G. NON TECHNICAL SUMMARY (NTS)

Project title: Multisensory integration: Olfaction and Metabolism
Duration of project - years: 5
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: **YES**
(b) translational or applied research with one of the following aims:
(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants: **NO**
(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants: **NO**
(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes: **NO**

(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b): **NO**
(d) protection of the natural environment in the interests of the health or welfare of man or animals: **NO**
(e) research aimed at preserving the species of animal subjected to regulated procedures as part of the programme of work: **NO**
(f) higher education or training for the acquisition, maintenance or improvement of vocational skills: **NO**
(g) forensic inquiries: **NO**

Keywords:
Olfaction, Metabolism, Neuroscience, Brain circuits

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

This project has 4 objectives that aim to determine how changes in the level of hunger alter brain activity responsible for processing odours and how these changes result in altered perception, feeding behaviour and metabolism.

1) We will use advanced brain imaging methods to observe how the neural circuitry responsible for processing odours is altered when the metabolic state is changed from hungry to satiated. Depending on the neuronal population of interest we will use mice expressing genetically encoded indicators of neural activity restricted to a single cell type or we will use alternate methods to express indicators in the neurons of interest. Prior to an experiment an animal will be fasted overnight to induce hunger, it will then be anaesthetised and a small window, to allow imaging of the brain, will be placed in the skull. The activity of the labelled neurons will be recorded in response to odours before and after manipulation of satiety by inflating the stomach or injection of for example glucose. At the end of the experiment the animal will be humanely killed under anaesthesia. The imaging technique to be used enables the activity of hundreds of neurons to be recorded simultaneously, a refinement which will reduce the number of animals required.

2) We will then use the same experimental paradigm described in 1 together with pharmacological tools to determine the molecules and receptors responsible for sensing satiety and which cause the resulting changes in brain activity.

3) To link the observed changes in neural activity to altered olfactory perception, feeding behaviour and metabolism we will use genetic strategies to selectively block the molecules we identified in aim 2 in the specific neural population identified in 1 & 2. This will be achieved with intracranial injections of viruses designed to selectively knock down the receptor of interest and will be performed under stable and balanced anaesthesia. Animals are expected to recover quickly and will be given painkillers and post-operative care just like people recovering in hospital, they are unlikely to experience any adverse effects. Once the receptor of interest has been knocked down we will determine whether this manipulation affects olfactory sensitivity using simple behavioural tests and will also determine whether metabolism is altered. This will be determined by giving the mice a high-fat diet and monitoring food intake, weight gain and energy expenditure. The results from this work will describe how changes in the activity of a brain circuit cause altered perception and feeding behaviour and may identify potentially druggable targets to reduce food consumption.

4) To determine the brain circuit changes that occur during learning of a food odour and whether food odours are more susceptible to modulation by satiety, we will use the same imaging strategies as described in 1 but will perform the experiments in awake animals to allow comparison of neural activity before and after learning. Similar to the experiments in aims 1& 2 this requires implantation of a device to stabilize the head and placing a window over the brain area to be imaged. This will all be performed under stable and balanced anaesthesia and with post-operative pain relief mice recover from this surgery quickly and are unlikely to experience any adverse effects. Once the mice have recovered they will be trained to remain with their head fixed on a treadmill where, under their own volition, they are free to groom, run/walk, remain stationary or drink from a water spout. Mice quickly become accustomed to this protocol and during these periods neural activity will be imaged in response to delivery of odours. ,This will allow us to reveal how the activity of the brain changes as a mouse learns to associate an odour with food reward e.g. sugar.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?:
Identifying molecular targets that modulate the neural circuitry in the olfactory bulb responsible for the control of feeding and metabolic health could lead to drug therapies to improve metabolic health. Additionally the insights gained from studying how neural activity is altered during learning will have broad benefit to the neuroscience community as this is still an outstanding question.

What types and approximate numbers of animals do you expect to use and over what period of time?:
Genetically altered mice ~ 610 used in experiments over a 5 year period. Around 2000 will be bred to achieve the correct genotypes

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?:
There are negligible adverse effects expected. The aspect of the project with moderate severity involves recover from general anaesthesia after surgery which is classed as moderate severity. The animals will be euthanised at the end of experimental protocols

Application of the 3Rs

Replacement:

To study multi-sensory integration there are no alternatives but to use animal models. Furthermore,

the ability to measure neural activity in defined components of the neural circuitry is only afforded in animal models.

Reduction:
Our aim is to reduce the number of animal experiments whenever possible. All experimental parameters are monitored and recorded to ensure the reproducibility of the experiments. The methods we use record from 100s of neurons simultaneously which will reduce the number of animals used.

Refinement:

These studies aim to characterise the properties of the neural circuitry of the olfactory bulb and how it responds to altered metabolic state with the hope of a better understanding of olfaction and metabolism in humans. The organisation of the mammalian olfactory bulb is distinctly different to invertebrates or even other vertebrates. The observation that olfaction and metabolic health are linked has been made in both humans and rodents.

The genetic amenability of mice also means that many genetic tools are available to study circuit function. This project relies on such lines of mice, which express an optical reporter of neural activity in defined cell types.