



Home Office

## NON-TECHNICAL SUMMARY

# Impact of sensory and electrical activity on neuronal function

### 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
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

### Key words

neuroscience, plasticity, olfaction, dopamine, neurogenesis

### Animal types

### Life stages

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Mice

adult, pregnant, neonate, juvenile, 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?**

To investigate how different activity-induced plastic changes in shape and electrical activity impact neurons and neural networks, how they are employed to sustain different behavioural needs, and how they could be harnessed to counteract disease. The project will mainly focus on the olfactory system, the part of the brain that encodes smells, and on a class of neurons called dopaminergic, which are important for sensory processing, movement and motivation.

**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?**

In all living organisms the ability to sense and react to the environment is fundamental to survival. Animals must constantly sample the ever-changing environment via their sensory organs (e.g. eyes, nose, skin, ears). They then need to compute the resulting information in brain cells (neurons) and circuits to generate an appropriate behavioural output. When successful, this process, known as neuronal plasticity, underlies processes such as development, learning, memory, adaptation.

With this work I aim to investigate how brain cells and brain circuits respond to changes in input that they receive. This will enhance our understanding of the fundamental principles of how the brain controls behaviour.

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

Chiefly, outputs will include generation of new knowledge, which will be timely and freely disseminated as publications in open-access journals, full datasets, and conference presentations.

In the medium to long term, the proposed work on how the brain cells and circuits in the olfactory system change to adapt to incoming odour stimuli promises to uncover novel strategies for treating smell disorders.

Moreover, the part of the project that focuses on brain cells called "dopaminergic" is relevant for disorders such as Parkinson's disease that involve specific pathology to that cell type, and that is associated with early loss of smell. While the work presented in the project is not directly translatable to the clinic, it will hopefully inform future diagnostic and therapeutic approaches.

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

The proposed project comprises basic neuroscience research, with primary benefits coming from the generation of new knowledge.

By changing the inputs coming from the environment in a controlled way, and adopting and state-of-the-art technology to interact with brain cells, the proposed project will significantly enhance our knowledge of how the environment shapes brain function.

Given its multidisciplinary nature, the work presented in this proposal will be of great interest to a variety of researchers. These include scientists working on, for instance, different brain areas, brain cell development, the five senses and how they influence brain cell function. Moreover, the outputs will inform translational work done by clinician scientists interested in cell transplantation, Parkinson's and other dopaminergic and/or neurodegenerative disorders. Indeed, I have already secured two collaborations, one with a mathematician interested in generating computer models of the brain, and one with a neurologist whose lab works on translational neuroscience.

I therefore will do my absolute best to ensure that the resulting raw data (physiology, morphology, and behaviour), analysis and manuscripts will be fully and promptly available.

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

I am a strong supporter of open science. Since I have been actively involved in deciding where to publish, I have opted for open source journals or paid the extra fee to make the paper immediately accessible.

I am also in favour of data sharing: I have uploaded the entire dataset of my most recent work (over 40GB, raw data and analysis) to the online repository Dryad. I also am the proud first author of two papers which include ONLY negative findings.

I plan to continue along this path for all research performed in my own laboratory, to cost open science and data sharing into all grant applications, publish negative findings, and, importantly, share full datasets.

### **Species and numbers of animals expected to be used**

- Mice: 6350

## **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 is looking at how neurons born during different stages of the animal's life respond to changes in incoming stimuli from the environment. To achieve this goal, the project will use mice of different ages and developmental stages. These include a small number of pregnant dams carrying developing embryos and pups (to investigate the developmental origin of various cell types), and adult

mice (to investigate how mature neurons respond to change in incoming stimuli, and how these changes drive animal behaviour).

Mice will be used to: a) take full advantage of the transgenic mouse lines that have been developed in recent years; b) build upon the extensive existing mouse literature covering development, shape, physiology and behaviour.

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

Only transgenic mice which carry no harmful genetic mutations (for instance, having specific neurons coloured in green or red) will be bred in this project. Each mouse will then be used for only one experiment, lasting, on average, a few days, and never more than 4 months. Experiences may include being exposed to more or less odours, and performing a behavioural smell or motor tasks. A minority of mice will be subjected to a single brain surgery, with post-operative care and monitoring and full recovery necessary before undergoing further testing.

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

The vast majority of animals (92%) will be experiencing a series of mild procedures: transgenic breeding, blocking one nostril, behavioural testing.

Less than 1% of animals (20 in total) will undergo vasectomy to generate and maintain transgenic lines.

5.5% of mice will undergo recovery surgery to label, manipulate or make visible individual brain cells; we do not expect adverse effects. In the extremely unlikely scenario of surgical complications, animals will be immediately killed with an appropriate painless method.

1.6% of mice will receive a single intraperitoneal injection which will result in degeneration of the cells in the nose and a transient loss of their sense of smell. The mice will regenerate these cells, and they within a month or less they will be able to smell as before the injection. We do not expect adverse effects and we will make sure that food and water are easily accessible, that the mice do not have troubles eating and do not lose weight.

A small number of animals (less than 1%, 30 mice in total) will undergo recovery surgery to inject specific substances which kill a subset of dopaminergic brain cells in the midbrain. They may suffer slight difficulties in moving around, associated with early onset of Parkinson's disease which is brought about by the surgical injection. These are expected to be minimal (imperfect posture, walking and coordination), and not lasting longer than a few weeks. Animals will be closely monitored, and adjustments will be made to make sure that food and water will be always within reach.

### **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)?**

Mild: 92%

Moderate: 8%

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

- Killed
- Kept alive
- 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?**

The proposed research necessitates experiments on animals because, to answer the important questions in this proposal there are currently no possible non-animal-based approaches. On one hand, it is not possible to study how learning at the behaviour level changes without using a living animal, for example, we cannot make individual cells learn to recognise different odours and behave differently as a result of this learning. On the other hand, in order to understand what drives this learning and behavioural adaptation, we need to investigate how brain cells change their shape and function at the microscopic level, something that we cannot do in a human at the necessary granular level of single current in individual neurons. To link the two levels of understanding - cells and whole organism - and to relate the findings to what happens in the human brain, we need to use small mammals such as mice. Moreover, mice are macrosmatic and rely on scents to drive their behaviour, making them the ideal model to focus on how the environment shapes plasticity, learning and behaviour in the olfactory and dopaminergic networks.

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

I considered the following options:

- in silico models such as computer simulations
- invertebrates, such as worms (*Caenorhabditis elegans*) and flies (*Drosophila melanogaster*)
- human-derived stem cells
- brain organoids, which are three-dimensional clusters of stem cells resembling a small brain-like structure

These methods are used in other fields in biology and neuroscience which study processes happening in single cells, or simple behaviours in insects. Unfortunately they are not suitable to answer the kind of questions that I am asking because I need to link changes at the cell levels with complex behaviours and pathologies.

I maximise the use of non-animal methods for many of my experiments. When doing so, I obtain brain tissue from mice that are painlessly killed.

Moreover, I am setting up a collaboration with computational neuroscience. The data gathered from the animal work included in this project will hopefully be instrumental to attempt to generate computer simulations of plastic olfactory network, to be used for future studies.

### **Why were they not suitable?**

Our understanding of how the brain changes in response to changes in the environment is improving, but is not yet at the point where we can build realistic mathematical models to simulate and investigate different scenarios.

This means that we need a better characterisation of the mechanisms and effects of plasticity in real neuronal circuits, which is precisely the goal of the proposed research. Human-derived stem cells or brain organoids are not currently used for integrated investigations of multiple forms of plasticity in realistic neural networks, and human-derived cultures of the brains cells that encode for smells are also not currently available. In addition, of course, such cultures would not be able to address the multi-level analysis (i.e., from cellular changes to behavioural changes) which is central to this project's success.

In short, there are no viable alternatives to animal use if the project is to meet its stated aims.

## **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?**

Animal numbers were estimated based on my extensive experience with these experiments, by using previously-collected data, and by consulting with colleagues specifically trained in statistics.

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

I used online reference tools (NC3Rs Experimental Design Assistant), and made sure that my plan adhered to all best practices in experimental design. These include:

- using control littermates to minimize breeding mice that are not then used for experiments;
- including animals of same age and sex in all groups to minimize variability;
- randomised group assignment;

- blinding: the scientists will not know information which may influence or bias the results until all analysis is completed;
- appropriate statistical analysis (e.g., multilevel mixed model analysis with mouse as the subject variable).

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

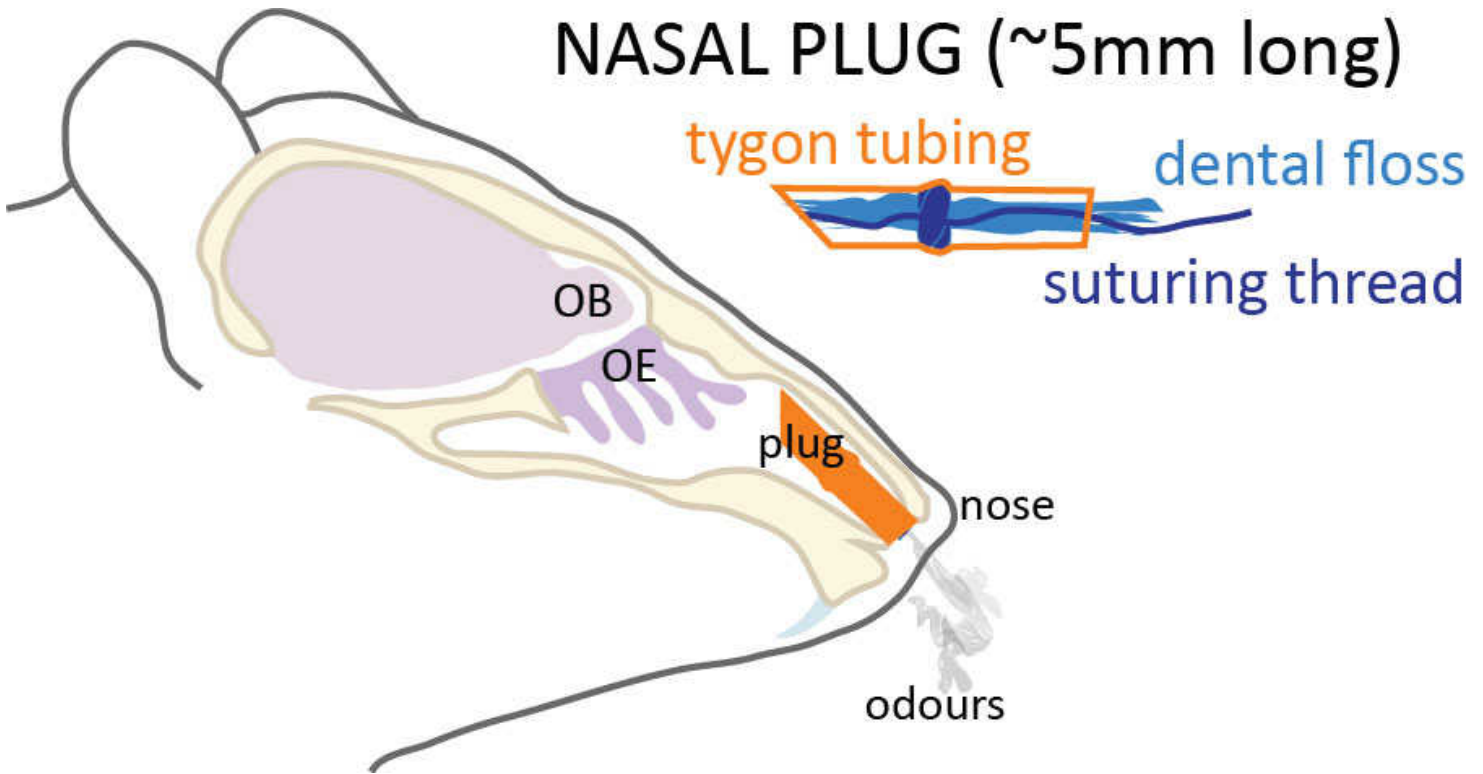
- Refinement of surgical techniques and of behavioural testing, as well as high quality animal welfare, will minimise suffering and distress. This in turn will reduce inter-animal variability and thus decrease the number of animals needed to achieve a meaningful result.
- Longitudinal recording of the same brain cells achieved via chronic imaging, which will reduce the number of used animals and increase statistical power.
- For new experimental approaches, small pilot studies to determine appropriate animal numbers.
- Efficient breeding system to minimize generation of surplus animals.
- Tissue-sharing among laboratory members, and, when feasible, with other laboratories.
- Generation of computer models based on the data that we collect.

## 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.**

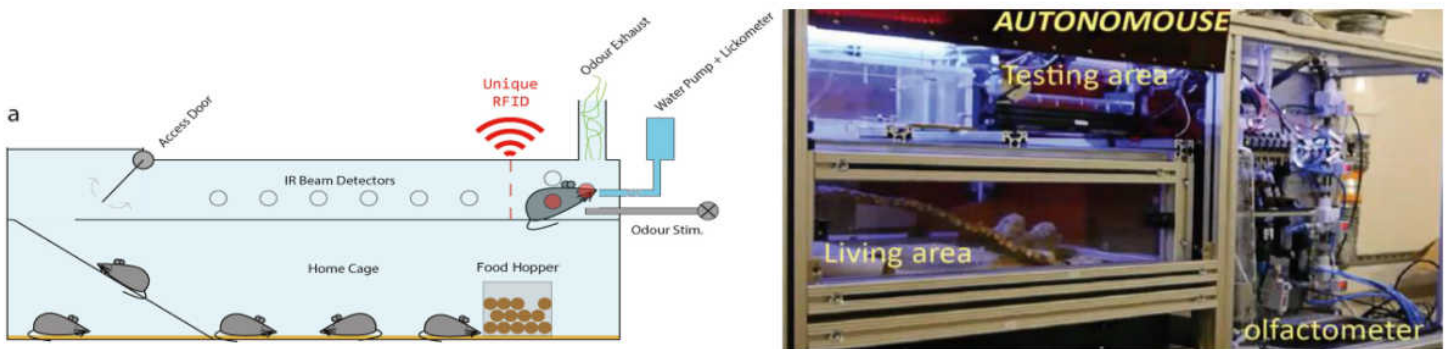
**Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.**

**Sensory deprivation:** this will be achieved by my choice of occlusion of one nostril via a removable plastic plug, rather than the painful and irreversible surgical nose closure typically used in this type of research. This technique is now routinely employed in the various laboratories. In addition, suffering will be minimised by carrying out the procedure under appropriate anaesthesia, preparing custom-made plugs of appropriate size which' as illustrated in the figure below, blocks the airflow without damaging the olfactory epithelium (OE) or olfactory bulb (OB) in the brain. Lubricant will be used to facilitate plug insertion.



**Surgery:** all procedures will be carried out aseptically in the designated surgical suites of the animal facilities. Appropriate anaesthesia and pain medication will be provided before and after surgery at doses and frequencies agreed in advance with the veterinarian. I have over ten years' experience in performing recovery surgery in mice, and I have had a very low incidence of complications. I will have in place clear protocols detailing how often the animal will have to be checked, how to assess pain and distress, and how to proceed if surgical complications occur (immediate humanely kill for serious complications, swift consultation with the veterinarian and intervention for minor complications).

**Behavioural testing:** traditionally behaviour has been tested in rodents by means of a go- no go task: water-deprived mice are presented with a series of smells/images/sounds, and only rewarded with a drop of water for some of them (targets). Mice learn reasonably quickly to lick the water spout exclusively when target stimuli are presented, and such lick measures are used to quantify performance, learning rates and memory consolidation. While extremely effective and well characterized, this methodology has important caveats – first and foremost, water deprivation. In order to overcome these limitations and to provide a more ethical way to test smelling behaviour in mice, an automated testing apparatus called AutoNOMouse has been recently developed.





Briefly, colonies of up to 20 mice of the same sex share a large two-story cage. On the lower floor they have access to nesting material, environmental enrichment and unrestricted food. When they want to drink they need to go to the first floor and access a separate compartment, where only one mouse at the time can enter after having its identification under-the-skin microchip read at the door flap (similar to house cats with their cat doors). Both the water port and the odour delivery spout are located in this separate compartment, and the mouse will have to perform an olfactory go/no-go task in order to receive water. Testing is therefore self-initiated, and mice will do as many sessions a day as they want. Mice are neither water restricted nor single housed: they do not drop in weight and do not appear stressed, and thus the performance variability across days and individual is reduced. Moreover, the live monitoring of how individual animals engage with the task guarantees that water intake will be closely monitored. If on any given day a mouse fails to engage with the task or makes lots of mistakes and thus does not drink enough, the experimenter will be promptly alerted by the software and water will be manually given to the animal to avoid dehydration. Animals do not live in the AutoMouse all their lives, but they are placed there with their littermates for the duration of testing, and then returned to their normal home cage.

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

The choice of mouse as the animal model ensures that I am using the most appropriate and simplest species possible for the proposed research. How the brain processes smells, and how brain cells change their shape and function in response to inputs from the environment are all best understood in rodents, providing a wealth of background information that will prove essential for the project's success. In addition, many of the experiments outlined above would not be possible without the availability of key mouse transgenic lines.

Throughout my work I will address the 3Rs requirement for refinement by minimising animal suffering. Moreover, the majority of the work will be performed on tissue acquired from mice that had been killed under terminal anaesthesia.

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

Mice will be group housed and provided with appropriate enrichment such as nesting material and toy wheels, and I will adopt tunnel handling. To minimize the single housing of stud males, they will be kept in low numbers and rotated among partner females. Each female will not carry more than 6 litters, and no mouse will be kept for more than one year. In line with the HO GAA Framework, genetic identification of littermates will be carried out by collecting small ear samples for DNA analysis (second/third week of age); these small pieces of tissue are the by-product of identifying the mice and therefore by combining identification and genotyping we will minimise procedure done to the animals.

For surgical interventions, we will employ pain relief medication, surgical care and observation sheets, use of grimace scale or other body scoring tools. Instead of the normal hard food pellet hanging from the cage ceiling, softened food pellets will be left on the cage floor after surgery, to facilitate eating during recovery.

Before behavioural testing, mice will be acclimatised to the new environment, and handled for a few days by the person performing the experiment.

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

I will routinely discuss care and husbandry with my Named Persons, check the 3Rs Search Tool, and keep updated on the PREPARE guidelines, ARRIVE version 2 guidelines and the 2017 LASA guidelines for surgery.

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

I will regularly visit the NC3Rs website, the Norecopa website and meet with the named persons in my facility to ensure best practice. Moreover, I will regularly interact with colleagues and collaborators, in the UK and internationally, working on similar topics, to exchange notes and keep up to date with the latest developments.