IRISH MEDICINES BOARD
NON-TECHNICAL PROJECT SUMMARY

NON-TECHNICAL PROJECT SUMMARY DETAILS
Project reference number: V010/2013
Project title: Research investigation of the neurochemical and behavioural consequences of psychotropic drug administration in the rat
Duration of the project work (months): 36 months
Project keywords: Olfactory bulbectomy; rat; antidepressant modelling; behaviour; neurochemistry
Purpose of the project under Article 5 of Directive 2010/63/EU: Translational and applied research; human nervous and mental disorders
Project objectives, scientific unknowns or scientific or clinical needs being addressed: Models of depression are of growing importance as methods of identifying antidepressant properties of substances and in the development of novel antidepressant treatments. This study will explore the functioning of the central serotonergic system in the Olfactory Bulbectomy rat model of depression. Olfactory bulbectomy (OB) is a surgical procedure which removes the olfactory bulbs, an area in of the brain responsible for the sense of smell. This procedure results in changes in behaviour and the chemistry of the brain that are similar to clinical symptoms of depression experienced in humans. Serotonin is a principal chemical used to transfer signals from cells of the brain (neurons) to cells throughout the body. Serotonin function is altered in depression and can lead to subsequent changes in behaviour and influence mood. For this reason, serotonin is the target of action for many currently marketed antidepressants. The first part of this study will explore the major components involved in transmission of serotonin in neurons, construct a time course for the development of such changes and investigate the ability of chronic antidepressant treatment to treat the depressive effects induced by the OB rat model. The first set of studies will provide a unique and complete overview of the components of the serotonergic system in the OB rat model and will lead to an improved understanding of how these relate to those observed clinically in humans. The second part of this study will investigate the effect of gender in the OB model, firstly to examine whether changes in the OB behavioural syndrome exist between male and female subjects, and secondly whether OB has any effects on reproductive performance and / or on the development of the resultant offspring. Such studies have considerable clinical relevance with the high prevalence of depression amongst females and also address concerns that depression in pregnancy or the neonatal period can have long term consequences on the developing offspring. The anti-depressant effects of the opioidergic system in the OB model will also be investigated to determine if opioid-like substances, such as morphine, have any anti-depressant properties in the OB model in order to search for new clinically effective antidepressants.

The third and final part of this study will investigate variability in psychotropic or mood altering drug responses through behavioural studies. Typically results from these types of behavioural pharmacological studies vary between laboratories therefore this part of the study will explore the possible sources of variation in relation to these pharmacological assays. The expected sources of variation include extensive environmental enrichment being provided to the animals and variations in the housing of animals, for example light variations, type of bedding used, etc. In addition, recently introduced legislation regarding animal welfare will require improvements in bedding and environmental conditions. The project objective will explore the above parameters to evaluate whether any change in baseline and drug-induced behavioural responses are observed.

Potential benefits likely to derive from this project:

Studies involving these preclinical models are expected to have the following benefits:

1. Provide a comprehensive evaluation of the role of the central serotonergic system in the OB model of depression, at a variety of levels, and whether these changes are modified by effective antidepressant treatment.
2. Identification of differences in the OB model between males and females, which could have an important bearing on the preclinical evaluation of antidepressants in the future.
3. Examination of whether the offspring born to mothers who have received olfactory bulbectomy are altered in their development and therefore determine if the OB model will be useful as a model of maternal depression.
4. Contribution to understanding the role of the central opioidergic system as a novel target for antidepressant drugs, potentially opening up a new treatment option to complement the existing drug strategies.
5. Creation of a single test battery for evaluating antidepressant activity for future drugs which would draw on several distinct behavioural and neurochemical endpoints, and facilitate the production of a robust and reliable test system for future drugs. This would lead to future drugs being evaluated in an efficient manner and therefore reduce the number of animals required for future studies and the time taken for such evaluation.
6. Aid in the identification of environmental parameters that may impact results of drug-induced behaviour studies used in the evaluation of drug properties. This information will contribute to refining future behavioural pharmacological experimental designs.

Species and approximate number of animals expected to be used:

1300 rats

Expected adverse effects on the animals, the expected level of severity and the fate of the animals:

Rats will undergo surgery to remove the olfactory bulb in order to facilitate depressive-like behaviour, which has a moderate severity classification. Associated adverse effects, although rare, may include surgical infections and initial weight loss after surgery.

Rats will be individually housed for the duration of the behavioural studies. Social isolation of the rats is classed as moderate in severity. Animals will be positioned to have visual contact with other animals in order to reduce any distress experienced.

For the purpose of the study, animals will be separated into a number of groups. One group of animals will undergo a series of behavioural tests, the first of which are of short duration and which are classed as being mild in severity and do not involve any pain to the animals. Animals will undergo an additional behavioural test, called the forced swim test, in which animals are placed in a cylinder filled with water and from which they cannot escape. This procedure can cause stress to the animal and so has a severity classification of severe. In order to minimise the distress caused, animals will be dried

and kept warm after being in the water and this procedure will only be performed by trained and experienced personnel. Finally two behavioural tests of moderate severity will be performed; the first of which involves animals being placed on a heated surface in order to examine their ability to react to a painful stimulus. Exposure to the heated surface will be minimised to a maximum exposure time of 40 seconds in order to avoid any damage to tissues. The second moderate procedure involves challenging the rat's territorial behaviour; an unfamiliar or intruder rat will be placed in the home cage of a resident rat in order to observe changes in territorial behaviour. The exposure will be moderately stressful for both rats and so duration of exposure will be minimised in order to avoid unnecessary suffering and distress. Associated adverse effects may include injury due to attacks from the rats.

Finally, offspring from pregnant mothers who have previously undergone the OB surgery will be examined to determine the effect of maternal OB surgery on the behaviour and development of pups. These tests all have a mild severity classification. Adverse effects associated with each of the procedures above include mild distress to the pups as result of being separated from their mothers for the duration of these procedures. For this reason, the procedures will be performed for a maximum time of two minutes in order to ensure the time separated from mothers is kept to a minimum.

At the end of the study all animals will be euthanised humanely.

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Replacement - why animals need to be used for this project and why non-animal alternatives could not be used:

The purpose of these studies is to evaluate animal behaviour and central molecular and neurochemical signalling. By its nature, animal behaviour as a resultant of a complex interplay between environmental cues and the interaction of a range of central neurotransmitter pathways cannot be evaluated except in an intact mammalian organism. For this reason, the use of a non-animal alternative is not possible.

Reduction - how the use of minimum numbers of animals can be assured:

Data from previous studies and literature reviews was used to determine the minimum number of animals required. Statistical methods were then employed in order to provide further information on the minimum number of animals required to display objective results. Resulting data will be analysed statistically using appropriate methods decided upon by the nature of the data.

Refinement - justification for the choice of species, why the animal model(s) used are the most refined and general measures to be taken to minimise harm to the animals:

The laboratory rat was selected for this study because it has been extensively studied and accepted as an animal model of the clinical conditions described in this project therefore there is a rich background in its utilisation. In addition, the olfactory bulbectomised rat model has been recognised as a valuable model in the development of novel antidepressant treatments.

The depression model used has been refined by introducing automated technology for the evaluation of behavioural signals and by incorporating relevant endpoints to gain the most possible information from the animals used.

A number of methods were incorporated into the study design to minimise any unnecessary distress or suffering to the animals. Examples include:

- Use of only trained and experienced personnel throughout the study
- Use of antibiotics after surgery
- Animals will be given pain relief before and after surgery
- Surgery will be performed under anaesthesia and in sterile conditions by trained and

experienced personnel.

- Administration of drugs with minimal adverse effects at site of injection
- Limiting the duration of behavioural procedures to ensure the minimum possible distress
- Drying and keeping animals warm after being placed in water
- Minimising separation times of pups from mother

Should any animal welfare issues arise, a veterinarian will be consulted.