UNIVERSITY OF CAMBRIDGE
University Biomedical Services

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NR 2023/50

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 (under my name)

Name of applicant: Ilkem Sevgili

Applicant's e-mail: is487@cam.ac.uk

Applicant's mobile number: 07523648259

Applicant's qualifications and experience:
4th year- PhD Student in Clinical Neurosciences, University of Cambridge; Master of Research (MRes) in Bioengineering, Imperial College London, Bachelor of Science (BSc) in Molecular Biology and Genetics, Gebze Technical University, TR

Applicant's professional address (University of Cambridge Department):
Clifford Allbutt Building (6 th floor), Department of Clinical Neurosciences, Hills Road, Cambridge Biomedical Campus, Cambridge, CB2 0AH

Name of applicant's supervisor (if applicable):

Prof Manohar Bance

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:

Anne McLaren Building (AMB)

Species, number, sex and strain of animal(s):

Pregnant Sprague-Dawley (S-D) female rat order for the purpose of pup collection, at least one pregnant female per month and the maximum of 2 per month until the project end date of end of June 2024. All the pups from the pregnant rat will be used.

The source of the animal(s):

Charles River labs

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:
We require rat litters supplied on a regular basis at a specific developmental point (P0-P9) for primary cell culture purposes, so we will be ordering time-mated pregnant female Sprague-Dawley (S-D) rats. If there are unneeded S-D pups of the right age available, we may consider using them as well.

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

Please give details of accommodation in which the animal(s) will be kept:

Rat cages provided by AMB

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

Pregnant S-D female rats may be housed individually or up to 3 per cage.

If individually, why is this necessary, and how long will your animals be individually housed?
A pregnant female may be kept individually until she litters (1 week on average)

If applicable, how will your animals be identified?

No identification needed

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?

Bance's group grant code:
RRAG/188
William Demant Foundation, peer-reviewed
Ends 30/06/2024 (£20K)

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 animals will have had no procedures conducted during life and will be killed by Schedule1.

Please provide the scientific background and context for this request.

We have 2 main projects that requ ire animal use. 1. Bance group who will collect cochlear spiral ganglion cells (SGNs) from the cochlea for the purposes of studying SGN firing parameters, developing SGNs from human pluripotent stem cells (hiPSCs), building a cochlea-on-a-chip, and investigating mechanisms of hearing loss in vitro and in vivo. Bance group will also collect dorsal root ganglion cells from the spinal cord to study the electrophysiological features of these neurons. 2. Brains will be extracted and primary brain cells will be cultured from these P0-3 rat pups. This developmental time window is optimal for primary cell culture. Brain cells of interest for our group constitute the central and peripheral astroglia. They will also be used for a number of projects in collaboration with Dr Kotter's group, which include generation of human astrocytes and microglia, and study of demyelinating disorders - see below for details. Briefly, the first project sets out to investigate how different regional astroglia affect the maturation and electrophysiological profile of iPSC induced neurons in the coculture.

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

Cochlear spiral ganglion cells - used by Yi-Lin Yu and Ilkem Sevgili Dorsal root ganglion cells (DRGs)- used by Dr Paul Charlesworth Brain tissue - used by Yi-Lin Yu 1. For the cochlear projects, Prof Bance's group are aiming to explore the possibility of generating implantable devices to prevent or restore hearing loss, and an in vitro cochlea-on-chip model to serve as a key platform for evaluating both current and novel processing strategies to improve patient outcomes, gaining a better understanding of how cochlear implants interact with SGNs. Main questions: 1. Is it possible to recover hearing loss by establishing synapses between SGNs and glutamatergic induced neurones in implantable cochlear devices, in vitro and, ultimately, in vivo? 2. What are the similarities and differences between rat SGNs and hiPSCderived SGNs and DRGs? 3. What are the electrical and chemical stimulation parameters that can induce SGN and DRG firing in vitro? 4. How can we develop a cochlea-on-a-chip model to study SCH behaviour in vitro? 5. Can we regenerate degenerated SGNs?

There are 4 main projects that will benefit from the brain primary cells. 1. Astroglial co-culture - used primarily for the project led by Dr Yu to study regional differences in astroglial co-cultures with iPSC-induced neurons Main questions: a. What are the differences of electrophysiology between iPSC-induced neurons co-cultured with

regional astroglia in the rat nervous system? b. How regional astroglia affect the maturation profile of iPSC-induced neurons in the co-culture? c. How regional astroglia affect the neurite outgrowth of iPSC-induced neurons in the co-culture?

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

For the cochlear SGN projects, Prof Bance's group are aiming to explore the possibility of generating implantable devices to prevent or restore hearing loss, and an in vitro cochlea-on-chip model to serve as a key platform for evaluating both current and novel processing strategies to improve patient outcomes, gaining a better understanding of how cochlear implants interact with SGNs.
For the astroglial co-culture project, we expect to understand the differences of maturation and electrophsyiological profile in iPSC-induced neurons co-cultured with regional astroglia to test a novel therapeutic intervention with a translational ootential.

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

Previously, the dissection and culture of SGNs are optimized. Some electrophysiological recordings are made from SGNs using patch-clamp. These cells are used in calcium imaging and cocultures studies were carried out. Although we optimized our techniques using these cells, now we need to acquire data and make characterise the electrophysiological properties of SGNs.

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)

Pups: Overdose of an anaesthetic agent (Pentabarbitone) followed by death confirmation by the exsanguination.

Adult rats: Anaesthetic injection or exposure to CO2 gas (no tissue will be used from adult animals, other methods can also be used); followed by exsanguination to confirm death. The mother rat will be killed after all the pups have been collected (not immediately after birth but after pup collection), unless requested by other groups.

The adult rat may be culled straight after the pup collection; adult rat tissues of interest may be shared with other users. The Schedule 1 procedures named here will be carried out by the AMB staff experienced in these techniques.

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: 01/11/2023

End date: 30/06/2024

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).

N/A

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.

After Bance group dissects the cochlea and spinal cord, the rest of the tissue can be shared with any group that is interested in the remaining tissues.

We will email the 3Rs list to offer the tissues if they are not used by Bance group (ubs-3rs-enquiry@lists.cam.ac.uk)

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: I. Sevgili Date: 11/10/2023

Print Name: Ilkem Sevgili

Signature of NACWO: J. Soffe Date: 16.10.23

Print Name: Julie Soffe

Signature of NVS: A Phillips MRCVS Date: 16/10/23

Print Name: Amelia Phillips MRCVS

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