Home Office

NON-TECHNICAL SUMMARY

Proteotoxicity in development and ageing

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Proteotoxicity, Development, Ageing, Neurodegeneration, Therapy

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant, aged

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The aim of this project is to understand how proteotoxicity (pathology that develops due to damaged proteins) alters development and ageing.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Ultimately, this project will lead to the identification of potential treatment targets and therapies for proteotoxicity induced disorders such as neurodegeneration (disease caused by loss of function of brain cells or loss of brain cells). There is no cure for neurodegenerative disorders and thus, finding a new common target is at utmost importance.

What outputs do you think you will see at the end of this project?

A successful outcome would be the identification of potential treatment targets and therapies for neurodegeneration via targeting proteotoxicity. The dissemination of the results will consist of presentations at international conferences and publishing important discoveries in high impact publications. Additionally our lab will engage in communication and outreach activities for general public in science fairs and by visiting schools.

Who or what will benefit from these outputs, and how?

It has been shown that proteotoxicity caused by protein misfolding and aggregation is connected with various types of health problems, including neurodegeneration, cancer (condition where cells in a specific part of the body grow and reproduce uncontrollably), muscle degeneration, cardiovascular disease (conditions affecting the heart or blood vessels), and autoimmune disorders (condition arising from an abnormal immune response to a functioning body part). Therefore, it is important to characterise the underlying mechanisms of proteotoxicity that contribute to these diseases and to identify potential targets for their prevention and treatment.

One proteotoxicity induced disease is neurodegeneration. Neurodegenerative diseases are incurable and result in progressive loss of function or death of brain cells called neurons. Neurodegenerative diseases include Alzheimer's disease and other dementias, Parkinson's disease, Huntington's disease, motor neurone disease, Creutzfeldt-Jakob disease and multiple sclerosis. Dementia is a group of related symptoms associated with an ongoing decline of brain functioning that results in impaired ability to remember, think, or make decisions that interferes with doing everyday activities.

The dementias are responsible for the greatest burden of disease, with Alzheimer's disease representing over 60% to 70% of cases. Neurodegenerative diseases are strongly linked with age, and the UK and other European countries have an increasingly ageing population. Currently 16% of the European population is over 65, with this figure expected to reach 25% by 2030.

Dementias are the biggest health challenge of our generation; one in three people born today will develop the disease. With one million people in the UK predicted to have dementia by 2025 and the current cost of £26 billion a year to the UK economy, there is a huge financial and societal impact, yet we are still without treatment.

Short term benefits: if we successfully answer each of the research questions we will be able to understand how proteotoxicity alters development and ageing.

Long term benefits: ultimately, such testing steps will lead to the identification of potential drug targets and therapies for proteotoxicity induced diseases such as neurodegeneration via targeting proteotoxicity. A successful therapeutic strategy for the treatment of neurodegeneration would be at utmost importance for patients, enhancing both lifespan and quality of life. Apart from the scientific and societal impact, the economic value of effective neuroprotective therapies for neurodegeneration would be significant.

How will you look to maximise the outputs of this work?

We will provide open access to our publications, and we will have responsible management of research data in line with the FAIR principles. All information about results, tools, and codes required to validate the obtained conclusions will be completely available through public repositories such as GEO (https://www.ncbi.nlm.nih.gov/gds/), Github (https://github.com) and Mendeley Data (https://data.mendeley.com) in order to ensure the reproducibility and transparency of our findings. Also, in line with the "early and open sharing" philosophy, the primary research manuscripts generated during the proposed project will be available from bioRxiv pre-print server (http://www.biorxiv.org/) as soon as we submit it to a peer-reviewed journal, which preferentially will be an open peer-reviewed journal, also to increase the transparency and reproducibility of generated outputs. Finally, we will implement a thorough process to ensure the robustness of the obtained results and conclusions by including all relevant negative results in our publications and performing robust and unbiased statistical analysis. The data will be disseminated by presentations at national and international scientific meetings and seminars.

Species and numbers of animals expected to be used

- Mice: 14000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

This project uses mouse as an experimental animal to study the role of proteotoxicity in development and ageing. Unfortunately, in-vitro studies (method to study the behaviour of animal cells in controlled environment that is outside of their normal biological context) cannot be used to understand how

genetic modifications result in normal or abnormal physiological processes (the way in which a living organism or bodily part functions). We have created new mouse lines with specific mutations in genes that disrupt one of the stress response pathway that is called proteotoxicity. Preliminary data shows, that one of the mouse line with impaired proteotoxic stress response developed a movement disorder. The mice were otherwise healthy and without any body weight loss. As proteotoxicity is connected with various types of health problems, our aim is to understand how impairing proteotoxic stress pathway alters development and ageing. Therefore our aim is to phenotype (describe the set of observable characteristics or traits of an organism) these new genetically altered mice throughout their lifespan, from embryonic stage to adulthood to the age of 24 months.

Typically, what will be done to an animal used in your project?

Some animals are expected to develop movement disorder (e.g. tremor with impaired posture and balance). Movement disorder will be permanent, although it is not expected to be fatal and animals will be killed before any symptoms become life-threatening. In summary, mice will be aged up to the age of 24 months. Some animals are expected to develop dementia that affects the brain and causes problems with memory, thinking, and behaviour.

Typically mice will go through behaviour testing. For example, cognitive abilities will be tested using object recognition test, and motor behaviour will be evaluated using rotarod. Additionally, mice can be imaged using non-invasive imaging (MRI) and have blood samples taken approximately in every three months, for example at the age of 1 month, 4 month, 7 month etc.

Typically at the end of experiment, mice will be injected of cell labelling agents using standard routes (subcutaneous, intraperitoneal). And thereafter mice will be killed humanely at specific time points and tissues will be collected for RNA and protein analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

The vast majority of animals are not expected to show harmful phenotypes that are more than mild and transient.

Some animals will undergo surgical embryo transfer and they will experience transient post-operative pain and discomfort. Animals undergoing non-surgical embryo transfer will experience mild transient discomfort and no lasting harm.

Animals undergoing surgery for vasectomy will experience short-lived post-operative pain and discomfort.

Some genetically altered animals will develop movement disorder (e.g. partial hind limb paralysis, abnormal gait, involuntary movements, tremor, impaired posture and balance).

Some animals will develop dementia which means that they will have problems with memory, thinking, and behaviour (1) due to a gene mutation that causes damaged proteins to build up in the brain or (2) due to an injection of disease-causing substances like beta amyloid.

Then animals with dementia will be treated with novel neuroprotective therapies using use Adeno-associated virus-mediated gene therapy.

Some genetically altered mice will be maintained for ageing until they reach a maximum of 24 months of age. Animal that live beyond 12 months may experience conditions associated with ageing such as weight loss, loss of appetite, reduced movement, skin and eye problems, coat changes, mallocclusion (teeth are not aligned properly), and spontaneous tumours. Additionally, growth and development abnormalities may be observable for certain strains.

Motor behaviour and cognitive tests will place the mice shortly in an unfamiliar environment.

During non-invasive imaging e.g. MRI under isoflurane anaesthesia will take no longer than 30 minutes. Mice will have no more than eight anaesthetics throughout their lifetime and only one in any 24hr period. In case during MRI with isoflurane anaesthesia mice do not fully recover from the anaesthesia within 24 hours (eating, drinking and return to normal behaviour) they will be killed by a Schedule 1 method.

During blood sample collection from superficial vessels animals will experience mild and transient discomfort.

Animals will experience mild and transient discomfort from intraperitoneal and subcutaneous injection of cell labelling agent.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice:

1) Mild   60%

2) Sub-threshold   20%

3) Moderate   20%

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

In-vitro research (cell culture, brain organoids (three-dimensional tissue cultures that are derived from stem cells), brain slice culture) and computational methods are used in parallel with in vivo studies. Although in-vitro assays alone cannot adequately model the complete array of molecular, cellular, developmental, physiological, and behavioural interactions necessary to fully understand how genetic modifications result in normal or abnormal brain physiology and that can only be studied in whole organisms[1]. Zebrafish has become a model organism in neuropharmacology, although they are not mammals and thus are not as closely related to humans as a mouse is[2]. Moreover, nematode worm, fruit fly or other non-protected animal alternatives do not replicate system level neurological changes that occur during human neurodevelopment, ageing and neurodegeneration[3].

1.   Barré-Sinoussi, F. & Montagutelli, X. Animal models are essential to biological research: issues and perspectives. Future Sci. OA 1, fso.15.63 (2015).

2.   Kalueff, A. V., Stewart, A. M. & Gerlai, R. Zebrafish as an emerging model for studying complex brain disorders. Trends Pharmacol. Sci. 35, 63–75 (2014).

3.   Arora, S. & Ligoxygakis, P. Beyond Host Defense: Deregulation of Drosophila Immunity and Age-Dependent Neurodegeneration. Front. Immunol. 11, 1574 (2020).

Which non-animal alternatives did you consider for use in this project?

1.   We considered to use human stem cells derived organoids to study neurodevelopment, although organoids cannot be used to study motor and cognitive behaviour.

2.   In parallel to animal work we will use primary neurons that can be isolated directly from animal brain tissue. We will use primary cell culture to study the molecular and cellular consequences of impaired proteotoxic stress response. Although, primary neurons model immature (neonatal) neurons and therefore they cannot be used to study neurodegeneration, motor and cognitive behaviour. To create mouse primary neuronal or mixed culture, neonatal mice are needed.

3.   To define therapeutic targets we will use mouse brain slice culture, although for this mouse brains are needed. However, the animals can be spared from pharmacological treatments.

Why were they not suitable?

Non-animal alternatives will be used in parallel to animal experiments, although they alone cannot be used to study neurodevelopment, neurodegeneration, motor and cognitive behaviour, this can only be done in whole animals. Moreover, cells are cultured in an environment that is very different from that in a live animal. Furthermore, cells in culture experience a variable and abnormal oxygen tension, are usually cultured in high concentrations of glucose, growth factors, survival factors and secreted substances derived from cows. Although brain organoids can be used to study neurodevelopment, they are still unable to model complex interaction of multiple cell types.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We will be studying at least 10 different mouse lines with different mutations in proteotoxic stress pathways over 5 years. Mice are needed to generate homozygous cohorts (two of the same mutant alleles are inherited).

Estimation of numbers will be informed by an analysis of published work and preliminary experiments and with assistance from a statistician. We will use power calculations.

Variability between experimental groups will be limited by using closely related mouse strains raised in a controlled environment, free from specific diseases, fed a uniform diet and matched for age and body weight. Imaging and analysis will be performed by blinded and experienced users.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We will regularly consult websites such as Norecopa (https://norecopa.no/) and NC3Rs (https://www.nc3rs.org.uk/) and read the NC3Rs e-newsletter. We will take advice from dedicated technicians within the animal units.

To minimise the number of animals examined to reach significance for any assay, we will minimise variance in phenotypic measures. This is done by using age and gender matched cohorts, born within a window of approximately seven days. Furthermore, genetic background is well known to influence phenotypic measures. Therefore, to minimise variance associated with segregating modifiers, mutations are generated and maintained on a defined and uniform genetic background. Before newly generated mice strains undergo behavioural assessment, analysis will be carried out on the target gene(s) to ensure it is appropriately modified.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Where there is a need to use alternative genetic backgrounds for certain tests, pilot studies may be employed to provide baseline data and experiment feasibility. Where new strains are created; a small colony will be generated on the breeding protocol of this licence initially to assess for lethality and welfare prior to larger cohorts going through the developmental/phenotyping steps of this protocol. Wherever possible, multiple experiments will be performed on the tissues collected from an individual mouse. For example, in addition to the brain, the spleen can be used to isolate T cells for toxicology assays (to understand the harmful effects of the environment), heart can be used to study changes in cardiomyocytes (heart cells), the bone marrow can be flushed and frozen down, multiple tissues can be collected for DNA/RNA/protein extraction for expression analysis and embryos harvested from females for establishing embryonic stem (ES) cell lines.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use mouse as an experimental model because the genome of the mouse has been well characterised. This all allows us to genetically manipulate the genome and thereby study the role of proteotoxicity in brain development and ageing, induced by individual gene mutations.

In order to define the consequences of impaired proteotoxic stress response in mice, animals with specific mutations in the proteotoxic stress response pathway will be used.

Then in order to understand how proteotoxicity alters development and ageing, we will use either neurodegenerative mouse models with gene mutations that cause damaged proteins to build up in the brain or we will induce neurodegeneration by injecting damaged proteins like beta amyloid directly to the brain or body.

To identify potential treatment targets and therapies for neurodegeneration via targeting proteotoxicity, we will use harmless viruses such as Adeno-associated virus-mediated gene therapy which enables us to overexpress individual genes in the brain.

To assess motor behaviour, we will perform tests such as Catwalk, Rotarod, and Grip strength and to assess cognitive behaviour, we will perform Barnes maze test and novel object recognition test.

Additionally to define which brain region is affected, we will perform in vivo MRI analysis. Additionally we will collect blood to test biomarkers and we will inject cell labelling agents before culling mice. Blood collection and dosing will be done using the minimum possible volumes and frequencies.

Similar studies investigating the role of proteotoxicity in brain development and ageing cannot be performed in humans or in other non-animal models.

Why can't you use animals that are less sentient?

There is detailed knowledge and comparison of mouse physiology and genetics which allows to compare mouse physiology to human physiology. Moreover our aim is to define the consequences of impaired proteotoxic stress response to motor and cognitive behaviour. This cannot be done using lower vertebrates such as zebrafish because they are not mammals and thus are not as closely related to humans as a mouse is. Thus, we will use mouse brain to model proteotoxicity of human brain. The mouse represents the lowest level of sentience to investigate the role of proteotoxicity in development and ageing in mammalian brain.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The procedures carried out in this protocol will be refined to a high extent, prioritising the welfare of the animal.

We will ensure that the animals are regularly monitored by experienced workers so as not to exceed pre-determined endpoints that might increase the amount of distress caused.

Animals are housed according to the best recommendations and enrichment, and nesting material will be added to cages and where possible mice will not be singly housed. We will ensure there is acclimatisation to handling and procedures and optimal handling and interaction with the animals to maximise their welfare.

Mice will be monitored using a scoring system. Mice will be monitored weekly, although when needed e.g. after detecting weight loss, mice will be monitored daily.

Mice injected with harmless viruses or disease-causing substances will be monitored twice daily for 5 days and thereafter once daily for 7 days.

When needed, there will be intervention to prevent further worsening of body conditioning such as providing food on the cage floor, including mash and gel packs. Mice cages will be required to display health observation cards to notify technicians of scoring to ensure counter measures remain in place. Where weight loss is not improved by these measures, mice will be culled. In all cases, weight loss is expected to be gradual.

All procedures will be continually evaluated, reviewed and refined to minimise and reduce experimental duration, animal numbers and suffering while maintaining or improving scientific benefits. To facilitate this, we will have regular meetings within the lab after every series of experiments, and we will also have similar discussions with our close collaborators.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will refer to LASA guidance for surgery and aseptic techniques https://www.lasa.co.uk/current_publications/. We will use guidelines (PREPARE) prior to initiating any experimental study to aid in the planning of each stage (https://norecopa.no/PREPARE), and guidelines (ARRIVE) to help in the design, analysis and reporting of all studies (www.arriveguidelines.org).

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly consult websites such as Norecopa (https://norecopa.no/) and NC3Rs (https://www.nc3rs.org.uk/) and read the NC3Rs e-newsletter. We will take advice from NVS, NACWO and technicians within the animal units. Additional support will be given by ATLA (Alternatives to

Laboratory Animals) Journal: https://journals.sagepub.com/home/atla, and the LASA Guidelines: https://www.lasa.co.uk/current_publications/.