UNIVERSITY OF CAMBRIDGE
University Biomedical Services

UNIVERSITY OF CAMBRIDGE AWERB REQUEST

Application for non-regulated use of animals in scientific procedures

(To be completed electronically)

Is this a repeat request – Yes/~~No~~

Name of applicant: Sebastian Timmler
Applicant's e-mail: st794@cam.ac.uk
Applicant's mobile number: +44 7423385515
Applicant's qualifications and experience: PhD in molecular neuroscience Home office PIL course (ABC) Certificate number: 54690 PIL I65781689 Experience in schedule1 procedures and perfusion.
Applicant's professional address (University of Cambridge Department): Wellcome - MRC Stem Cell Institute Jeffrey Cheah Biomedical Centre University of Cambridge Biomedical Campus Puddicombe Way Cambridge CB2 0AW
Name of applicant's supervisor (if applicable): Ragnhildur Thora Karadottir
Department in which the animal(s) are to be kept (if applicable) or address of the Place Other than Licensed Establishment (POLE) where the work will be undertaken: AMB (rat and mouse work), CAF (occasional mouse work if surplus stock animals are there)
Species, number, sex and strain of animal(s): Species; Rat Number: Maximum 40 pregnant females for embryonic dissections and litters used at E14-16, E18-19, or postnatal P0-P2). Sex: Females (+offspring) Strain: CD or SD Species; Mouse Number: Up to 6 pregnant females + litters Sex: Females (+offspring) Strain: C57bl/6, CD-1 These numbers are based on the assumption of 1 rat used for embryonic timepoints and one rat used for post natal timepoints, per week, for 20 weeks of the year.

The source of the animal(s):

Successful cell culture experiments require exact timing of pregnancy. Therefore, rats will be purchased from Charles River that guarantees correct breeding dates. Additionally, mice may be bred in the facility.

Could your work be undertaken using surplus stock animals and if so have you attempted to access surplus stock animals: Yes/ No

If you have answered 'No', please explain why:
For the rats there aren't stock animals kept within the biofacilities close to the JCBC. For the mice there is the possibility of using surplus stock animals, biofacilities (such as CAF and AMB) will be approached when the experiments are due to be carried out.

If you have answered 'Yes', which UBS biofacilities have you approached:

Please give details of accommodation in which the animal(s) will be kept:
Animals will be kept in standard cages with ad libitum access to food and water.

Will your animals be individually or group housed (if applicable)?

Animals may be delivered individually housed or in groups, animals may be individually housed for mating or littering.

If individually, why is this necessary, and how long will your animals be individually housed?

See above, there might not be suitable cagemates available. The likely maximum amount of time for being individually housed would be 12 days (assuming delivery of an untimed mated female rat, generally approx E12-14 and being left to litter down at E21-23). For animals used at embryonic timepoints, the female will generally be used within 1-3 days of delivery.

If applicable, how will your animals be identified?
Animals will be identified by ear clip or by cage cards, most animals will not need to be individually identified.

If it is proposed to use animals which are currently under Project Licence authority, please give details (for example, has anything been done to the animals before they are used for this project):
N/A

**If animals are to be cared for by individuals other than Animal Technicians employed by the University please give details (*e.g. qualifications and*

***experience*):**

N/A

Source, amount and duration of funding for this project. Has your funding been peer reviewed and if so, by whom?

European Research Council, €1 999 729 , until 31/5/23, peer reviewed by the ERC PSAG/182.

A "regulated procedure" is defined as any experimental or other scientific procedure applied to a protected animal that may have the effect of causing that animal pain, suffering, distress or lasting harm.

"Pain, suffering, distress and lasting harm" encompass any material disturbance to normal health (defined as the physical, mental and social well-being of the Animal). They include disease, injury and physiological or psychological discomfort, whether immediately (such as at the time of an injection) or in the longer term.

Regulated procedures may be acts of commission (such as dosing or sampling) or of deliberate omission (such as withholding food or water).

Why do you believe this research project does not require Project Licence authority under the Animals (Scientific Procedures) Act 1986. Have discussions with the Home Office inspector taken place (if so, please give details):

Wild type rats and mice will be used and sch1 culled for tissues. No regulated procedures will be undertaken.

Please provide the scientific background and context for this request.

Myelin is essential for normal brain function, as it provides fast signal transmission, promotes synchronisation of neuronal signals and helps to maintain neuronal function. Alterations in myelination are increasingly being implicated as a mechanism for sensory-motor learning. The importance of myelin becomes evident in diseases, such as multiple sclerosis (MS), where myelin damage causes cognitive and motor disability. Moreover, recent studies have highlighted the contribution of myelin to many diseases that were previously considered to be 'neuronal', such as dementia, schizophrenia, autism and bipolar disorder. Despite the profound importance of myelin, there are serious deficits in our understanding of how myelination is regulated and to what extent myelin is plastic; which are impediments for understanding both the functional connectivity of the central nervous system (CNS) and white matter disease. We, and others, have shown that myelination can be regulated by neuronal activity, thus we hypothesize that neuronal activity is a driver of myelin plasticity, similar to synaptic plasticity.

Myelination is a complex process that can be modelled by ex vivo primary culture of cells from rodents. Tissue requirements are based on average litter sizes and the need for primary cultures weekly as cell lines are not suitable for generating myelinating co-cultures. Large numbers of cultures are required to test multiple factors. In addition since the development of electrical properties, synapses and myelination in cultures is variable larger n numbers are required in order to give sufficient statistical power to address our research questions. Similarly each litter can only count as an n=1 despite yielding more cells than may be necessary for a single experiment, as the whole litter needs to be processed in one experiment, thus not meeting the requirements for technical replicates. Cultures may therefore be shared between projects to minimise overall animal use, and experiments with different test substances or stimulation patterns and controls will run in parallel, to give an n=1 for multiple different experiments at once. This work will be conducted by several researchers working under the same grant, though with different research aims and tissues/cells will be shared between researchers. We have shared tissues/cultures with Sarah Crisp (who has submitted her own non reg form), and there is the possibility of sharing blood (from the unused mothers) with Emma Rawlins group (as we have done for our transgenic rats). We have previously approached Fiona Love to try and share tissues but unfortunately this was not possible due to the numbers of cells required by each group.

What are the scientific objectives or questions this request aims to address?

We aim to determine mechanisms that mediate neuronal activity dependent myelination, and identify to what extent myelin is plastic.

What scientific outputs do you expect, who will benefit and why?

The outcome of this proposal will break new ground in our understanding of myelin plasticity, and has the potential to provide novel therapeutic strategies for myelin regeneration in white matter diseases such as MS.

If this is a repeat request, please give a summary of what you have achieved under the previous application

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2022 achievements

In 2022 I established two primary cell culture models: 1. Myelinating co-culture system where neurons can be stimulated using optogenetics (channelrhodopsin 2) 2. Dorsal root ganglion cell culture system that efficiently separates cell bodies and axons. Additionally, I am providing oligodendrocyte precursor cells that I don't need

to a collaboration partner in the engineering department (Shery Huang). They establish a myelination assay based on measuring the impedance of conductive polysterene fibres that are myelinated by oligodendrocytes.

1. Myelinating co-culture with stimulation

I improved the Chr2+ rates in DRG cultures to >90% by switching from electroporation transfection to a lentivirus system (Fig. 1). Finally, I optimized stimulation conditions, staining, imaging and analysis parameters. Preliminary results show an increase of myelinating oligodendrocytes in cultures that were stimulated at 1 Hz (Fig. 1). However, this needs further replication and stimulation at different frequencies.

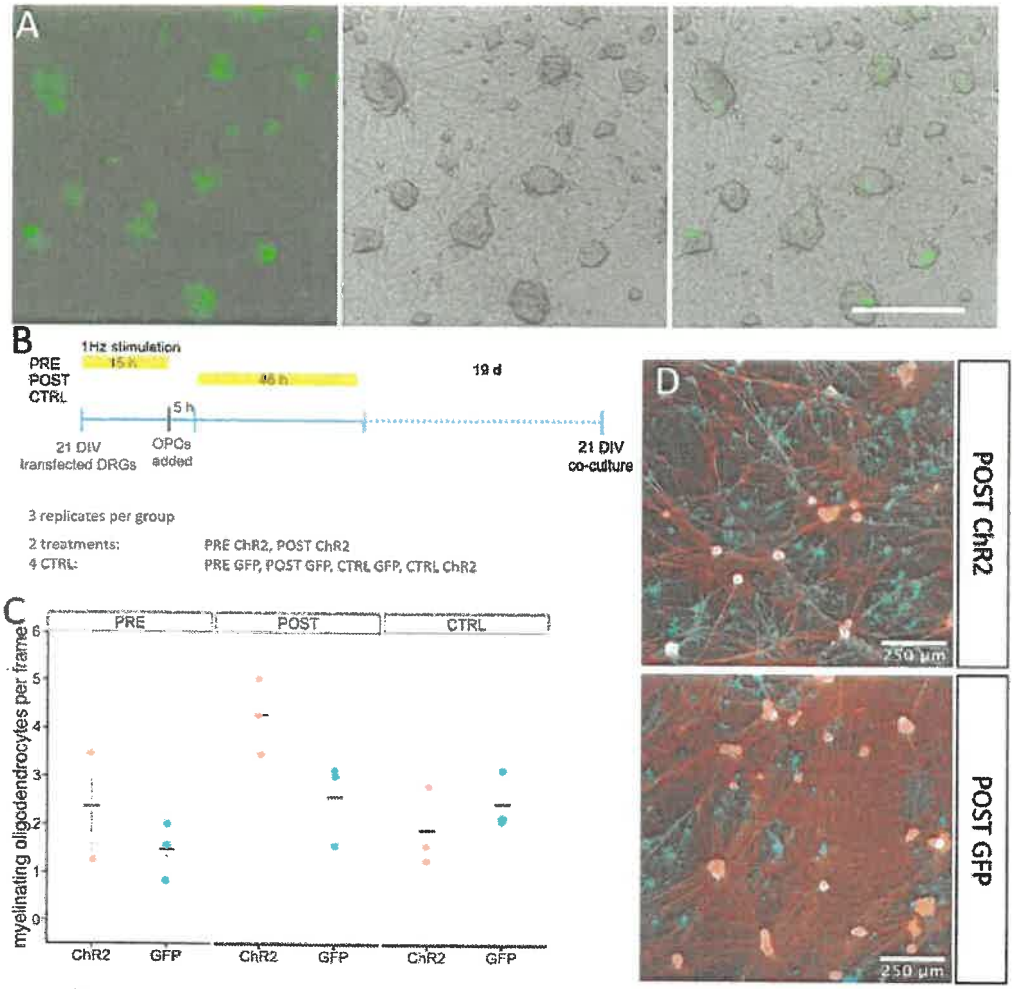

Figure 1 Primary myelinating co-culture. A Lentivirus infected DRG cells (ChR2-YFP, phase contrast, merge). B culture and stimulation protocol C preliminary results D representative images of co-cultures (red = neurofilament, cyan = MBP)

2. DRG system for proteomics

Obtaining sufficient protein material from axons without cell body contamination to perform quantitative mass spectroscopy is challenging. I tried campenot chambers and

microfluidic devices. Finally, I achieved the highest protein amounts (~100 ug) using a hanging filter insert system (Fig. 2). I am currently stimulating similar cultures and isolate protein to send it for mass spectroscopy.

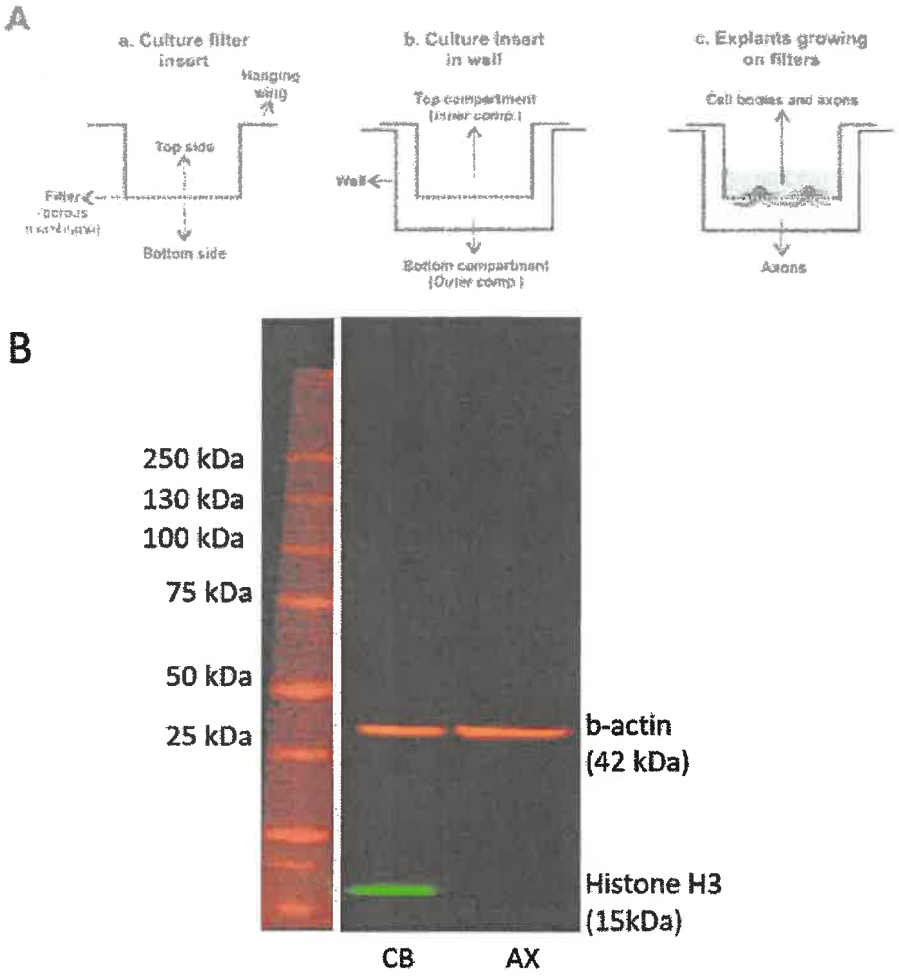

Figure 2 Cell-culture system to gain high amounts of pure axonal proteins A cell culture filter insert system B Western blot for actin and Histone H3 (nuclear protein) to assess purity of protein lysate. CB = cell body, AX= axon

While I successfully established my model systems, I am now in the application and data acquisition phase. Therefore, I will need additional embryonal tissue in 2023.

Killing a protected animal for a scientific purpose at a designated establishment does not require a licence if a method listed in Schedule 1 is used. However, if an alternative method is used, the killing is a regulated procedure and requires personal and project licence authority.
Please describe precisely the Schedule 1 method you intend to use and the secondary method which must be taken to ensure the animal is dead. Guidance on the operation of ASPA (Page 125-126)

For embryos, the mother will be culled by CO2 exposure (with cervical dislocation as the secondary method), then E14-E19 pups will be removed from the uteri and decapitated, exsanguination follows as a result and can be used as the secondary method.
For P0-P2 rat pups, they will be culled via cervical dislocation with exsanguination (by decapitation) as a secondary method.

Please note that animals not subject to the Animals (Scientific Procedures) Act 1986, may be subject to the Protection of Animals Act 1911 and Animal Welfare Act 2006. You may be required to produce relevant authorities and or licences.

Expected duration of the project (Please note for projects or requests for materials that extend beyond 12 months a new application will be required every 12 months).
In relation to the whole project or sourcing request, please provide the planned start and end date.
Start date: 01/01/23

End date: 31/12/2023

If not humanely killed, what will happen to the animals at the termination of the project?(Any animals housed at a University facility being rehomed or released into the wild must first be examined by an NVS to ensure they are in good health).

Through the animal ordering process, the AMB NACWO will always be informed about expected animals that are not further needed (females that littered down to get P0-2 pups for mixed glia culture, 50% of rats). These rats and mice can be used further for wildtype breeding or tissue collection. In the past, we used rats for breeding and offered them to another research group for blood collection. Additionally, those rats or mice can be used for S1 training for AMB technicians and other rat users. Rats or mice will be culled either by dislocation of neck then exsanguination or CO2 and then neck dislocation.

Every possibility should be taken to share excess animals and tissues. If animals or tissues will be obtainable for others to use, please indicate below and explain what will available.

N/A

Please note that if while working with animals you notice any material change in any of your animals or any unexpected adverse effects that are more than minor and transient you must contact a UBS NACWO, NVS and the Director for Governance and Welfare immediately.

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Signature of Applicant: _____ Date: 20/12/2022

Print Name: Sebastian Timmler

Signature of NACWO: _____ Date: 16/1/23

Print Name: Martin Rice

Signature of NVS: _____ Date: 13/01/2023

Print Name: MICHAEL PLANCHE

Signature of Chairperson: _____ Date: _____
of the UBS AWERB Committee

Print Name: _____

Please return this completed form to the Named Animal Care and Welfare Officer (NACWO) for your Department who will progress the application through the AWERB Process.

Work can begin when this form is signed and agreed by the NVS and NACWO