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: Julia Monika Becker
Applicant's e-mail: jb942@cam.ac.uk
Applicant's mobile number: 00447871826795 or 00441716588800
Applicant's qualifications and experience: Qualified veterinary surgeon - MRCVS, PIL holder, trained in the required schedule 1 methods
Applicant's professional address (University of Cambridge Department): Franze lab, Anatomy Building, Department of Physiology, Development and Neuroscience, University of Cambridge, CB2 3DY
Name of applicant's supervisor (if applicable): Prof Kristian Franze
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: CAF
Species, number, sex and strain of animal(s): Rat, up to 100 animals, both sexes, predominantly Lister Hooded and Sprague Dawley, but possibly also other strains
The source of the animal(s): Envigo and Charles River. Also surplus animals from other groups with the university.
Could your work be undertaken using surplus stock animals and if so have you attempted to access surplus stock animals: Yes **If you have answered 'No', please explain why:** **If you have answered 'Yes', which UBS biofacilities have you approached:** I regularly approach Anatomy and Physiology to enquire if any surplus animals are available and have a record of using those in the past. I have also used in the past and will use in the future the UBS enquiry email list to find animals across all sites within the University.
Please give details of accommodation in which the animal(s) will be kept:

Conventional or IVC caging

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

If individually, why is this necessary, and how long will your animals be individually housed?
Might be necessary for short periods (a few days) when animals are taken one by one and the last animal remains in the cage alone at the end of the experiment. Might also be necessary for surplus male exbreeder rats if those cannot be socially housed or no suitable partner for group housing is available.

If applicable, how will your animals be identified?
Numbers painted on the tails if required.

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 unless such animal might become available as surplus animals. If this were the case, I can't give details here because I don't know yet which animals might become available.

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: *Consolidator Award (772426)*, 06/2018 – 05/2023 ***The integration of mechanical and chemical signals in neuronal guidance*** (Franze, PI) Amount: € 2,468,520

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):

These rats will have had no procedures during life and will be killed by Schedule 1. The spinal cord and potentially other organs will be dissected out and used for AFM or will be processed for histological analyses.

No discussions with the HO inspector have taken place.

Please provide the scientific background and context for this request.
Injuries or diseases of the spinal cord constitute devastating conditions that may lead to loss of limb movement, sensation and bladder control. Despite intense research, treatment is still very limited. Most research to date has focused on biochemical signalling. However, some more recent studies have hinted that the mechanical environment plays an important role in the development of the nervous system. In vitro, neurons display distinct growth behaviour depending on their mechanical environment. Furthermore, it has been shown that the mechanical properties of damaged or diseased spinal cord differ from healthy tissue which might influence regeneration attempts. Improving our understanding of the mechanical properties of healthy and diseased spinal cord tissue will open new avenues to improve recovery after damage to this delicate organ.

What are the scientific objectives or questions this request aims to address?
Using atomic force microscopy (AFM), a cutting-edge technique which allows us to very precisely measure stiffness maps of biological tissues, we will investigate the stiffness of spinal cord tissue.
I will more closely investigate the stiffness of spinal cord tissue in various anatomical planes, the effect of age on spinal cord stiffness and how different measurement techniques influence the readout. This will help resolve contradictions in the literature and allow us to gain a better and more global understanding of spinal cord mechanics. I might also use/optimise histological methods.
To help reduce animal numbers and in line with the 3 Rs, I might give tissue and organs which I do not require to other users after the animal has been killed.

What scientific outputs do you expect, who will benefit and why?
A better understanding on the effect of age and measurement techniques on spinal cord tissue. This will benefit the entire biomechanical community as there's ongoing debate about these topics in the field.
We have optimized our technique and setup so that the quality of the data we can produce exceeds what has previously been published, thus we can provide more in-depth data than is currently available.
Overall, this might aid other scientists who develop computational models of the spinal cord as we can provide more systematic and in-depth data. Eventually, it will help patients with spinal cord injury as the field makes advances and discovers new avenues for the treatment of the damaged spinal cord.

If this is a repeat request, please give a summary of what you have achieved under the previous application
I investigated how the force and speed of an AFM measurement affected the measured stiffness of healthy grey and white matter in the rat. I found that even though increasing these parameters generally led to an increase in the measured stiffness, grey and white matter were affected to a different degree. This led to a decrease in the ratio of grey to white matter stiffness. For very low forces and speeds relevant for cellular mechanosensing, I found grey matter to be stiffer than

white matter in all three anatomical planes. Next, I assessed how spinal cord tissue stiffness measured with such low forces and speeds changes with the age of the rats. There were no significant differences between the sexes, albeit between Lister Hooded and Sprague Dawley rats. While grey matter stiffness was relatively stable throughout life, white matter stiffness sharply decreased in the first four weeks of life, before remaining constant until old age. The decrease of white matter stiffness correlated well with an increase in the amount of white matter, which suggests that myelination might play a key role in white matter softening.

Further experiments are necessary to fill gaps in the study population for the age dataset and to assess how age-related stiffness changes affect the horizontal and the sagittal plane (so far on the transverse plane has been studied).
Furthermore, I want to examine how the temperature of the tissue affects the measured stiffness, as various temperatures are used in the literature which might contribute to the differences in the findings.

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)

Inhalation anaesthesia with isoflurane followed by intraabdominal injection of pentobarbitone, confirmation of death by exsanguination.

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: ASAP

End date: 30.06.2023

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).

I will use all animals as animals are bought in small batches. Animals will be killed by Schedule 1 method.

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.

All tissues other than spinal cord will be available for other users after the dissection is finished. No deviation from the experimental protocol (method of euthanasia, post-mortem perfusion with ice-cold slicing cerebrospinal fluid) is possible, though, so other users would need to assess if this is suitable for their needs. Please do pass my details on to researchers who are potentially interested.

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.

Signature of Applicant: Julia Becker Date: 02/02/2023

Print Name: Julia Becker

Signature of NACWO: Gemini Chu Date: 02/02/2023

Print Name: Gemini Chu

Signature of NVS: S. Kimpton Date: 17/01/2023

Print Name: Sophie Kimpton

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