Private & confidential: *Please be aware that the contents of this form may be made public resulting from the "Freedom of information Act". Personal details will not be released.*

G: NON-TECHNICAL SUMMARY (NTS)

Please attach the Non-technical Summary as generated by your application in ASPeL.

Word limit; 1000 words

Project Title	CARDIAC CONDUCTION SYSTEM IN HEALTH AND DISEASE
Key Words	Heart, Heart failure, Pacemaker, Mouse models
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(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.

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

The *cardiac conduction system* is the electrical wiring system of the heart and it initiates the heartbeat and controls the heart's rhythm. The *cardiac conduction system* consists of three parts, (i) the sinus node, the pacemaker of the heart, which initiates the electrical impulse that causes the heart to beat, (ii) the atrioventricular node, which transmits the impulse from the top to the bottom of the heart and (iii) the His-Purkinje system, which transmits the impulse to the two powerful pumping chambers of the heart. It can be life threatening when the system goes wrong, because the heart does not beat or the heart beat is irregular. At the moment, the only treatment is the implantation of an artificial pacemaker in the chest and these are not without problems (for example, the battery runs down). We aim to study why the *cardiac conduction system* goes wrong. If we can understand why it goes wrong, we can design new treatment strategies. For example, our work over the last 10 or more years has shown that the system goes wrong because it loses 'ion channels'. Ion channels are the molecules responsible for the electrical impulse.

In this project, we want to discover why ion channels are being lost and our work is showing that it is regulatory molecules called 'micro-RNAs' and 'transcription factors' that are responsible. We have also shown that, in two cases, if we knock out the regulatory molecule responsible (we did it using an 'anti-micro-RNA'), we can reverse the *cardiac conduction system* disease. Using mice and rats as models, we will investigate *cardiac conduction system* disease in veteran athletes, heart failure patients, the elderly, obese patients and patients with thyroid problems. Strangely, veteran athletes who have been exercising at a high level for decades are more likely to need an artificial pacemaker. Heart failure patients have *cardiac conduction system* disease and it has been shown to increase the likelihood of them dying. The elderly frequently have *cardiac conduction system* disease and artificial pacemakers are mainly fitted to the elderly. Obese patients frequently have abnormal heart rhythms and some can be life threatening. Patients with thyroid problems have abnormal heart rates. In addition, we aim to study why heart rhythm problems occur at particular times of the day or night and why shift work, jetlag and insomnia etc. (which all affect the body's 'circadian rhythm') disturb the *cardiac conduction system*.

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)?

1. Advancement of current understanding of the mechanisms that control the cardiac conduction system in health and disease

2. Identification of 'druggable' small molecule therapies for cardiac conduction system disease that could ultimately lead to improvement in human health.

What types and approximate numbers of animals do you expect to use and over what period of time?

We anticipate using approximately 4390 mice over a period of five years.

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?

When studying disease, adverse effects are possible, but every effort is made to avoid them. With exercise training (severity categorised as moderate), mice are swim trained for up to 60 minutes twice a day for 1 month. The mice are constantly watched to ensure that no mouse cannot cope (in the worst case, drown). Mice are natural swimmers and our (extensive) experience is that the mice cope very well and become fitter and sleeker. For heart failure, under anaesthesia, the mice undergo surgery to tie a band around the major artery coming from the heart, the aorta. The heart, therefore, has to pump blood against a higher resistance and this leads to heart failure over six to eight weeks (severity categorised as moderate). Following surgery, the mice are given an analgesic (pain killer). After surgery, the mice are carefully monitored and up to 5% of the mice will display signs of suffering and have to be humanely killed immediately. After six to eight weeks, the mice are carefully monitored for signs of heart failure (for example, loss of body weight, inactivity) and at this point the mice will be humanely killed. For studying the day-night ('circadian') rhythm in the heart, mice are humanely killed at different times of day and night to take heart tissue. Mice are nocturnal and are active at night. To avoid working at night, the mice can be made to be active during our day and sleep during our night by altering the lighting of their cages. Experience shows that this has no discernible effect on the mice (severity categorised as moderate). For studying shift work or jet lag etc. when the body's 'circadian rhythm' is disturbed, the mice will alternate between three days at UK time, three days at Chinese time, back to three days at UK time etc. for 30 days. Experience shows that this 'jet lag' has no discernible effect on the mice apart from small irregularities in the heart's rhythm (severity categorised as moderate). To study ageing, mice up to the age of 27 months will be obtained from a breeder. On arrival, the mice will be humanely killed. At the age of 27 months (equivalent to 80 years for a human), half of all mice will have died of natural causes. The severity is categorised as moderate. To study obesity, mice will be raised on a diet high in fat for up to five months. Generally, the mice are not expected to have health and welfare problems, although there is a risk of skin abrasions, which can be treated (severity categorised as moderate). In some cases, animals will be fitted with a telemetry system to record the ECG or a mini-pump to deliver a drug. In both cases, this will involve a small operation under anaesthesia. Post-surgery the animals will receive an analgesic. There is a small risk (1% or less) of wound infection or wound breakdown. During the course of the work, animals will be anaesthetised to allow the recording of an echocardiogram or ECG. Other than unconsciousness, this is not expected to cause adverse effects. At the end, all animals will be humanely killed.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

This study aims to model human cardiac conduction dysfunction (that involves the integrated response of cells and organs) and identify the key underlying genetic changes. There is no viable alternative to using a small mammal as:

1. It is virtually impossible to procure human cardiac conduction system tissue

2. Cells isolated from the cardiac conduction system do not proliferate and are not amenable to culture for more than 48 hours

Therefore, the use of animal models alongside *in vitro* and *in silico* work is required. In this study, mouse models will be utilized. Mice are highly used in physiological research as they provide a comparable model of human cardiac physiology and cell function. The murine genome has been well characterised, and genetic manipulation can be performed with relative ease. This allows for the investigation of gene function within the context of the whole organ. While non-mammalian models are available, such as zebrafish, there are key differences in their cardiovascular physiology compared to humans (including incomplete separation of ventricles and circulatory physiology).

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

We always take measures to ensure that the minimum number of animals will be used. We have carried out these types of studies many times before and are highly experienced understanding the variability typically encountered and the number of animals needed to observe a statistically significant difference. Furthermore, power calculations are routinely carried out to ensure that we are using the minimum number of animals necessary. Where it is possible, we will use non-animal alternatives such as cell lines or computer models to test the validity of hypotheses before working with animals.

Refinement

Explain the choice of animals and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Refinement

The physiology of the mouse is similar to that of the human. It is a popular laboratory species and because it is commonly studied by others, we know a great deal about it (for example, where the *cardiac conduction system* is in the heart). This means that many techniques are readily available and are refined to be as minimally invasive as possible for the mouse. We know what drugs to use, how to administer them, at what concentrations and what adverse effects are possible.

General measures to minimise welfare costs to the animals

Animals will be group housed wherever possible. We will use full analgesia following surgery. We will conduct daily monitoring of animals at non-critical times. We will monitor animals more frequently following surgery and if an animal deteriorates. We shall regularly record body weight, respiratory rate, grooming, coat condition, ocular and nasal discharge, piloerection, hunched appearance, activity and other factors to allow us to accurately detect suffering and determine when an animal will need to be humanely killed. All animals will be humanely killed at the end of the study.