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Calcium Signaling involved in Neuronal Death and Dysfunction in SCA2

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Spinocerebellar ataxias are relatively common neurodegenerative diseases of the human nervous system. Although the most common mutations leading to SCAs are related to expansions of CAG DNA repeats in the coding region of genes, other mutations have pointed to the importance of genes related to voltage-gated calcium channels (CACNA1a, SCA6) and intracellular calcium signaling (I3PR1, SCA15/16). The background for this program project came from human studies showing that normal genetic variation in the CACNA1a channel modified age of onset in SCA2 patients and from studies in genetically engineered mice indicating that presence of mutant ATXN2 in PNs resulted in enhanced calcium release from intracellular stores via abnormal interaction of ATXN2 with the Ins3P receptor.

This PPG utilizes the existing collaborations of the investigator group and will harness the unique and diverse expertise in mouse models, calcium signaling and cerebellar physiology to examine SCA2 pathogenesis at the molecular, cellular, and circuit level, to delineate normal cerebellar aging and its relationship to calcium handling, and to identify novel disease-modifying treatments.

The administrative core is based at the University of Utah and will ensure the appropriate financial and academic interactions. The animal core headed by d. Scoles will use the vivarium facilities at the University of Utah, generate animal models, cross existing models into the appropriate genetic background, and distribute animals to the laboratories of the principal investigators.

Project 1 (Pulst): Genetic approach to studies of calcium signaling perturbed in SCA2 and in normal aging *in vivo*. (Pharmacologic and genetic targeting of the I3P receptor in Purkinje cells.)

The Pulst group has developed a number of mouse models examining onset and progression of Purkinje neuron (PN) degeneration in mice expressing mutant ATXN2 under control of the Pcp2 (L7) promoter. These models have been used to examine the effect of modulating CACNA1a-mediated calcium signaling using genetic intercrosses in collaboration with the Otis laboratory. In collaboration with the Bezprozvanny laboratory, we have utilized ATXN2 transgenic mice to examine the effects of blocking abnormal Ins3PR function on disease progression *in vivo*.

We are now proposing to expand the use of genetic intercrosses and transduction using viral vectors to examine the effects of I3PR signaling on SCA2 disease initiation and progression. This will be accomplished in a PN-centric models and using mouse models expressing BAC-transgenes that may resemble the human disease more closely.

SA1 Define PC dysfunction morphology, biochemistry, cell death in normal aging & SCA2 pathology

H1: Normal PN aging and ATXN2$_{exp}$-mediated PN degeneration are related to abnormal calcium-signaling or reduced calcium buffering capacity.

Approach: We will examine progression of cerebellar dysfunction in BAC transgenic mice expressing various ATXN2 repeat expansions at different copy number numbers. We will determine the earliest biochemical and morphological changes and correlate them with onset and progression of functional abnormalities. We will examine changes in RNA expression of genes involved in calcium homeostasis using qPCR, both in aged wildtype and in transgenic animals. The time-course of changes established in SA1 will be compared with abnormalities recorded in PN culture (project 2) and in the cerebellar slice (project 3).

SA2: Genetic interaction of I3PR1 loss of function and mutant ATXN2 *in vivo*.

H2: Signaling through ITPR1 is critical in SCA2 pathogenesis and normal aging.

Significance: As the human disease SCA15 demonstrates, loss of one copy of ITPR1 can result in late-onset ataxia. Therefore, these intercrosses will answer the crucial question whether downregulation of ITPR1 will ameliorate the phenotype in the presence of mutant ATXN2. Furthermore, ITPR1+/- animals have been reported as normal, but these animals may show subtle abnormalities in cerebellar functioning in older age.

Approach: Although many proteins are involved in calcium-signaling or buffering in PNs, due to the time and costs involved in mouse genetic interaction experiments, we will focus on ITPR1. We will cross our ATXN2$_{exp}$ transgenic mouse lines with mice that have on copy of ITPR1 inactivated by a loss of function mutation. The resulting crosses will be compared by biochemical, morphologic criteria developed in SA1. We expect that loss of one ITPR1 copy will ameliorate ataxia in transgenic animals.

We will compare phenotypes between animals targeting ATXN2$_{exp}$ expression to PNs only and those lines expressing ATXN2$_{exp}$ under control of the ATXN2 promoter. We will also determine whether heterozygosity for ITPR1 loss-of-function alters PN aging in collaboration with project 3.

SA3: Evaluation of Dantrolene in new SCA2 mouse models

H3: Dantrolene treatment is effective after symptom onset and in ATXN2$_{exp}$-BAC transgenic models.

Significance: Dantrolene is a drug approved for human use in the acute treatment of malignant hyperthermia and the chronic treatment of spasticity. It is also effective in animal models of HD, SCA3, and SCA2.

Approach: In Pcp2-ATXN2$_{exp}$ mice, dantrolene delays development of ataxia and cell loss if started at 2 months of age, prior to abnormal motor performance on the rotarod. We will test dantrolene at different doses prior to and at symptom onset in Pcp2-ATXN2$_{exp}$ mice. We will then test whether dantrolene can

delay progression in a new $ATXN2_{exp}$ BAC mouse model. At the end of treatment, cerebellar slices from these animals will be examined in project 3 (Otis).

Project 2 (Bezprozvanny/Kim): Pathological role of InsP3R-mediated Ca2+ signaling in SCA2.

SA1. $InsP_3R1$-mediated Ca^{2+} signaling and synaptic plasticity in Purkinje neurons (PNs) in SCA2 animal models.

2-photon Ca^{2+} imaging will be used to study $InsP_3R$-mediated Ca^{2+} signaling in WT and SCA2 mice at different ages. mGluR-evoked Ca^{2+} signals in synaptic compartments of PC cells will be evaluated. Also InsP3 uncaging experiments will be performed. Cerbellar LTD experiments will be performed with slices from WT and SCA2 mice. Based on previous in vitro experiments and studies with PC cultures (Liu at al, (2009) J Neurosci 29, 9148-62.) **our prediction** is that $InsP_3R1$-mediated Ca^{2+} signals will be supranormal in PC cells from SCA2 mice. It is also expected that cerebellar LTD will be abnormally enhanced in SCA2 neurons as cerebellar LTD depends of Ca^{2+} release.

SA2. $InsP_3R1$-mediated Ca^{2+} signaling as potential target for SCA2 treatment.

$InsP_3$ 5-phosphatase (5PP) dephosphorylates $InsP_3$ to $InsP_2$. Overexpression of exogenous 5PP in cells provides an effective and highly specific way to reduce $InsP_3R$-mediated Ca^{2+} signaling. We will express 5PP in PC cells of WT and SCA2 animals by AAV-mediated infection. Catalytically inactive 5PP will serve as a negative control. The phenotype of AAV-5PP injected mice will be evaluated in Ca^{2+} imaging and LTD experiments (SA1) and in behavioral and neuropathological experiments (Liu at al, JN-2009; Kasumu and Bezprozvanny, (2010) Cerebellum). **Our prediction** is that overexpression of 5PP will normalize Ca^{2+} signals and alleviate phenotype of SCA2 mice.

SA3. Role of endogenous PC Ca^{2+} buffers in SCA2 pathogenesis.

Neuronal Ca^{2+} buffering capacity is reduced with aging. We previously proposed that reduction in endogenous Ca^{2+} buffering capacity increases susceptibility of aging neurons to Ca^{2+} excitotoxicity (Kasumu and Bezprozvanny, (2010) Cerebellum). Parvalbumin (PV) and calbindin (CB) Ca^{2+} buffering proteins are abundantly expressed in PC cells. PV is a major Ca^{2+} buffer in somatic-related Ca^{2+} events while CB is a major Ca^{2+} buffer in dendrites and spines in PC cells. To determine the importance of endogenous Ca^{2+} buffering, we will cross SCA2 mouse model with CB and PV KO mice. We already obtained both CB and PV KO mice from Dr. Beat Schwaller (University of Fribourg, Switzerland). **Our prediction** is that SCA2 phenotype will be accelerated in the absence of CB or PV. These experiments may also help to dissect an importance of cytosolic or dendritic Ca^{2+} buffering in the context of SCA2 pathology.

SA4. Structural studies of $InsP_3R$-Atxn2 complex.

The reason for supranomal $InsP_3R$ activity in SCA2 is pathogenic association between Atxn2exp and $InsP_3R$ carboxy-terminal region (Liu at al, JN-2009). To understand this interaction at molecular level we will solve crystal structure of polyQ-expanded region of Atxn2 in complex with $InsP_3R$ carboxy-terminal region. In these studies we will use the same approach as in previous structural studies of polyQ region of Huntingtin protein (Kim at al, (2009) Structure 17, 1205-12). Obtained **structural information** can be used to develop small molecule inhibitors of $InsP_3R1$ association with Atxn2exp. Such inhibitors are expected to normalize Ca^{2+} signaling in SCA2 PC cells and may provide leads for therapeutic development for SCA2 and other polyQ-expansion disorders.

P3 (Otis) - Probing mGluR-IP3R-related dysfunction in the cerebellar circuit

SA1. mGluR-linked excitability of Purkinje neurons (PNs) in SCA2^{exp} mice. Extracellular and current clamp recordings from Purkinje neurons will be used to test whether mGluR-mediated excitability is altered in L7/pcp2-SCA2^{exp} mice. Synaptically-evoked mGluR and DHPG-evoked effects on spontaneous spiking will be measured at various ages in brain slice recordings. Recognizing the tight link between Group I mGluRs and endocannabinoid signaling we will examine endocannabinoid mediated short term plasticity at parallel fiber, climbing fiber and inhibitory interneuron synapses on Purkinje neurons.

SA2. Examine afferent-induced pauses in simple spike firing. Climbing fibers afferents are known to elicit brief pauses in PN spontaneous firing and these pauses are thought to be a signature of learned movements, i.e. conditioned responses, in associative motor learning paradigms (McCormick and Thompson, Steuber, Hausser, and DeSchutter). We hypothesize that hyperactive IP3R signaling may strongly enhance calcium-activated potassium conductances which underlie these pauses. We will stimulate CFs electrically and with optogenetic methods and measure the effects on spontaneous firing. Similarly strong parallel fiber input will be tested to see if it is more effective at disrupting regular firing.[This is an interesting issue in light of the Khodakhah results with the positive SK channel modulators Ebio-1 and chlorzoxazone in EA-2 models – his data argue these are therapeutic – if Ilya is correct about excessive calcium they should make things worse in SCA mice]

SA3. Purkinje neuron integration of patterns of parallel fiber input. Using adaptive optical techniques to deliver complicated spatial patterns of input we will test whether dendritic integration in individual PNs becomes impaired in SCA^{exp} animals. Patterned stimulation will be accomplished by using SLM technology and a 405 nm diode laser for multisite glutamate uncaging (Lutz et al., 2008; Nikolenko et al., 2008). Alternatively we will make use of the Thy1ChR2 mouse line and a 488 laser.

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Notes:

Stefan – I assume the BAC SCA2 model you suggest will be a global SCA2^{exp} mouse (or rat)?If so we could also look at molecular layer interneuron mGluR-IP3R signaling as we have shown (Karakossian and Otis, 2004) that these feedforward inhibitory neurons have group 1 mGluRs which are very likely PLC-coupled. This would also give us the possibility of examining cell-autonomous vs. non-autonomous mechanisms within the circuit.

Synergy with Ilya-We will obviously help him with his brain slice experiments that are directed at measuring intracellular calcium signals and mGluR-mediated, TRPC3 conductance. Ilya- if you would like to examine EAAT4 glutamate transporter mediated signals to test whther there is an SCA5-like impairment of glutamate uptake (or a compensatory increase to offset the hyperactive mGluR cascade) I can help with this.

The circuit excitability experiments proposed in my SAs are directly related to Ilya's experiments synergy abounds I think.

If our mGluR-IP3R and pause-Ca-activated K channel experiments work we could use the 5PP viral reagent to see if this "normalizes" the circuit behavior. We could also look at these circuit phenomena in CB or PV KO mice.

Synergy with Stefan-The BAC SCA2 mouse will be a very interesting counterpoint to the L7/pcp2 mouse with regard to circuit mechanisms. It opens up possibilities of looking at interneuron-PN signaling and of looking at whether IP3Rs are also dysfunctional in the interneurons.

We could also of course examine these circuit phenomena in any mice with altered calcium signaling genes.

Other things: Stefan – if you get the rat working we could certainly try associative motor learning with optogenetic methods. For technical reasons this is much easier in rats -I can expand on this more on Friday