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NON-TECHNICAL SUMMARY

Deciphering neural circuits that integrate visual and somatosensory cues for avian flight

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

sensory neurobiology, multisensory integration, bird, neuroanatomy, locomotion

Animal types	Life stages
Taeniopygia guttata	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?

Flying birds integrate sensory information to support diverse strategies for manoeuvrability and stabilisation that do not rely on feedback from the ground. The brainstem and cerebellum are key brain sites for complex sensory integration vital for numerous cognitive processes and behaviours. The functional organisation of sensory information in the avian brainstem and cerebellum is still poorly understood. This project seeks to characterise sites in the cerebellum that receive visual and tactile signals to understand their role in translating sensory stimulation into fine motor control.

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?

Investigating the timing and integration of sensory signals in the cerebellum will inform our understanding of neural circuits and mechanisms that control stabilisation, steering, and fine motor control. This project will also provide insight into the principles of multisensory integration that are prerequisite for biomedical applications (e.g., rehabilitation devices and prostheses) and engineered systems that must rapidly prioritise and process parallel streams of sensory information. Studying the underlying mechanisms of multimodal integration that control avian flight will have direct input for biomedical and bio-inspired technologies.

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

Outputs will include novel insights and publications related to the neuroanatomy and neurophysiology of the avian cerebellum. Characterising the neurophysiological principles underpinning avian sensorimotor control will provide insights into these conserved circuits in other species, including humans, that are involved in visuomotor behaviours.

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

In the short term, new insights into the functional circuitry of the cerebellum will guide new research aims and funding applications to support further work building on these findings.

In the long term, basic science discoveries and publications arising from this project will contribute to a broader understanding of avian flight control. These data can be used by other neuroscientists, biomedical researchers, engineers, and members of the private sector to design research questions and engineered systems related to sensorimotor control. The proposed research will inform translational research on clinical conditions, neurodegenerative diseases, and syndromes involving visuomotor dysfunction. A better understanding of the functional organisation of the cerebellum will aid researchers and clinicians in these fields.

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

Outputs of this project will be maximised through internal collaborations and dissemination of new knowledge at national and international conferences. We will employ cutting edge neural recording techniques. This expertise can be applied in other animal systems, and we will form collaborations to ensure that these techniques are broadly available. National and international conferences provide important opportunities to discuss new findings and research pathways with colleagues, present data, and to establish new collaborations.

Species and numbers of animals expected to be used

- Other birds: No answer provided

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 have expertise identifying and recording from discrete sensory brain regions in small birds. We will use zebra finches (T. guttata), a captive species that perform rapid sensorimotor transformations. Many visual nuclei are highly conserved between avian and mammalian brains, so the work proposed here will be directly relevant to understanding critical sensorimotor pathways underlying human health and disease. Thus, birds are an effective model for understanding general principles of brain anatomy and function. Zebra finches are an appropriate model for this work because (1) they are a common laboratory model for avian neuroscience used by an active community of researchers with tools and resources for exploring neurophysiological questions; (2) my previous work has defined relevant midbrain-cerebellar visual pathways; 3) my pilot studies have identified stereotaxic coordinates for key regions and proof-of-concept for neurophysiological recordings in adult zebra finches, which reduces the chance of experimental failure.

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

Animals will undergo non-recovery surgical procedures that allow access to the brain for neural recordings. Animals will be under surgical anaesthesia for these procedures, and euthanised at the end of the relevant protocol or if signs of pain or discomfort cannot be managed with anaesthetics. Animals will be anaesthetised at the start of the procedure using an injectable anaesthetic, and depth of anaesthesia will be monitored throughout. All procedures begin by surgically accessing the brain, followed by using electrophysiological tools to identify and record from visually-responsive neurons and/or somatosensory neurons in discrete brain regions. During a subset of experiments, a pharmacological agent (e.g., tetrodotoxin and/or muscimol) will be injected into discrete sensory regions of the brainstem. Subsequently, neural recordings in the cerebellum will be performed as described above. Procedures typically last 6-12 hours but can last longer. After neural recordings are completed, the animal will be administered an anaesthetic overdose and transcardially perfused.

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

Our protocols are based on well-established procedures that have undergone considerable refinement. Surgical anaesthesia will be induced at the start of the procedure. All procedures are non-recovery and anaesthesia will be monitored regularly. Animals will not suffer more than transient pain and distress and no lasting harm from handling and the initial injection, and there will be no cumulative effect from repeated injections as these are non-recovery procedures.

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)?

All procedures carried out under the proposed licence will be non-recovery procedures under general anaesthesia. Animals may be used for non-regulated procedures prior to their use on this licence.

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

- Killed

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 research addresses gaps in our understanding of how vertebrates fuse visual motion and tactile information in the cerebellum to support complex locomotion and navigation. The research requires measurement of neural activity from multisensory sites. There is no alternative to live animals for studying these sensorimotor circuits. Developing computer models to make predictions about multisensory integration requires empirically observed activity as an input. Simultaneous recordings of neural activity during sensory stimuli allows us to characterise population-level responses and inform models that will generate future avenues of research.

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

There are no non-animal alternatives that can address systems neuroscience questions about how neurons respond to sensory stimuli.

Why were they not suitable?

In all the protocols to be used, alternatives are not available that replicate the response of neurons in discrete brain regions to complex sensory stimuli or how this activity is impacted by pharmacologically blocking specific inputs.

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 extensive experience performing experiments using electrophysiology in avian species, including zebra finches, hummingbirds, and pigeons. Using this experience and the large body of published literature in this field we estimated yields for (1) the number of birds in which we will find responsive cells and (2) the number of sites in each bird that will have responsive cells. Using these estimates we calculated how many birds will be required to record the appropriate number of cells.

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

Minimum animal numbers will be used to obtain statistically significant results, based upon power calculations, our extensive experience, and the literature. We will make every effort to improve experimental design and ensure that maximum scientific output is achieved with the smallest number of experiments consistent with scientific soundness. We perfuse all brain tissue so that immunohistochemistry studies can be performed to support other studies. Collaboration with colleagues will ensure that animal tissues are used as effectively as possible, avoiding unnecessary experimental duplication. Presentation order of visual stimuli will be randomised.

We have used the PREPARE guidelines and NC3R tools to guide our experimental design. We use power calculations and expected yield (from the literature and experience) to ensure the minimum numbers of animals are used in the programme. The following power calculation determines the minimum sample size (number of cells) required to gain power above 0.8 using the pwr package (1.3-0) in R v4.0.3. For k = 3 groups, effect size f = 0.25, a = 0.05, power = 0.8; n = 53 cells are required for each group. Based on other neural recording studies using similar techniques in the literature, and this power calculation, we expect to need recordings from 60-100 visually responsive or tactile-sensitive neurons per region to have enough statistical power (mixed-effects regression models).

We will record multiple individual cells, across multiple recording sites, in each bird. Thus, we estimate an effective n at each level to approximate the number of animals. Based on prior experience, and a significant body of literature in the avian visual system, I anticipate requiring 20 individuals for each study to achieve this number of cells. Target size and accessibility (depth, surrounding vasculature) affects success rate for each individual (i.e., whether a target will be found). We expect to identify visually responsive or tactile-sensitive cells in 80% of birds. On average, we expect to record from 6 sites/individual (1-2 cells/site with a glass/tungsten electrode) with an expected success rate of 60% for finding visually responsive cell(s) at a given site. Thus, we expect to record 3-5 visually responsive cells per individual. To record 80 cells, we use the following equation to calculate the number of required individuals:

(target number of cells) / (expected cell yield/individual) = number of individuals

80 cells / (0.8 * 0.6 * 9 cells) = 18 individuals

Protocol 1: We expect to need a maximum 20 birds for each study, and to perform eleven studies on this project licence (maximum 220 individuals total). Other studies in birds using similar approaches have achieved a similar yield.

We are investigating the implementation of tools that may increase the yield of each recording site -- for example, using cutting-edge neural recording technology (high-density probes; single multi-valued recording) to collect rich datasets from each individual, thus reducing the number of animals required, keeping with the 3Rs objectives. Recent studies with these probes have acquired 10-25 individual cells per site, and >200 units for multi-site network analyses. Typical recordings yield 6-10 single units per site with a 32-channel array.

Protocol 2: Up to 10 animals will be used in Protocol 2 for control tissues and setting up immunohistochemistry or other in vitro assays.

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

I have expertise in performing electrophysiological recordings in zebra finches and other avian species and have mapped the locations of several visual and somatosensory brain regions. This expertise has the potential to increase the number of successful recording tracks, thus reducing animal numbers.

We are able to use relatively few animals because we rely on response properties of cells when placing our injections and determining recording sites. We will increase the yield of each recording site by using cutting-edge neural recording technology (high-density probes; single multi-valued recording) to collect rich datasets from each individual, keeping with the 3Rs objectives.

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 procedures described in the proposed project are terminal and carried out under general anaesthesia, minimising pain, suffering, distress, or lasting harm to the animals.

Adult, male zebra finches will be sourced from a local commercial or other approved supplier in the UK. Zebra finches are a common avian model for neuroscience studies. The neural activity of the zebra finch song system is well-described. The project described in this licence proposal will address

gaps in the literature related to the neural mechanisms involved in visual motion processing and tactile sensing. The elaborate visual system of birds and their reliance on visual inputs, rather than feedback from the ground, during flight, makes birds an ideal model for studies of visual motion processing. It is also thought that somatosensory inputs are used for flight guidance, but the mechanisms governing the processing and integration of these inputs lack significant study. These birds will also be used because of their future potential for pharmacological and behavioural studies using complex sensory stimuli as a challenge during flight. In addition, these studies will be foundational for selecting the most salient sensory cues for future studies.

Animals will be under general anaesthesia, a small craniotomy performed, and then neurons in brain regions of interest will be recorded using electrophysiological techniques while sensory stimuli are delivered (e.g., videos of moving dots or gratings, airstreams, feather deflections, etc...). Visual and somatosensory neurons respond to these stimuli in animals under general anaesthesia. Anaesthetic depth will be monitored regularly, and supplemental doses given as needed.

Pharmacological blockade via nano-scale injections of inhibitory receptor agonists or channel blockers to discrete brain regions will be used to study necessity and sufficiency of midbrain-cerebellar projections. A small injection of dye may be used to mark recording sites and then the animal will be given an overdose of anaesthesia prior to undergoing a transcardial perfusion with saline and fixative. In some cases, animals may be euthanised by a Schedule 1 method instead of undergoing transcardial perfusion.

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

This is not a developmental study. Studying visual and tactile processing networks requires a fully developed neural system to understand how adult visual and tactile neural networks process information relevant for locomotion. Systems neuroscience studies, like this one, seek to experimentally open feedback loops to understand how specific stimuli affect neural activity in key regions, and how this activity impacts other nuclei within a neural pathway. The avian visual system has structures homologous to the mammalian visual system and these studies will provide insights into basic principles of midbrain-cerebellar network organisation and function that are applicable to other systems.

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

Local or topical anaesthetic will be used at the site of incision in addition to general anaesthesia.

For husbandry-based refinements, we will provide toys in the aviary for enrichment and cuttle bone to maintain bill health.

Handling stress will be minimal as birds typically will be handled for less than a minute prior to induction of anaesthesia.

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

We will plan, conduct, and record our experiments so that we are able to publish our results following the ARRIVE and PREPARE guidelines.

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

We will have regular contact with AWERB, the NACWO and animal technicians at the housing facility to review current approaches and whether there are any new 3Rs opportunities. AWERB and the NVS will be consulted regularly to ensure the most refined approaches are being used. LASA guidelines are being used for preparing for surgical procedures. We will use NC3R newsletters and other resources to stay abreast of advances in the 3Rs.

We have the unique advantage of wide-ranging expertise and state-of-the-art veterinary resources. We will take advantage of these resources to work with specialist anaesthesiologists to optimise protocols.