BIOGRAPHICAL SKETCH

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NAME: Julie A Siegenthaler

eRA COMMONS USER NAME (credential, e.g., agency login): siegenthaler

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Mount Holyoke College, South Hadley MA	B.A.	05/2000	Neuroscience & Behavior
SUNY Upstate Medical Univ, Syracuse NY	Ph.D.	07/2005	Neuroscience
SUNY Upstate Medical Univ, Syracuse NY	Postdoctoral	05/2006	Developmental Neurobiology
University of California, San Francisco San Francisco CA	Postdoctoral	06/2012	Developmental Neurobiology

A. Personal Statement

My research goal is to elucidate signaling between non-neural structures, namely meninges and cells of the neuro-vasculature, and the brain required for normal brain development and adult brain function. Projects in my lab use mouse genetics, ex vivo and culture models, and animal models of adult brain injury (stroke and traumatic brain injury) to address these important questions in CNS biology. The origins of this proposal on meningeal-neural signaling in the adult hippocampus can be found in my graduate work at SUNY Upstate Medical University when I first studied molecular and cellular mechanisms of neocortical development. There I identified TGFβ signaling in neocortical progenitors as a key signal in corticogenesis and an important target of a pervasive neuro-developmental teratogen, alcohol exposure. I began my post-doctoral researcher at a time when the meninges, a neural-crest and mesoderm-derived structure that encases the brain, was beginning to be recognized as a rich source of signals for the developing brain. I was fortunate to be an integral part of characterizing mice with mutations in the meningeal-expressed gene *Foxc1* and developing these mice as a model to study how the meninges regulate brain development. I discovered that Retinoic acid (RA) synthesized by the meninges regulates symmetric and asymmetric divisions in neocortical progenitors (Siegenthaler et al., 2009 *Cell*). In my independent group at UC Denver we have made important progress toward understanding how meningeal-derived RA functions in cerebrovascular development and blood-brain barrier formation (Bonney et al., 2016 *J of Neurosci*; Mishra et al., 2016 *Dev Bio;* Bonney at al., 2017 *eNeuro*). In this application, we are applying our expertise in meninges-neural signaling to the adult brain specifically looking at the influence of RA from the meninges on neural stem cells in the adult hippocampal niche.

Our meninges-neural signaling project is complemented by our projects on RA in neuro-vascular development and in the chronic phase of stroke injury. My work in neuro-vascular development has been supported by a K99/R00 “Pathway to Independence” grant from NINDS and more recently an R01 grant from NINDS. Supported by funding from this award we discovered a novel interaction between the Retinoic Acid and Wnt signaling pathways in brain endothelial cells that is required for vascular growth and stabilization in the developing CNS. Our project on brain pericytes, a vital support cell for the brain endothelium, led to our discovery that a pericyte subpopulation activated following brain injury (referred to as “A” pericytes or perivascular stromal cells) is a source of RA for damaged brain areas and potentially promotes neural recovery. This work has been supported by grants from the American Heart Association, the Brain Research

Foundation and a pilot grant from the Colorado Clinical and Translational Sciences Institute to identify PSC activation signals.

In addition to the scientific and technical expertise needed to carry out this research, I have and continue to gain experience in training and mentoring young researchers. As a post-doctoral fellow I helped mentor 3 graduate students and now in my own lab I am currently mentoring two graduate students from the Cell Biology, Stem Cells and Development PhD program at the University of Colorado, School of Medicine in addition to numerous undergraduates and high school students in my own lab as summer research interns. I consider teaching young scientists how to conduct research and become independent thinkers as one of the most rewarding aspects of my career. Importantly, these experiences have helped develop my approach as a lead investigator and will aid me as I guide my team in carrying out the research outlined in this proposal.

B. Positions and Honors

Positions and Employment

08/00-07/05	Graduate Student, Department of Neuroscience and Physiology, SUNY Upstate Medical Syracuse, NY
08/05-4/05	Postdoctoral fellow, Department of Neuroscience and Physiology, SUNY Upstate Medical Syracuse, NY
05/06-06/12	Postdoctoral fellow, Department of Neurology, University of California, San Francisco, San Francisco, CA
07/12-Present	Assistant Professor, Career Track, Department of Pediatrics, Section of Developmental Biology, University of Colorado, School of Medicine-Anschutz Medical Campus, Aurora, CO

Other Experience and Professional Memberships

2006-Present	Member, Society for Neuroscience
2014-Present	Member, North American Vascular Biology Organization

Honors and Awards

2006	Fetal Alcohol Syndrome Study Group Travel Award (Research Society on Alcoholism)
2007-2009	Lawrence M. Brass, M.D. Stroke Research Postdoctoral Fellowship Award (AHA/AAN)
2010-2015	K99/R00 Pathway to Independence Award (NS070920) National Institutes of Health/NINDS

C. Contribution to Science

1. I performed my PhD thesis research with Dr. Michael Miller in Department of Neuroscience and Physiology at SUNY Upstate Medical University. During my graduate studies, I investigated the molecular mechanisms underlying Fetal Alcohol Syndrome (FAS) associated defects in neocortical development. Using telencephalon explant assays, we found that Transforming growth factor-β (TGFβ) signaling was involved in promoting both progenitor cell cycle exit and neocortical migration and that these functions of TGFβ were impaired by alcohol exposure. We identified induction of CKI p21 as a mechanism by which TGFβ1 promotes neuronal progenitors to differentiate and that TGFβ1 promoted neuron migration through up-regulation of cell adhesion molecule NCAM and β-integrins. Together this work provided important new insight into how a pregnant mother's alcohol consumption perturbs normal brain development through modulation of TGFβ1.

a. Siegenthaler, JA and Miller, MW (2004) "Transforming growth factor β1 modulates cell migration in rat cortex: effects of ethanol." *Cerebral Cortex*, 14: 791-802.

b. Mooney, SM, Siegenthaler, JA, and Miller, MW (2004) "Ethanol induces heterotopias in organotypic cultures of rat cerebral cortex." *Cerebral Cortex*, 14: 1071-1080.

c. Siegenthaler, JA and Miller, MW (2005) "Ethanol disrupts cell cycle regulation in the developing cortex: effects on TGFβ1." *Journal of Neurochemistry*, 95: 902-912.

d. Siegenthaler, JA and Miller, MW (2005) "TGFβ1 promotes cell cycle exit through the CKI p21 in the developing cerebral cortex." *Journal of Neuroscience*, 25(38):8627-8636.

2. I completed a one year postdoctoral training in Dr. Michael Miller's laboratory where I focused my efforts on the microencephaly phenotype of mice heterozygous for the forkhead transcription factor Foxg1. *FOXG1* is a disease gene for Rett syndrome of which microcephaly and developmental delay is a major feature. Foxg1 is critical for specification of the dorsal (neocortex) and ventral (striatum) telencephalon as KO mice for *Foxg1* lack both of these brain structures. We found that heterozygosity of *Foxg1*, of which some Rett syndrome patients have, caused reduced production of intermediate progenitor cells in the neocortex and reduced neuron generation. We identified loss of Foxg1-mediated inhibition of CKI p21 as a mechanism leading to premature exit of ventricular zone progenitors from the cell cycle. We also identified a mechanism by which generation of Cajal-Retizus cells, an early born neuron that resides in the marginal zone and secretes Reelin, is regulated by TGFβ signaling. We showed that Cajal-Retzius cells are generated in areas of the forebrain that do not express Foxg1. This was required as Foxg1 has been shown to be an inhibitor of TGFβ signaling and we showed that TGFβ signaling is needed to induce CKI p21 in Foxg1-negative regions and promote cell cycle exit of Cajal-Retzius cells. Our work on Foxg1 in telencephalic development not only provided knowledge regarding the cause of microcephaly in human patients with *FOXG1* mutations but also outlined a novel mechanism by which TGFβ signaling regulates birth of Cajal-Retzius cells during early brain development.

a. Siegenthaler, JA and Miller, MW (2007) "Generation of Cajal-Retzius neurons in mouse forebrain is regulated by transforming growth factor-β-Fox signaling pathways." *Developmental Biology,* 313: 35-46*.*

b. Siegenthaler*, JA, Tremper-Wells*, B, and Miller, MW (2008) "Foxg1 haplo-insufficiency reduces the population of cortical intermediate progenitor cells: effect of increased p21 expression." *Cerebral Cortex,* 8:1865-1875. *Co-first authors

3. I joined Dr. Sam Pleasure's lab in the Department of Neurology at UCSF in 2006 as a post-doctoral fellow where I embarked on an incredible research 'odyssey' to understand how signals made by the meninges, a neural-crest derived structure, are needed to regulate neocortical development. We found that mice with mutations in the forkhead transcription factor *Foxc1* fail to form meninges around the neocortex and, as a result, have enlarged cerebral hemispheres. We showed that these defects in neocortical development result from failure of neocortical progenitors to switch from symmetric to asymmetric, neuron generating divisions due to lack of meningeal derived Retinoic Acid. This work represented a significant advancement in our understanding of corticogenesis, solidified the meninges as a major regulator of neocortical development and identified defects in meningeal development as a possible cause of focal cortical dysplasia in human patients.

a. Zarbalis*, K, Siegenthaler*, JA, Choe, Y, May, SR, Peterson, AS, Pleasure, SJ (2007) "A novel hypomorphic *Foxc1* allele with disruption of meningeal differentiation causes cortical dysplasia and skull defects." *PNAS* 104:14002-14007. *Co-first authors

b. Siegenthaler, JA, Ashique, AM, ,Zarbalis, K, Patterson, KP, Hecht, JH, Kane, MA, Folias, AE, Choe, Y, May, SR, Kume, T, Napoli, JL, Peterson, AS, Pleasure, SJ (2009) "Retinoic acid from the meninges regulates cortical neuron generation." *Cell*, 139:597-609.

c. Hecht, JH, Siegenthaler, JA, Patterson, KP, Pleasure, SJ (2010) "Primary cellular meningeal defects cause neocortical dysplasia and dyslamination." *Annals of Neurology* 68(4):454-64.

d. Siegenthaler, JA and Pleasure, SJ (2011) "We have got you covered: how the meninges control brain development." *Current Opinion in Genetics and Development*. 21(3): 249-55.

4. Supported AHA/AAN grant and later by my K99-R00 Pathway to Independence Award and an AHA Beginning Grant-in-Aid, I focused the latter half of my post-doctoral work and now work in my independent position on brain pericyte function in vascular development and the response of pericyte subpopulations to brain injury. I have shown that Foxc1 has important roles in pericytes during brain vascular development. Using pericyte-conditional *Foxc1* KOs, we show that Foxc1 is needed to regulate pericyte proliferation. Also, *Foxc1*-deficient pericytes fail to inhibit endothelial cell proliferation leading to dysplastic, hemorrhage-prone vessels in the conditional mutants. This phenotype is particularly relevant in light of several publications describing cerebrovascular bleeds in patients with point mutations in or deletions that include *FOXC1*. Our lab has also taken the lead on characterizing the developmental origins and functions of a poorly understood but important pericyte subtype termed perivascular stromal cells (PSCs). PSCs have recently been shown to have a differential response to brain injury. Unlike pericytes that die in the injury core, PSCs proliferate to increase numbers and are the source of most fibrotic scar material. We have shown these cells are potentially derived

from the meninges during post-natal brain development and that PSCs may have a separate function in the injury site, specifically a source of bioactive signals like RA that have the potential to be neuroprotective and anti-inflammatory.

a. **Siegenthaler, JA**, Choe, Y., Patterson, K., Hsieh, I., Li, D., Jaminet, S., Daneman, R., Kume, T., Huang, E. and Pleasure, S. (2013). Foxc1 is required by pericytes during fetal brain angiogenesis. *Biology Open: The Company of Biologists* 0:1-13 DOI: 10.1242; bio.20135009.

b. **Siegenthaler, JA**, Sohet, F and Daneman, R (2013) "Sealing off the CNS: cellular and molecular regulation of blood brain barriergenesis." *Current Opinion in Neurobiology.* 23(6): 1057-64.

c. Kelly, KK, MacPherson ,A, Grewal, H, Strnad , Jones, J, Yu , J, Pierzchalski , K, Kane, M, Herson, P, **Siegenthaler, JA** (2016) "Col1a1+ perivascular cells in the brain are a source of retinoic acid following stroke." *BMC Neuroscience* 17(49) DOI: 10.1186/s12868-016-0284-5

5. An important direction from my work on RA in corticogenesis has been exploring the function of RA signaling in brain vascular development. Using several animal models of RA deficiency and targeted disruption of RA signaling in brain endothelial cells, my lab has uncovered a previously unknown interaction between RA and the WNT pathway. Endothelial WNT signaling is absolutely required for development of the CNS vasculature however no pathways have been identified upstream of WNT in the CNS vasculature. We have found that RA is required to promote endothelial WNT signaling specifically in the neocortex via inhibiting expression soluble WNT inhibitors by cortical neural progenitors. Surprisingly, we have found that RA is required in brain endothelial cells to directly inhibit WNT signaling to prevent ectopic expression of WNT target Sox17. These discoveries are providing important insight into signaling mechanisms underlying brain vascular development that we believe can be applied to maintenance and improved regrowth of blood vessel in brain pathologies.

a. Bonney*, S, Harrison-Uy*, S, Mishra, S, MacPherson, A, Choe, Y, Li, D, Jaminet, SC, Fruttiger, M, Pleasure, SJ and **Siegenthaler, JA** (2016) "Diverse functions of retinoic acid in brain vascular development." *Journal of Neuroscience* 36(29):7786-801. *Co-first authors

b. Mishra, S, Choe, Y, Pleasure, SJ, **Siegenthaler JA** (2016) "Cerebrovascular defects in *Foxc1* mutants correlate with aberrant WNT and VEGFA pathways downstream of retinoic acid from the meninges." *Developmental Biology* doi: 10.1016/j.ydbio.2016.09.019.

c. Bonney, S and **Siegenthaler JA** (2017) "Differential Effects of Retinoic Acid Concentrations in Regulating Blood-Brain Barrier Properties." May 26;4(3) DOI: 10.1523/ENEURO.0378-16.2017.

Complete List of Published Work in My Bibliography:

https://www.ncbi.nlm.nih.gov/sites/myncbi/julie.siegenthaler.1/bibliography/40688331/public/?sort=date&direction=ascending

D. Research Support

Ongoing

R01NS098273-01 – NIH/NINDS 6/1/2016-5/31/2021

Title: "Retinoic acid in the development of the CNS vasculature"

Projects in this grant will identify a role for RA signaling in controlling brain endothelial cell and pericyte proliferation required for vascular stability through regulation of WNT signaling and its target Sox17. Further, we will elucidate the function of RA in retinal vascular development and identify a role for RA deficiency in the developmental vascular pathology retinopathy-of-prematurity.
Role: Principal Investigator

Completed

Colorado Clinical and Translational Sciences Institute (CCTSI) -CNS Pilot Grant 1/1/16-12/31/16

Title: **"**Identification of signals that activate perivascular stromal cells and initiate fibrosis following CNS injury"

Projects in the grant will test potential ischemic cell types underlying activation of pro-fibrotic perivascular cell types following stroke injury and identify molecular pathways underlying cellular transformation that lead to fibrotic scar deposition.

Role: Principal Investigator

AHA 14BGIA18440006 Beginning Grant-in-Aid 1/1/14-12/31/15

Title: “Specification and function of perivascular stromal cells (PSCs) in response to stroke”

Projects in the proposal seek to characterize the role of the transcription factor Foxc1 in the response of a pericyte sub-population, PSCs, following stroke injury.
Role: Principal Investigator

R00 NS070920 - NINDS 9/1/12-8/31/15

Title: “Forebrain Angiogenesis in *Foxc1* mutants”

The goal of the projects outlined in this grant are to determine the role of Wnt signaling in formation of the perineural vascular plexus, establish downstream targets of Foxc1 in fetal brain pericytes and determine how disruptions in neocortical development negatively impact neurovascular patterning and stability.
Role: Principal Investigator

Brain Research Foundation Seed Grant (BRFSG-2014-11) 6/1/14-5/31/15

Title: “Activation of fibrotic scar forming cells following traumatic brain injury”

This proposal seeks to establish the presence of pro-fibrotic PSCs following a mild and severe closed-head injury model in adult mice. Also propose to look at role of immune cell derived leukotrienes in PSC activation.
Role: Principal Investigator