Title: Establishing the Combinatorial Code of Protocadherins in Neurite Outgrowth
Keywords: Axon guidance, cadherins, combinations, primary culture
Background and Rationale: Axon guidance and target recognition are essential for establishing the nervous system of animals. The specificities of neural circuits are achieved through selective adhesion and signaling, dictated by a variety of guidance cues. Billions of neurons must form trillions of synapses; however, only hundreds of guidance molecules have been discovered. To explain how this relatively small number of cues can enable the formation of trillions of connections, axon guidance cue molecules must be working in combination to guide growth cones to their appropriate targets [1]. Although this combinatorial model of axon guidance has been proposed for over fifty years, its complexities have been difficult to study and consequently, it remains poorly understood [1,2]. To date, nearly all studies have focused on the function of a single guidance cue at a time, resulting in a failure to understand how combinations of cues affect neuronal growth [3]. Thus, determining the impact of these combinations on neuronal function is essential for understanding how the nervous system develops.

The goal of my honors thesis was to demonstrate that combinations can influence axon guidance. Using the delta-protocadherin (pcdh) family of guidance cues, I showed that *pcdh19* and *n-cadherin (ncdh)* in combination results in a dramatic increase in neurite outgrowth in olfactory sensory neurons (OSNs) (Figure 1). I then demonstrated that *pcdh10* disrupts *pcdh19*'s ability to increase outgrowth. This showed that combinations of cues can increase (*pcdh19 + ncdh)* or decrease *(pcdh19 + pcdh10)* neurite outgrowth. The combinatorial model could contribute to our understanding of the far-reaching and evolutionarily conserved roles of the pcdhs. My research is amongst the first to examine the functional impact of cadherin combinations. I will further this preliminary work by investigating additional combinations.

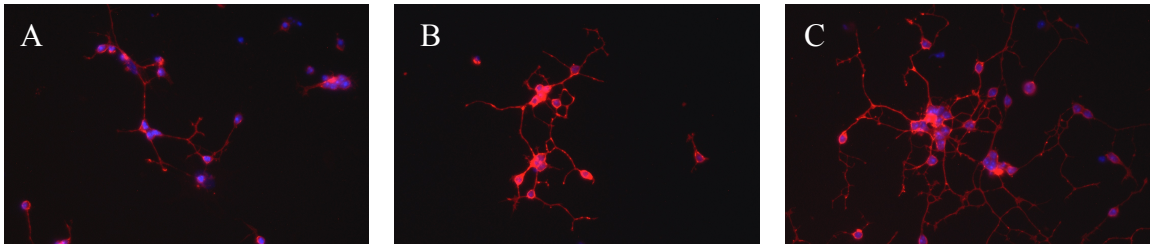

Figure 1: Primary culture of OSNs demonstrating effects of A) *pcdh19* B) *ncdh* C) *ncdh + pcdh19*

Hypothesis: I hypothesize that different combinations of protocadherins produce phenotypes of neurite outgrowth distinct from each protocadherin alone. Combinations that affect neurite outgrowth exert their effects because guidance cues physically bind one another.

Aim 1: Establish how combinations of pcdhs affect neurite outgrowth in OSNs

In my thesis, I tested 7 of the 54 possible pairwise combinations of the delta-pcdhs. In this study, I will test the remaining 47 combinations to establish a hierarchy of neurite outgrowth in OSNs. The simple bipolar structure and highly organized olfactory system make OSNs ideal candidates to study the combinatorial model of axon guidance. Primary culture of mammalian OSNs will be used to identify if combinations of pcdhs affect neuronal outgrowth. Each pcdh will be tested individually and in combination with other members. OSNs will be grown on coverslips that have been coated with the pcdhs being tested before being fixed, stained, and analyzed using ImageJ. **When applied in combination, it is expected that specific combinations of pcdhs will alter the neurons' phenotype in a reproducible manner.** Combinations will be compared to establish a hierarchy of effects on neuronal outgrowth including: neurite length, branching,

cell count and clumping, allowing me to define the specific effect of each combination. I will then ask if these combinations exert their effects by binding to one another or by acting independently on neurons.

Aim 2: Coimmunoprecipitations of pcdhs to establish binding partners

I previously showed using coimmunoprecipitation (Co-IP) that *pcdh19* and *ncdh* bind to one another, suggesting the augmented neurite outgrowth phenotype observed in OSNs is caused by this physical binding. I will test the combinations identified in Aim 1 that clearly influence neurite outgrowth by employing Co-IP. This strategy uses protein specific antibodies to indirectly identify a protein bound to the target protein. **It is expected that pairs of pcdhs that result in a distinct neurite outgrowth phenotype physically bind one another.** If a positive result is observed, this will suggest a physical binding that causes a conformational change affecting axon pathfinding. If combinations of pcdhs do not bind to one another, it is likely both axon guidance cue molecules are exhibiting an independent effect that is additive.

Aim 3: Establish effect of combinations of pcdhs on outgrowth in cortical neurons

I will expand this study to investigate the role of combinations in the central nervous system (cortical neurons) as well as the peripheral nervous system (OSNs). I will utilize a new microfluidic system that is more sensitive and flexible than coverslips to study the effect of these combinations on the outgrowth of mammalian cortical neurons [4]. This system will need to be optimized, but will ultimately allow me to determine the directionality of axons and growth rate, in addition to the parameters measured in Aim 1. **It is expected that combinations of pcdhs will result in distinct phenotypes in neurite outgrowth in cortical neurons.** Combinations will augment, inhibit or have no effect on neurite outgrowth. This study will identify if the same combinations of guidance cue molecules can have different effects in different neuronal subtypes. A hierarchy of neuronal outgrowth, branching, directionality and clumping will be established in cortical neurons. Should this microfluidic system fail, I will test the effect of combinations on cortical neurons using coverslips.

Personal Qualifications: This work is a continuation of my thesis and later work in Dr. [redacted]'s lab that piloted this approach to primary culture of OSNs. I have the technical skills, including CoIPs, neuronal outgrowth analysis, OSN and cortical primary culture to complete this study.

Intellectual Merit and Broader Impact: This study will be one of the first of its kind to clearly define how combinations of protocadherins interact during development to establish a proper functioning nervous system, which is essential to our knowledge of neuronal development. Additionally, these aims will serve as a platform to test the remaining plethora of combinations of axon guidance cues. Continuing my involvement with youth and disability advocacy, I will present this research to a variety of groups at my future academic institution. LEND is a leadership training program uniquely bringing policy makers, clinicians, and families of children with neurodevelopmental disabilities together. I will present the importance of the combinatorial model to this wide range of individuals committed to becoming leaders in the community. The combinatorial model will also be presented in a hands-on approach at Brain Awareness Week, a nationwide program aimed at introducing elementary students to neuroscience. This project will therefore enable me to blend my scientific interests and community involvement.

References:

[1] [redacted]
[2] [redacted]. [3] [redacted]
[4] [redacted]