G: NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed project clearly using non-technical terms which will be understandable to a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary may render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at http://scienceandresearch.homeoffice.gov.uk/animal-research/).

(WORD LIMIT: 1000 WORDS)

Please complete the following:

Project Title (max. 50 characters)	Postnatal light effects on circadian function
Key Words (max. 5 words)	Circadian system, development, environmental light, early life experience, neurodevelopmental programming
Expected duration of the project (yrs)	5
Purpose of the project (as in section 5C(3)[1]	

Basic research	**Yes**	No
Translational and applied research	Yes	**No**
Regulatory use and routine production	Yes	**No**
Protection of the natural environment in the interests of the health or welfare of humans or animals	Yes	**No**
Preservation of species	Yes	**No**
Higher education or training	Yes	**No**
Forensic enquiries	Yes	**No**

[1] Delete Yes or No as appropriate.

	Maintenance of colonies of genetically altered animals[2]	**Yes**	No
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Postnatal light experience exerts strong effects on developing animals, altering their future daily (circadian) behaviour under different light environments. Importantly, the long-term alterations in circadian behaviour are linked to modifications at the level of the brain clock in mice. Moreover, in addition to the biological clock, abnormal postnatal light experience has long-term effects on the regulation of the stress axis and can lead to a depressive behaviour in adulthood. However, the mechanisms underlying these effects are still unclear. This project aims at determining: 1) the molecular and neural mechanisms behind the long-term effects of early light exposure on the developing circadian system, and 2) the links between postnatal light experience and later-life health and wellbeing.		
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)?	The characterisation of the effects of postnatal light environment on both central and peripheral clocks, behaviour and neural activity is key for a better understanding of the mechanisms behind the interaction between environment and the circadian system during development. In addition, the results of this project will be critical to understanding the health-related significance of postnatal light environment alterations and are the first step towards the design of improved husbandry conditions in animal facilities and better care for preterm infants in Neonatal Intensive Care Units.		
What species and approximate numbers of animals do you expect to use over what period of time?	These experiments can only be conducted in animals, as they involve studying the long-term effects of early life experiences on behaviour and physiology, and mice are the species with lowest sensitivity commensurate with the scientific aims. The use of a combination of in vivo, in vitro and ex vivo techniques will allow us to maximise the		

[2] At least one additional purpose must be selected with this option.

	amount of data collected from each animal, and hence animal numbers will be minimised. We plan to use approximately 2850 mice over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most of the procedures included in this project are mild and therefore, minimal pain or suffering is predicted. Possible sources of distress may be the exposure of animals to constant light or constant darkness during long periods of time, implantation of temperature-monitoring systems, behavioural testing and blood sampling. Animals will be continually monitored for signs of distress and adequate analgesia and anaesthesia will be provided when necessary. At the end of all the experiments, mice will be culled and their tissues (e.g. brain) collected for either organotypic tissue culture or protein/mRNA quantification through immunohistochemistry, qPCR or any other suitable technique. Blood may also be collected to determine the expression of key circadian and stress markers. Animals will be culled by an appropriate and humane method at the end of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is essential that the research is conducted on animals since it involves studying the effects of early environment or experience on the later life of the animal. It is not possible to replicate these effects in vitro, nor do we have sufficient baseline data to run realistic simulation exercises. Consequently, there is no realistic alternative to the use of intact animals to address this objective.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have used our long experience in the field of circadian rhythm development to statistically design the experiments included in this project to use the minimum number of animals to obtain strong, well-sampled results. Furthermore, the use of a combination of in vivo, in vitro and ex vivo techniques will allow us to maximise the amount of data obtained from each animal. This wide range of

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<tr><td></td><td>experimental readouts, targeting different levels of organisation (behavioural, physiological, tissue level and molecular), is a key approach of our integrative project.</td></tr>
<tr><td>3. Refinement

Explain the choice of species 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.</td><td>As a lower order mammal, mouse studies have a general applicability but low species sensitivity. In addition, the opportunities for genetic modification are greater in mice than any other vertebrate species, and we now have a wealth of data showing the long-term effects of postnatal light environment on mouse circadian function, stress levels, physiology and behaviour. For all these reasons, we believe that mouse is the species which will provide us with the most satisfactory results.

The transgenic mice included in this project do not show any alterations in their daily rhythms and are not known to be associated with any neuropathic pain.

Most of the procedures included in this project are mild and therefore, no pain or suffering is predicted. Possible sources of distress may be the exposure of animals to constant light or constant darkness during long periods of time, intraperitoneal implantation of temperature-monitoring systems and blood sampling. Animals will be continually monitored for signs of distress and adequate analgesia and anaesthesia will be provided when necessary.</td></tr>
<tr><td>For Office Use Only</td><td></td></tr>
<tr><td>Will the project be subject to Retrospective Assessment?[1]</td><td><table><tr><td>Yes</td><td>No</td><td>Date due[3]:</td></tr></table></td></tr>
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[3] The retrospective assessment should be completed, agreed with the establishment AWERB, and submitted to the Home Office within 3 months of this date (or when the project terminates if earlier).