



Home Office

NON-TECHNICAL SUMMARY

Using zebrafish to understand the function of genes involved in protein clearance pathways in health and disease

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

Zebrafish, Neurodegeneration, Dementia, Autophagy, protein clearance

Animal types

Zebra fish (*Danio rerio*)

Life stages

Embryo and egg, Neonate, Juvenile, Adult

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?

When nerve cells in the brain die, this causes dementia. This is often caused by too much protein building up in the nerve cells. We want to understand the processes that control how cells get rid of unwanted proteins as this will help us understand the causes of dementia and will help us to discover possible treatments.

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?

There are over 900,000 people in the UK who have dementia and this number is increasing because people are living longer. At present, there are no known treatments that slow down or reduce the loss of nerve cells in the brain (which is the main cause of dementia). Our work will help to understand why proteins build up causing nerve cells to die. We will use this to understand who is at greater risk of getting dementia and also to find possible medicines that can be used to treat the disease. This work will help other scientists understand the causes of dementia so that they can diagnose the disease and also to design (small molecules which are like the active ingredients in medicines) to treat patients.

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

Our work will help to understand why proteins build up causing nerve cells to die. We will generate new scientific information that will allow scientists and doctors to understand who is at greater risk of getting dementia. In this work, we will also find compounds that reduce the number of nerve cells which die in zebrafish models of disease. These compounds can then be tested in other animal models and maybe developed to be used in patients. We will share this knowledge with other scientists and medical professionals in peer-reviewed publications and at seminars and conferences.

Where our work generates large amounts of data that is useful to other scientists, we will put this on websites which they can access for free. We will also make zebrafish with genetic alterations which may be useful to other researchers. We will share these with other research scientists.

To achieve these outputs, we need to take a small amount of tissue from the tail fin of adult fish (a biopsy) to perform genetic identification. This is a mild procedure and is performed under anaesthesia. After taking the biopsy, fish will be singly housed for up to 14 days.

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

In the short-term, our work will generate new data to help us understand why proteins build-up and cause nerve cells to die. We will identify new compounds which improve the disease read-outs that we observe in our zebrafish models. The next step will be for researchers to test whether these compounds work in other animal models and whether they are safe to use in man. In the longer term, researchers in pharmaceutical companies will use this information to develop these, or similar compounds and test whether they can prevent or slow the diseases which cause dementia in patients. These diseases are often referred to as neurodegeneration or neurodegenerative diseases. This term means that it is a disease where nerve cells (neurons) die.

We are particularly interested in how cells of the immune systems (the cells in the body which normally fight disease) may play different roles in neurodegeneration. There is already evidence that sometimes they might make the disease better and sometimes they might make it worse. In the short-term, our work will help scientists understand how immune cells might make neurodegeneration worse or better. In the longer term, this will help doctors know when to treat patients with different medicines so that they can be sure that the treatments are effective and will not accelerate or worsen the disease.

There are many different factors that increase or decrease a person's likelihood of developing dementia. The genetic work that we do will help identify the genetic factors which alter someone's risk of developing these diseases. In the long term, this genetic information will help doctors tailor the correct treatment to the patient.

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

We will publish our work in free-to-view journals and will present our findings at seminars and conferences. We will ensure that our work will reach a wider audience as we will also talk to patient groups and schools and give media interviews.

Where we have tested compounds and they are not successful or if genetic alterations do not cause the expected changes in our disease models, we will share our results with the research community to prevent others from adopting these approaches.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 46,600

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 will use zebrafish. To study neurodegeneration and identify potential treatments, we need to study this in a vertebrate animal with all the cell type that are present in man. We estimate that we will use approximately 46,600 zebrafish over the 5 years of this project (approximately 9,320 per year).

Most of the animals used in this project will be embryos or larvae (juveniles). We use this stage of animals as this allows us to perform protocols of lower severity than at older stages. It also allows us to use microscopes to visualise disease changes without the need to perform invasive procedures.

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

Most of the procedures carried out in this work are unlikely to cause any pain or suffering (these are termed “mild” procedures). There are some procedures which involve the use of genetically altered strains of fish which develop the early stages of disease (termed “moderate” procedures). The experiments on these fish are typically performed on unlicensed stages (i.e. within the first 5 days after fertilisation) but there will be some occasions where we need to observe them for longer. The second type of moderate procedure is used to investigate whether drugs (compounds which are like medicines) or genetic alterations affect the progression of the disease. In some cases, the drug or genetic alteration might make the disease worse. Drugs are typically administered by adding them to the water in which the fish live. Any animals showing a worsening of the disease will be culled by a humane method. For drug treatments, we find out the amount of drug that it is safe to give without causing harmful effects using unlicensed stages. Occasionally, when treating older stages, some will show signs of toxicity, such as increased heart rate or failure to swim in the correct position. We expect 90% of these experiments will be performed at larval/juvenile stages (up to approximately 20 days post-fertilisation, d.p.f.). Fish will be killed by a humane method as soon as any toxic effect is observed. In moderate procedures, we need the fish to show some signs of neurodegeneration so that we can investigate the causes of disease and test ways of slowing or preventing the disease.

To maintain the breeding colony of fish, we need to identify the adult fish which have genetic alterations. Where the genetic alteration has a fluorescent marker, we can breed adult fish in pairs and then screen the larvae to look for this marker. For other genetic alterations, we need to take a small amount of tissue from the tail fin of adult fish (a biopsy) and use this tissue for genetic identification. This is a mild procedure and is performed under anaesthesia. In both these cases, the adult/parent fish will be singly housed for up to 14 days.

When zebrafish larvae are being reared to adulthood, food is given from 5 d.p.f. Zebrafish eggs are laid with a supply of yolk and this lasts for longer than 5 days, but food is provided from 5 d.p.f. to help the larvae transition to eating. For some experiments where we use larvae from 5 d.p.f. – 10 d.p.f., we will not give food. Previous work has shown that larvae are viable and develop normally without food to 10 d.p.f. It is necessary to withhold food in some of our studies of disease, where feeding could cause size and growth differences and would therefore introduce bias. In addition, we will not provide food in drug treatment studies on larvae between 5 - 10 d.p.f. For such studies, larvae are typically kept small dishes with between 1 - 10 ml of liquid. Food is not given for the duration of the experiment (i.e. for up to 5 days) as it would cause a build-up of waste in the liquid which would affect the water quality and therefore affect survival. Importantly, larvae will be culled at the end of the period for which food has been withheld (at no later than 10 d.p.f.).

All animals will be humanely killed at the end of procedures.

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

Since we are investigating neurodegenerative disease, in some cases, the animals will develop the early signs of disease. We expect these signs to be changes in body shape, neurodegeneration, behavioural changes. For mild procedures, fish will be killed by a humane method at the first signs of these disease changes. For moderate procedures, fish showing clinical signs (e.g. neuronal and other tissue degeneration, difficulty swimming, altered morphology, failure to feed or breathing difficulties) will be culled at the point when they show clinical signs that will prevent survival and cannot be alleviated.

There are no expected adverse effects as a result of single housing.

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)?

Zebrafish; Mild procedures: 61 %

Zebrafish; Moderate procedures: 39%

What will happen to animals used in 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?

To investigate dementia, the disease must be studied in neurons surrounded by the other cell types that the brain needs to function normally. These studies need to be performed in a vertebrate animal so that the animal model has all the cell types and connections that are present in the human brain. We need to study possible treatments in living animals so that we can be sure that the drugs reach the blood circulation and make it into the brain.

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

The majority of the work in our lab is performed in vitro (i.e. typically about 20 scientists work on cells grown in incubators whereas 6 scientists work on animal models). In our previous project licence, we replaced compound testing in zebrafish, instead performing these experiments on cells grown in the lab. The work in this new PPL application will focus on the validation of targets that have already undergone extensive validation in cells grown in the lab. These cells are often grown in dishes where every cell is identical (these are called cell lines). These have benefits because they are all identical and all behave the same way, but they are quite different from neurons. Therefore, our group have

established technologies to make and grow neurons in the lab (these are made from cells which are capable of turning into every different cell type in the body) and have developed collaborations to perform studies in mixtures of cells which can be grown in the lab in small clumps (organoids). These organoids contain lots of different cell types and so are more similar to the tissues in our bodies. Scientists hope that these organoids can be used to replace some animal studies and so we are investigating this in our lab.

Why were they not suitable?

Many of these approaches have been suitable. In our previous project licence, we used 20-25,000 less animals than anticipated by replacing zebrafish assays with cell-based assays. However, we still need to perform some animal studies. When we carry out experiments in cells, we can see whether they are alive or dead and whether proteins build up or not. However, we also need to test genetic alterations and compound treatments in living animals to see if they are able to prevent or slow disease. We need to check whether they change the speed at which disease symptoms appear and whether they can stop the severe parts of the disease from happening. We cannot measure these things in cells.

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?

The estimated number of animals for collection of gametes or for the generation, breeding and maintenance and generation of genetically altered (GA) lines is based on the different GA lines that are needed to achieve the project aims. We have 20 years of experience of using zebrafish and know how many adult animals we will need to maintain for each GA line in order to generate the number of experimental offspring for these experiments.

Although the aims of this project licence are different from those of our previous one, many of the techniques that we will use are ones that we have used previously and have been optimised to use the smallest number of animals to achieve meaningful and statistically significant results. In our previous licence, we used approximately 20,000 less animals than we originally anticipated as we were able to replace animal experiments with cell experiments. We have taken this into account in planning the number of animals needed in this application.

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

Where appropriate, we have used the NC3R's Experimental Design Assistant to design experiments. We regularly perform analysis on both living animals and on their tissue after they are humanely killed to reduce the number of animals that are used. For example, the brain can be imaged using fluorescent microscopy in living animals and/or behavioural analysis can be performed. We can then look at markers of disease in tissue samples from the same animals after they have been humanely killed.

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

We use control groups in all of our experiments. These are animals that are normal and do not undergo any procedures or experimental steps, so that they are the normal animals that we compare our experimental animals with. The control group are siblings from the same batch of fertilised eggs as the experimental group. Experimental groups are blinded for analysis. Pilot studies are performed for new analysis methods to determine the effect size (this is how big the changes are that we expect to see in our experiments). Once we know the effect size, we can work out how many animals to use in an experiment (the sample size). This allows us to work out how many animals we will need to use to be able to see whether the disease has been improved or rescued.

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 genetically altered (GA) zebrafish for this project. Zebrafish are small tropical fish that have many advantages as an animal model for this work. For example, one pair of adults produces 100-200 fertilised eggs per breeding and young fish are transparent allowing internal organs such as the brain to be seen without surgery. Also, there is a high level of similarity between the genes and tissues in man and other vertebrates. We have carefully developed genetically modified zebrafish which have aspects of human disease but in which we have limited the severity of the disease, e.g. by expressing the disease-causing gene in only one cell type in the eye (in the rod cells, which are light receptors in the eye). When these cells degenerate, only one type of cell dies (causing night blindness) whereas if this disease-causing gene were expressed throughout the brain, the animal would have ill health and reduced lifespan. We also use a special technique so that parent lines do not express disease-causing proteins and do not have any signs of disease. The disease protein is only expressed when two fish with different genetic alterations are mated together. Following mating, only the offspring produce the disease protein and show disease pathology. Using such lines, we only generate offspring for experimental purposes and the adult animals that are kept to maintain a breeding colony are viable and healthy, with no sign of disease. Many of our existing GA lines and any new transgenic lines that are generated in this project will carry a fluorescent marker in a cell type that is not needed for our

work (e.g. green fluorescent protein expressed in the pancreas or red fluorescent protein expressed in the heart). This allows us to identify the GA fish by fluorescence microscopy in 1- or 2-day old embryos and means that we do not need to take tissue biopsies from adults to identify the fish carrying the genetic alteration.

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

We have refined many of our experiments to use GA zebrafish where the disease manifests at unlicensed stages (i.e. prior to 5 days post-fertilisation). We will use these models in preference to those that require us to perform licensed procedures in all instances where they allow us to meet our scientific aims.

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

We use record sheets to monitor animals and regularly assess whether these need to be modified to see whether we should increase or alter the monitoring intervals to ensure we identify any adverse events at the earliest opportunity.

Zebrafish are a social species - we have refined our protocols to reduce the number of fish which are kept in single tanks when we are identifying transgenic founders. In most cases, adult fish will be returned to group tanks within 3 days. However, for some genetic alterations, the adults need to be kept for up to 14 days, for example, when identifying transgenic founders. This will only be in a very small number of cases (we estimate, less than 10% of the fish which undergo genetic identification). In this case, by housing we house these individuals with wildtype fish with a different pigmentation pattern (e.g. if transgenic fish is "stripy", it can be housed with "spotty" wildtype fish). After performing fin clipping (taking a small tissue biopsy from the tail fin), fish are singly housed in an adapted shared tank which is separated by dividers meaning the fish are aware that there are other fish nearby.

Our animal facility staff are testing different types of enrichment (such as using plastic plants and artificial grass) to encourage females to breed and to improve husbandry. Enrichment (e.g. plastic plants and artificial grass) will be used for singly housed fish.

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

We use the following guidelines:

ARRIVE guidelines for reporting of your experimental data, <https://arriveguidelines.org/>,

NC3Rs for zebrafish welfare <https://www.nc3rs.org.uk/3rs-resources/zebrafish-welfare> and reproducibility <https://www.nc3rs.org.uk/3rs-resources/zebrafish-welfare>.

PREPARE guidelines, <https://norecopa.no/prepare>

NORECOPA guidance on classification of severity of procedures (10.1258/la.2011.010181)

FELASA guidance on pain management in zebrafish (<https://doi.org/10.1177/00236772231198733>)

and are awaiting the publications from FELASA working groups on "Severity classification in zebrafish and their larvae",

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

We receive regular emails from the Named Information Officer at our institution. These contain details about advances in the 3Rs and are sent to all personal licence holders. In addition, we have regular meetings with our animal care technicians and vet who are part of additional information networks. We share best practice with other zebrafish research groups within our institution and with the wider zebrafish community using researcher forums such as ZFIN - the zebrafish information network (www.zfin.org) and ZHA - zebrafish husbandry association (<https://zhaonline.org/>). We discuss any advances at our weekly lab meetings and implement trials of improved husbandry and new experimental methods, where appropriate.