31**(2-3): 120-125.
- Ariza, M, Serra-Grabulosa, JM, Junque, C, Ramirez, B, Mataro, M, Poca, A, Bargallo, N and Sahuquillo, J (2006). "Hippocampal head atrophy after traumatic brain injury." Neuropsychologia **44**(10): 1956-1961.
- Ashton, RS, Conway, A, Pangarkar, C, Bergen, J, Lim, KI, Shah, P, Bissell, M and Schaffer, DV (2012). "Astrocytes regulate adult hippocampal neurogenesis through ephrin-B signaling." Nat Neurosci **15**(10): 1399-1406.
- Atkins, CM (2011). "Decoding hippocampal signaling deficits after traumatic brain injury." Transl Stroke Res **2**(4): 546-555.
- Baker, NL, Carlo Russo, V, Bernard, O, D'Ercole, AJ and Werther, GA (1999). "Interactions between bcl-2 and the IGF system control apoptosis in the developing mouse brain." Brain Res Dev Brain Res **118**(1-2): 109-118.
- Bareyre, F, Wahl, F, McIntosh, TK and Stutzmann, JM (1997). "Time course of cerebral edema after traumatic brain injury in rats: effects of riluzole and mannitol." J Neurotrauma **14**(11): 839-849.
- Barha, CK, Ishrat, T, Epp, JR, Galea, LA and Stein, DG (2011). "Progesterone treatment normalizes the levels of cell proliferation and cell death in the dentate gyrus of the hippocampus after traumatic brain injury." Exp Neurol **231**(1): 72-81.
- Bartlett, WP, Li, XS, Williams, M and Benkovic, S (1991). "Localization of insulin-like growth factor-1 mRNA in murine central nervous system during postnatal development." Dev Biol **147**(1): 239-250.

- Becker, D, Zahn, N, Deller, T and Vlachos, A (2013). "Tumor necrosis factor alpha maintains denervation-induced homeostatic synaptic plasticity of mouse dentate granule cells." Front Cell Neurosci **7**: 257.
- Bekinschtein, P, Kent, BA, Oomen, CA, Clemenson, GD, Gage, FH, Saksida, LM and Bussey, TJ (2013). "BDNF in the dentate gyrus is required for consolidation of "pattern-separated" memories." Cell Rep **5**(3): 759-768.
- Bekinschtein, P, Kent, BA, Oomen, CA, Clemenson, GD, Gage, FH, Saksida, LM and Bussey, TJ (2014). "Brain-derived neurotrophic factor interacts with adult-born immature cells in the dentate gyrus during consolidation of overlapping memories." Hippocampus **24**(8): 905-911.
- Birdsill, AC, Walker, DG, Lue, L, Sue, LI and Beach, TG (2011). "Postmortem interval effect on RNA and gene expression in human brain tissue." Cell Tissue Bank **12**(4): 311-318.
- Blaiss, CA, Yu, TS, Zhang, G, Chen, J, Dimchev, G, Parada, LF, Powell, CM and Kernie, SG (2011). "Temporally specified genetic ablation of neurogenesis impairs cognitive recovery after traumatic brain injury." J Neurosci **31**(13): 4906-4916.
- Bliss, TV and Collingridge, GL (1993). "A synaptic model of memory: long-term potentiation in the hippocampus." Nature **361**(6407): 31-39.
- Boldrini, M, Fulmore, CA, Tartt, AN, Simeon, LR, Pavlova, I, Poposka, V, Rosoklija, GB, Stankov, A, Arango, V, Dwork, AJ, Hen, R and Mann, JJ (2018). "Human Hippocampal Neurogenesis Persists throughout Aging." Cell Stem Cell **22**(4): 589-599 e585.
- Bolkvadze, T and Pitkanen, A (2012). "Development of post-traumatic epilepsy after controlled cortical impact and lateral fluid-percussion-induced brain injury in the mouse." J Neurotrauma **29**(5): 789-812.
- Bolos, M, Perea, JR, Terreros-Roncal, J, Pallas-Bazarra, N, Jurado-Arjona, J, Avila, J and Llorens-Martin, M (2018). "Absence of microglial CX3CR1 impairs the synaptic integration of adult-born hippocampal granule neurons." Brain Behav Immun **68**: 76-89.
- Bondi, CO, Cheng, JP, Tennant, HM, Monaco, CM and Kline, AE (2014). "Old dog, new tricks: the attentional set-shifting test as a novel cognitive behavioral task after controlled cortical impact injury." J Neurotrauma **31**(10): 926-937.
- Bondy, CA, Bach, MA and Lee, W-H (1992). "Mapping of brain insulin and insulin-like growth factor receptor gene expression by in situ hybridization." Neuroprotocols **1**(3): 240-249.
- Borges, K, McDermott, D, Irier, H, Smith, Y and Dingledine, R (2006). "Degeneration and proliferation of astrocytes in the mouse dentate gyrus after pilocarpine-induced status epilepticus." Exp Neurol **201**(2): 416-427.
- Bozdagi, O, Tavassoli, T and Buxbaum, JD (2013). "Insulin-like growth factor-1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay." Mol Autism **4**(1): 9.
- Bramlett, HM and Dietrich, WD (2015). "Long-Term Consequences of Traumatic Brain Injury: Current Status of Potential Mechanisms of Injury and Neurological Outcomes." J Neurotrauma **32**(23): 1834-1848.

- Brenner, LA, Betthausen, LM, Homaifar, BY, Villarreal, E, Harwood, JE, Staves, PJ and Huggins, JA (2011). "Posttraumatic stress disorder, traumatic brain injury, and suicide attempt history among veterans receiving mental health services." *Suicide Life Threat Behav* **41**(4): 416-423.
- Brenner, LA, Ignacio, RV and Blow, FC (2011). "Suicide and traumatic brain injury among individuals seeking Veterans Health Administration services." *J Head Trauma Rehabil* **26**(4): 257-264.
- Bromley-Brits, K, Deng, Y and Song, W (2011). "Morris water maze test for learning and memory deficits in Alzheimer's disease model mice." *J Vis Exp*(53).
- Buch, S (2014). "Growth factor signaling: implications for disease & therapeutics." *J Neuroimmune Pharmacol* **9**(2): 65-68.
- Buckmaster, PS and Lew, FH (2011). "Rapamycin suppresses mossy fiber sprouting but not seizure frequency in a mouse model of temporal lobe epilepsy." *J Neurosci* **31**(6): 2337-2347.
- Burghardt, NS, Park, EH, Hen, R and Fenton, AA (2012). "Adult-born hippocampal neurons promote cognitive flexibility in mice." *Hippocampus* **22**(9): 1795-1808.
- Buss, RR, Sun, W and Oppenheim, RW (2006). "Adaptive roles of programmed cell death during nervous system development." *Annu Rev Neurosci* **29**: 1-35.
- Butler, CR, Boychuk, JA and Smith, BN (2015). "Effects of Rapamycin Treatment on Neurogenesis and Synaptic Reorganization in the Dentate Gyrus after Controlled Cortical Impact Injury in Mice." *Front Syst Neurosci* **9**: 163.
- Butler, CR, Boychuk, JA and Smith, BN (2016). "Differential effects of rapamycin treatment on tonic and phasic GABAergic inhibition in dentate granule cells after focal brain injury in mice." *Exp Neurol* **280**: 30-40.
- Butler, CR, Boychuk, JA and Smith, BN (2017). "Brain Injury-Induced Synaptic Reorganization in Hilar Inhibitory Neurons Is Differentially Suppressed by Rapamycin." *eNeuro* **4**(5).
- Bye, N, Carron, S, Han, X, Agyapomaa, D, Ng, SY, Yan, E, Rosenfeld, JV and Morganti-Kossmann, MC (2011). "Neurogenesis and glial proliferation are stimulated following diffuse traumatic brain injury in adult rats." *J Neurosci Res* **89**(7): 986-1000.
- Cameron, HA and McKay, RD (2001). "Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus." *J Comp Neurol* **435**(4): 406-417.
- Can, A, Dao, DT, Arad, M, Terrillion, CE, Piantadosi, SC and Gould, TD (2012). "The mouse forced swim test." *J Vis Exp*(59): e3638.
- Cantu, RC (2001). "Posttraumatic Retrograde and Anterograde Amnesia: Pathophysiology and Implications in Grading and Safe Return to Play." *J Athl Train* **36**(3): 244-248.
- Cao, Y, Gunn, AJ, Bennet, L, Wu, D, George, S, Gluckman, PD, Shao, XM and Guan, J (2003). "Insulin-like growth factor (IGF)-1 suppresses oligodendrocyte caspase-3 activation and increases glial proliferation after ischemia in near-term fetal sheep." *J Cereb Blood Flow Metab* **23**(6): 739-747.
- Carlson, CJ, White, MF and Rondinone, CM (2004). "Mammalian target of rapamycin regulates IRS-1 serine 307 phosphorylation." *Biochem Biophys Res Commun* **316**(2): 533-539.

- Carlson, SW, Madathil, SK, Sama, DM, Gao, X, Chen, J and Saatman, KE (2014). "Conditional overexpression of insulin-like growth factor-1 enhances hippocampal neurogenesis and restores immature neuron dendritic processes after traumatic brain injury." J Neuropathol Exp Neurol **73**(8): 734-746.
- Carlson, SW and Saatman, KE (2018). "Central Infusion of Insulin-Like Growth Factor-1 Increases Hippocampal Neurogenesis and Improves Neurobehavioral Function after Traumatic Brain Injury." J Neurotrauma.
- Carracedo, A, Ma, L, Teruya-Feldstein, J, Rojo, F, Salmena, L, Alimonti, A, Egia, A, Sasaki, AT, Thomas, G, Kozma, SC, Papa, A, Nardella, C, Cantley, LC, Baselga, J and Pandolfi, PP (2008). "Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer." J Clin Invest **118**(9): 3065-3074.
- Castellano, E and Downward, J (2011). "RAS Interaction with PI3K: More Than Just Another Effector Pathway." Genes Cancer **2**(3): 261-274.
- Chen, B, Dowlatshahi, D, MacQueen, GM, Wang, JF and Young, LT (2001). "Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication." Biol Psychiatry **50**(4): 260-265.
- Chen, C, Ma, TZ, Wang, LN, Wang, JJ, Tu, Y, Zhao, ML, Zhang, S, Sun, HT and Li, XH (2016). "Mild hypothermia facilitates the long-term survival of newborn cells in the dentate gyrus after traumatic brain injury by diminishing a pro-apoptotic microenvironment." Neuroscience **335**: 114-121.
- Chen, S, Atkins, CM, Liu, CL, Alonso, OF, Dietrich, WD and Hu, BR (2007). "Alterations in mammalian target of rapamycin signaling pathways after traumatic brain injury." J Cereb Blood Flow Metab **27**(5): 939-949.
- Chen, Y and Swanson, RA (2003). "Astrocytes and brain injury." J Cereb Blood Flow Metab **23**(2): 137-149.
- Chiaretti, A, Antonelli, A, Riccardi, R, Genovese, O, Pezzotti, P, Di Rocco, C, Tortorolo, L and Piedimonte, G (2008). "Nerve growth factor expression correlates with severity and outcome of traumatic brain injury in children." Eur J Paediatr Neurol **12**(3): 195-204.
- Chou, A, Morganti, JM and Rosi, S (2016). "Frontal Lobe Contusion in Mice Chronically Impairs Prefrontal-Dependent Behavior." PLoS One **11**(3): e0151418.
- Cicerone, K, Levin, H, Malec, J, Stuss, D and Whyte, J (2006). "Cognitive rehabilitation interventions for executive function: moving from bench to bedside in patients with traumatic brain injury." J Cogn Neurosci **18**(7): 1212-1222.
- Cifu, DX, Keyser-Marcus, L, Lopez, E, Wehman, P, Kreutzer, JS, Englander, J and High, W (1997). "Acute predictors of successful return to work 1 year after traumatic brain injury: a multicenter analysis." Arch Phys Med Rehabil **78**(2): 125-131.
- Clelland, CD, Choi, M, Romberg, C, Clemenson, GD, Jr., Fragniere, A, Tyers, P, Jessberger, S, Saksida, LM, Barker, RA, Gage, FH and Bussey, TJ (2009). "A functional role for adult hippocampal neurogenesis in spatial pattern separation." Science **325**(5937): 210-213.
- Cohen, J, Blethen, S, Kuntze, J, Smith, SL, Lomax, KG and Mathew, PM (2014). "Managing the child with severe primary insulin-like growth factor-1 deficiency (IGFD): IGFD diagnosis and management." Drugs R D **14**(1): 25-29.

- Collin, C, Miyaguchi, K and Segal, M (1997). "Dendritic spine density and LTP induction in cultured hippocampal slices." J Neurophysiol **77**(3): 1614-1623.
- Conover, JC and Todd, KL (2017). "Development and aging of a brain neural stem cell niche." Exp Gerontol **94**: 9-13.
- Copps, KD and White, MF (2012). "Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2." Diabetologia **55**(10): 2565-2582.
- Coronado, VG, McGuire, LC, Sarmiento, K, Bell, J, Lionbarger, MR, Jones, CD, Geller, AI, Khoury, N and Xu, L (2012). "Trends in Traumatic Brain Injury in the U.S. and the public health response: 1995-2009." J Safety Res **43**(4): 299-307.
- Cortez, SC, McIntosh, TK and Noble, LJ (1989). "Experimental fluid percussion brain injury: vascular disruption and neuronal and glial alterations." Brain Res **482**(2): 271-282.
- Dajani, DR and Uddin, LQ (2015). "Demystifying cognitive flexibility: Implications for clinical and developmental neuroscience." Trends Neurosci **38**(9): 571-578.
- Dash, PK, Mach, SA and Moore, AN (2001). "Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury." J Neurosci Res **63**(4): 313-319.
- Dash, PK, Orsi, SA and Moore, AN (2006). "Spatial memory formation and memory-enhancing effect of glucose involves activation of the tuberous sclerosis complex-Mammalian target of rapamycin pathway." J Neurosci **26**(31): 8048-8056.
- Dauth, S, Grevesse, T, Pantazopoulos, H, Campbell, PH, Maoz, BM, Berretta, S and Parker, KK (2016). "Extracellular matrix protein expression is brain region dependent." J Comp Neurol **524**(7): 1309-1336.
- Day, LB, Weisand, M, Sutherland, RJ and Schallert, T (1999). "The hippocampus is not necessary for a place response but may be necessary for pliancy." Behav Neurosci **113**(5): 914-924.
- Dayer, AG, Ford, AA, Cleaver, KM, Yassaee, M and Cameron, HA (2003). "Short-term and long-term survival of new neurons in the rat dentate gyrus." J Comp Neurol **460**(4): 563-572.
- DeKosky, ST, Taffe, KM, Abrahamson, EE, Dixon, CE, Kochanek, PM and Ikonovic, MD (2004). "Time course analysis of hippocampal nerve growth factor and antioxidant enzyme activity following lateral controlled cortical impact brain injury in the rat." J Neurotrauma **21**(5): 491-500.
- Deller, T, Haas, CA, Naumann, T, Joester, A, Faissner, A and Frotscher, M (1997). "Up-regulation of astrocyte-derived tenascin-C correlates with neurite outgrowth in the rat dentate gyrus after unilateral entorhinal cortex lesion." Neuroscience **81**(3): 829-846.
- Deng, W, Aimone, JB and Gage, FH (2010). "New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory?" Nat Rev Neurosci **11**(5): 339-350.
- Dennis, CV, Suh, LS, Rodriguez, ML, Kril, JJ and Sutherland, GT (2016). "Human adult neurogenesis across the ages: An immunohistochemical study." Neuropathol Appl Neurobiol **42**(7): 621-638.
- DeWitt, DS and Prough, DS (2009). "Blast-induced brain injury and posttraumatic hypotension and hypoxemia." J Neurotrauma **26**(6): 877-887.

- Dietrich, WD, Truettner, J, Zhao, W, Alonso, OF, Busto, R and Ginsberg, MD (1999). "Sequential changes in glial fibrillary acidic protein and gene expression following parasagittal fluid-percussion brain injury in rats." J Neurotrauma **16**(7): 567-581.
- Dikmen, SS, Corrigan, JD, Levin, HS, Machamer, J, Stiers, W and Weisskopf, MG (2009). "Cognitive outcome following traumatic brain injury." J Head Trauma Rehabil **24**(6): 430-438.
- Dillon, SE, Tsivos, D, Knight, M, McCann, B, Pennington, C, Shiel, AI, Conway, ME, Newson, MA, Kauppinen, RA and Coulthard, EJ (2017). "The impact of ageing reveals distinct roles for human dentate gyrus and CA3 in pattern separation and object recognition memory." Sci Rep **7**(1): 14069.
- Divall, SA and Radovick, S (2013). "Growth Hormone and Treatment Controversy; Long Term Safety of rGH." Curr Pediatr Rep **1**(2): 128-132.
- Dixon, CE, Kochanek, PM, Yan, HQ, Schiding, JK, Griffith, RG, Baum, E, Marion, DW and DeKosky, ST (1999). "One-year study of spatial memory performance, brain morphology, and cholinergic markers after moderate controlled cortical impact in rats." J Neurotrauma **16**(2): 109-122.
- Draznin, B (2006). "Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85alpha: the two sides of a coin." Diabetes **55**(8): 2392-2397.
- Duan, X, Chang, JH, Ge, S, Faulkner, RL, Kim, JY, Kitabatake, Y, Liu, XB, Yang, CH, Jordan, JD, Ma, DK, Liu, CY, Ganesan, S, Cheng, HJ, Ming, GL, Lu, B and Song, H (2007). "Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain." Cell **130**(6): 1146-1158.
- Eakin, K, Rowe, RK and Lifshitz, J (2015). Modeling Fluid Percussion Injury: Relevance to Human Traumatic Brain Injury. Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. F. H. Kobeissy. Boca Raton (FL).
- Eriksson, PS, Perfilieva, E, Bjork-Eriksson, T, Alborn, AM, Nordborg, C, Peterson, DA and Gage, FH (1998). "Neurogenesis in the adult human hippocampus." Nat Med **4**(11): 1313-1317.
- Evans, SJ, Choudary, PV, Neal, CR, Li, JZ, Vawter, MP, Tomita, H, Lopez, JF, Thompson, RC, Meng, F, Stead, JD, Walsh, DM, Myers, RM, Bunney, WE, Watson, SJ, Jones, EG and Akil, H (2004). "Dysregulation of the fibroblast growth factor system in major depression." Proc Natl Acad Sci U S A **101**(43): 15506-15511.
- Fanselow, MS and Dong, HW (2010). "Are the dorsal and ventral hippocampus functionally distinct structures?" Neuron **65**(1): 7-19.
- Farioli-Vecchioli, S, Sarauli, D, Costanzi, M, Pacioni, S, Cina, I, Aceti, M, Micheli, L, Bacci, A, Cestari, V and Tirone, F (2008). "The timing of differentiation of adult hippocampal neurons is crucial for spatial memory." PLoS Biol **6**(10): e246.
- Faul, M and Coronado, V (2015). "Epidemiology of traumatic brain injury." Handb Clin Neurol **127**: 3-13.
- Feeney, C, Sharp, DJ, Hellyer, PJ, Jolly, AE, Cole, JH, Scott, G, Baxter, D, Jilka, S, Ross, E, Ham, TE, Jenkins, PO, Li, LM, Gorgoraptis, N, Midwinter, M and Goldstone, AP (2017). "Serum insulin-like growth factor-I levels are associated

- with improved white matter recovery after traumatic brain injury." Ann Neurol **82**(1): 30-43.
- Feliciano, DM, Quon, JL, Su, T, Taylor, MM and Bordey, A (2012). "Postnatal neurogenesis generates heterotopias, olfactory micronodules and cortical infiltration following single-cell Tsc1 deletion." Hum Mol Genet **21**(4): 799-810.
- Feng, Z and Levine, AJ (2010). "The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein." Trends Cell Biol **20**(7): 427-434.
- Ferguson, PL, Smith, GM, Wannamaker, BB, Thurman, DJ, Pickelsimer, EE and Selassie, AW (2010). "A population-based study of risk of epilepsy after hospitalization for traumatic brain injury." Epilepsia **51**(5): 891-898.
- Flanagan, SR (2015). "Invited Commentary on "Centers for Disease Control and Prevention Report to Congress: Traumatic Brain Injury in the United States: Epidemiology and Rehabilitation"." Arch Phys Med Rehabil **96**(10): 1753-1755.
- Fleminger, S and Ponsford, J (2005). "Long term outcome after traumatic brain injury." BMJ **331**(7530): 1419-1420.
- Forslund, MV, Arango-Lasprilla, JC, Roe, C, Perrin, PB, Sigurdardottir, S and Andelic, N (2014). "Multi-level modelling of employment probability trajectories and employment stability at 1, 2 and 5 years after traumatic brain injury." Brain Inj **28**(7): 980-986.
- Friedland, D and Swash, M (2016). "Post-traumatic amnesia and confusional state: hazards of retrospective assessment." J Neurol Neurosurg Psychiatry **87**(10): 1068-1074.
- Frischknecht, R, Heine, M, Perrais, D, Seidenbecher, CI, Choquet, D and Gundelfinger, ED (2009). "Brain extracellular matrix affects AMPA receptor lateral mobility and short-term synaptic plasticity." Nat Neurosci **12**(7): 897-904.
- Gao, X and Chen, J (2013). "Moderate traumatic brain injury promotes neural precursor proliferation without increasing neurogenesis in the adult hippocampus." Exp Neurol **239**: 38-48.
- Gao, X, Deng-Bryant, Y, Cho, W, Carrico, KM, Hall, ED and Chen, J (2008). "Selective death of newborn neurons in hippocampal dentate gyrus following moderate experimental traumatic brain injury." J Neurosci Res **86**(10): 2258-2270.
- Garcia-Martinez, JM, Perez-Navarro, E, Gavaldà, N and Alberch, J (2006). "Glial cell line-derived neurotrophic factor promotes the arborization of cultured striatal neurons through the p42/p44 mitogen-activated protein kinase pathway." J Neurosci Res **83**(1): 68-79.
- Garthe, A, Behr, J and Kempermann, G (2009). "Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies." PLoS One **4**(5): e5464.
- Garthe, A and Kempermann, G (2013). "An old test for new neurons: refining the Morris water maze to study the functional relevance of adult hippocampal neurogenesis." Front Neurosci **7**: 63.
- Garza-Lombo, C and Gonsebatt, ME (2016). "Mammalian Target of Rapamycin: Its Role in Early Neural Development and in Adult and Aged Brain Function." Front Cell Neurosci **10**: 157.
- Ge, S, Pradhan, DA, Ming, GL and Song, H (2007). "GABA sets the tempo for activity-dependent adult neurogenesis." Trends Neurosci **30**(1): 1-8.

- Ge, S, Sailor, KA, Ming, GL and Song, H (2008). "Synaptic integration and plasticity of new neurons in the adult hippocampus." *J Physiol* **586**(16): 3759-3765.
- Gong, C, Wang, TW, Huang, HS and Parent, JM (2007). "Reelin regulates neuronal progenitor migration in intact and epileptic hippocampus." *J Neurosci* **27**(8): 1803-1811.
- Gotz, M and Barde, YA (2005). "Radial glial cells defined and major intermediates between embryonic stem cells and CNS neurons." *Neuron* **46**(3): 369-372.
- Greve, MW and Zink, BJ (2009). "Pathophysiology of traumatic brain injury." *Mt Sinai J Med* **76**(2): 97-104.
- Griesbach, GS, Hovda, DA, Molteni, R, Wu, A and Gomez-Pinilla, F (2004). "Voluntary exercise following traumatic brain injury: brain-derived neurotrophic factor upregulation and recovery of function." *Neuroscience* **125**(1): 129-139.
- Gunn, RM and Hailes, HC (2008). "Insights into the PI3-K-PKB-mTOR signalling pathway from small molecules." *J Chem Biol* **1**(1-4): 49-62.
- Guo, D, Zeng, L, Brody, DL and Wong, M (2013). "Rapamycin attenuates the development of posttraumatic epilepsy in a mouse model of traumatic brain injury." *PLoS One* **8**(5): e64078.
- Guzowski, JF, McNaughton, BL, Barnes, CA and Worley, PF (1999). "Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles." *Nat Neurosci* **2**(12): 1120-1124.
- Hall, ED, Sullivan, PG, Gibson, TR, Pavel, KM, Thompson, BM and Scheff, SW (2005). "Spatial and temporal characteristics of neurodegeneration after controlled cortical impact in mice: more than a focal brain injury." *J Neurotrauma* **22**(2): 252-265.
- Han, J, Kim, HJ, Schafer, ST, Paquola, A, Clemenson, GD, Toda, T, Oh, J, Pankonin, AR, Lee, BS, Johnston, ST, Sarkar, A, Denli, AM and Gage, FH (2016). "Functional Implications of miR-19 in the Migration of Newborn Neurons in the Adult Brain." *Neuron* **91**(1): 79-89.
- Han, J, Wang, B, Xiao, Z, Gao, Y, Zhao, Y, Zhang, J, Chen, B, Wang, X and Dai, J (2008). "Mammalian target of rapamycin (mTOR) is involved in the neuronal differentiation of neural progenitors induced by insulin." *Mol Cell Neurosci* **39**(1): 118-124.
- Harrington, LS, Findlay, GM, Gray, A, Tolkacheva, T, Wigfield, S, Rebholz, H, Barnett, J, Leslie, NR, Cheng, S, Shepherd, PR, Gout, I, Downes, CP and Lamb, RF (2004). "The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins." *J Cell Biol* **166**(2): 213-223.
- Hart, T, Brenner, L, Clark, AN, Bogner, JA, Novack, TA, Chervoneva, I, Nakase-Richardson, R and Arango-Lasprilla, JC (2011). "Major and minor depression after traumatic brain injury." *Arch Phys Med Rehabil* **92**(8): 1211-1219.
- Hart, T, Kozlowski, AJ, Whyte, J, Poulsen, I, Kristensen, K, Nordenbo, A and Heinemann, AW (2014). "Functional recovery after severe traumatic brain injury: an individual growth curve approach." *Arch Phys Med Rehabil* **95**(11): 2103-2110.
- Hartman, NW, Lin, TV, Zhang, L, Paquelet, GE, Feliciano, DM and Bordey, A (2013). "mTORC1 targets the translational repressor 4E-BP2, but not S6 kinase 1/2, to regulate neural stem cell self-renewal in vivo." *Cell Rep* **5**(2): 433-444.

- Hatton, J, Kryscio, R, Ryan, M, Ott, L and Young, B (2006). "Systemic metabolic effects of combined insulin-like growth factor-I and growth hormone therapy in patients who have sustained acute traumatic brain injury." J Neurosurg **105**(6): 843-852.
- Hatton, J, Rapp, RP, Kudsk, KA, Brown, RO, Luer, MS, Bukar, JG, Chen, SA, McClain, CJ, Gesundheit, N, Dempsey, RJ and Young, B (1997). "Intravenous insulin-like growth factor-I (IGF-I) in moderate-to-severe head injury: a phase II safety and efficacy trial." J Neurosurg **86**(5): 779-786.
- Hawryluk, GW and Manley, GT (2015). "Classification of traumatic brain injury: past, present, and future." Handb Clin Neurol **127**: 15-21.
- Hayward, NM, Tuunanen, PI, Immonen, R, Ndode-Ekane, XE, Pitkanen, A and Grohn, O (2011). "Magnetic resonance imaging of regional hemodynamic and cerebrovascular recovery after lateral fluid-percussion brain injury in rats." J Cereb Blood Flow Metab **31**(1): 166-177.
- Heigele, S, Sultan, S, Toni, N and Bischofberger, J (2016). "Bidirectional GABAergic control of action potential firing in newborn hippocampal granule cells." Nat Neurosci **19**(2): 263-270.
- Hill, JL, Kobori, N, Zhao, J, Rozas, NS, Hylin, MJ, Moore, AN and Dash, PK (2016). "Traumatic brain injury decreases AMP-activated protein kinase activity and pharmacological enhancement of its activity improves cognitive outcome." J Neurochem **139**(1): 106-119.
- Holland, MC, Mackersie, RC, Morabito, D, Campbell, AR, Kivett, VA, Patel, R, Erickson, VR and Pittet, JF (2003). "The development of acute lung injury is associated with worse neurologic outcome in patients with severe traumatic brain injury." J Trauma **55**(1): 106-111.
- Horster, H, Garthe, A, Walker, TL, Ichwan, M, Steiner, B, Khan, MA, Lie, DC, Nicola, Z, Ramirez-Rodriguez, G and Kempermann, G (2017). "p27kip1 Is Required for Functionally Relevant Adult Hippocampal Neurogenesis in Mice." Stem Cells **35**(3): 787-799.
- Huat, TJ, Khan, AA, Pati, S, Mustafa, Z, Abdullah, JM and Jaafar, H (2014). "IGF-1 enhances cell proliferation and survival during early differentiation of mesenchymal stem cells to neural progenitor-like cells." BMC Neurosci **15**: 91.
- Hung, CM, Garcia-Haro, L, Sparks, CA and Guertin, DA (2012). "mTOR-dependent cell survival mechanisms." Cold Spring Harb Perspect Biol **4**(12).
- Hunt, RF, Scheff, SW and Smith, BN (2010). "Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy." J Neurophysiol **103**(3): 1490-1500.
- Hunt, RF, Scheff, SW and Smith, BN (2011). "Synaptic reorganization of inhibitory hilar interneuron circuitry after traumatic brain injury in mice." J Neurosci **31**(18): 6880-6890.
- Hurtado-Chong, A, Yusta-Boyo, MJ, Vergano-Vera, E, Bulfone, A, de Pablo, F and Vicario-Abejon, C (2009). "IGF-I promotes neuronal migration and positioning in the olfactory bulb and the exit of neuroblasts from the subventricular zone." Eur J Neurosci **30**(5): 742-755.
- Husson, EC, Ribbers, GM, Willemse-van Son, AH, Verhagen, AP and Stam, HJ (2010). "Prognosis of six-month functioning after moderate to severe traumatic brain

- injury: a systematic review of prospective cohort studies." J Rehabil Med **42**(5): 425-436.
- Hyde, LA, Hoplight, BJ and Denenberg, VH (1998). "Water version of the radial-arm maze: learning in three inbred strains of mice." Brain Res **785**(2): 236-244.
- Ibrahim, S, Hu, W, Wang, X, Gao, X, He, C and Chen, J (2016). "Traumatic Brain Injury Causes Aberrant Migration of Adult-Born Neurons in the Hippocampus." Sci Rep **6**: 21793.
- Idris, N, Neill, J, Grayson, B, Bang-Andersen, B, Witten, LM, Brennum, LT and Arnt, J (2010). "Sertindole improves sub-chronic PCP-induced reversal learning and episodic memory deficits in rodents: involvement of 5-HT(6) and 5-HT (2A) receptor mechanisms." Psychopharmacology (Berl) **208**(1): 23-36.
- Ishikawa, R, Fukushima, H, Frankland, PW and Kida, S (2016). "Hippocampal neurogenesis enhancers promote forgetting of remote fear memory after hippocampal reactivation by retrieval." Elife **5**.
- Jacotte-Simancas, A, Costa-Miserachs, D, Coll-Andreu, M, Torras-Garcia, M, Borlongan, CV and Portell-Cortes, I (2015). "Effects of voluntary physical exercise, citicoline, and combined treatment on object recognition memory, neurogenesis, and neuroprotection after traumatic brain injury in rats." J Neurotrauma **32**(10): 739-751.
- Jagger, J, Vernberg, K and Jane, JA (1987). "Air bags: reducing the toll of brain trauma." Neurosurgery **20**(5): 815-817.
- Jaworski, J, Spangler, S, Seeburg, DP, Hoogenraad, CC and Sheng, M (2005). "Control of dendritic arborization by the phosphoinositide-3'-kinase-Akt-mammalian target of rapamycin pathway." J Neurosci **25**(49): 11300-11312.
- Jenkins, LW, Peters, GW, Dixon, CE, Zhang, X, Clark, RS, Skinner, JC, Marion, DW, Adelson, PD and Kochanek, PM (2002). "Conventional and functional proteomics using large format two-dimensional gel electrophoresis 24 hours after controlled cortical impact in postnatal day 17 rats." J Neurotrauma **19**(6): 715-740.
- Jessberger, S, Aigner, S, Clemenson, GD, Jr., Toni, N, Lie, DC, Karalay, O, Overall, R, Kempermann, G and Gage, FH (2008). "Cdk5 regulates accurate maturation of newborn granule cells in the adult hippocampus." PLoS Biol **6**(11): e272.
- Jessberger, S, Romer, B, Babu, H and Kempermann, G (2005). "Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells." Exp Neurol **196**(2): 342-351.
- Jessberger, S, Zhao, C, Toni, N, Clemenson, GD, Jr., Li, Y and Gage, FH (2007). "Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling." J Neurosci **27**(35): 9400-9407.
- Jinde, S, Zsiros, V and Nakazawa, K (2013). "Hilar mossy cell circuitry controlling dentate granule cell excitability." Front Neural Circuits **7**: 14.
- Jinnou, H, Sawada, M, Kawase, K, Kaneko, N, Herranz-Perez, V, Miyamoto, T, Kawaue, T, Miyata, T, Tabata, Y, Akaike, T, Garcia-Verdugo, JM, Ajioka, I, Saitoh, S and Sawamoto, K (2018). "Radial Glial Fibers Promote Neuronal Migration and Functional Recovery after Neonatal Brain Injury." Cell Stem Cell **22**(1): 128-137 e129.
- Johnson, VE, Meaney, DF, Cullen, DK and Smith, DH (2015). "Animal models of traumatic brain injury." Handb Clin Neurol **127**: 115-128.

- Joinson, C, O'Callaghan, FJ, Osborne, JP, Martyn, C, Harris, T and Bolton, PF (2003). "Learning disability and epilepsy in an epidemiological sample of individuals with tuberous sclerosis complex." Psychol Med **33**(2): 335-344.
- Jones, DM, Tucker, BA, Rahimtula, M and Mearow, KM (2003). "The synergistic effects of NGF and IGF-1 on neurite growth in adult sensory neurons: convergence on the PI 3-kinase signaling pathway." J Neurochem **86**(5): 1116-1128.
- Joseph D'Ercole, A and Ye, P (2008). "Expanding the mind: insulin-like growth factor I and brain development." Endocrinology **149**(12): 5958-5962.
- Justova, V, Lacinova, Z, Melenovsky, V, Marek, J, Holly, JM and Hass, T (2001). "The changes of IGF binding proteins after rhGH administration to patients totally dependent on parenteral nutrition." Growth Horm IGF Res **11**(6): 407-415.
- Kappeler, L, De Magalhaes Filho, C, Dupont, J, Leneuve, P, Cervera, P, Perin, L, Loudes, C, Blaise, A, Klein, R, Epelbaum, J, Le Bouc, Y and Holzenberger, M (2008). "Brain IGF-1 receptors control mammalian growth and lifespan through a neuroendocrine mechanism." PLoS Biol **6**(10): e254.
- Karelina, K, Hansen, KF, Choi, YS, DeVries, AC, Arthur, JS and Obrietan, K (2012). "MSK1 regulates environmental enrichment-induced hippocampal plasticity and cognitive enhancement." Learn Mem **19**(11): 550-560.
- Kempermann, G and Gage, FH (2002). "Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis." Brain Res Dev Brain Res **134**(1-2): 1-12.
- Kempermann, G, Gast, D, Kronenberg, G, Yamaguchi, M and Gage, FH (2003). "Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice." Development **130**(2): 391-399.
- Kempermann, G, Song, H and Gage, FH (2015). "Neurogenesis in the Adult Hippocampus." Cold Spring Harb Perspect Biol **7**(9): a018812.
- Kernie, SG, Erwin, TM and Parada, LF (2001). "Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice." J Neurosci Res **66**(3): 317-326.
- Kilincaslan, A, Kok, BE, Tekturk, P, Yalcinkaya, C, Ozkara, C and Yapici, Z (2017). "Beneficial Effects of Everolimus on Autism and Attention-Deficit/Hyperactivity Disorder Symptoms in a Group of Patients with Tuberous Sclerosis Complex." J Child Adolesc Psychopharmacol **27**(4): 383-388.
- Kim, B, Leventhal, PS, Saltiel, AR and Feldman, EL (1997). "Insulin-like growth factor-I-mediated neurite outgrowth in vitro requires mitogen-activated protein kinase activation." J Biol Chem **272**(34): 21268-21273.
- Kim, JY, Duan, X, Liu, CY, Jang, MH, Guo, JU, Pow-anpongkul, N, Kang, E, Song, H and Ming, GL (2009). "DISC1 regulates new neuron development in the adult brain via modulation of AKT-mTOR signaling through KIAA1212." Neuron **63**(6): 761-773.
- Kim, S, Lee, SH, Kim, JH, Jeong, YW, Hashem, MA, Koo, OJ, Park, SM, Lee, EG, Hossein, MS, Kang, SK, Lee, BC and Hwang, WS (2006). "Anti-apoptotic effect of insulin-like growth factor (IGF)-I and its receptor in porcine preimplantation embryos derived from in vitro fertilization and somatic cell nuclear transfer." Mol Reprod Dev **73**(12): 1523-1530.
- Kim, WR, Kim, Y, Eun, B, Park, OH, Kim, H, Kim, K, Park, CH, Vinsant, S, Oppenheim, RW and Sun, W (2007). "Impaired migration in the rostral migratory

- stream but spared olfactory function after the elimination of programmed cell death in Bax knock-out mice." J Neurosci **27**(52): 14392-14403.
- Kishi, T, Yoshimura, R, Ikuta, T and Iwata, N (2017). "Brain-Derived Neurotrophic Factor and Major Depressive Disorder: Evidence from Meta-Analyses." Front Psychiatry **8**: 308.
- Kjelstrup, KG, Tuvnes, FA, Steffenach, HA, Murison, R, Moser, EI and Moser, MB (2002). "Reduced fear expression after lesions of the ventral hippocampus." Proc Natl Acad Sci U S A **99**(16): 10825-10830.
- Kleindienst, A, McGinn, MJ, Harvey, HB, Colello, RJ, Hamm, RJ and Bullock, MR (2005). "Enhanced hippocampal neurogenesis by intraventricular S100B infusion is associated with improved cognitive recovery after traumatic brain injury." J Neurotrauma **22**(6): 645-655.
- Kleinknecht, KR, Bedenk, BT, Kaltwasser, SF, Grunecker, B, Yen, YC, Czisch, M and Wotjak, CT (2012). "Hippocampus-dependent place learning enables spatial flexibility in C57BL6/N mice." Front Behav Neurosci **6**: 87.
- Knierim, JJ (2015). "The hippocampus." Curr Biol **25**(23): R1116-1121.
- Knoth, R, Singec, I, Ditter, M, Pantazis, G, Capetian, P, Meyer, RP, Horvat, V, Volk, B and Kempermann, G (2010). "Murine features of neurogenesis in the human hippocampus across the lifespan from 0 to 100 years." PLoS One **5**(1): e8809.
- Konigs, M, Engenhorst, PJ and Oosterlaan, J (2016). "Intelligence after traumatic brain injury: meta-analysis of outcomes and prognosis." Eur J Neurol **23**(1): 21-29.
- Kopczak, A, Stalla, GK, Uhr, M, Lucae, S, Hennings, J, Ising, M, Holsboer, F and Kloiber, S (2015). "IGF-I in major depression and antidepressant treatment response." Eur Neuropsychopharmacol **25**(6): 864-872.
- Kotulska, K, Borkowska, J, Mandra, M, Roszkowski, M, Jurkiewicz, E, Grajkowska, W, Bilka, M and Jozwiak, S (2014). "Congenital subependymal giant cell astrocytomas in patients with tuberous sclerosis complex." Childs Nerv Syst **30**(12): 2037-2042.
- Krishnan, V and Nestler, EJ (2011). "Animal models of depression: molecular perspectives." Curr Top Behav Neurosci **7**: 121-147.
- Kuhn, HG, Dickinson-Anson, H and Gage, FH (1996). "Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation." J Neurosci **16**(6): 2027-2033.
- Kumar, V, Zhang, MX, Swank, MW, Kunz, J and Wu, GY (2005). "Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways." J Neurosci **25**(49): 11288-11299.
- Kurland, D, Hong, C, Aarabi, B, Gerzanich, V and Simard, JM (2012). "Hemorrhagic progression of a contusion after traumatic brain injury: a review." J Neurotrauma **29**(1): 19-31.
- Langlois, JA, Rutland-Brown, W and Wald, MM (2006). "The epidemiology and impact of traumatic brain injury: a brief overview." J Head Trauma Rehabil **21**(5): 375-378.
- Laplante, M and Sabatini, DM (2009). "mTOR signaling at a glance." J Cell Sci **122**(Pt 20): 3589-3594.
- Laplante, M and Sabatini, DM (2012). "mTOR signaling in growth control and disease." Cell **149**(2): 274-293.

- Larphaveesarp, A, Ferriero, DM and Gonzalez, FF (2015). "Growth factors for the treatment of ischemic brain injury (growth factor treatment)." Brain Sci **5**(2): 165-177.
- Le Greves, M, Le Greves, P and Nyberg, F (2005). "Age-related effects of IGF-1 on the NMDA-, GH- and IGF-1-receptor mRNA transcripts in the rat hippocampus." Brain Res Bull **65**(5): 369-374.
- Le Greves, M, Zhou, Q, Berg, M, Le Greves, P, Fholenag, K, Meyerson, B and Nyberg, F (2006). "Growth hormone replacement in hypophysectomized rats affects spatial performance and hippocampal levels of NMDA receptor subunit and PSD-95 gene transcript levels." Exp Brain Res **173**(2): 267-273.
- Lee, C and Agoston, DV (2010). "Vascular endothelial growth factor is involved in mediating increased de novo hippocampal neurogenesis in response to traumatic brain injury." J Neurotrauma **27**(3): 541-553.
- Lee, DY (2015). "Roles of mTOR Signaling in Brain Development." Exp Neurobiol **24**(3): 177-185.
- Lee, TK, Chen, BH, Lee, JC, Shin, MC, Cho, JH, Lee, HA, Choi, JH, Hwang, IK, Kang, IJ, Ahn, JH, Park, JH, Choi, SY and Won, MH (2018). "Agedependent decreases in insulinlike growth factorI and its receptor expressions in the gerbil olfactory bulb." Mol Med Rep **17**(6): 8161-8166.
- Leunissen, I, Coxon, JP, Caeyenberghs, K, Michiels, K, Sunaert, S and Swinnen, SP (2014). "Subcortical volume analysis in traumatic brain injury: the importance of the fronto-striato-thalamic circuit in task switching." Cortex **51**: 67-81.
- Li, Y, Sun, W, Han, S, Li, J, Ding, S, Wang, W and Yin, Y (2017). "IGF-1-Involved Negative Feedback of NR2B NMDA Subunits Protects Cultured Hippocampal Neurons Against NMDA-Induced Excitotoxicity." Mol Neurobiol **54**(1): 684-696.
- Liu, L, Brown, JC, 3rd, Webster, WW, Morrisett, RA and Monaghan, DT (1995). "Insulin potentiates N-methyl-D-aspartate receptor activity in Xenopus oocytes and rat hippocampus." Neurosci Lett **192**(1): 5-8.
- Liu, W, Ye, P, O'Kusky, JR and D'Ercole, AJ (2009). "Type 1 insulin-like growth factor receptor signaling is essential for the development of the hippocampal formation and dentate gyrus." J Neurosci Res **87**(13): 2821-2832.
- Lovblad, KO, Schaller, K and Vargas, MI (2014). "The fornix and limbic system." Semin Ultrasound CT MR **35**(5): 459-473.
- Lowenstein, DH, Thomas, MJ, Smith, DH and McIntosh, TK (1992). "Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus." J Neurosci **12**(12): 4846-4853.
- Lu, D, Mahmood, A, Qu, C, Goussev, A, Schallert, T and Chopp, M (2005). "Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury." J Neurotrauma **22**(9): 1011-1017.
- Lu, D, Qu, C, Goussev, A, Jiang, H, Lu, C, Schallert, T, Mahmood, A, Chen, J, Li, Y and Chopp, M (2007). "Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury." J Neurotrauma **24**(7): 1132-1146.
- Lyeth, BG, Jenkins, LW, Hamm, RJ, Dixon, CE, Phillips, LL, Clifton, GL, Young, HF and Hayes, RL (1990). "Prolonged memory impairment in the absence of

- hippocampal cell death following traumatic brain injury in the rat." Brain Res **526**(2): 249-258.
- Maas, AI, Marmarou, A, Murray, GD, Teasdale, SG and Steyerberg, EW (2007). "Prognosis and clinical trial design in traumatic brain injury: the IMPACT study." J Neurotrauma **24**(2): 232-238.
- Madathil, SK, Carlson, SW, Brelsfoard, JM, Ye, P, D'Ercole, AJ and Saatman, KE (2013). "Astrocyte-Specific Overexpression of Insulin-Like Growth Factor-1 Protects Hippocampal Neurons and Reduces Behavioral Deficits following Traumatic Brain Injury in Mice." PLoS One **8**(6): e67204.
- Madathil, SK, Evans, HN and Saatman, KE (2010). "Temporal and regional changes in IGF-1/IGF-1R signaling in the mouse brain after traumatic brain injury." J Neurotrauma **27**(1): 95-107.
- Magri, L and Galli, R (2013). "mTOR signaling in neural stem cells: from basic biology to disease." Cell Mol Life Sci **70**(16): 2887-2898.
- Mahmood, A, Lu, D, Qu, C, Goussev, A, Zhang, ZG, Lu, C and Chopp, M (2007). "Treatment of traumatic brain injury in rats with erythropoietin and carbamylated erythropoietin." J Neurosurg **107**(2): 392-397.
- Mairet-Coello, G, Tury, A and DiCicco-Bloom, E (2009). "Insulin-like growth factor-1 promotes G(1)/S cell cycle progression through bidirectional regulation of cyclins and cyclin-dependent kinase inhibitors via the phosphatidylinositol 3-kinase/Akt pathway in developing rat cerebral cortex." J Neurosci **29**(3): 775-788.
- Mander, BA, Rao, V, Lu, B, Saletin, JM, Lindquist, JR, Ancoli-Israel, S, Jagust, W and Walker, MP (2013). "Prefrontal atrophy, disrupted NREM slow waves and impaired hippocampal-dependent memory in aging." Nat Neurosci **16**(3): 357-364.
- Marais, L, Stein, DJ and Daniels, WM (2009). "Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats." Metab Brain Dis **24**(4): 587-597.
- Marklund, N and Hillered, L (2011). "Animal modelling of traumatic brain injury in preclinical drug development: where do we go from here?" Br J Pharmacol **164**(4): 1207-1229.
- Martin, LA, Tan, SS and Goldowitz, D (2002). "Clonal architecture of the mouse hippocampus." J Neurosci **22**(9): 3520-3530.
- Matsuo, R, Ago, T, Kamouchi, M, Kuroda, J, Kuwashiro, T, Hata, J, Sugimori, H, Fukuda, K, Gotoh, S, Makihara, N, Fukuhara, M, Awano, H, Isomura, T, Suzuki, K, Yasaka, M, Okada, Y, Kiyohara, Y and Kitazono, T (2013). "Clinical significance of plasma VEGF value in ischemic stroke - research for biomarkers in ischemic stroke (REBIOS) study." BMC Neurol **13**: 32.
- Meehan, WP, 3rd, Mannix, RC, O'Brien, MJ and Collins, MW (2013). "The prevalence of undiagnosed concussions in athletes." Clin J Sport Med **23**(5): 339-342.
- Meikle, L, Talos, DM, Onda, H, Pollizzi, K, Rotenberg, A, Sahin, M, Jensen, FE and Kwiatkowski, DJ (2007). "A mouse model of tuberous sclerosis: neuronal loss of Tsc1 causes dysplastic and ectopic neurons, reduced myelination, seizure activity, and limited survival." J Neurosci **27**(21): 5546-5558.
- Mendoza, MC, Er, EE and Blenis, J (2011). "The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation." Trends Biochem Sci **36**(6): 320-328.

- Meng, Y, Chopp, M, Zhang, Y, Liu, Z, An, A, Mahmood, A and Xiong, Y (2014). "Subacute intranasal administration of tissue plasminogen activator promotes neuroplasticity and improves functional recovery following traumatic brain injury in rats." PLoS One **9**(9): e106238.
- Miller, JF, Neufang, M, Solway, A, Brandt, A, Trippel, M, Mader, I, Hefft, S, Merkow, M, Polyn, SM, Jacobs, J, Kahana, MJ and Schulze-Bonhage, A (2013). "Neural activity in human hippocampal formation reveals the spatial context of retrieved memories." Science **342**(6162): 1111-1114.
- Miltiadous, P, Stamatakis, A, Koutsoudaki, PN, Tiniakos, DG and Stylianopoulou, F (2011). "IGF-I ameliorates hippocampal neurodegeneration and protects against cognitive deficits in an animal model of temporal lobe epilepsy." Exp Neurol **231**(2): 223-235.
- Molina, DP, Ariwodola, OJ, Linville, C, Sonntag, WE, Weiner, JL, Brunso-Bechtold, JK and Adams, MM (2012). "Growth hormone modulates hippocampal excitatory synaptic transmission and plasticity in old rats." Neurobiol Aging **33**(9): 1938-1949.
- Morales, DM, Marklund, N, Lebold, D, Thompson, HJ, Pitkanen, A, Maxwell, WL, Longhi, L, Laurer, H, Maegele, M, Neugebauer, E, Graham, DI, Stocchetti, N and McIntosh, TK (2005). "Experimental models of traumatic brain injury: do we really need to build a better mousetrap?" Neuroscience **136**(4): 971-989.
- Morganti, JM, Jopson, TD, Liu, S, Riparip, LK, Guandique, CK, Gupta, N, Ferguson, AR and Rosi, S (2015). "CCR2 antagonism alters brain macrophage polarization and ameliorates cognitive dysfunction induced by traumatic brain injury." J Neurosci **35**(2): 748-760.
- Mori, T, Wang, X, Jung, JC, Sumii, T, Singhal, AB, Fini, ME, Dixon, CE, Alessandrini, A and Lo, EH (2002). "Mitogen-activated protein kinase inhibition in traumatic brain injury: in vitro and in vivo effects." J Cereb Blood Flow Metab **22**(4): 444-452.
- Moser, E, Moser, MB and Andersen, P (1993). "Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions." J Neurosci **13**(9): 3916-3925.
- Muhic, M, Vardjan, N, Chowdhury, HH, Zorec, R and Kreft, M (2015). "Insulin and Insulin-like Growth Factor 1 (IGF-1) Modulate Cytoplasmic Glucose and Glycogen Levels but Not Glucose Transport across the Membrane in Astrocytes." J Biol Chem **290**(17): 11167-11176.
- Myers, CE, Bermudez-Hernandez, K and Scharfman, HE (2013). "The influence of ectopic migration of granule cells into the hilus on dentate gyrus-CA3 function." PLoS One **8**(6): e68208.
- Nakase-Richardson, R, Sepehri, A, Sherer, M, Yablon, SA, Evans, C and Mani, T (2009). "Classification schema of posttraumatic amnesia duration-based injury severity relative to 1-year outcome: analysis of individuals with moderate and severe traumatic brain injury." Arch Phys Med Rehabil **90**(1): 17-19.
- Neuberger, EJ, Swietek, B, Corrubia, L, Prasanna, A and Santhakumar, V (2017). "Enhanced Dentate Neurogenesis after Brain Injury Undermines Long-Term Neurogenic Potential and Promotes Seizure Susceptibility." Stem Cell Reports **9**(3): 972-984.

- Ng, T, Hor, CH, Chew, B, Zhao, J, Zhong, Z, Ryu, JR and Goh, EL (2016). "Neuropilin 2 Signaling Is Involved in Cell Positioning of Adult-born Neurons through Glycogen Synthase Kinase-3beta (GSK3beta)." J Biol Chem **291**(48): 25088-25095.
- Nieto-Estevez, V, Defterali, C and Vicario-Abejon, C (2016). "IGF-I: A Key Growth Factor that Regulates Neurogenesis and Synaptogenesis from Embryonic to Adult Stages of the Brain." Front Neurosci **10**: 52.
- Nieto-Estevez, V, Oueslati-Morales, CO, Li, L, Pickel, J, Morales, AV and Vicario-Abejon, C (2016). "Brain Insulin-Like Growth Factor-I Directs the Transition from Stem Cells to Mature Neurons During Postnatal/Adult Hippocampal Neurogenesis." Stem Cells **34**(8): 2194-2209.
- Nikolaeva, I, Crowell, B, Valenziano, J, Meaney, D and D'Arcangelo, G (2016). "Beneficial Effects of Early mTORC1 Inhibition after Traumatic Brain Injury." J Neurotrauma **33**(2): 183-193.
- Ning, R, Xiong, Y, Mahmood, A, Zhang, Y, Meng, Y, Qu, C and Chopp, M (2011). "Erythropoietin promotes neurovascular remodeling and long-term functional recovery in rats following traumatic brain injury." Brain Res **1384**: 140-150.
- Niv, F, Keiner, S, Krishna, Witte, OW, Lie, DC and Redecker, C (2012). "Aberrant neurogenesis after stroke: a retroviral cell labeling study." Stroke **43**(9): 2468-2475.
- Nixon, K, Kim, DH, Potts, EN, He, J and Crews, FT (2008). "Distinct cell proliferation events during abstinence after alcohol dependence: microglia proliferation precedes neurogenesis." Neurobiol Dis **31**(2): 218-229.
- O'Kusky, J and Ye, P (2012). "Neurodevelopmental effects of insulin-like growth factor signaling." Front Neuroendocrinol **33**(3): 230-251.
- Ocrant, I, Valentino, KL, Eng, LF, Hintz, RL, Wilson, DM and Rosenfeld, RG (1988). "Structural and immunohistochemical characterization of insulin-like growth factor I and II receptors in the murine central nervous system." Endocrinology **123**(2): 1023-1034.
- Orozco, OE, Walus, L, Sah, DW, Pepinsky, RB and Sanicola, M (2001). "GFRalpha3 is expressed predominantly in nociceptive sensory neurons." Eur J Neurosci **13**(11): 2177-2182.
- Osier, ND and Dixon, CE (2016). "The Controlled Cortical Impact Model: Applications, Considerations for Researchers, and Future Directions." Front Neurol **7**: 134.
- Otaegi, G, Yusta-Boyo, MJ, Vergano-Vera, E, Mendez-Gomez, HR, Carrera, AC, Abad, JL, Gonzalez, M, de la Rosa, EJ, Vicario-Abejon, C and de Pablo, F (2006). "Modulation of the PI 3-kinase-Akt signalling pathway by IGF-I and PTEN regulates the differentiation of neural stem/precursor cells." J Cell Sci **119**(Pt 13): 2739-2748.
- Paliouras, GN, Hamilton, LK, Aumont, A, Joppe, SE, Barnabe-Heider, F and Fernandes, KJ (2012). "Mammalian target of rapamycin signaling is a key regulator of the transit-amplifying progenitor pool in the adult and aging forebrain." J Neurosci **32**(43): 15012-15026.
- Palmer, TD, Willhoite, AR and Gage, FH (2000). "Vascular niche for adult hippocampal neurogenesis." J Comp Neurol **425**(4): 479-494.

- Parent, JM, Yu, TW, Leibowitz, RT, Geschwind, DH, Sloviter, RS and Lowenstein, DH (1997). "Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus." J Neurosci **17**(10): 3727-3738.
- Park, J, Zhang, J, Qiu, J, Zhu, X, Degtrev, A, Lo, EH and Whalen, MJ (2012). "Combination therapy targeting Akt and mammalian target of rapamycin improves functional outcome after controlled cortical impact in mice." J Cereb Blood Flow Metab **32**(2): 330-340.
- Patel, AD, Gerzanich, V, Geng, Z and Simard, JM (2010). "Glibenclamide reduces hippocampal injury and preserves rapid spatial learning in a model of traumatic brain injury." J Neuropathol Exp Neurol **69**(12): 1177-1190.
- Patnaik, A, et al. (2016). "First-in-human phase I study of copanlisib (BAY 80-6946), an intravenous pan-class I phosphatidylinositol 3-kinase inhibitor, in patients with advanced solid tumors and non-Hodgkin's lymphomas." Ann Oncol **27**(10): 1928-1940.
- Peineau, S, Taghibiglou, C, Bradley, C, Wong, TP, Liu, L, Lu, J, Lo, E, Wu, D, Saule, E, Bouschet, T, Matthews, P, Isaac, JT, Bortolotto, ZA, Wang, YT and Collingridge, GL (2007). "LTP inhibits LTD in the hippocampus via regulation of GSK3beta." Neuron **53**(5): 703-717.
- Perera, TD, Dwork, AJ, Keegan, KA, Thirumangalakudi, L, Lipira, CM, Joyce, N, Lange, C, Higley, JD, Rosoklija, G, Hen, R, Sackeim, HA and Coplan, JD (2011). "Necessity of hippocampal neurogenesis for the therapeutic action of antidepressants in adult nonhuman primates." PLoS One **6**(4): e17600.
- Perluigi, M, Di Domenico, F and Butterfield, DA (2015). "mTOR signaling in aging and neurodegeneration: At the crossroad between metabolism dysfunction and impairment of autophagy." Neurobiol Dis **84**: 39-49.
- Phillips, C (2017). "Brain-Derived Neurotrophic Factor, Depression, and Physical Activity: Making the Neuroplastic Connection." Neural Plast **2017**: 7260130.
- Pierce, JE, Smith, DH, Trojanowski, JQ and McIntosh, TK (1998). "Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats." Neuroscience **87**(2): 359-369.
- Pleasant, JM, Carlson, SW, Mao, H, Scheff, SW, Yang, KH and Saatman, KE (2011). "Rate of neurodegeneration in the mouse controlled cortical impact model is influenced by impactor tip shape: implications for mechanistic and therapeutic studies." J Neurotrauma **28**(11): 2245-2262.
- Pomerance, M, Gavaret, JM, Jacquemin, C, Matricon, C, Toru-Delbauffe, D and Pierre, M (1988). "Insulin and insulin-like growth factor 1 receptors during postnatal development of rat brain." Brain Res **470**(1): 77-83.
- Popescu, C, Anghelescu, A, Daia, C and Onose, G (2015). "Actual data on epidemiological evolution and prevention endeavours regarding traumatic brain injury." J Med Life **8**(3): 272-277.
- Popken, GJ, Dechert-Zeger, M, Ye, P and D'Ercole, AJ (2005). "Brain development." Adv Exp Med Biol **567**: 187-220.
- Rabinowitz, AR and Levin, HS (2014). "Cognitive sequelae of traumatic brain injury." Psychiatr Clin North Am **37**(1): 1-11.

- Radomski, KL, Zhou, Q, Yi, KJ and Doughty, ML (2013). "Cortical contusion injury disrupts olfactory bulb neurogenesis in adult mice." BMC Neurosci **14**: 142.
- Rastogi, R, Jiang, Z, Ahmad, N, Rosati, R, Liu, Y, Beuret, L, Monks, R, Charron, J, Birnbaum, MJ and Samavati, L (2013). "Rapamycin induces mitogen-activated protein (MAP) kinase phosphatase-1 (MKP-1) expression through activation of protein kinase B and mitogen-activated protein kinase kinase pathways." J Biol Chem **288**(47): 33966-33977.
- Ratcliff, G, Colantonio, A, Escobar, M, Chase, S and Vernich, L (2005). "Long-term survival following traumatic brain injury." Disabil Rehabil **27**(6): 305-314.
- Reener, DV, Hisel, RD, Hoffman, JM, Kryscio, RJ, Lusk, BT and Geddes, JW (2011). "A multi-mode shock tube for investigation of blast-induced traumatic brain injury." J Neurotrauma **28**(1): 95-104.
- Reuveni, H, Flashner-Abramson, E, Steiner, L, Makedonski, K, Song, R, Shir, A, Herlyn, M, Bar-Eli, M and Levitzki, A (2013). "Therapeutic destruction of insulin receptor substrates for cancer treatment." Cancer Res **73**(14): 4383-4394.
- Rhim, JH, Luo, X, Gao, D, Xu, X, Zhou, T, Li, F, Wang, P, Wong, ST and Xia, X (2016). "Cell type-dependent Erk-Akt pathway crosstalk regulates the proliferation of fetal neural progenitor cells." Sci Rep **6**: 26547.
- Robel, S, Berninger, B and Gotz, M (2011). "The stem cell potential of glia: lessons from reactive gliosis." Nat Rev Neurosci **12**(2): 88-104.
- Robinson, C, Apgar, C and Shapiro, LA (2016). "Astrocyte Hypertrophy Contributes to Aberrant Neurogenesis after Traumatic Brain Injury." Neural Plast **2016**: 1347987.
- Rockich, KT, Hatton, JC, Kryscio, RJ, Young, BA and Blouin, RA (1999). "Effect of recombinant human growth hormone and insulin-like growth factor-1 administration on IGF-1 and IGF-binding protein-3 levels in brain injury." Pharmacotherapy **19**(12): 1432-1436.
- Rola, R, Mizumatsu, S, Otsuka, S, Morhardt, DR, Noble-Haeusslein, LJ, Fishman, K, Potts, MB and Fike, JR (2006). "Alterations in hippocampal neurogenesis following traumatic brain injury in mice." Exp Neurol **202**(1): 189-199.
- Rong, Z, Pan, R, Chang, L and Lee, W (2015). "Combination treatment with ethyl pyruvate and IGF-I exerts neuroprotective effects against brain injury in a rat model of neonatal hypoxic-ischemic encephalopathy." Int J Mol Med **36**(1): 195-203.
- Rosenfeld, RG (2006). "Molecular mechanisms of IGF-I deficiency." Horm Res **65 Suppl 1**: 15-20.
- Rosset, C, Netto, CBO and Ashton-Prolla, P (2017). "TSC1 and TSC2 gene mutations and their implications for treatment in Tuberous Sclerosis Complex: a review." Genet Mol Biol **40**(1): 69-79.
- Rozas, NS, Redell, JB, Hill, JL, McKenna, J, 3rd, Moore, AN, Gambello, MJ and Dash, PK (2015). "Genetic activation of mTORC1 signaling worsens neurocognitive outcome after traumatic brain injury." J Neurotrauma **32**(2): 149-158.
- Rozengurt, E, Soares, HP and Sinnet-Smith, J (2014). "Suppression of feedback loops mediated by PI3K/mTOR induces multiple overactivation of compensatory pathways: an unintended consequence leading to drug resistance." Mol Cancer Ther **13**(11): 2477-2488.

- Rubin, RD, Watson, PD, Duff, MC and Cohen, NJ (2014). "The role of the hippocampus in flexible cognition and social behavior." Front Hum Neurosci **8**: 742.
- Rubovitch, V, Edut, S, Sarfstein, R, Werner, H and Pick, CG (2010). "The intricate involvement of the Insulin-like growth factor receptor signaling in mild traumatic brain injury in mice." Neurobiol Dis **38**(2): 299-303.
- Ryu, JR, Hong, CJ, Kim, JY, Kim, EK, Sun, W and Yu, SW (2016). "Control of adult neurogenesis by programmed cell death in the mammalian brain." Mol Brain **9**: 43.
- Saatman, KE, Contreras, PC, Smith, DH, Raghupathi, R, McDermott, KL, Fernandez, SC, Sanderson, KL, Voddi, M and McIntosh, TK (1997). "Insulin-like growth factor-1 (IGF-1) improves both neurological motor and cognitive outcome following experimental brain injury." Exp Neurol **147**(2): 418-427.
- Saatman, KE, Duhaime, AC, Bullock, R, Maas, AI, Valadka, A, Manley, GT, Workshop Scientific, T and Advisory Panel, M (2008). "Classification of traumatic brain injury for targeted therapies." J Neurotrauma **25**(7): 719-738.
- Saha, B, Peron, S, Murray, K, Jaber, M and Gaillard, A (2013). "Cortical lesion stimulates adult subventricular zone neural progenitor cell proliferation and migration to the site of injury." Stem Cell Res **11**(3): 965-977.
- Saha, R, Knapp, S, Chakraborty, D, Horovitz, O, Albrecht, A, Kriebel, M, Kaphzan, H, Ehrlich, I, Volkmer, H and Richter-Levin, G (2017). "GABAergic Synapses at the Axon Initial Segment of Basolateral Amygdala Projection Neurons Modulate Fear Extinction." Neuropsychopharmacology **42**(2): 473-484.
- Sahay, A, Scobie, KN, Hill, AS, O'Carroll, CM, Kheirbek, MA, Burghardt, NS, Fenton, AA, Dranovsky, A and Hen, R (2011). "Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation." Nature **472**(7344): 466-470.
- Samueli, S, Abraham, K, Dressler, A, Groppe, G, Muhlebner-Fahrngruber, A, Scholl, T, Kasprian, G, Laccone, F and Feucht, M (2016). "Efficacy and safety of Everolimus in children with TSC - associated epilepsy - Pilot data from an open single-center prospective study." Orphanet J Rare Dis **11**(1): 145.
- Sasaoka, T, Ishiki, M, Wada, T, Hori, H, Hirai, H, Haruta, T, Ishihara, H and Kobayashi, M (2001). "Tyrosine phosphorylation-dependent and -independent role of Shc in the regulation of IGF-1-induced mitogenesis and glycogen synthesis." Endocrinology **142**(12): 5226-5235.
- Schachar, RJ, Park, LS and Dennis, M (2015). "Mental Health Implications of Traumatic Brain Injury (TBI) in Children and Youth." J Can Acad Child Adolesc Psychiatry **24**(2): 100-108.
- Scharfman, H, Goodman, J, Macleod, A, Phani, S, Antonelli, C and Croll, S (2005). "Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats." Exp Neurol **192**(2): 348-356.
- Scharfman, H, Goodman, J and McCloskey, D (2007). "Ectopic granule cells of the rat dentate gyrus." Dev Neurosci **29**(1-2): 14-27.
- Scharfman, HE (2016). "The enigmatic mossy cell of the dentate gyrus." Nat Rev Neurosci **17**(9): 562-575.
- Scharfman, HE, Goodman, JH and Sollas, AL (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." *J Neurosci* **20**(16): 6144-6158.
- Scharfman, HE, Sollas, AE, Berger, RE, Goodman, JH and Pierce, JP (2003). "Perforant path activation of ectopic granule cells that are born after pilocarpine-induced seizures." *Neuroscience* **121**(4): 1017-1029.
- Scheff, SW, Baldwin, SA, Brown, RW and Kraemer, PJ (1997). "Morris water maze deficits in rats following traumatic brain injury: lateral controlled cortical impact." *J Neurotrauma* **14**(9): 615-627.
- Schmidt-Hieber, C, Jonas, P and Bischofberger, J (2004). "Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus." *Nature* **429**(6988): 184-187.
- Schober, ME, Block, B, Beachy, JC, Statler, KD, Giza, CC and Lane, RH (2010). "Early and sustained increase in the expression of hippocampal IGF-1, but not EPO, in a developmental rodent model of traumatic brain injury." *J Neurotrauma* **27**(11): 2011-2020.
- Schoch, KM, Madathil, SK and Saatman, KE (2012). "Genetic manipulation of cell death and neuroplasticity pathways in traumatic brain injury." *Neurotherapeutics* **9**(2): 323-337.
- Schumacher, R, Mosthaf, L, Schlessinger, J, Brandenburg, D and Ullrich, A (1991). "Insulin and insulin-like growth factor-1 binding specificity is determined by distinct regions of their cognate receptors." *J Biol Chem* **266**(29): 19288-19295.
- Selassie, AW, Zaloshnja, E, Langlois, JA, Miller, T, Jones, P and Steiner, C (2008). "Incidence of long-term disability following traumatic brain injury hospitalization, United States, 2003." *J Head Trauma Rehabil* **23**(2): 123-131.
- Selenica, ML, Benner, L, Housley, SB, Manchec, B, Lee, DC, Nash, KR, Kalin, J, Bergman, JA, Kozikowski, A, Gordon, MN and Morgan, D (2014). "Histone deacetylase 6 inhibition improves memory and reduces total tau levels in a mouse model of tau deposition." *Alzheimers Res Ther* **6**(1): 12.
- Sengupta, S, Peterson, TR and Sabatini, DM (2010). "Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress." *Mol Cell* **40**(2): 310-322.
- Seo, DO, Carillo, MA, Chih-Hsiung Lim, S, Tanaka, KF and Drew, MR (2015). "Adult Hippocampal Neurogenesis Modulates Fear Learning through Associative and Nonassociative Mechanisms." *J Neurosci* **35**(32): 11330-11345.
- Seto, B (2012). "Rapamycin and mTOR: a serendipitous discovery and implications for breast cancer." *Clin Transl Med* **1**(1): 29.
- Shah, A, Jhavar, SS and Goel, A (2012). "Analysis of the anatomy of the Papez circuit and adjoining limbic system by fiber dissection techniques." *J Clin Neurosci* **19**(2): 289-298.
- Shah, OJ and Hunter, T (2006). "Turnover of the active fraction of IRS1 involves raptor-mTOR- and S6K1-dependent serine phosphorylation in cell culture models of tuberous sclerosis." *Mol Cell Biol* **26**(17): 6425-6434.
- Shapiro, LA (2017). "Altered Hippocampal Neurogenesis during the First 7 Days after a Fluid Percussion Traumatic Brain Injury." *Cell Transplant* **26**(7): 1314-1318.
- Sherer, M, Struchen, MA, Yablon, SA, Wang, Y and Nick, TG (2008). "Comparison of indices of traumatic brain injury severity: Glasgow Coma Scale, length of coma and post-traumatic amnesia." *J Neurol Neurosurg Psychiatry* **79**(6): 678-685.

- Shi, L, Linville, MC, Tucker, EW, Sonntag, WE and Brunso-Bechtold, JK (2005). "Differential effects of aging and insulin-like growth factor-1 on synapses in CA1 of rat hippocampus." *Cereb Cortex* **15**(5): 571-577.
- Shiurba, RA, Spooner, ET, Ishiguro, K, Takahashi, M, Yoshida, R, Wheelock, TR, Imahori, K, Cataldo, AM and Nixon, RA (1998). "Immunocytochemistry of formalin-fixed human brain tissues: microwave irradiation of free-floating sections." *Brain Res Brain Res Protoc* **2**(2): 109-119.
- Shively, S, Scher, AI, Perl, DP and Diaz-Arrastia, R (2012). "Dementia resulting from traumatic brain injury: what is the pathology?" *Arch Neurol* **69**(10): 1245-1251.
- Shukitt-Hale, B, McEwen, JJ, Szprengiel, A and Joseph, JA (2004). "Effect of age on the radial arm water maze-a test of spatial learning and memory." *Neurobiol Aging* **25**(2): 223-229.
- Skeberdis, VA, Lan, J, Zheng, X, Zukin, RS and Bennett, MV (2001). "Insulin promotes rapid delivery of N-methyl-D- aspartate receptors to the cell surface by exocytosis." *Proc Natl Acad Sci U S A* **98**(6): 3561-3566.
- Slaughter, B, Fann, JR and Ehde, D (2003). "Traumatic brain injury in a county jail population: prevalence, neuropsychological functioning and psychiatric disorders." *Brain Inj* **17**(9): 731-741.
- Sleiman, SF, Henry, J, Al-Haddad, R, El Hayek, L, Abou Haidar, E, Stringer, T, Ulja, D, Karuppagounder, SS, Holson, EB, Ratan, RR, Ninan, I and Chao, MV (2016). "Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body beta-hydroxybutyrate." *Elife* **5**.
- Snyder, JS, Choe, JS, Clifford, MA, Jurling, SI, Hurley, P, Brown, A, Kamhi, JF and Cameron, HA (2009). "Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice." *J Neurosci* **29**(46): 14484-14495.
- Song, H, Stevens, CF and Gage, FH (2002). "Astroglia induce neurogenesis from adult neural stem cells." *Nature* **417**(6884): 39-44.
- Song, J, Zhong, C, Bonaguidi, MA, Sun, GJ, Hsu, D, Gu, Y, Meletis, K, Huang, ZJ, Ge, S, Enikolopov, G, Deisseroth, K, Luscher, B, Christian, KM, Ming, GL and Song, H (2012). "Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision." *Nature* **489**(7414): 150-154.
- Sorrells, SF, Paredes, MF, Cebrian-Silla, A, Sandoval, K, Qi, D, Kelley, KW, James, D, Mayer, S, Chang, J, Auguste, KI, Chang, EF, Gutierrez, AJ, Kriegstein, AR, Mathern, GW, Oldham, MC, Huang, EJ, Garcia-Verdugo, JM, Yang, Z and Alvarez-Buylla, A (2018). "Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults." *Nature* **555**(7696): 377-381.
- Spalding, KL, Bergmann, O, Alkass, K, Bernard, S, Salehpour, M, Huttner, HB, Bostrom, E, Westerlund, I, Vial, C, Buchholz, BA, Possnert, G, Mash, DC, Druid, H and Frisen, J (2013). "Dynamics of hippocampal neurogenesis in adult humans." *Cell* **153**(6): 1219-1227.
- Stallock, J, Molyneaux, K, Schaible, K, Knudson, CM and Wylie, C (2003). "The pro-apoptotic gene Bax is required for the death of ectopic primordial germ cells during their migration in the mouse embryo." *Development* **130**(26): 6589-6597.

- Sun, W, Winseck, A, Vinsant, S, Park, OH, Kim, H and Oppenheim, RW (2004). "Programmed cell death of adult-generated hippocampal neurons is mediated by the proapoptotic gene Bax." J Neurosci **24**(49): 11205-11213.
- Taib, T, Leconte, C, Van Steenwinckel, J, Cho, AH, Palmier, B, Torsello, E, Lai Kuen, R, Onyeomah, S, Ecomard, K, Benedetto, C, Coqueran, B, Novak, AC, Deou, E, Plotkine, M, Gressens, P, Marchand-Leroux, C and Besson, VC (2017). "Neuroinflammation, myelin and behavior: Temporal patterns following mild traumatic brain injury in mice." PLoS One **12**(9): e0184811.
- Talos, DM, Jacobs, LM, Gourmaud, S, Coto, CA, Sun, H, Lim, KC, Lucas, TH, Davis, KA, Martinez-Lage, M and Jensen, FE (2018). "Mechanistic target of rapamycin complex 1 and 2 in human temporal lobe epilepsy." Ann Neurol **83**(2): 311-327.
- Tang, YP, Shimizu, E, Dube, GR, Rampon, C, Kerchner, GA, Zhuo, M, Liu, G and Tsien, JZ (1999). "Genetic enhancement of learning and memory in mice." Nature **401**(6748): 63-69.
- Tashiro, A, Sandler, VM, Toni, N, Zhao, C and Gage, FH (2006). "NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus." Nature **442**(7105): 929-933.
- Tate, DF and Bigler, ED (2000). "Fornix and hippocampal atrophy in traumatic brain injury." Learn Mem **7**(6): 442-446.
- Taupin, P (2007). "BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation." Brain Res Rev **53**(1): 198-214.
- Tavazoie, SF, Alvarez, VA, Ridenour, DA, Kwiatkowski, DJ and Sabatini, BL (2005). "Regulation of neuronal morphology and function by the tumor suppressors Tsc1 and Tsc2." Nat Neurosci **8**(12): 1727-1734.
- Taylor, CA, Bell, JM, Breiding, MJ and Xu, L (2017). "Traumatic Brain Injury-Related Emergency Department Visits, Hospitalizations, and Deaths - United States, 2007 and 2013." MMWR Surveill Summ **66**(9): 1-16.
- Thirone, AC, Paez-Espinosa, EV, Carvalho, CR and Saad, MJ (1998). "Regulation of insulin-stimulated tyrosine phosphorylation of Shc and IRS-1 in the muscle of rats: effect of growth hormone and epinephrine." FEBS Lett **421**(3): 191-196.
- Titus, DJ, Wilson, NM, Freund, JE, Carballosa, MM, Sikah, KE, Furones, C, Dietrich, WD, Gurney, ME and Atkins, CM (2016). "Chronic Cognitive Dysfunction after Traumatic Brain Injury Is Improved with a Phosphodiesterase 4B Inhibitor." J Neurosci **36**(27): 7095-7108.
- Tolcher, AW, Patnaik, A, Papadopoulos, KP, Rasco, DW, Becerra, CR, Allred, AJ, Orford, K, Aktan, G, Ferron-Brady, G, Ibrahim, N, Gauvin, J, Motwani, M and Cornfeld, M (2015). "Phase I study of the MEK inhibitor trametinib in combination with the AKT inhibitor afuresertib in patients with solid tumors and multiple myeloma." Cancer Chemother Pharmacol **75**(1): 183-189.
- Tucker, LB, Burke, JF, Fu, AH and McCabe, JT (2017). "Neuropsychiatric Symptom Modeling in Male and Female C57BL/6J Mice after Experimental Traumatic Brain Injury." J Neurotrauma **34**(4): 890-905.
- Um, SH, Frigerio, F, Watanabe, M, Picard, F, Joaquin, M, Sticker, M, Fumagalli, S, Allegrini, PR, Kozma, SC, Auwerx, J and Thomas, G (2004). "Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity." Nature **431**(7005): 200-205.

- Unal-Cevik, I, Kilinc, M, Gursoy-Ozdemir, Y, Gurer, G and Dalkara, T (2004). "Loss of NeuN immunoreactivity after cerebral ischemia does not indicate neuronal cell loss: a cautionary note." Brain Res **1015**(1-2): 169-174.
- van Praag, H, Schinder, AF, Christie, BR, Toni, N, Palmer, TD and Gage, FH (2002). "Functional neurogenesis in the adult hippocampus." Nature **415**(6875): 1030-1034.
- Vaynman, S, Ying, Z and Gomez-Pinilla, F (2004). "Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition." Eur J Neurosci **20**(10): 2580-2590.
- Veilleux, A, Houde, VP, Bellmann, K and Marette, A (2010). "Chronic inhibition of the mTORC1/S6K1 pathway increases insulin-induced PI3K activity but inhibits Akt2 and glucose transport stimulation in 3T3-L1 adipocytes." Mol Endocrinol **24**(4): 766-778.
- Villasana, LE, Kim, KN, Westbrook, GL and Schnell, E (2015). "Functional Integration of Adult-Born Hippocampal Neurons after Traumatic Brain Injury(1,2,3)." eNeuro **2**(5).
- Vink, R, Mullins, PG, Temple, MD, Bao, W and Faden, AI (2001). "Small shifts in craniotomy position in the lateral fluid percussion injury model are associated with differential lesion development." J Neurotrauma **18**(8): 839-847.
- Voermans, NC, Petersson, KM, Daudey, L, Weber, B, Van Spaendonck, KP, Kremer, HP and Fernandez, G (2004). "Interaction between the human hippocampus and the caudate nucleus during route recognition." Neuron **43**(3): 427-435.
- Vorhees, CV and Williams, MT (2014). "Assessing spatial learning and memory in rodents." ILAR J **55**(2): 310-332.
- Wahane, SD, Hellbach, N, Prentzell, MT, Weise, SC, Vezzali, R, Kreutz, C, Timmer, J, Kriegstein, K, Thedieck, K and Vogel, T (2014). "PI3K-p110-alpha-subtype signalling mediates survival, proliferation and neurogenesis of cortical progenitor cells via activation of mTORC2." J Neurochem **130**(2): 255-267.
- Walter, KH, Barnes, SM and Chard, KM (2012). "The influence of comorbid MDD on outcome after residential treatment for veterans with PTSD and a history of TBI." J Trauma Stress **25**(4): 426-432.
- Wan, X, Harkavy, B, Shen, N, Grohar, P and Helman, LJ (2007). "Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism." Oncogene **26**(13): 1932-1940.
- Wang, C, Hu, Z, Zou, Y, Xiang, M, Jiang, Y, Botchway, BOA, Huo, X, Du, X and Fang, M (2017). "The post-therapeutic effect of rapamycin in mild traumatic brain-injured rats ensuing in the upregulation of autophagy and mitophagy." Cell Biol Int **41**(9): 1039-1047.
- Wang, J, Elewaut, D, Veys, EM and Verbruggen, G (2003). "Insulin-like growth factor 1-induced interleukin-1 receptor II overrides the activity of interleukin-1 and controls the homeostasis of the extracellular matrix of cartilage." Arthritis Rheum **48**(5): 1281-1291.
- Wang, JM, Hayashi, T, Zhang, WR, Sakai, K, Shiro, Y and Abe, K (1999). "Insulin-like growth factor-1 affects expressions of cyclin-dependent kinase 5 and its activator p35 in perfused rat brain." Neurosci Lett **277**(1): 17-20.

- Wang, X, Gao, X, Michalski, S, Zhao, S and Chen, J (2016). "Traumatic Brain Injury Severity Affects Neurogenesis in Adult Mouse Hippocampus." J Neurotrauma **33**(8): 721-733.
- Wang, X, Seekaew, P, Gao, X and Chen, J (2016). "Traumatic Brain Injury Stimulates Neural Stem Cell Proliferation via Mammalian Target of Rapamycin Signaling Pathway Activation." eNeuro **3**(5).
- Warner, MA, Youn, TS, Davis, T, Chandra, A, Marquez de la Plata, C, Moore, C, Harper, C, Madden, CJ, Spence, J, McColl, R, Devous, M, King, RD and Diaz-Arrastia, R (2010). "Regionally selective atrophy after traumatic axonal injury." Arch Neurol **67**(11): 1336-1344.
- Washington, PM, Forcelli, PA, Wilkins, T, Zapple, DN, Parsadanian, M and Burns, MP (2012). "The effect of injury severity on behavior: a phenotypic study of cognitive and emotional deficits after mild, moderate, and severe controlled cortical impact injury in mice." J Neurotrauma **29**(13): 2283-2296.
- Watson, MR, Fenton, GW, McClelland, RJ, Lumsden, J, Headley, M and Rutherford, WH (1995). "The post-concussional state: neurophysiological aspects." Br J Psychiatry **167**(4): 514-521.
- Wheless, JW (2015). "Use of the mTOR inhibitor everolimus in a patient with multiple manifestations of tuberous sclerosis complex including epilepsy." Epilepsy Behav Case Rep **4**: 63-66.
- Whiteneck, GG, Gerhart, KA and Cusick, CP (2004). "Identifying environmental factors that influence the outcomes of people with traumatic brain injury." J Head Trauma Rehabil **19**(3): 191-204.
- Whiting, MD, Baranova, AI and Hamm, RJ (2006). Cognitive Impairment following Traumatic Brain Injury. Animal Models of Cognitive Impairment. E. D. Levin and J. J. Buccafusco. Boca Raton (FL).
- Wiener, CD, de Mello Ferreira, S, Pedrotti Moreira, F, Bittencourt, G, de Oliveira, JF, Lopez Molina, M, Jansen, K, de Mattos Souza, LD, Rizzato Lara, D, Portela, LV, da Silva, RA and Oses, JP (2015). "Serum levels of nerve growth factor (NGF) in patients with major depression disorder and suicide risk." J Affect Disord **184**: 245-248.
- Wildburger, R, Zarkovic, N, Leb, G, Borovic, S, Zarkovic, K and Tatzber, F (2001). "Post-traumatic changes in insulin-like growth factor type 1 and growth hormone in patients with bone fractures and traumatic brain injury." Wien Klin Wochenschr **113**(3-4): 119-126.
- Witgen, BM, Lifshitz, J, Smith, ML, Schwarzbach, E, Liang, SL, Grady, MS and Cohen, AS (2005). "Regional hippocampal alteration associated with cognitive deficit following experimental brain injury: a systems, network and cellular evaluation." Neuroscience **133**(1): 1-15.
- Wrigley, S, Arafa, D and Tropea, D (2017). "Insulin-Like Growth Factor 1: At the Crossroads of Brain Development and Aging." Front Cell Neurosci **11**: 14.
- Xavier, GF, Oliveira-Filho, FJ and Santos, AM (1999). "Dentate gyrus-selective colchicine lesion and disruption of performance in spatial tasks: difficulties in "place strategy" because of a lack of flexibility in the use of environmental cues?" Hippocampus **9**(6): 668-681.

- Xiong, Y, Mahmood, A and Chopp, M (2013). "Animal models of traumatic brain injury." *Nat Rev Neurosci* **14**(2): 128-142.
- Yagita, Y, Kitagawa, K, Ohtsuki, T, Takasawa, K, Miyata, T, Okano, H, Hori, M and Matsumoto, M (2001). "Neurogenesis by progenitor cells in the ischemic adult rat hippocampus." *Stroke* **32**(8): 1890-1896.
- Yang, LY, Greig, NH, Huang, YN, Hsieh, TH, Tweedie, D, Yu, QS, Hoffer, BJ, Luo, Y, Kao, YC and Wang, JY (2016). "Post-traumatic administration of the p53 inactivator pifithrin- α oxygen analogue reduces hippocampal neuronal loss and improves cognitive deficits after experimental traumatic brain injury." *Neurobiol Dis* **96**: 216-226.
- Ye, P, Hu, Q, Liu, H, Yan, Y and D'Ercole A, J (2010). "beta-catenin mediates insulin-like growth factor-I actions to promote cyclin D1 mRNA expression, cell proliferation and survival in oligodendroglial cultures." *Glia* **58**(9): 1031-1041.
- Ye, P, Popken, GJ, Kemper, A, McCarthy, K, Popko, B and D'Ercole, AJ (2004). "Astrocyte-specific overexpression of insulin-like growth factor-I promotes brain overgrowth and glial fibrillary acidic protein expression." *J Neurosci Res* **78**(4): 472-484.
- Ye, P, Umayahara, Y, Ritter, D, Bunting, T, Auman, H, Rotwein, P and D'Ercole, AJ (1997). "Regulation of insulin-like growth factor I (IGF-I) gene expression in brain of transgenic mice expressing an IGF-I-luciferase fusion gene." *Endocrinology* **138**(12): 5466-5475.
- Yellinek, S, Cohen, A, Merkin, V, Shelef, I and Benifla, M (2016). "Clinical significance of skull base fracture in patients after traumatic brain injury." *J Clin Neurosci* **25**: 111-115.
- Yu, S, Kaneko, Y, Bae, E, Stahl, CE, Wang, Y, van Loveren, H, Sanberg, PR and Borlongan, CV (2009). "Severity of controlled cortical impact traumatic brain injury in rats and mice dictates degree of behavioral deficits." *Brain Res* **1287**: 157-163.
- Yu, TS, Zhang, G, Liebl, DJ and Kernie, SG (2008). "Traumatic brain injury-induced hippocampal neurogenesis requires activation of early nestin-expressing progenitors." *J Neurosci* **28**(48): 12901-12912.
- Zhang, J, Moats-Staats, BM, Ye, P and D'Ercole, AJ (2007). "Expression of insulin-like growth factor system genes during the early postnatal neurogenesis in the mouse hippocampus." *J Neurosci Res* **85**(8): 1618-1627.
- Zhang, W, Wang, W and Kuang, L (2018). "The relation between insulin-like growth factor 1 levels and risk of depression in ischemic stroke." *Int J Geriatr Psychiatry* **33**(2): e228-e233.
- Zhao, C, Teng, EM, Summers, RG, Jr., Ming, GL and Gage, FH (2006). "Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus." *J Neurosci* **26**(1): 3-11.
- Zhao, Z, Loane, DJ, Murray, MG, 2nd, Stoica, BA and Faden, AI (2012). "Comparing the predictive value of multiple cognitive, affective, and motor tasks after rodent traumatic brain injury." *J Neurotrauma* **29**(15): 2475-2489.
- Zheng, W, ZhuGe, Q, Zhong, M, Chen, G, Shao, B, Wang, H, Mao, X, Xie, L and Jin, K (2013). "Neurogenesis in adult human brain after traumatic brain injury." *J Neurotrauma* **30**(22): 1872-1880.

- Zheng, WH and Quirion, R (2004). "Comparative signaling pathways of insulin-like growth factor-1 and brain-derived neurotrophic factor in hippocampal neurons and the role of the PI3 kinase pathway in cell survival." J Neurochem **89**(4): 844-852.
- Zhou, H, Chen, L, Gao, X, Luo, B and Chen, J (2012). "Moderate traumatic brain injury triggers rapid necrotic death of immature neurons in the hippocampus." J Neuropathol Exp Neurol **71**(4): 348-359.
- Zhou, M, Li, W, Huang, S, Song, J, Kim, JY, Tian, X, Kang, E, Sano, Y, Liu, C, Balaji, J, Wu, S, Zhou, Y, Zhou, Y, Parivash, SN, Ehninger, D, He, L, Song, H, Ming, GL and Silva, AJ (2013). "mTOR Inhibition ameliorates cognitive and affective deficits caused by Disc1 knockdown in adult-born dentate granule neurons." Neuron **77**(4): 647-654.
- Zhou, Z, Chen, H, Zhang, K, Yang, H, Liu, J and Huang, Q (2003). "Protective effect of nerve growth factor on neurons after traumatic brain injury." J Basic Clin Physiol Pharmacol **14**(3): 217-224.
- Zhu, G, Chow, LM, Bayazitov, IT, Tong, Y, Gilbertson, RJ, Zakharenko, SS, Solecki, DJ and Baker, SJ (2012). "Pten deletion causes mTorc1-dependent ectopic neuroblast differentiation without causing uniform migration defects." Development **139**(18): 3422-3431.
- Zhu, X, Park, J, Golinski, J, Qiu, J, Khuman, J, Lee, CC, Lo, EH, Degtrev, A and Whalen, MJ (2014). "Role of Akt and mammalian target of rapamycin in functional outcome after concussive brain injury in mice." J Cereb Blood Flow Metab **34**(9): 1531-1539.

VITA

Erica L. Littlejohn, Ph.D.

Spinal Cord and Brain Injury Research Center
Department of Physiology
College of Medicine, University of Kentucky

Citizenship: USA

Educational Experience

2011-2018 University of Kentucky: Lexington, Kentucky
 Doctor of Philosophy in Physiology

2003-2008 **University of Iowa: Iowa City, Iowa**
 B.S. in Microbiology

Professional Experience

2012-2018 **Predoctoral Graduate Research Assistant**—Lexington, KY
 Ph.D. Mentorship and training under Kathryn E. Saatman, Ph.D.

2016 **Instructor for Anatomy and Physiology course**, Bluegrass Community
 and Technical College, Lexington, KY

2016 **University of Kentucky Area Health Education Center (AHEC)**
 Physiology Lecturer —Lexington, KY

2015-2018 **Mentor for NIH Kentucky Bridge to a Biomedical Doctorate for**
 Appalachian Students —Lexington, KY

- 2015 **University of Kentucky Course Facilitator** —Lexington, KY
- 2011 **Mentor for Illinois Mathematics and Science Academy-Student Inquiry and Research (SIR) Student** — Chicago IL
- 2008-2011 **Research Technologist** — Chicago IL

Selected Awards

- 2018 **National Neurotrauma Society Diversity Grant**
- 2017 **Neuroscience Scholars Program Professional Development Award**
- 2016 **Annual Central Kentucky Multicultural Opportunities, Strategies and Institutional Inclusiveness Consortium Award**
- 2016 **University of Kentucky Center for Graduate and Professional Diversity Initiatives Essence Award**
- 2016 **Shane Carlin and Annie Sit Inclusion Award**
- 2015 **Inaugural Thomas V. Getchell, Ph.D. Memorial Award**
- 2015 **National Neurotrauma Society Symposium Travel Award**
- 2011 **Illinois Mathematics and Science Academy Mentor Appreciation Award**

Service

- 2015-2018 **University of Kentucky Graduate School Diversity Advisory Council, Graduate Student Representative**
- 2015-2016 **Representative for Physiology Graduate Students**
- 2014-2016 **University of Kentucky Black Graduate and Professional Student Association**
President

Professional Membership

2012 to present **Bluegrass Chapter of the Society for Neuroscience**

2012 to present **National Neurotrauma Society**

2012 to present **Society for Neuroscience**

Manuscripts:

Mascarenhas JB, **Littlejohn EL**, Wolsky RJ, Young KP, Nelson M, Salgia R, Lang D. (2010). "PAX3 and SOX10 activate MET receptor expression in melanoma". *Pigment Cell & Melanoma Research*. Volume 23, p 225-237

Mascarenhas JB, Young KP, **Littlejohn EL**, Yoo BK, Salgia R, Lang D. "PAX6 is expressed in pancreatic cancer and actively participates in cancer progression through activation of the MET tyrosine kinase receptor gene." *Journal of Biological Chemistry*. Volume 284, 27524-27532

Grants:

- 2017 **Lyman T. Johnson Fellowship**, “Insulin-like growth factor-1 overexpression mediates hippocampal remodeling and plasticity following tbi”
- 2015-2017 **NIH T32 trainee**, “Neurobiology of CNS Injury and Repair” (Drs. Ed Hall and Jim Geddes, co-PI)
- 2012-2015 **NIH Research Supplement Award trainee**, “Insulin-like growth factor-1 overexpression” (Drs. Kathryn Saatman)

Speaking Engagements:

- 2017 **Littlejohn EL** “Insulin-like growth factor-1 overexpression mediates hippocampal remodeling and plasticity following TBI”. Kentucky Spinal Cord and Head Injury Research Trust Symposium, Louisville, KY
- 2017 **Littlejohn EL** “The need for student-led activism: The model, tools, and a modern approach”. Inaugural Sister Circle Forum: The Power of Your Reach, University of Kentucky, Lexington, KY
- 2016 **Littlejohn EL** “UK Call to Action”. Inaugural Town Hall on Inclusion and Diversity. University of Kentucky, Lexington, KY
- 2013 **Littlejohn EL** “How I did it”. GEM Consortium, Statewide Kentucky STEM Undergraduates at GRADLAB —Lexington, KY

Abstracts and Presentations:

*Denotes presenter

*Insulin-like growth factor-1 overexpression enhances neurogenesis and activates the mTOR pathway after moderate TBI. Littlejohn EL**, Scott DA, DeSana AJ, Juras AJ, and Saatman KE. Society for Neuroscience (SFN) Annual Meeting. Washington, DC. November 11-15, 2017.

Insulin-like growth factor-1 overexpression enhances neurogenesis and activates the mTOR pathway after moderate TBI **Littlejohn EL***, Scott DA, DeSana AJ, Saatman KE. *Journal of Neurotrauma* (Vol. 34, No. 13, pp. A137-A137). Snowbird, UT. July 7-12, 2017.

Insulin-like growth factor-1 overexpression mediates hippocampal remodeling and plasticity following TBI. **Littlejohn EL***, DeSana AJ, Juras AJ, and Saatman KE. The 23rd Annual Kentucky Spinal Cord and Head Injury Research Trust Symposium (KSCHIRT). Louisville, KY. May 17-18, 2017 (Oral)

Insulin-like growth factor-1 overexpression mediates long-term survival and migration of immature neurons in the hippocampus following TBI. **Littlejohn EL***, DeSana AJ, Madathil SK, Stewart TM, Saatman KE. Society for Neuroscience (SFN) Annual Meeting. San Diego, CA. November 12-16, 2016

Insulin-like growth factor-1 mediates regional alterations to the mTOR signaling pathway in the hippocampus following TBI. **Littlejohn EL***, Madathil SK, DeSana AJ, Stewart TM, Saatman KE. *Journal of Neurotrauma* (vol. 33, no. 13, pp. A130-a130). Lexington, KY. June 26-29, 2016.

Intranasal delivery of human insulin-like growth factor-1 mediates regional alterations to the mTOR signaling pathway in the hippocampus following TBI. **Littlejohn EL***, Sama AM, Stewart TM, Saatman KE. 31st Annual BGSFN Spring Neuroscience Research Day (BGSFN) Annual Meeting. Lexington Convention Center, Lexington, KY. April 21, 2016

Insulin-like growth factor-1 Overexpression Promotes Survival of Adult-born Neurons After Traumatic Brain Injury. **Littlejohn EL***, Madathil SK, Stewart TM, Saatman KE. National Neurotrauma Symposium (NNS) Annual Meeting. Santa Fe Convention Center, Santa Fe, NM. June 28-2, 2015

Rin GTPase deficiency promotes neuroprotection following traumatic brain injury. **Pannell M***, Cai W, Brelsfoard JM, Carlson SW, Littlejohn EL, Stewart TM, Saatman KE, Andres DA. (2015) *The FASEB Journal*. 29, 727.15

Inhibition of PAX3 Leads to Growth Arrest and a Change in Morphology of Melanoma cells. **Littlejohn EL**, Kubic JD, Mascarenhas JB, Smithberger E, Lang D*. American Association for Cancer Research (AACR) Annual Meeting. Orange County Convention Center, Orlando, FL. April 2-6, 2011.

Pax3 Contributes to Cancer Phenotype of Melanoma. **Littlejohn EL***, Kubic JD, Ludvik AE, Sun DX and Lang D. University of Chicago Department of Medicine Research Symposium. Duchossois Center for Advanced Medicine at the University of Chicago, Chicago, IL. March 11, 2010.