Title: Establishing the Homeostatic and Physiological role of KCC3 and NKCC1 in Peripheral Nerves
Keywords: Locomotion, Proprioception, Peripheral Nerves, KCC3, NKCC1, SuperClomeleon

Background & Significance: Organisms must move within and respond to their environment. Locomotion can be studied at the organism, organ/tissue, and cellular levels. On a cellular level, cell response is done in part through ion channels and transporters, which play important roles in a variety of physiological functions, including neurotransmission, cell contraction, epithelial transport and pH regulation. The ability of cells to maintain and regulate their ion concentration and volume depends on swelling or shrinkage and is essential for proper function. Such homeostasis and changes in cell volume[1] are mediated by two major families of proteins that respond to changes in cell volume, the K-Cl cotransporters **(**KCCs) and the Na-K-2Cl cotransporters (NKCCs), which have opposing functions in ion transport. While KCC moves Cl^- out of the cell and is activated by cell swelling, NKCC transports Cl^- into the cell and is activated by cell shrinkage[2] (Figure 1). These two transporters regulate intracellular Cl^- ($[Cl^-]_i$) homeostasis in neurons where the Cl^- concentration is critical in modulating inhibitory GABA/glycine neurotransmission. Specifically, in central neurons, the function of the KCC family member KCC2 is critical in maintaining a low $[Cl^-]_i$, thereby facilitating GABA-mediated hyperpolarizing Cl^- currents and synaptic inhibition[3,4]. In adult peripheral neurons, NKCC1 increases $[Cl^-]_i$ levels, leading to GABA depolarization[5,6].

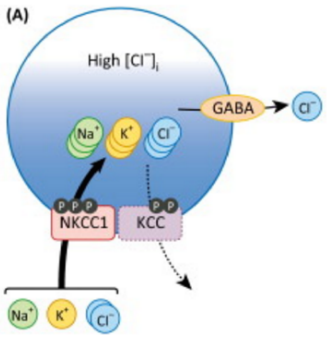

Figure 1. Reciprocal function of NKCC1 and KCC3 affects Cl^- homeostasis and cell volume.

Work from our laboratory has shown that loss of function mutations in the KCC family member KCC3, results in a severe locomotion phenotype in mice[7]. The neurons giving rise to this locomotion deficit are parvalbumin expressing proprioceptive neurons in dorsal root ganglia (DRG), a population that represents 10-25% of sensory neurons[8]**. While there is no question that a genetic link between KCC3 and proprioception exists, the molecular mechanism of Cl^- and water homeostasis in these cells has never been explored. A role for KCC3 in sensory neurons is novel and intellectually challenging as sensory neurons are known to highly express NKCC1, a transporter recognized to reciprocally mediate Cl^- and water transport. Understanding the role and mechanism of Cl^- homeostasis in these neurons is critical for understanding peripheral nerve function, proprioception, and locomotion.** Therefore, my proposed project will uncover the fundamental biology of K-Cl and Na-K-2Cl cotransporters in proprioceptive sensory neurons.

Hypothesis: I hypothesize that NKCC1 is expressed in parvalbumin-positive (PV+) proprioceptive sensory neurons and will participate in accumulation of intracellular Cl^-, and that KCC3 will constitute a major pathway for Cl^- movement across the neuronal membrane in conditions of mild swelling and inhibition of NKCC1. I predict that the primary role of KCC3 is to maintain the volume and integrity of the proprioceptive fibers, and in doing so, protect the control of locomotor activity by these cells

Aim 1. Determine basal intracellular chloride concentration of parvalbumin expressing sensory proprioceptive neurons and determine if NKCC1 affects this concentration.

As previously done in the laboratory[6] I will harvest DRG neurons from Pvalb-CRE[8] x SuperClomeleon (Rosa26:LSL:SpClomeleon)[9] mice, which are PV+, to assess basal $[Cl^-]_i$. SuperClomeleon is a highly sensitive, ratiometric Cl^- sensor that detects changes in intracellular Cl^- within the physiological range[10]. First, I will stain PV+ sections with an NKCC1 antibody to determine NKCC1 expression. To determine NKCC1 contribution to the $[Cl^-]_i$, neurons will first be

incubated with or without (control) a NKCC-specific inhibitor. Then, $[Cl^-]_i$ will be manipulated in sensory neurons by sustained applications of GABA neurotransmitter, which leads to Cl^- leakage, as previously accomplished with central neurons[11]. I anticipate that these protocols will induce a drop in $[Cl^-]_i$, and the participation of NKCC1 in the maintenance or restoration of $[Cl^-]_i$ levels will be assessed by comparing neurons exposed to GABA in the presence or absence of the cotransporter inhibitor. **I anticipate that proprioceptive DRG neurons, like other sensory neurons, will express NKCC1 and accumulate Cl^- through its activity.** Next, I will establish whether $[Cl^-]_i$ will be lower compared to other neurons, due to co-expression of KCC3.

Aim2. Characterize the role of KCC3 in the regulation of intracellular Cl^- and cell volume

Following Aim 1, the role of KCC3 in regulating Cl^- levels will be assessed. Experiments similar to the NKCC1 inhibitor experiments will be done using a KCC-specific inhibitor[12]. Response of neurons to sustained or repeated GABA application will be assessed in the presence or absence of the KCC inhibitor. Experiments will also be done using DRG neurons isolated from mice that carry PV-CRE, SuperClomeleon, and KCC3 floxed alleles. In this system, cells expressing the Cl^- sensor will be devoid of KCC3. Because the function of NKCC1 is inhibited and KCC3 is activated by cell swelling, I will test $[Cl^-]_i$ regulation under control isosmotic (310 mOsM) conditions as well as hypotonic (280 mOsM or 240 mOsM) conditions. To measure cell volume, I will utilize the Cl^- insensitive fluorescence of the CFP portion of Superclomeleon. We have gathered preliminary data obtained with SuperClomeleon expressed in HEK293 cells to show that CFP fluorescence decreases with cell swelling (through dilution) and recovers upon regulatory volume decrease. **It is expected that the absence of KCC3 in these neurons will disrupt Cl^- regulation under swelling conditions and prevent the neurons from maintaining their volume under conditions that lead to water influx**.

Broader Impact:

Locomotion is vital for many, if not most life forms, whether it is to seek food, reproduce, or escape a predator. Proper locomotion is also vital for humans in their daily life activities. Locomotion involves many organs and physiological systems in the body, where all organs play a synchronous role in coordinating body movement. Proprioception is a large component of locomotion. It involves sensory fibers traveling from muscles to the spinal cord to provide cues to motor neurons on how to respond and fine tune muscle contraction for optimal posture. This study would be the first in the field that seeks to understand the role of KCC3 and a related cotransporter, NKCC1, in Cl^- and volume homeostasis in this subset of peripheral neurons. Although molecular in nature, our studies relate to a broader physiological system and to ethology with animal locomotion behavior. This work would allow me contribute to three major disciplines: Neuroscience (modulation of inhibitory synaptic transmission), Physiology (sensory perception), and Biology (locomotion). I plan on impacting the scientific community through this work by mentoring both high school and undergraduate students (especially underrepresented students in the community) that express interests in science and technology through [redacted] IMSD: [redacted] and the [redacted] Summer Science Academy, respectively. I also plan to present my research findings at multiple meetings.

References:

1. [redacted]