Home Office

NON-TECHNICAL SUMMARY

Adaptive sound representation in cortico-subcortical loops: hearing vs listening.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

hearing, subcortical, cortical, circuit, statistics

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

Retrospective assessment

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

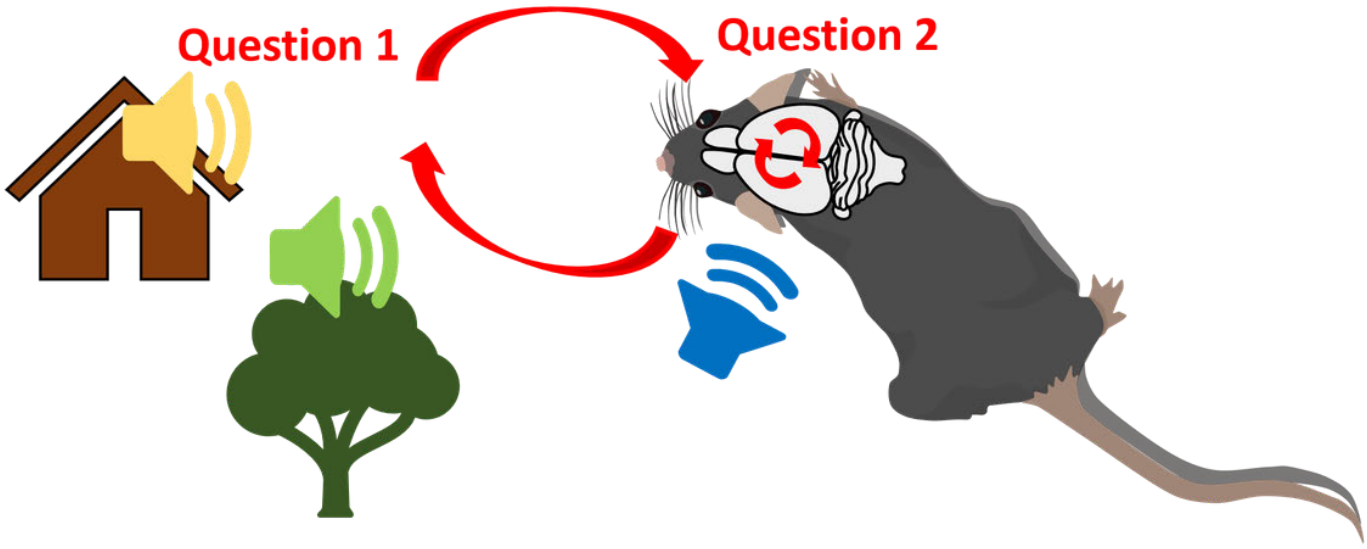
Objectives and benefits

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?

The main aim is to characterize the sound features and patterns that are relevant for a mammal interacting with its environment, and their representation along the hierarchy of the auditory system, from peripheral regions to more central ones.

There are two scientific questions: 1) to characterize how the acoustic environment determines mice' use of the space, and 2) to characterize how sound is represented in the brain depending on its meaning and under specific mental states.



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?

We have meagre understanding of the brain mechanisms that lead to complex listening by the brain (interpretation of the acoustic scene), beyond hearing the responses to clean and loud sounds in a quiet background. The capacity to interpret complex acoustic scenes deteriorates with age, stress and mental diversity and this is exacerbated as the world around us becomes louder and people live longer.

It is crucial that we understand the impact that sound has on our life and why. This will have consequences at several levels. It will impact how we design public spaces, from schools to work and social environments. It will determine how we prevent and respond to hearing loss, a phenomenon that is on the increase and known to correlate with cognitive and psychological decline. It will also help us understand the consequences of human activities on other animals (including the oceans).

Downloaded: 9:16am, 10 Feb 2026

Sound is something that the brain processes automatically, even when we are not paying attention. Understanding how this is done will give us also invaluable information on automatic brain processes that impact attention, stress, and hearing in general. This information is relevant for the design of, for example, music that helps us concentrate or relax. The brain can use patterns in the sounds to dissociate between background and foreground sounds and thus help the listener focus on relevant information. Understanding how the brain performs this function will help us develop smarter hearing aids for background sound suppression.

To understand the circuit underlying these processes and the mechanisms involved we need to perform the experiments in animals. Through these basic research experiments, we will obtain new information on how mammals interact with the acoustic surroundings and the underlying brain mechanisms.

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

Data: we will have a better understanding of 1) the auditory brain structures that are crucial in sound processing, and the detection and storage of sound patterns as the animal interacts with the environment, 2) the interactions between these structures and higher other structures involved in decision making and long-term memory, which presumably convey predictions and memories that help with sound interpretation, and 3) how the environmental acoustics impact behaviours (nest making, curiosity, resting, playing, socializing) in mice living in groups in an spatially and sensory complex environment.

Publications and conference proceedings: we expect to publish our results in peer reviewed scientific journals. We will aim to publish whenever possible in open access journals and have links through platforms such as F1000.

Collaborations: we expect to establish collaborations within and outside the department and to share our data and know-how with colleagues.

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

The hearing research community and the wider scientific community will gain a better understanding of brain mechanisms and circuits active in animals as they interact with their environment, as well as the processing of acoustic environmental statistics.

The medical community can eventually benefit when our results are embedded in the wider knowledge and used to develop therapies. This will take place at several different disciplines. The data will be useful to understand hearing impairments in ageing and stressed populations, who will benefit through the development of smart hearing aids with smart filtering algorithms that separate background from foreground or noise from signal. The data will also impact our understanding of phenomena such acoustic overload in autism, tinnitus, or acoustic hallucinations in schizophrenia, potentially helping to develop preventive or palliative therapies. Because of the strong behavioural and cognitive load of this research, the data will impart a wider perspective on cognitive decline resulting from hearing impairments.

Downloaded: 9:16am, 10 Feb 2026

Society will benefit from the knowledge itself, since understanding how our brain works and interacts with the environment is understanding who we are. It will also benefit through the derived medical advances, and potentially for the influence the findings might have on the design of the acoustic environments for humans and animals. For example, by understanding how sounds impact where animals chose to nest, play or socialize, we can design better social spaces for learning, working and playing. This would have a strong impact on young, neurodiverse, ageing, or hearing deficient populations. As the world becomes louder and louder, understanding the effect sound has on our peace of mind, our sleep and our hearing, as well as on the brain structures that process it, becomes increasingly important.

The veterinary industry would also benefit since many of the ailments that affect humans also affect the animals that live with them. Like our anxiety levels, milk or egg production, for example, are likely affected by the quality of soundscape the animal lives in.

The natural world will benefit from a better understanding of the impact that sound has on the ecosystem of animals and their behaviour within. We are only now starting to realize the negative effects of an increase in sound level, even in the relative remote ecosystem of the oceans.

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

Through publications, conference posters and lectures, collaborations, discussions with colleagues and medical staff, dissemination in schools and public events.

We will aim to communicate also negative results or unsuccessful approaches, as this will reduce the use of animals and save future researchers both time and effort.

Species and numbers of animals expected to be used

- Mice: 6460

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.

Mice are the most suitable species for the questions addressed in these experiments. Mice are a model of choice within systems neuroscience research, an area of neuroscience interested in how groups of neurons work together to perform specific functions like perception, movement, and cognition. As mammals, their brains are composed of the same structures as the human brain and they display complex behaviours, including learning, decision making and behavioural adaptation to changing conditions. This means that they are a suitable species on which to study learning and adaptation to the environment. Their rapid and efficient breeding as well as their rapid growth into adulthood makes it possible to answer certain questions with relative ease and speed, making them

better suited than other, bigger species for certain questions. The existence of numerous mouse mutant models, as well as viral techniques designed to generate genetic modifications in mice makes them suitable to test hypotheses through manipulations, again making them more appropriate than other animals. Their innate curiosity makes them suitable for behavioural research and questions about learning. Finally, the small brain size makes different brain regions easily accessible. For example, the thalamus is only 1.5-2 mm away from the surface of the brain. This means that with a single 4mm long electrode we can record simultaneously from the superficial cortex, the underlying hippocampus, and the even deeper thalamus.

We intend to use mainly juvenile and adult mice to better understand the function of the developed brain. In some experiments we will also use pregnant and lactating females, as well as their offspring, for behavioural experiments aimed to understand how the acoustic environment impacts the behaviour of mice living in natural social structures.

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

We will use two typical experiments in objective 1 and 2 respectively.

In a typical experiment in objective 1, we will place a group of mice in an environment with several compartments and complex sensory stimulation. This is referred to as enriched environment, to differentiate it from the standard housing cages. We will observe how each animal explores this enriched environment, the social interactions between cohabiting mice, and how they make nests as a group. We will also study how they react to small changes in the compartment-specific soundscape. We will analyse individual and group behaviour using established algorithms for the analysis of complex multimodal data (e.g. deep and machine learning tools). In some of these experiments, animals will be implanted with very small chips for detection, much like the ones pets carry.

In a typical experiment in objective 2, we will start by handling the mouse (5-10 minutes per day for 4 days) so that it habituates to the experimenter and the experimental room.

If we want the brain of the mouse to express certain genes, we might start by performing a virus injection into the desired structure through a craniotomy (a small hole in the skull). We might then train the mouse in a behavioural task that involves discriminating sounds and learning which ones are coupled with reward. For this we typically water restrict the mouse such that it only receives water during the task and immediately after. We make sure the animal obtains sufficient water every day and maintains a healthy body weight and physiology. Behaviour will be assessed through sensors positioned in the behavioural apparatus, that detect the position of the animal and its response (e.g. a nosepoke into the water delivery tube).

If we are measuring neuronal activity, we might fixate the head of the mouse. With this aim, we would fix a head-holder (a small metal bar) on the skull of the mouse using dental cement under anaesthesia (approx. 1 hour). The mouse will receive pre (2 days, habituation to pain medication) and post-operative (3-5 days, pain medication, hydration, warmth) care appropriate for this procedure. After a few recovery days (min. 5 days), we will start behavioural training under head-fixation, meaning that the head-holder will be fixed to a holder bar such that the head of the mouse is in a fixed position relative to the speaker used to present sounds, or the screen used to present images. When we deem that the behavioural performance has reached the level that we want to study, we will prepare the

Downloaded: 9:16am, 10 Feb 2026

animal for neuronal activity sampling (measuring the activity of neurons in the brain). We will make a small craniotomy under anaesthesia so that we can access the brain using electrodes (approx. 4mm long) or through an optic fibre that is either placed over or inserted painlessly into the brain. We will take measurements for approx. 2 hours/day for approx. 5 days. At the end of each measurement the probe will be taken out, the brain surface carefully cleaned and covered with a protective sheath, and the mouse will be returned to its cage until the next day.

After completion of any experiment animals will be humanely killed.

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

Water restriction causes little stress to the mouse because they have evolved to adapt to environments with irregular food and water availability (Toth and Gardiner, 2000 PMID: 11487246) and are accustomed to once-a-day occurrences (nychthemeral rhythms, Rowland, 2007 PMID: 17536615). In fact, some evidence suggests that water restriction caused mild reduction in home-cage activity and occasionally (<5%) postural changes and task-related hyperactivity, in animals going through a protocol similar to ours (Goltstein et al., 2018; PMID: 30212542) and that, within experimental constrains, mice tolerate water restriction better than food restriction (Tucci et al. 2006, PMID: 16870275). During water restriction, mice might eat less (dehydration-related anorexia), meaning that the mild weight loss (approx. 10%) observable in the initial days of restriction might be partly caused by a reduced food intake.

Behavioural tasks can be stressful initially, as the animal learns to understand what is expected from them. We design our tasks such that the great majority of animals show a healthy learning curve and can perform about 75% correct. This minimizes the frustration in the mice. Participating in a well-designed behavioural task can act as a form of enrichment.

Implantation of the head-holder is a minor surgical procedure. Mice recover quickly and will be given pre- and post-operative care including nutrients and pain medication, much like people recovering in hospital.

The effect of surgeries with craniotomy (small hole in the skull) varies depending on the procedure and the length of the surgery. If the surgery involves only a fast craniotomy for future recording of neuronal activity in the brain, the animal recovers its body weight, posture, and activity levels within 24 hours (for example in animals that had a prior head-holder implantation). If the surgery is longer and involves skin opening, cleaning of exposed skull and craniotomy followed by injections, recovery of body weight, posture and activity levels is typically observed within 48 hours. Either way, pre- and post-operative care in the form of analgesia is essential. Animals need to be monitored, and the pain and physiological conditions managed for 3-5 days after surgery.

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: 75%

moderate: 25%

What will happen to animals used in this project?

- Killed
- Used in other projects
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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?

Because we are studying the adaptive responses of nerve pathways to changes in the environment during different mental states (awake, alert, asleep, naive vs proficient in a behavioural task), we need a live animal that is interacting with the sounds and the environment in which the sounds are played. For this reason, we cannot use alternative methods such as brain slices, or organ cultures.

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

There are two general categories for non-animal alternatives used in systems neuroscience: in-vitro (cultures of cells or organs outside the body, e.g. organoids) and in-silico (computer models).

In-vitro: We inform ourselves about advances that happen in the in-vitro field. To date these approaches are unfortunately not suitable for our experiments because they do not allow as to study the interactions between brain regions, since the cultures do not replicate faithfully these regions and their connections, and they do not interact with the environment like animals constantly do. For example, organoids are a relatively recent development. An organoid is a very small, three-dimensional, self-organizing structure grown in a lab, typically from stem cells, that mimics a specific organ. These organoids however do not replicate the very complex structure of brain regions, let alone their interactions.

In-silico: We are endeavouring to address some of the questions raised here in parallel on a large-scale using computer simulations. Our group co-operates with theoretical research groups in the field of computational neurobiology. This collaboration often solves problems that would otherwise require a large number of additional experiments to answer using physiological methods. Additional animal experiments are thus avoided.

Why were they not suitable?

Because we are interested in the interactions that take place while the animal is actively interacting with the environment the methods mentioned above cannot be used on their own. For example, even if it were possible to generate organoids that closely mimic the brain regions we are interested in, these

would not have sensors (ears, eyes) that would integrate information and generate behaviours. Computer simulations, while very useful to predict and guide future research, can only simulate activity based on real data obtained from living brains.

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?

To ensure that experiments are adequately statistically powered we rely on our experience as well as on exhaustive literature searches to better understand the effect sizes obtained by colleagues performing similar experiments.

To manage the colonies of genetically altered mice, we will obtain advice from the internal colony management group.

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

Electrophysiology with multi-electrode silicon probes: because of their high number of contact points, the use of these electrode types to sample neuronal activity increases the number of cells we record at any given time, something that has dramatically reduced the number of animals we use to address a specific question..

Recording the neuronal activity in more than one brain regions: By recording from several regions simultaneously in a given mouse, across several days, we can obtain more data with less animals.

Head-fixed neuronal activity sampling and manipulation: by maintaining a fixed position between the animal's sensory organs (ears, eyes...) and both the sensory stimulation devices (speaker/screen) and/or reward output (e.g. nose-poke) we decrease the variability in behavioural and neuronal activity outcomes, requiring less trials per stimulus-outcome combination and in the long run, less animals.

Sampling and recording non-task related behavioural outputs such as pupil size, whisker and facial movement, and/or full body images. This gives us additional information that complements the neuronal and task-related behavioural sampling, allowing us to obtain more information from a single recording and an individual mouse.

We design our experiments well ahead of time, with the help if NC3Rs, planning a few stages in advance, sometimes covering time windows of many months (e.g. if experiment 1 leads to outcome A, we will carry out experiment 2 not 3; and so on). We typically use excel sheets and have now started to explore EDA (Experimental Design Assistant, NC3Rs).

Downloaded: 9:16am, 10 Feb 2026

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 guidelines to design and plan experiments (e.g. https://norecopa.no/prepare).

We carry out extensive literature searchers and discuss plans within (weekly) and outside the lab (conferences, zoom calls, departmental seminars), with colleagues that are experts in the field.

We carry out technical or experimental pilot studies with approx. 4 mice to obtain preliminary data that will guide the design of the experiment. This prevents that use a large group of mice and then find out that the protocol design was not optimal for the learning of the task.

We randomize the stimuli (sounds) presented, their reward location (side), and the order in which they are placed to ensure there are no confounded effects that would force us to run more experiments in the future.

We plan control experiments very carefully, whether for behavioural comparison or neural activity measurements. We use for example within subject controls (comparing the behaviour/activity in the same animal on different days or sessions) for comparisons across time, diminishing thus the number of animals relative to an across-subject comparison (where the behaviour/activity is measured at different times but on different animals).

Computational modelling in collaboration with expert labs: this allows us to decide the next experimental direction based on predictions derived from modelling.

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 mice from both genders and ages ranging between neonates and adult.

We will use methods that are well established in the field of systems neuroscience:

- Behaviour: we will use 1) automatized behaviours in enclosures designed for a group of animals to live in for weeks at a time. The behaviour of each mouse will be detected through cameras and/or presence sensors (e.g. identifying chip implanted under the skin, nosepoke) and the performance in a task will be volitional; and 2) single-mouse acute behaviour where a single mouse will be tested for a restricted amount of time and its behaviour motivated through water reward.

- Neuronal activity sampling: We will measure the activity of brain neurons using different types of sensors that are sensitive to either changes in voltage or calcium concentration. We will take these measurements in either freely moving animals, head-fixed mice, or anaesthetized mice.

- Techniques to manipulate the activity of neurons: We will manipulate neuronal activity with light or with drugs, also in either freely moving animals, head-fixed mice, or anaesthetized mice.

- Genetically altered mice: mice with modifications in specific brain cells or specific brain pathways will be used to sense or manipulate the activity of specific brain cells and regions.

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

Because we are studying the adaptive responses of brain pathways and regions to changes in the environment during different mental states (asleep, awake, behaving, passive), we need a live animal that is interacting with the sounds and the environment in which the sounds are played.

We cannot use non-mammalian animals because they do not have the same brain regions whose function we are trying to understand. We cannot use embryos or very young animals as they are not yet interacting with their environment.

We are, however, endeavouring to address some of the questions raised here in parallel on a large-scale using computer simulations. Our group co-operates with theoretical research groups in the field of computational neurobiology. This collaboration often solves problems that would otherwise require a large number of additional experiments to answer using physiological methods. Additional animal experiments are thus avoided. However, as explained above, the remaining questions cannot be answered without animal experiments, and these remain necessary to deliver the project outputs and thereby benefits.

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

As described above, a decisive contribution to minimising the number of animals is achieved by the advanced experimental methodology in our research project. Many of the experiments we are endeavouring to carry out were not even methodologically possible 10 years ago. We use novel refined experimental paradigms in both the electrophysiological recordings and in the behavioural paradigms.

We ensure that animals are acclimatized to their surroundings for at least 1 week. We now use tunnel handling throughout, as this has been demonstrated to decrease the stress of the mouse.

Behaviourally, the Audiobox and the Audioworld are both naturalistic volitional behavioural paradigms, where animals live in groups over days with little contact with the experimenter. The animals are thus constantly in a setting where they can choose when the eat or drink, who they socialize with, where they sleep and when, which is on contrast with other types of behaviours (also necessary for certain questions) where the animal is trained on its own, during the day (mice are nocturnal), and motivated by thirst or hunger. These behavioural designs allow us to study the behaviour of mice in settings that favour their participation in the task as well as their wellbeing.

Downloaded: 9:16am, 10 Feb 2026

For behaviours that are performed under head-fixation using water reward (where the animals is under water restriction), we have optimized the level of habituation to the experimenter that we do before the actual experiment begins, as well as the method for training to head-fixation which is performed over several days. The level of water restriction is monitored carefully on a daily basis and the weight and appearance of the mouse noted. We use the minimal restriction necessary to keep the weight above 85% of the expected weight and make sure that the animals receive at least 1ml of water per day. They typically obtain this or more through their participation in the task. if this is not the case (during the first sessions) we top up the amount until 1ml. Typically the animals remain closer to 90% than 85%. We are always optimizing these methods and are now considering using more palatable food to ensure that the animals do not lose their appetite during the initial days of water restriction. We are currently considering using other liquid rewards that do not require water restriction, for example we are considering giving the animals acidified water ad libitum and normal, more palatable water, as reward during the behavioural task.

Electrophysiology with multi-electrode silicon probes: the use of these electrode types to record the activity of neurons in the brain, increases the number of cells we record at any given time, decreasing the length of an experiment and thus decreasing the harm to the mouse.

The protocols we use for pain management before, during, and after surgery are optimized through discussions with our NVS. For example, we now include analgesic in the water of the animals starting 2 days before surgery so that the animal is habituated to its taste and is not suspicious of it after surgery, when it most needs it. This means that the animal's pain and discomfort are minimized. The same is true for protocols designed to ensure the correct temperature, and hydration of the animal during and after surgery, when the animal is less likely to be able to control these itself. We also sterilize the instruments and clean the wounds carefully minimizing the likelihood of post-operative infections.

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

We will consult:

- colleagues' publications

- databases where this information is regularly updated, such as Alternatives to Laboratory Animals Journal (ATLA), LASA and NC3Rs.

- databases such as PREPARE or ARRIVE, which offer suggestions on adaptations one can perform to increase the wellbeing of the animal.

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

We will stay in communication with academic colleagues as well as colleagues in the animal facility (Named Animal Care and Welfare Officer or NACWO, Named Veterinary Surgeon or NVS, and Named Information Officers or NIOs).

Downloaded: 9:16am, 10 Feb 2026

We will consult with databases where this information is regularly updated, such as Alternatives to Laboratory Animals Journal (ATLA)

Downloaded: 9:16am, 10 Feb 2026