OVERVIEW/ABSTRACT

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder after Alzheimer's disease. The prevalence in industrialized countries is estimated to be about 0.3% of the general population and around 2% among individuals 65 years of age and older (de Rijk et al 2000, Strickland & Bertoni 2004). The motor symptoms of Parkinson's disease (PD), such as tremor, rigidity, akinesia and posture instability, are mainly caused by the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) (Bernheimer et al 1973, de Rijk et al 2000, Fearnley & Lees 1991, Strickland & Bertoni 2004). In addition to the hallmark motor phenotypes, disturbances of smell, sleep, mood, and gastrointestinal function may predict Parkinson's disease by 5 or more years (Goldman & Postuma 2014). Importantly, these symptoms occur before the onset of DA neuron degeneration, suggesting that dysfunction of DA neurons precedes their loss. Indeed, *the **central hypothesis** of our efforts is that dysfunction in the membrane properties of DA neurons in the SNpc is a common prodromal state of PD and causes selective vulnerability*. Identifying the basis of these changes in membrane properties will lead to novel insights into the etiology of PD, potential molecular markers for early detection, and possible pathways for novel early intervention.

The **goal** of the SEED grant is to combine the expertise of three labs – Riessland (genetics), Plotkin (calcium imaging & *in vivo* experiments) and Wollmuth (electrophysiology) – to establish an inter-laboratory pipeline to assess the impact of various PD-related genes on membrane properties and cell vulnerability *in vitro* and *in vivo*. Numerous genes have been associated with PD (Nalls et al 2019). Our efforts will focus mainly on *KCNS3* (Nalls et al 2019, Zheng et al 2021) as well as *SATB1* (Nalls et al 2019), and *NR1D1* (Brichta et al 2015). *KCNS3* encodes a K^+ channel subunit that affects membrane properties in other cell types and preliminary genetic data indicate that its expression is altered in SATB1-induced senescence, a pathway for cell death in DA neurons, and in a circadian rhythm manner (*NR1D1* is a circadian rhythm gene). The specific **objectives** of the SEED grant are (i) to publish our first joint manuscript and (ii) to generate additional key preliminary data. Once the objectives of the SEED grant are attained, which we anticipate will take 10-14 months, we will submit a multi-PI NIH R01.

Faculty involved in this project. Our SEED grant encompasses three faculty with diverse expertise. Dr. Markus Riessland is a new faculty who is the nucleating factor for our joint efforts. He is an expert in human genetics and PD and has extensive experience with human stem cell-derived DA neurons. Dr. Joshua Plotkin is an expert in local networks in brain function and notably brings expertise in Ca^{2+} imaging. Dr. Lonnie Wollmuth is an expert in synaptic transmission and membrane physiology and has extensive experience in characterizing membrane properties in diverse neuron types. It is this combination of expertise – genetics, stem cells, Ca^{2+} imaging and membrane physiology – that will allow integrated questions to be assayed.

General scientific questions. A hallmark of DA neurons in the substantia nigra is that they display 'pacemaking' that is they fire action potentials without synaptic input (Figure 1) (Kang & Kitai 1993a, Kang & Kitai 1993b, Nedergaard et al 1993, Yung et al 1991). This pacemaking is fundamental to how these DA neurons regulate brain circuits, including voluntary movement, action selection, future movement, and reward-based learning (da Silva et al 2018, Schultz 2007). Notably, pacemaking 'stresses' DA neurons in part because pacemaking in general requires considerable energy but also because in SNpc DA neurons it is associated with the influx of Ca^{2+} via voltage-gated Ca^{2+} channels (VGCCs) (Chan et al 2007, Surmeier et al 2012). While intracellular Ca^{2+} is a key signaling molecule, its imbalance can induce cell stress and potentially cell death. Thus, anything that perturbs pacemaking may represent a pathway for initiating cell death in these neurons. Hence, our central hypothesis.

Experimentally, we will characterize the membrane properties of human stem cell-derived DA neurons. These stem cell-derived DA neurons are advantageous since they are human, display many properties of SNpc DA neurons including pacemaking, and can be manipulated to knock-out specific genes.