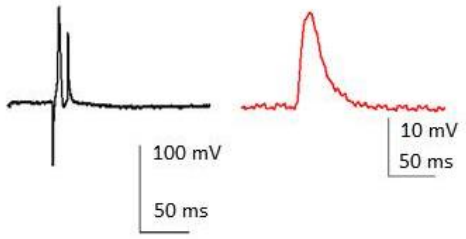
Effect of SUMOylation on presynaptic function in superior cervical ganglion (SCG) neurons

S15183 Japan Society for the Promotion of Science Fellowship Award. Professor Gary Stephens research visit to Professor Sumiko Mochida, Tokyo Medical University, March 28th - April 22nd 2016.

Background. SUMOylation is a prominent post-translational modification biological process mediated by attachment of small ubiquitin-related modifier (SUMO) proteins to their molecular targets. Recent evidence, reviewed by us and others, suggests that SUMOylation pathways are not solely confined to the nucleus, but can also affect cytoplasmic and plasma membrane proteins, including ion channels and receptors (Silveirinha et al., 2013 J Neurochem 127:580-591). The hypothesis tested here was that SUMOylation modulates such proteins to affect synaptic function.

Methods. We took advantage of the SCG neuron model of synaptic transmission uniquely available in Professor Mochida's laboratory in Tokyo Medical University, whereby cells maintained in long-term culture can form synaptic couples between each other. Current injection into the presynaptic partner produces an action potential which generates an excitatory postsynaptic potential, as measured using dual microelectrode electrophysiolgy (Figure 1). SCG neurons are also amenable to injection of cDNA (or other agents) into the presynaptic partner cell. Here, we investigated the injection of cDNA for the 97 amino acid SUMO-1-YFP protein, or SUMOΔGG-YFP, an 'inactive' 95 amino acid form of the protein that lacks two glycine residues and is unable to be conjugated by the UBC9 enzyme. SCG neurons in 4-5 week culture were injected with cDNA and YFP fluorescence investigated after 48 hours using confocal microscopy.

Figure 1. Synaptically-coupled SCG neuron



Results We found that SUMO-1 protein was distributed throughout the cell body, including the nucleus and cytoplasm and showed clear evidence of being transported to axons, dendrites and also as punctuate 'dots', consistent with the synaptic contacts seen in SCG neurons in long-term culture (Figure 2A; typical example from n=6 separate cells). By contrast, SUMOΔGG protein was largely confined to the cell body and, in particular, nuclear staining was prominent (Figure 2B; typical example from n=3 separate cells). Preliminary electrophysiological recordings showed that it was possible to record synaptic transmission between SUMO-injected SCG neurons (Figure 3).

Figure 2. cDNA-injected SCG neurons

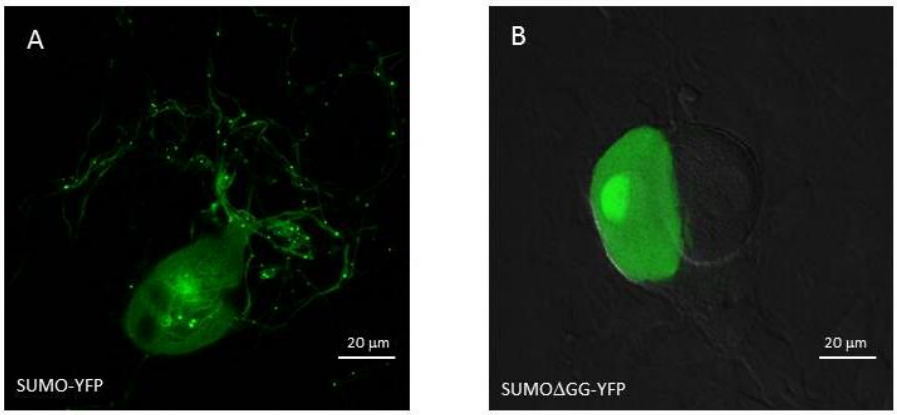
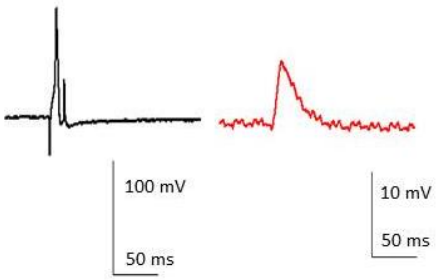
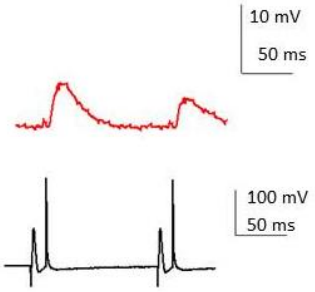


Figure 3. SUMO-injected SCG neuron



Conclusions/future work. These data suggest a differential distribution of SUMO-1 versus SUMOΔGG in SCG neurons. The findings represent proof-of-concept pilot data whereby the effects of SUMO-1-and SUMOΔGG on synaptic function can be investigated. In particular, we will investigate effects of SUMOylation using paired pulse protocols (Figure 4); such recordings are commonly used to determine presynaptic calcium dynamics and to measure synaptic plasticity.

Figure 4. Paired pulse recording in SCG neuron



Additional outcomes. This research visit also included a visit to Otsuka Pharmaceuticals, Tokushima, Japan. Otsuka (8th April, 2016), where Professor Stephens presented a research seminar entitled "Mechanism of action of levetiracetam, an anti-epileptic SV2A ligand"**.** Otsuka Pharmaceuticals supply levetiracetam as a major anti-epileptic drug in Japan; this research seminar discussed our previous work describing proposed mechanisms of action, whereby levetiracetam acts on presynaptic calcium channels and also affects SV2A (Vogl et al., 2012 Mol Pharmacol 82:199-208; Vogl et al., 2015 Eur J Neurosci. 41:398-409). The latter study also represents original work conducted in collaboration with Prof Mochida and Dr Shota Tanifuji and partly supported by a JSPS Postdoctoral Fellowship award to Christian Vogl.

Professor Sumiko Mochida, Professor Gary Stephens and Dr Shota Tanifuji at Tokyo Medical University in April 2016.