1. Project title

Variability in diameter of axonal endoplasmic reticulum tubules

2. Title, name, department and email address of project supervisor

Dr Cahir O'Kane (Reader in Genetics), Department of Genetics,
c.okane@gen.cam.ac.uk

3. Contact details of any co-supervisors

n.a.

4. Project Description (500 words maximum)

Endoplasmic reticulum (ER) in axons has a number of striking features, whose functional consequences are not fully understood. It is physically continuous throughout axons, over distances that are enormous on a subcellular scale. It is therefore a potential channel for long-distance communication within neurons, independent of action potentials or physical transport, earning it the term a "neuron within a neuron" (1,2). A further feature is its tiny diameter – ER tubules typically have an outer diameter of 60-80 nm, but in axons, this is around 40 nm, and often smaller - too small for the lumen to be visible (5-7). Therefore, despite the continuity of ER membrane, paradoxically lumen continuity appears constrained. The reasons for this are unknown, but could include constrained diffusion through the ER lumen. Indeed, fluorescence recovery after photobleaching (FRAP) of a lumen GFP marker shows that axonal ER tubule diameter is limiting for lumen diffusion - mutant tubules with an average diameter that is 50% greater than wild type show FRAP recovery around twice as fast as wildtype tubules (3)

Understanding the constraints on lumen diffusion, and ultimately being able to model it in healthy and perhaps in diseased axons, requires understanding of the variation in dimensions of ER tubules along their length. A simple model of ER tubules as a hosepipe of uniform diameter may not be helpful, if someone puts their foot on the hosepipe and constricts it. This project has two aims designed to assess the potential for diffusion along the narrow axon ER lumen, one dry and one wet; the balance between these will depend on how much lab access is possible this summer.

First we will use existing serial EM sections (7), to measure the diameters of individual ER tubules along their length, and quantify their variability. We are primarily interested in wildtype axons, but would also like to quantify mutants with larger tubules - these mutants affect genes homologous to those that cause the axon degeneration disease, hereditary spastic paraplegia. This will give a better basis for modelling diffusion through the axonal ER lumen, with potential consequences for the interconnectedness of Ca2+ stores through the axon. Second, we aim to follow single molecule movement through the ER lumen in axons; K Chahwala has generated transgenic flies carrying constructs used for tracking single molecule movement through non-neuronal ER lumen (4), and we will try to establish tracking of these in *Drosophila* axons (collaboration with Edward Avezov, Dementia Research Institute).

1. Berridge, M. (1998) Neuron 21, 13.
2. Berridge, M. J. (2002) Cell Calcium 32, 235.
3. Chahwala and O'Kane (2020) https://www.youtube.com/watch?v=NUw9dp5ml-E&feature=youtu.be
4. Holcman et al (2018) Nat Cell Biol 20, 1118.

5. Terasaki, M. et al. (2018). J Cell Sci 131: jcs210450
6. Wu et al (2017) PNAS 114, E4859.
7. Yalçın, B. et al. (2017) Elife 6,e23882.

5. Short summary (max 80 words) along with key aims/tasks of the project

Endoplasmic reticulum (ER) forms a continuous intracellular tubular nanostructure along the length of axons. Axonal ER has an unusually narrow diameter, which constrains diffusion along its length; larger tubules, caused by loss of genes whose loss causes axon degeneration disease, allow faster diffusion. We will explore how uniformly this diffusion constraint is distributed along tubules, by (1) measuring how tubule diameter varies along their length, and (2) developing tracking of single molecules inside tubules.

Key aims and tasks:
i. Use existing serial EM sections to manually measure individual tubule diameters along successive sections
ii. Assess parameters, e.g. variance, interquartile range, to describe variability along length of individual tubules, and can be used to statistically compare variation across tubules, axons and genotypes
iii. Test object segmentation and automated measurements, for consistency with manual measurements
iv. Laboratory access permitting, use confocal microscopy to test efficiency of in vivo labeling and diffusion of lumenal markers

6. Links to relevant supporting information (optional)

Chahwala and O'Kane (2020) https://www.youtube.com/watch?v=NUw9dp5ml-E&feature=youtu.be - (prize for best poster presentation at 2020 meeting of Cambridge Centre for Physics in Biology)

7. Specific details for your project, as applicable. This could include, for example:

- essential knowledge, skills and attributes that would be advantageous;
- whether the project can be conducted remotely or if it is in-person or lab-based;
- duration (from 6 to 8 weeks maximum)

Essential skills: numeracy, basic statistical knowledge
Desirable experience, but training will be given: image analysis; knowledge of subcellular organisation; fluorescence/confocal microsopy; statistical software; basic genetics

Remote/in-person: The project has a major component that can be conducted remotely, and a component that is lab-based, if distancing/occupancy constraints allow lab access over the summer. If lab access is not practicable, the entire project can be remote.

Preferred duration: 8 weeks, with dates flexible.