16 March 2015

Project title	**Circadian control of behaviour and metabolism**		
Key words	Obesity, diabetes, clock, biological rhythms, physiology		
Expected duration of the project (years)	5		
Purpose of the project	Basic research	Yes	
	Translational and applied research		No
	Regulatory use and routine production		No
	Protection of the natural environment in the interests of the health or welfare of humans or animals		No
	Preservation of species		No
	Higher education or training		No
	Forensic enquiries		No
	Maintenance of colonies of genetically altered animals	Yes	
Objectives of the project	The aim of this project is to further our understanding of the physiological and neural circuits which govern metabolism and energy balance. This work will also examine specifically how our body clocks (the circadian system) contribute to the regulation of metabolism and energy balance, determine the extent to which clock disruption may contribute to metabolic disease.		
Potential benefits likely to derive from this project	This work looks directly at the pathophysiology of metabolic disease. At present we have few successful or effective treatment options for patients. Therefore, the potential benefits of this research are high.		
Species and approximate numbers of animals expected to be used, and anticipated period of time	Approx. 14000 mice (including breeding) over 5 years		
Expected adverse effects and the likely/expected level of severity. What will happen	The majority of the research falls into the mild category, being confined to breeding, routine monitoring of behaviour, and environmental manipulations (diet, light, and ambient temperature). Studies in the moderate category include the surgical implantation of physiological		

to the animals at the end.	monitoring devices, slow release pumps, and other surgical techniques such as intraneural injection and removal of the adrenal glands. The animals recover well from these procedures, with adverse effects limited to the immediate postoperative recovery. As we are studying normal physiological and behavioural processes that govern circadian timing and metabolism, it is critical for our work that the animals are as healthy as possible. As soon as the scientific objectives have been reached, animals will be killed by an accepted and humane method.
Application of the 3 Rs	
1. Replacement Why do animals need to be used, and why non-animal alternatives cannot be used.	Many tissue and organ systems contribute to energy homeostasis, metabolism, and circadian rhythmicity, and therefore the pathways and events involved cannot be modelled within an in vitro cell or tissue culture setting. In-vitro assays do however offer a powerful model to test how genetic alteration of clock function may impact on the core circadian oscillator, the re-setting characteristics of this oscillator to biochemical stimuli, and the role of metabolic-acting drugs and compounds on clock function. By using lines of genetically modified mice expressing a clock gene reporter, it is possible to define key responses using an in-vitro model and use these data to inform the design of subsequent in-vivo experiments. Whenever possible stable cell lines will be used to investigate clock and metabolic coupling (for example in 3T3-Li adipocytes and rhythms in lipid metabolism).
2. Reduction How the use of minimum numbers of animals will be assured	To ensure that we use the minimal number of animals, careful consideration is given to experimental design. Based on our experience of the animal models and techniques used, we use power analysis calculations to determine the minimum number of animals required. Generally, group sizes of 6-8 are required to achieve 80% power assuming an effect size of 30-50%. Simultaneous recording of multiple physiological responses offers a significant reduction to the number of animals used, as well as increased statistical power. Novel technology for in vivo recording of gene transcription significantly reduces animal use, through lessening the need for serial tissue sampling. Similarly, non-invasive technology (EchoMRI) will allow serial assessment of body composition, again reducing the need for large numbers of terminal dissection.

	Whenever possible stable cell lines will be used. In particular, use of circadian clock-reporter lines allow extensive use of in-vitro assays on isolated cells and tissue (e.g. to test the effect of clock acting drugs prior to any application in vivo).
3. Refinement Reasons for the choice of species and why the animal model(s) to be used are the most refined, having regard to the objectives. General measures to be taken to minimise welfare costs (harms) to the animals.	Our studies focus on mice. Mice offer unparalleled opportunities for investigations of the underlying genetic mechanisms involved in circadian timing and physiological function. Many pathways involved in normal regulation of metabolism, as well as the pathological events associated with obesity are well conserved between mice and humans. The principal mechanism for ensuing minimal welfare cost is close monitoring of the animals, understanding the normal physiology of the animals, and optimal design of all animal experiments regardless of expected severity.