## 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.

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.

**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.

**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