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NON-TECHNICAL SUMMARY

Pathways of filopodial formation

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

cell motility, filopodia, cell biology, actin cytoskeleton

Animal types	Life stages
Xenopus laevis	adult, 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 aim of the project is to understand how the cells make finger-like projections, called filopodia.

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?

Filopodia are produced when cells move, and they are thought to be sensory, in helping cells find the directions they need to go or how to make contacts with other cells. As a fundamental structure of cells they are widely important for health and disease, in the brain, in cancer and infection as three examples. In the brain, filopodia are produced when nerve cells make connections with each other, which is important for learning, behaviour and mental health. 3-4% of children in the UK are affected by neurodevelopmental disorders, and more than half of those referred to child and adolescent mental health services are found to have a neurodevelopmental disorder (such as autism). A second example is the spread of cancer cells outside the original organ that they came from, called metastasis. Cancer causes 28% of UK deaths and metastasis is estimated to be the cause of 90% of them. Metastatic cancer cells have many filopodia and we do not understand what these filopodia are doing or how to change them. Filopodia are also formed when viruses infect cells, e.g. human immunodeficiency virus and the COVID virus where they are assumed to spread viral infection around the body. If we can understand and control how cells make or turn off filopodia we might be able to come up with new therapies in the future in any of these areas or others.

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

We will discover new information about filopodia. We will understand more about where filopodia form, how they reach certain lengths, how long they last for, and how they are suppressed.

The main output of the information will be publications reporting our findings. We will also present our findings at local, national and international scientific meetings. We will generate data that supports future funding applications. There may be new intellectual property that supports a patent application and we may generate data that supports clinical trials in the future.

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

In the short to medium term, other researchers studying filopodia and related aspects of cell biology will benefit from the outputs by being able to make new discoveries in their own work using the information we found out. In the long term the information we obtain may be useful information for people working on disease and in the very long term may benefit patients. Our data may lead to new ideas for therapies and the scientific tools that we create may be the foundation for new approaches to control filopodia (such as antiviral medicines or anti-cancer drugs).

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

We will disseminate our work in conferences and collaborate with other researchers. We will also disseminate our work by making short videos for a lay audience and present our work at events such as festivals for the general public. We will make the computer code that we use publicly available and upload our manuscripts to a preprint server. Our work will be published open access so anyone can read it for free. Where our work is directly applicable to disease we will engage with patient charities. We will consider publishing unsuccessful approaches too, for example in supplementary information of other papers or as preprints.

Species and numbers of animals expected to be used

- Xenopus laevis: 400

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.

We are using adult female African Clawed Frogs and their tadpole stages before they are independently feeding. We are using adult females to obtain their eggs. We use the tadpole stages where nerve cells are moving from the eyes to the brain of the tadpole as this is a well-understood system where filopodia are important and is a native biological system with immature animals.

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

The female adult frogs are injected with a hormone produced by horses during pregnancy. The frogs are injected under the skin at the bottom of their backs, just above their legs, into a place that contains a sac of fluid, which helps the hormone spread through the body. The hormone causes the egg cells to mature into eggs, mimicking what would happen to the frogs when they produce their eggs normally (e.g. in the spring). The immature egg cells finish their growing process, become eggs and are then able to be fertilized by sperm. A second injection 3-10 days later, of a hormone produced during human pregnancy and purified from urine (the hormone which is detected by pregnancy tests), makes the frogs lay the eggs. The differences between the horse and human hormones have the different effects in maturing the eggs or inducing laying.

After they are injected the frogs are put back into tanks of fresh water. After 5-15 hours the frogs are placed in a specific salt water solution for no more than 20 hours that keeps the eggs fresh once they are laid so that we can use them for several hours after laying. The salt water solution keeps the conditions outside similar to inside the frog's body. When the frogs have finished laying they are put back into fresh water. The frogs undergo the procedure more that 3 months apart. Some frogs lay better eggs than others, if a frog lays bad eggs on more than one occasion and needs to be killed, we will do so humanely.

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

No adverse effects are expected beyond transient discomfort of injection. Very rarely, in 0.1% of superovulation procedures, the frogs do not properly lay their eggs and develop a hard, bloated

abdomen and become lethargic (become egg bound). This can be a delayed response up to a week after the procedure. Rarely there are mild stress responses that may be related to vibrations or the salt water such as sloughing of their skin in larger patches.

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)?

Xenopus Mild 100%

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?

We use animals to make extracts from the frog eggs and to understand filopodia during tadpole development.

Filopodia are small cellular projections which come and go very fast and use similar building blocks as other processes going on inside cells. This makes filopodia hard to study as we cannot see them for long enough and it is difficult distinguish which effect comes from which process in the cell. Extracts from frog eggs can be used to make structures similar to filopodia outside cells which are more stable and not confused by other processes. We use frog egg extracts because they allow us to attain very high levels of sophisticated molecular information, like which types of the building blocks are needed and which ones are used together.

To convert our insights using extracts back to real filopodia we need to use cells. We use cell lines for some of our work however the filopodia produced by cell lines are not fulfilling a proper biological function so we don't know if what we are seeing is meaningful. The nerve cells in frog tadpoles are a well-understood model that use filopodia for finding their way and connecting to other cells. We use tadpole development to provide a biological context where filopodia are important.

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

Tissue culture cells and fruit flies.

Why were they not suitable?

Cells grown in dishes do not recapitulate the normal filopodia growth and movements, so do not directly replace the tadpole experiments, which themselves replace experiments with mammals. Our experiments with extracts cannot yet be done with tissue culture cells as the extracts that can be made are too dilute. Mammalian brain extracts have been used but these are not as effective as frog egg extracts. The microscopy of the filopodia in fruit flies is not as fast or clear to see as the frog nerve cell filopodia so we would be unable to answer the objectives for this particular project using flies.

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 have estimated based on our previous use and design of anticipated experiments over the next 5 years.

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

Other licence holders have been able to superovulate frogs that lay good eggs 40 times, so we will do this to reduce the numbers of animals used. Bad layers are specifically identified by monitoring quality, rather than after an arbitrary number of superovulations. If bad laying frogs are identified we will kill them humanely.

We will keep frogs in salt solution for a day (if they suffer no visible harms from doing so) as they continue to lay and we can continue to use the eggs thus reducing numbers of animals used.

We use a low amount of extracts for our work by optimising dilution, using a small volume and carefully calculating the levels of reagents we need to use which reduces numbers of animals needed. We also perform pilot experiments to check we are using the correct conditions.

To obtain tadpoles, we fertilize eggs from 1 or 2 frogs at a time and use numbers of embryos that we are confident we will have time toprocess.

We use control experiments to ensure each experiment is meaningful, collect multiple datasets from each experiment and carefully perform steps to minimise variability.

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

We share the eggs and sometimes the fertilized eggs with other groups so less frogs are used. We track the quality of the eggs and kill frogs that do not lay good eggs rather than inject them again.

We will trial a double ovulation procedure, where each frog lays twice, which would reduce the number of animals by up to half if successful. We will only use this procedure if it does not increase the harms for each animal.

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.

The project uses Xenopus laevis, an amphibian model. We will induce the frogs to lay eggs and do this on repeated occasions as this is similar to what occurs in the wild.

The eggs will be used in two ways. One way is fertilizing them to make tadpoles that are used in immature larval forms before they are protected. The cells of the tadpole that will later become eyes are removed and cultured to obtain nerve cells. As a final approach we may image filopodia from cells directly within unconscious anaethetized larvae (before they are protected).

The second way is crushing the eggs in a test tube to make extracts.

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

The use of frog egg extracts is specialised and optimised for the field of study. Because frog eggs are so large they can be easily broken open without too much damage, leading to experiments that cannot be performed with other cell extracts. The filopodia produced by the larval nerve cells we work with have a known function in connecting the eye to the brain and so give us a meaningful setting for working on filopodia. The cells give very high quality microscopy and a biologically relevant setting which are not possible to achieve in any other system. The nerve cell experiments share important reagents with the experiments with extracts and the two sets of work are used in combination to gain the most information with least animal harm.

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

We are transitioning to home-bred males and female animals to reduce the stress induced during traveling and adjusting to different environmental conditions. We keep handling to a minimum. All Xenopus are kept on dark backgrounds in environmentally enriched tanks. Using dark shelving and refuge tubes helps the animals to feel safe.

Frogs are monitored throughout the day while the technicians are in the room. If frogs appear stressed (excess skin shedding, overly jumpy, not eating) the NVS is engaged, and decisions are made as to whether or not it is best to humanely kill the frog. In the unlikely event a frog is in pain (a small number may develop a secondary bacterial infection colloquially called Red-Leg) the frog is closely monitored and if no improvement is seen within 48 hours the frog is humanely killed. Any frogs that have more that 20% body sores from diseases such as Red-Leg are euthanised immediately.

We work with a specialised Xenopus NACWO. All frogs are photographed for ID and health monitoring which is non- invasive and requires minimal handling, improving the clinical signs of the colony. We also monitor egg quality and keep the consistent good layers which reduces the overall number of animals held.

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

I am following PREPARE guidelines for planning experiments (https://norecopa.no/prepare) and ARRIVE guidelines to report my research (www.arriveguidelines.org). We will use published guidance on use of controls for using reagents that inhibit protein production (morpholino oligonucleotides) to ensure the best control experiments are used.

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

I will discuss with my colleagues in the Xenopus field and consider practical guidance from the Named Veterinary Surgeon and Named Animal Care and Welfare Officer at my institution. I will refer to material from the NC3Rs website https://nc3rs.org.uk/resource-hubs, 3Rs tools in house and external resources such as Norecopa https://norecopa.no/databases-guidelines.