



Home Office

NON-TECHNICAL SUMMARY

Understanding the roles of autophagy and related pathways in proteinopathies

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
 - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
 - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

autophagy, neurodegeneration, therapeutics, biomarkers, chaperones

Animal types

Life stages

Mice

adult, juvenile, neonate, pregnant, embryo

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 primary goal of our studies is the development of therapeutics and drugs for neurodegenerative diseases through modulation of cellular pathways important for protein clearance and correct folding.

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?

Proteinopathies are diseases where proteins within cells do not form or function correctly. This includes many of the major neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's disease. Although these diseases are caused by different types of genetic alterations, they show a common feature in neurons (nerve cells) and their supporting cells: proteins clump into large "aggregated" structures which are toxic and result in neuron death.

There is an urgent need for new drugs and therapies to slow, delay and prevent proteinopathy diseases. This project focuses on two areas that regulate proteins in cells. The first is the cellular processes that enable more rapid removal of proteinopathy proteins, particularly "autophagy" and related pathways that involve autophagy machineries. Autophagy literally means self-eating. The second is chaperone biology. Chaperones are a group of proteins that help other proteins to fold correctly so that they do not clump into aggregates.

Several studies have showed that modulating autophagy or chaperones can be beneficial to treat neurodegenerative diseases and help neurons to cope with protein aggregates.

Moreover, it has also been suggested by several research groups that, when autophagy and related pathways or chaperones function less efficiently in a cell, the nervous system is more prone to develop neurodegenerative diseases.

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

This work is expected to provide novel information and increase our knowledge about the cellular pathways that regulate protein control in cells in particular protein removal and proper folding. We will continue the work of our previous licence in identifying pathways regulating these processes and drug treatments, which improve the outcomes in Huntington's, Parkinson's and Alzheimer's disease mice.

Our work will be published in peer-reviewed scientific journals and presented to the global scientific community, providing pioneering information for discussion and review.

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

We are testing approaches and compounds for their therapeutic benefit directly in mouse models of neurodegenerative disease. In the long term, we hope that these studies will be used as the basis for further translational therapeutic trials in human patients. With increased population lifespan, the incidence of people experiencing these devastating disorders will continue to rise. As a result, there is an urgent need for increased understanding of how these diseases occur and progress, alongside the development of novel therapeutic treatments.

Our work has the potential to impact millions of people worldwide both at the social and economic levels.

- The latest figures for dementia alone, showing 55 million people worldwide living with dementia is set to rise to 78 million in 2030 and 139 million in 2050 (Alzheimer's Disease international).
- The current total cost of dementia in the UK is £34.7 billion. This is set to rise sharply to £94.1 billion by 2040. These costs is a sum of costs to the NHS, social care costs and costs of unpaid care (Alzheimer's Society UK).

At the second level, the main benefit from work carried out under this license will be scientific advancement, particularly in the areas of protein clearance and chaperone biology. Overall, we hope to identify new genes and compounds that allow us to better understand and manipulate neurodegeneration. This will benefit and guide future therapeutic studies.

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

Results that are not commercially sensitive will be submitted for publication in peer-reviewed scientific journals and presented at international conferences targeting broad scientific communities, in particular.

Successful compound therapeutics will be taken to the clinic, where possible, and unsuccessful treatments shared with the community to prevent others from adopting these approaches. In the longer term, the results produced across this licence regarding the physiology of protein clearance and chaperone biology will guide future approaches to understanding and treating proteinopathies.

Species and numbers of animals expected to be used

- Mice: 20,000

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.

Our choice of species for this licence is mice. They are the mammalian model of choice for neurodegeneration diseases as genetically modified models exist and have been well characterised, with an increasing pool of mice with engineered genetic changes or containing genes that allow one to monitor specific processes. Mammalian models are required to provide proof-of principle for therapeutics prior to human studies, and this may be provided with either genetic studies or pharmacological approaches.

We will mainly use adult and aged stage animals to study autophagy and related pathways and their involvement in human disease. In parallel to our work using animals, we will also isolate primary cells, including neurons, glial cells (supporting cells in the brain) and fibroblasts (connective tissue cells). For this reason, we will use mouse embryos or new-born mice.

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

Over the period of the license, we may generate new mouse lines using standard protocols for superovulation and embryo implantation into pseudo-pregnant females. We will also mate these mice or the mice we already have in house with our mouse models for neurodegeneration or with autophagy reporter mice that produce a fluorescent version of an autophagy protein. These animals will be bred in-house following standard Home Office GAA protocols and maintained by methods appropriate to their genetic alteration, with limited numbers coming from established facilities outside the UK.

We will administer compounds that modulate pathways important for protein clearance and folding through the least invasive method: intraperitoneal (into the cavity of the peritoneum), subcutaneous (under the skin), intravenous injection, food/drinking water, oral gavage (administration via a small tube fed down the throat into the stomach), intranasal (through the nose). However, occasionally we may need to perform surgery to implant a small plastic pump carrying the drug under the mouse skin or deliver the compound into the mouse brain via cannula (a tube) implantation using a three-dimensional coordinate system to locate the specific brain area (stereotactic surgery). Successful compounds will be further tested for their suitability for translation into patients. This will require blood sampling over a period of time to determine the absorption, distribution, metabolism, and elimination of drugs from the body (pharmacokinetic studies), to validate drug effects at concentrations and dosing regimens relevant to those that can be achieved in humans.

In some cases, we will perform food deprivation to induce autophagy. Animals will be maintained on a food deprivation schedule, which will typically occur over two days, and will receive 1.5 hours free access to food per day or sufficient food to maintain body weight at no less than 85% of its maximum body weight. We will ensure all mice get access to food in the feeding period by adding food inside the cage. Body weight will be monitored before and after food deprivation, as well as after the feeding period.

Our approach will include measurement of the levels of the respective defective protein, microscopic examination of tissues, behavioural analysis and ageing studies. To collect and preserve tissues for microscopic examination and biochemical purposes, we may require a non-recovery procedure, such as perfusion fixation (a widely used method that uses the circulatory system to distribute fixatives throughout the body). Moreover, some of our genetically altered models, such as our autophagy reporter mice that produce a fluorescent version of an autophagy protein, show variable fluorescent protein levels. For this reason, we will may need to perform tail tipping to check for protein levels, as

we have previously found a good correlation with the protein levels in brain. In addition to the microscopy and biochemical studies, we will use behavioural assessments to monitor mouse health and disease progression. The exact set of behavioural tests will be used is dependent on the mouse model being investigated. In each case, a set of behavioural tests will be established that enable the discrimination of the mouse model being studied from the normal animals, whilst causing the least distress to the animals tested.

To further understand their role in autophagy and chaperone modulation in a living organism, we will characterise genetically altered mice for the relevant genes or we will transiently modify gene expression by injecting small interfering RNA molecules or non-pathogenic viral particles both routinely used methods for transiently silencing a gene of interest. These injections will be either done into the tail vein or by stereotactic surgery into the mouse brain.

All mice will be humanely killed at the end of the experimental procedures.

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

New mouse lines will may be generated using standard protocols that are expected to result in no more than transient discomfort and no lasting harm.

Genetically altered animals that are mouse models of neurodegeneration show moderate age-dependent disease signs. 50% of mice born will not have the disease gene and are not expected to exhibit any harmful phenotype (characteristics). The remaining 50% carrying the disease gene are expected to have progressive neurodegeneration-related adverse effects, including subdued behaviour patterns even when provoked, hunching and piloerection, tremor, as well as weight loss.

When inducible transgenic systems will be used that allow for the activation of genes in specific cells and tissues at specific times, this will involve the administration of agents to modify transgene expression, an approach that is not expected to result in adverse effects.

When we will administer substances through injections and oral gavage mice (administration via a small tube fed down the throat into the stomach) may experience a mild discomfort, but they are expected to show normal behaviour immediately after the procedure. However, in cases when we have to implant a small plastic pump under the mouse skin or cannula in the mouse brain to deliver drugs this requires surgery and animals will experience short-lived post-operative pain and discomfort. When administering drugs through pumps under the skin we may need to replace them once (if needed) according to their duration, which varies from 1 to 6 weeks. Although, we generally use drugs at a safe concentration where no adverse effects are observed, we will closely monitor and if any adverse effects occur we will modify or stop the doses immediately.

Food deprivation is well tolerated in most cases, but it is expected to result in mild weight loss. The total period will not usually exceed 2 successive days but rarely (on a limited number of instances) may be extended to 4 days. Animals will be given a minimum of 1.5 hours free access to food per day or sufficient food to maintain body weight at no less than 85% of its maximum body weight.

Animals that will undergo surgery procedures are expected to have discomfort, therefore peri- and post-operative pain relief will be provided. Animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Mice are also expected to display weight loss.

For the ageing studies, mice will be allowed to approach the end of their natural lives. Although it is not possible to fully predict the adverse effects due to ageing for all animal strains, we expect that it is more likely to develop abdominal tumours.

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)?

Mouse: Sub-threshold 60%

Mouse: Mild 30%

Mouse: Moderate 10%

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?

There is no single alternative to assessing disease models in the context of a mammalian brain, especially if one wants to have a proof-of-principle model for testing potential therapeutics. Assessing genetic and pharmacological changes in the whole animal, the effects on behaviour and relevant outcomes like muscle strength, tremors and balance (depending on the disease) is crucial in order to allow added relevance to the human condition. As such, our cell culture and alternative model approaches guide all elements of our work that cannot be replaced. This includes both aspects of our aims for scientific advancement and therapeutic approaches.

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

In addition to the wide range of immortalised cell lines we have in the lab, over the period of our last licence, we have extended our use of primary cell culture lines from mice. We routinely culture a broad range of cell lines ranging from mouse embryonic neurons through to embryonic fibroblasts. These allow us to directly address many research questions for functional biology, as well as compound related studies.

We have also recently expanded our cell based assays in neurons derived from human induced pluripotent stem cells (iPSCs), cells that have been derived from somatic cells and that have the

capacity to self-renew and give rise to every other cell type in the body. Also, through collaboration we have access to organoids, self-organized three-dimensional tissue cultures that are derived from human induced pluripotent stem cells (iPSCs).

Why were they not suitable?

Cell based models, while they add support and can help strengthen hypotheses and mechanisms, cannot replace the need for mice, as described above. Cell culture models cannot easily represent long-term behaviour of specific types of neurons through various stages of development. Also, the cell signalling pathways may differ in cultured cells compared to neurons that do not divide. In addition, reversibility of the pathological disease process in an animal could rise from the interaction of different types of neurons and/or with glial cells. Such interactions cannot be fully studied using cell based models.

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?

Based on the studies we are planning to perform, our long-term experience and our current animal usage.

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

We have more than a decade of experience in designing, supervising and performing animal experiments and our experimental design is in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) and PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines. For our studies, we take advantage of the NC3Rs Experimental Design Assistant (EDA) tool.

Moreover, we have a track record of interacting with biostatisticians and we will continue taking their advice on the experimental design and methods of analysis.

We have used power calculations alongside a 15 years of experience when designing experiments. Group sizes to allow sufficient statistical power are dependent on the phenotype we are assessing. We have completed successful studies using sample sizes of approximately 20 animals per treatment group using behavioural approaches to assess disease progression against wild-type or placebo controls. For assessing phenotypes by microscopic examination of tissues smaller groups (generally n=6-10) are required.

We continuously review our studies to assess full results and amend our future plans accordingly.

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

We breed the minimum number of animals to address our objectives, following the Home Office Advice for standard GA mouse breeding protocols.

When testing new potential drugs in mice with limited information available for the long term use of these drugs in mice, we carry out pilot studies on a small group of non-genetically altered mice (starting with the lowest dose possible and then gradually increase dose) and we will carefully monitor for side effects.

We also maximise our use of animals beyond their lifecycle by routinely collecting and storing tissues for later re-use in other experiments as part of histological and biochemical experiments. This reduces the mice used and in cases where we collaborate with other scientists this reduction is maximised.

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.

All our studies are carefully designed having as top priority animal welfare.

In all our models, we have carefully established processes with monitoring to ensure that we minimise their suffering as a result of the disease or the experimental procedures.

We plan to study at least three mouse models for neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington disease. All these models typically over-express mutant proteins found in patients with familiar types of the respective disease and have age-dependent moderate disease signs. They show clear behavioural, biochemical and tissue changes that we can measure and aim to modify. Moreover, we will study several genetically altered mice to understand the function of autophagy and chaperons in mammals. Some of our mice have an inducible transgenic system that allows for the activation of genes in specific cells and tissues at specific times using agents to modify transgene expression.

When food deprivation will be applied, it will be only for short periods, which in most cases has no major adverse effects other than mild weight loss. Also, we use behavioural assessments to monitor mouse health and disease progression. Our assessment includes both non-regulated and regulated tests, most having no noticeable adverse effects.

When administering drugs, we will use the least invasive method and the majority of mice will not undergo surgery.

Any animal exhibiting signs of pain, distress or of significant ill health will be humanely killed.

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

Our project aims in developing therapeutic strategies to treat proteinopathies, the majority of them having a late onset. Accordingly, the neurodegeneration mouse models we study exhibit late-onset symptoms that progress over time, genetically altered animals are initially normal and then develop disease signs with age.

Work completed in the laboratory encompasses cell culture and zebrafish models. Over the last few years, we have substantially increased our efforts using zebrafish. We have been developing new models in the zebrafish that will allow us to use this system either in place of mice, or at least to allow us to refine the experiments that we subsequently carry out in mice. However, there is no single alternative to assessing disease models in the context of a mammalian brain, especially if one wants to have a proof-of-principle model for testing potential therapeutics.

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

All our studies are carefully designed, having animal welfare as top priority. In all our models, we have carefully established processes with monitoring to ensure that we minimise their suffering as a result of the disease.

To rapidly and accurately assess the health status of our animals we monitor weight alongside with Body Condition Scoring to define humane endpoints.

It is the aim of all the researchers in the group to communicate efficiently with the technicians who work with our animals. We realise that good communication with them is invaluable - giving them more information about our models and what we expect in each line allows them to inform us quickly and reliably if there is anything we should be aware of concerning the health of our animals. This effective communication allows us to act quickly to reduce the suffering of our animals. Also, we are always seeking advice from collaborators and colleagues regarding approaches that will refine our work with animals.

We will prioritise drugs where pharmacokinetics, brain concentration and dosing are known for mice. When testing drugs with limited information in the literature for their long-term use in mice, we will carry out pilot studies on a small group of non-genetically altered mice (starting with the lowest dose possible and then gradually increase dose), carefully monitoring for side effects. If adverse effects are identified, we may try to decrease drug dose or we will stop doses immediately.

Successful compounds will be tested for their suitability for translation into patients. This will require blood sampling over a period of time to determine the absorption, distribution, metabolism, and elimination of drugs from the body (pharmacokinetic studies), to validate drug effects at concentrations and dosing regimens relevant to those that can be achieved in humans. Compounds with acceptable

efficacy in mouse models will be subsequently tested if necessary (e.g. for novel compounds) for safety by assessing whether they have a toxic effect to body systems or organs prior to starting human studies.

It is important to note that constitutive increase in the self-eating pathway (autophagy) is generally beneficial to mouse health and increases lifespan (PMID: 29849149). Furthermore, if we use drugs then the alterations of autophagy will often be effectively transiently allowing return to normality when the drug drops below effective concentrations. For both of these reasons and also because the extensive experience we and others in the field have had testing autophagy inducing strategies in mice, we think that deleterious effects on other organ systems are very unlikely to occur as a consequence of enhancing autophagy.

Some of our studies will require surgical procedures. These are performed under general anaesthesia in aseptic conditions by trained staff. Local pain relief is applied and post-surgery monitoring is also carried out in the recovery room to ensure animals return to normal activity before being returned to normal housing. We favour the administration of pain relief in a flavouring, such as Nutella, which mice will find attractive to eat. This refinement reduces the need to handle animals to administer pain relief by injection. Following surgery and re-housing, they are monitored for activity, weight and wound healing for the next 7 days. We have now started using Nutella and peanut butter in mice that undergo surgery to help them maintain their weight.

In the cases that we will perform food deprivation to induce autophagy we will ensure that all mice get access to food in the feeding period by adding food inside the cage.

When we will use new-born mice to isolate primary cells, we will leave behind 1-2 pups (if in excess) to ensure mother is not getting stressed.

We continuously adapt our approaches to refine our animal studies. Some examples are listed below:

- Our Alzheimer's/tauopathy model shows a tendency to be hyperactive, a phenotype which is characterised by them running inside the cage. We noticed that this behaviour was occasionally causing the bedding to become tangled around the leg of the animal causing injury. We therefore changed the type of bedding used for softer material, which has prevented this injury.
- Our Parkinson's model shows hyperactivity when provoked, a phenotype which is characterised by them jumping or even having seizures upon disruption of the cage. We have noticed that this behaviour is improved when working in red light, we are therefore using the red light whenever possible.
- In some of our studies, we deliver drugs via an implanted small plastic pump under the mouse skin. We have observed that soaking these pumps in saline overnight prior to implantation reduces adhesions formed with the skin of the animals and have therefore adopted this as standard practice. Whenever suitable for our studies, we will favour the use of plastic pumps that can be refilled without the need of anaesthesia and therefore minimising animal suffer.
- We also deliver compounds via oral gavage (administration via a small tube fed down the throat into the stomach) - in this process we use flexible tubing for administration. Moreover, we dip the

tip in a sweet solution before starting the procedure to make it more palatable for the mice to swallow.

- We have started performing stereotactic surgeries using a three-dimensional coordinate system that enables us to study transient effects vs the long-term effect of gene modification (e.g. in a transgenic model) in the mouse brain - this may reduce possible adverse effects.

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

For our study design, we follow the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and we take advantage of the NC3Rs Experimental Design Assistant (EDA) tool.

For all our experiments involving surgery, we follow the "Guiding Principles for Preparing for and Undertaking Aseptic Surgery, 2nd Edition April 2017".

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

We are always seeking advice from collaborators, colleagues and the staff at the animal facility regarding approaches that will refine our work with animals and will have a positive impact in the 3Rs.

We also refer to the latest practical guidance from Laboratory Animal Science Association (LASA) that provides recommendations and advances in animal techniques.

We participate in the User's meetings hold regularly by the animal facility and we are members of the 3Rs enquiry list. Therefore, we get informed of any advances in the 3Rs.