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

Name of applicant: Sarah Crisp
Applicant's e-mail:sjc85@cam.ac.uk
Applicant's mobile number: 07718656655
Applicant's qualifications and experience: Current PIL (ABC) from April 2021. Previous personal license holder (held at UCL 2012- April 2019). Experience in performing schedule 1 procedures involving rats and mice (mainly embryos) since 2011.
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
Species, number, sex and strain of animal(s): Species; Rat Number: Maximum 96 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 20 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 48 weeks of the year.
The source of the animal(s): Animals will most likely be purchased from suppliers Charles River or Envigo, or if there is sufficient demand, bred within the facility
Could your work be undertaken using surplus stock animals and if so have you attempted to access surplus stock animals: 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 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)?
Where there might not be suitable cagemates available animals will be house individually, where possible they will be grouped housed.

If individually, why is this necessary, and how long will your animals be individually housed?
Where there might not be suitable cagemates available animals will be house individually. 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 1-5 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?

PSAG/182 – Thora Karadottir's peer-reviewed ERC grant (£1.8M, end date 30/11/23)

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.

The purpose of this project is to investigate disease mechanisms in neurological conditions associated with autoantibodies or thought to be autoimmune. These diseases include, but are not limited to autoimmune encephalitides, some acquired peripheral neuropathies, stiff person syndrome and some acquired demyelinating disorders.
Auotimmune neurological disorders can be modelled by ex vivo primary culture of cells from rodents. Typical readouts of the effects of autoantibodies on neuronal and non-neuronal cells of the nervous system include, but are not limited to, changes in protein localization and electrophysiological properties. Tissue requirements are based on average litter sizes and the need for primary cultures weekly as cell lines are not suitable for generating mature neuronal cultures or myelinating co-cultures. Large numbers of cultures are required to test samples from different patients with a range of neurological disorders. 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.

What are the scientific objectives or questions this request aims to address?
We aim to determine mechanisms that mediate neurological diseases associated with autoantibodies.

What scientific outputs do you expect, who will benefit and why?
While many studies report associations between autoantibodies and neurological disorders very few address whether and how autoantibodies disrupt neuronal and circuit function. These insights will directly inform treatment decisions in patients. Some of the studies aim to identify unknown antigenic targets of antibodies which could lead to the development of clinically useful assays for new diseases.

If this is a repeat request, please give a summary of what you have achieved under the previous application
We have almost completed our work to study the mechanisms of one particular

autoantibody (anti-DPPX) which is associated with the severe neurological syndrome in humans. Up to 10 pregnant rats are required now to complete this work. We have shown that the antibodies cause disease by disrupting a potassium current in neurons. The final steps of the work examine the consequence of loss of this current for action potential firing.
In a second line of investigation we have been setting up a novel method to identify antigenic targets of human autoantibodies targeting neurons. While progress has been made to optimise the technique we anticipate that further optimisation is required – we have succeeded in identifying targets but are working to reduce the background noise before applying the new method to identify antigenic targets in patients with antibodies to unknown targets.

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 (rat or mouse), the mother will be culled by CO2 exposure, death will be confirmed by cervical dislocation, then E14-E19 pups will be removed from the uteri and decapitated.
For P0-P2 rat pups, they will be culled via cervical dislocation with exsanguination (by decapitation) as a secondary method. I do not use post natal mouse pups.

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: 1/7/20

End date: 3/9/23

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).

All will be culled.

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.

I usually use all of the embryos when culturing from E15, and 6-7 from E18. I would be prepared to offer the remaining E18 embryos when available (though when I have

offered these previously there has been no interest). The maternal tissues could also be used – we have arranged this previously when approached by other users. I can post an email reminder on the 3Rs list (ubs-3rs-enquiry-request@lists.cam.ac.uk) that we sometimes have these tissues available to make arrangements with those who might have a use for them.

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: _____

Date: 24/10/22__

Print Name: Sarah Crisp

Signature of NACWO: _____ Date: 20.12.22

Print Name: Julie Gaide

Signature of NVS: _____ Date: 19/12/2022

Print Name: Michael Ramage

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