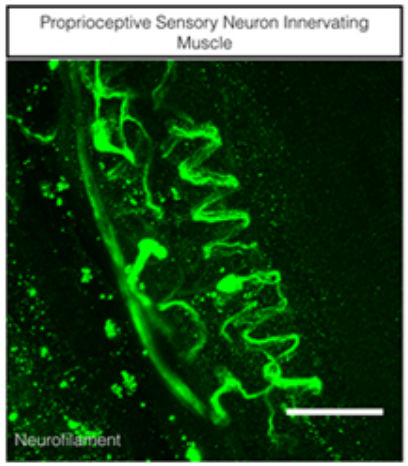

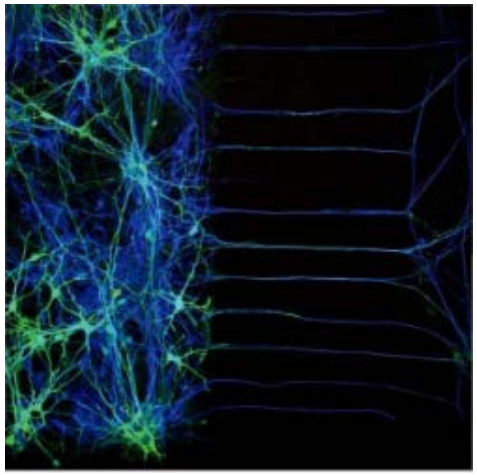

Taken in collaboration with Nikhil Sharma and David Ginty.

We recently found that developmental neuronal competition at the individual cell level involves a complex combination of events. We used mathematical modeling, biochemical, and genetic approaches to show that, in the PNS, the likelihood of neuronal survival is critically dependent on a sensitization process initiated by target-derived nerve growth factor (NGF) and strengthened by a series of positive-feedback loops (Science, 2008). We found that in addition to initiating expression of survival factors, the retrogradely transported NGF-TrkA signaling complex also increases expression of TrkA, thereby increasing the magnitude of NGF-TrkA pro-survival signaling. We also discovered that the duration of NGF-TrkA pro-survival signaling is variable, and regulated in an NGF-dependent manner via an unknown mechanism.

NGF also influences a complex protection/punishment mechanism affecting competition. We found that, in sympathetic neurons, NGF signaling induces neurons to increase secretion of unknown factors which, via p75 signaling, can kill or "punish" neighboring sympathetic neurons in a paracrine manner. High NGF-TrkA signaling protects neurons from this punishment cue while low NGF-TrkA signaling leaves cells vulnerable. In my lab we are working to not only understand the molecular basis for these competitive processes, we are also examining how this logic applies to other aspects of neural development, function, and disease.

Developmental Neurobiology Laboratory

Christopher Deppmann

Associate Professor
deppmann@virginia.edu
deppmannlab.com

Dept. of Biology, Biomedical Engineering, Cell Biology and Neuroscience
University of Virginia
Charlottesville, VA
434.924.7961

"Discovering how the nervous system is assembled during development and disassembled during pathology."

UNIVERSITY of VIRGINIA

SCHOOL of ENGINEERING & APPLIED SCIENCE

Competitive Signaling Pathways Underlying Neural Development

Over 50 years ago, Viktor Hamburger and Rita Levi-Montalcini put forth the neurotrophic-factor hypothesis, which describes neuronal competition for survival in the PNS. Their Nobel Prize-winning hypothesis, that neuronal survival during development is dependent on a limited supply of target-derived trophic factors, is a fundamental principle of nervous system organization, with an array of clinical applications. This hypothesis explains neuronal competition for survival at the population level; at the level of the individual cell, the basis of neuronal competition for survival is less clear. Delineating individual cellular competition has been our most significant accomplishment to date (*Science*, 2008). We found that, in the PNS, the likelihood of neuronal survival is critically dependent on a sensitization process initiated by target innervation and strengthened by a series of positive-feedback loops. We also found that some neurons, as they get stronger, secrete a "punishment" factor to expedite the death of weaker neighboring neurons. Importantly, strong neurons are impervious to this secreted death signal or are "protected from punishment." In our PNS model system, nerve growth factor (NGF), secreted from visceral targets such as the eye or salivary gland, acts through the receptor tyrosine kinase, TrkA to mediate neuron survival while brain derived neurotrophic factor (BDNF), secreted from neuronal cell bodies, acts through p75 in an autocrine/paracrine manner to expedite cell death. My lab is interested in a deeper understanding of not only the pathways underlying construction and destruction of the nervous system but also cross talk between these pathways (*Systems Biology of Apoptosis,* 2012). For most patterning events in the nervous system, we have yet to assign molecular determinants for self- activation and lateral inhibition. We have made headway on this problem in the sensory nervous system demonstrating that TNFR1 antagonizes TrkA to define the form and function of nociceptors (*Neuron,* 2014). We are also considering how TNFR family members rely on each other to promote refinement events in sympathetic, nociceptive and proprioceptive circuits (Cheng et al, *in prep)*

Long-Distance Endosome Signaling in Neural Development

NGF promotes its effects in part by binding to its cognate receptor tyrosine kinase, TrkA, on distal axons followed by internalization into a "signaling endosome" which travels retrogradely from the axon to cell body and dendrites (*Neuron,* 2010; *J. Neuro, 2012*). Regulation of signaling endosome function, trafficking, stability, and signaling figure prominently into the self-activation arm during nervous system development. Consistent with this we found that the NGF-TrkA signaling complex increases expression of TrkA, representing a feedback loop that increases the magnitude of NGF-TrkA pro-survival signaling (*Science*, 2008). We also found that the duration of NGF-TrkA pro-survival signaling is variable and regulated in an NGF-dependent manner. We found that the NGF-TrkA signaling endosome uses Coronin-1 to avoid degradation and endow the signaling endosome with signaling capabilities (*Nature Neuro,* 2014). This work also verifies the existence of a predicted feedback loop whereby NGF modulates its own signal duration, which we find is critical for competition for survival. We next examined how NGF dependent Coronin-1 expression influences another competitive developmental process: We speculate that axon growth and branching in neurons that are 'losing' the competition for neurotrophic factor will be slower to induce Coronin-1 expression and will experience a longer period of robust growth until they either find a sufficient concentration of NGF to support survival or are eliminated via apoptosis between E18.5 and P0 (*J. Neuro,* 2015*)*. We also have recently used these finding to model growth along intermediate target and have found dramatic differences in NT3- TrkA versus NGF-TrkA axon growth signaling (*MCN,* in revision).

Molecular Mechanisms of Axon Degeneration

The competitive signaling programs described above are widely appreciated to be involved in a type of developmental axon degeneration called pruning. Pruning shares several morphological similarities with both disease and injury induced degeneration. Because of these morphological similarities, we argue that they may share common molecular mechanisms despite having different etiologies. We reason that, like cancer, developmental programs required for nervous system assembly may be co-opted by pathology to disassemble the nervous system. We have begun to test this premise by examining injury-induced axon degeneration. As preamble for this, our collaborators and we have identified antibodies toward epitopes that are expressed specifically on degenerating axons (Sokolowski et al*., 2014*) and have developed software to automate quantification of degeneration (*Vacarri et al, 2012*). Using a novel *in vitro* model for axon injury, we found that p75NTR and TNFR1 do not play a large role in promoting axon degeneration after injury. However, the highly related protein, DR6 is required for injury induced degeneration both *in vitro* and *in vivo*. This appears to be working through a caspase 6 dependent pathway (Gamage et al,, 2017). We are now defining the ligands underlying this apparent coordination of axon degeneration (Gamage et al, *in prep*).

Technology Development: Magnetogenetics, Rapid Transgenesis and rapid developmental phenotyping

We are also building tools, which will enable next generation experiments related to development, function and pathology of the nervous system. In collaboration with Ali Guler's lab, we are building non-invasive magnetogenetic tools to better understand the signaling pathways underlying neural circuit assembly and function. The first of these tools, Magneto, is capable of remotely inducing the firing of neurons in awake and behaving animals (*Nat. Neuro, 2016*). We are finalizing the follow up tool, profx, which is capable of remotely inhibiting neurons and can even prevent siezures (Ottolini et al, *in prep*). We are also developing magnetically sensitive transcription factors as well as tools to remotely induce second messenger production (ie cAMP) (Kumar, *in prep*). We are developing a method to create rapid, low-cost knock out/in mice (and other model organisms) using CRISPR/Cas9d transgenesis by in vivo gene editing (Keeler et al, *in prep*). Finally, in collaboration with Prof. Eli Zunder we are developing techniques to rapidly assess neuronal identities, lineages and signaling states in the PNS and CNS.

RECENT RESEARCH DEVELOPMENTS

- In 2017 we uncovered a pathway by which degenerating axons communicate with each other.
- We have recently described how axons change their behavior as they grow toward final targets.
- We are now seeking to understand the role of the nervous system in fat loss during calorie

RECENT GRANTS

- "Extrinsic mechanisms governing injury-induced axon degeneration"
- NIH-R01 (National Institutes of Health-R01NS091617), PI: C. Deppmann, 2015-2020
- "CAREER: Emergent properties of systems matching in peripheral nervous system development"
- NSF-IOS-1453242, PI: Christopher Deppmann, 2015-2020
- "REU Site: Multi-Scale Systems Bioengineering"
- NSF-EEC-1560282, PI: Timothy Allen (Deppmann is one of 10 coinvestigators), 2016-2019
- "Understanding the spread of neurodegeneration"
- Owens Family Foundation, Co-PI (Deppmann and Lukens), 2016-2019
- "Magnetogenetic Approach for treating nervous system injury"
- Coulter Foundation, Co-PI (Deppmann, Guler, and Deal), 2016-2017

SEAS Research Information
Pamela M. Norris,
Executive Associate Dean for Research
University of Virginia
Box 400242
Charlottesville, VA 22903
pamela@virginia.edu
434.243.7683

UNIVERSITY of VIRGINIA | SCHOOL of ENGINEERING & APPLIED SCIENCE