HOME OFFICE LICENSING

Since the last meeting, the ERP Certificate Holder's Advisory Group has recommended seven applications for amendments to existing project licences and three applications for continuation project licences. The Committee is asked to receive and note these applications, which were:

Applications:

Project Title: **Parasitic Infections of Fish**

An application to continue work from an existing Project Licence. The application has been granted by the Home Office, and the project abstract is reproduced below for further information.

Project Title: **Cortical plasticity mechanisms**

An application to continue work from an existing Project Licence. The application has been granted by the Home Office, and the project abstract is reproduced below for further information.

Project Title: **Induction of Anti-Tumour Immunity**

An application to continue work from an existing Project Licence. The application has been granted by the Home Office, and the project abstract is reproduced below for further information.

Amendments:

Project Title: **Food & palatability: brain & behaviour**

An application to amend an existing Project Licence. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

Project Title: **The genetic control of cancer**

An application to amend an existing Project Licence. The application is currently with the Home Office, and the lay summary is reproduced below for further information.

Project Title: **Myeloid cells in inflammation and immunity**

An application to amend an existing Project Licence, dealt with by fast-track ERP. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

Project Title: **Modelling neuroendocrine and metabolic function**

An application to amend an existing Project Licence. The application has been granted by the Home Office, and the lay summary is reproduced below for further information.

Project Title: **Comparison of adenovirus subtypes for vaccination**

An application to amend an existing Project Licence. The application is currently with the Home Office, and the lay summary is reproduced below for further information.

Project Title: **Immune cell migration in health and disease**

An application to amend an existing Project Licence. The application has completed ERP and is being prepared for Home Office submission. The lay summary is reproduced below for further information.

Project Title: **Mechanisms of renal ischaemia-reperfusion injury.**

An application to extend an existing Project Licence. The licence was originally issued for only three years, but because of various administrative delays in appointments etc, work did not start until only nine months before the expiry date. Despite this, promising work was carried out and the project was positively received at retrospective review, taking into account the difficult circumstances. The application is for a two year extension.

BSO
01 Oct 2011

LAY SUMMARY AND ABSTRACTS FROM APPLICATIONS PROCESSED BY CHAG

Please NOTE – This is for information only – no assessment by the Committee is needed

Application 1 – Parasitic Infections of Fish

Abstract. This licence covers a series of experiments on the epidemiology of infectious disease in freshwater fish. Parasites pose a significant economic cost to aquaculture and as a potent evolutionary force they may threaten endangered fish populations. Understanding the basic biology of fish parasites is essential for more accurate diagnosis and design of control programmes to improve the health of fish stocks.

The species chosen for study are common, tropical and temperate freshwater fish that have relatively short generation times (6 weeks in the case of the guppy) and can easily be maintained under laboratory conditions. Gyrodactylids are obligate parasites that can not survive for any length of time away from the host (i.e. *in vitro* culture is not an option, although we have developed protocols for culturing other, less complex microparasites). Because gyrodactylids are ectoparasitic worms the entire course of infection can be monitored on a single fish (thus reducing the number of animals used, while still gaining quantitative data on population growth). Where possible, we seek to derive multiple endpoints from each cohort of animals, combining behavioural and infection studies with morphological and genetic studies. We are also developing models that will help identify key variables influencing host-parasite interactions. Such models can not replace animal use but they can help us reduce the number of variables that have to be tested empirically. An inherent problem in studying parasites is the huge intra-specific variability in host responses, necessitating large sample sizes, but appropriate experimental design ensures minimum numbers of animals are used to obtain statistically significant results.

During the course of this project, all experiments will follow a similar design in which fish (naïve to infection or with a known number of parasites) will be anesthetised (for approximately 3 min) and given a specific dose of parasites. The fish will then be monitored at regular intervals during the course of the infection to assess parasite population growth (the number of ectoparasitic worms can be countered on the surface of the fish) and possible effects on the host assessed (such as alterations in feeding and reproductive behaviour). Gyrodactylids cause high mortalities in wild and farmed fish (>90%) and a related species (namely *Gyrodactylus salaris*) has been defined as one of the most invasive fish parasitic worms having caused mass epidemics in Norwegian salmon. However, the work conducted under this licence is categorised as mild in severity because many fish shed their experimental load of parasites through induction of an immune response, and susceptible fish (which would die under natural conditions) are usually treated to remove potentially lethal doses of parasites. Therefore, the experimentally infected fish used in the current study are actually healthier than most wild or farmed fish. All fish are maintained in an enriched environment and our behavioural studies provide a further check on optimal maintenance conditions.

Application 2 – Cortical plasticity mechanisms

Abstract. Our long term aim is to understand experience-dependent synaptic plasticity properties in the cerebral cortex. Our study is divided into three main

sections 1. studies aimed at understanding the link between short term plasticity processes (lasting hours) that have generally been characterised at the cellular level with long term plasticity processes (lasting days and weeks) such as structural plasticity of dendritic spines that have been characterised at the whole animal level. 2. We will study the function of DISC1 (disrupted in schizophrenia gene 1) in development to understand its effect on adult plasticity as well as other key genes associated with the synapse and affected in schizophrenia and autism. 3. We will study mutants with enhanced cognition that show plasticity enhancements including the H-RasG12V mutant.

Experience-dependent plasticity is the process by which sensory experience affects the synaptic connections in the brain. It is therefore essential to study this process at the whole animal level because any reduced system (like cell culture) would lack any sensory apparatus or the complex neuronal circuitry to process those sensory signals. At present we do not have sufficient knowledge to use computer models to mimic the cortical circuitry nor the molecular mechanisms involved in mediating synaptic plasticity.

We will mainly study mice because they are the most commonly genetically mutated mammalian animal and they have a cerebral cortex that replicates many of the features of human cortex. We need the genetic mutations to see the cells of interest and to manipulate molecules involved in plasticity. We need to study cortex to understand cortical dysfunction in human disease and mental health conditions. The number of animals will be kept to a minimum by careful experimental design; for example, by using longitudinal experiments where possible so that each animal becomes its own control and ANOVA statistics to extract the most information from the study. Because the study will explore many possible plasticity factors both for disease and in our search for therapies, we will need to study many mouse lines. We expect to use approximately 2640 mice and 160 rats per year in these studies. Most of the mice will be used for breeding the non-harmful mutations we require for the experimental animals (1,600 mice per year) and there is no suffering for these animals. Suffering in the experimental animals is kept to a minimum in every case by the use of anaesthetics and analgesics. The mice will be studied with electrophysiological and cellular imaging techniques before and after altering their whisker complement to induce plasticity in the cortex.

The expected benefits of this study are that we will

1. learn the connection between long-term structural plasticity short term

2. understand how mutations present in schizophrenia, bipolar, autism affect synaptic plasticity in the cortex.

3. gain a major clue as to the mechanism by which AIDS related dementia occurs and its relationship to synaptic plasticity

4. uncover novel methods of enhancing plasticity for therapeutic benefit.

Application 3 – Induction of Anti-Tumour Immunity

Abstract. Our understanding of how the immune system interacts with tumours and how its activity can be best harnessed for the purpose of tumour rejection is incomplete. This project will examine the relationship between tumours and the immune system in detail with specific view to understanding how the immune system can be manipulated such that it is able to recognise and eliminate tumour

cells. To achieve this, experimental mice will be injected with carcinogens to induce tumours or with tumour cell-lines, and thereafter treating these animals with agents designed to boost anti-tumour responses. Simultaneously, components of the immune system that limit the magnitude of these responses will be suppressed. Interactions between the immune system and developing tumours depend on the integration of signals from a number of immune cell types and immune mediators. With this in mind, an animal model is necessary as there is currently no way to recreate these *in vivo* conditions in an *in vitro* setting. The mouse provides an excellent model in which to study the immune system. Mice are well characterised immunologically, and their immune systems closely resemble those of humans. In addition, a very large number of studies have previously utilised mice to study interactions between tumours and the mouse immune system. Thus, a large number of optimised reagents exist to facilitate designs of robust and reproducible experiments. As described above, mice used during the course of the study are subjected by injection to carcinogens and occasionally with tumour cell lines, a procedure that involves minimal restraint and little discomfort to the animal. Tumours are easily detectable and do not cause pain. Whilst there is promise that immunotherapy can be used as a successful means of treating cancer,

current methods remain sub-optimal. The overall aim of this work is to increase our understanding of how the immune system can be manipulated in order to inform the design of new immunotherapeutic strategies for the treatment of patients with cancer.

Amendment 1 PPL title: Food & palatability: brain & behaviour

Lay Summary:

The scientific purpose of the project: The overall purpose of this program of work is to understand the relationship between brain function and learnt and unlearnt responses to foods. Within this overall program, one of the detailed aims was to investigate the ways in which responding for food rewards in affected in animal models related to schizophrenia. At the time of writing, the focus of the project was on models related to either pharmacological or genetic manipulations, however, an ongoing collaboration with colleagues in the pharmaceutical industry has identified an additional model that is of particular interest – the methylazoxymethanol acetate (MAM) model.

The MAM model of schizophrenia relies upon the disruption of brain development by the administration of MAM (this temporarily disrupts cell division in a manner that is restricted to neuronal cells) to pregnant rats 17 days after conception. At this stage in development MAM exposure affects development of the frontal and temporal cortices, resulting in neuroanatomical and behavioural features resembling those in schizophrenia. In particular, pilot studies performed by my collaborators suggest that this treatment causes deficits in normal responding to rewards. These deficits are potentially related to the negative symptoms of schizophrenia such as anhedonia (an inability to experience pleasure from normally pleasurable life events such as eating). That is, the preliminary evidence suggests that the MAM might produce exactly the sort of effects that this project was hoping to discover and investigate.

Thus the reason for the application to change the licence is to support the use of this new animal model to assist in achieving the original goals of the project.

The application of the three Rs: Although the MAM treatment does influence the subsequent behaviour and brain function of the treated animals these effects are relatively subtle and there are no reports of distress or general ill health following the MAM treatment. Prior studies report no gross effects on litter size or birth weights, and while the adult weight of the rats is slightly reduced compared to controls (approximately 5-10%) there is no suggestion that this results in malnutrition. Moreover, there are no reports of significant adverse effects on the pregnant rats that are actually treated with MAM – that is, their bodyweight and general behaviour appears unaffected as is their maternal behaviour following the birth of the pups.

Thus, although the MAM treatment is not without impact, it does not produce any observable lasting distress or ill health in either the pregnant females treated with MAM or in their offspring that are to be the focus of behavioural investigation. This compares favourably with other potential animal models of schizophrenia – for example, although sub-chronic phencyclidine treatment model (which is already authorised for use on this project) produces no gross changes in health or well-being, it does involve repeated injections over an extended period, which is something the MAM model avoids.

In addition, in this project the behavioural assessment of food-related responses is used as an indication of whether normal motivational and affective responses are impaired. In comparison, other methods for investigating analogues of anhedonia in animal models rely on surgical and invasive techniques to assess the impact of directly stimulating the brain "reward" centres. So, the project as a whole represents a reduction in the severity of the techniques used to assess reward-related processes, and the addition of the MAM model does not affect this general reduction.

Animal usage: The original application envisaged that approximately 2000 adult rats would be used over the duration of the project. This number will not be increased by the proposed change – largely because the use of this model will partially replace other possible route of investigation. That said, the MAM model involves a pre-birth manipulation and so both male and female rats will be produced. However, because many experiments using the MAM model will involve only male animals (this allows for a reduction in variability during behavioural studies), there will be some discarding of female pups.

It should be noted that the breeding and actual MAM treatment will be contracted out to one of the main commercial suppliers of experimental animals. [redacted] have experience with this procedure and have confirmed that they are willing to supply the animals

. The use of a commercial supplier will both reduce the wastage inherent in maintaining a breeding program and take advantage of their expertise with the procedures involved to minimise any possibility for errors in the preparation of the animals.

Cost-benefit evaluation: The potential benefits of this additional work are clear. The majority of preclinical models of psychosis are aimed at the positive symptoms of schizophrenia such as hallucinations, delusions or motor disturbances. Possibly as a result the pharmaceutical treatments currently available for schizophrenia are generally more effective in the treatment of the positive symptoms than they are of the negative symptoms (such as anhedonia or the reduced sensitivity to external stimuli).

Applying the techniques developed in this project as a whole to the development and validation of preclinical models of schizophrenia in terms of negative

symptomatology could lead to the development of pharmacological treatments that are better targeted at these negative symptoms. This general program of work, and the specific investigation of the MAM model, is already supported by a collaboration with s38 which demonstrates the potential value of these lines of investigation.

It is true that the benefits of this project are uncertain and the most direct benefits lie sometime in the future. However, the development and validation of animal models of psychosis would be of great benefit to the development of clinical treatments for the disorder. Importantly, the experiments involved do not cause continued or general distress to the animals involved. Thus much can be achieved at relatively little cost.

Evidence of scientific value: Since the original project license was granted I have received funding for a BBSRC CASE studentship in collaboration with s38 Health and Safety s40 This funding. The use of the MAM model was a key feature of the application for this funding. Moreover, the preliminary results from the model were included in the doctoral thesis of a PhD student previously supported by a BBSRC CASE studentship in collaboration with s38 Health and Safety s40 This aspect of the doctoral thesis received particular commendation from the PhD examiners and is currently being prepared for publication.

Notes on location of changes to original PPL: The changes described above have resulted in the following changes to the PPL – these are highlighted by the use of red text.

S17(b) – Now described the MAM treatment and its relationship the scientific objectives of the project.

S17(c) – Additional references for the changes in 17(b) are supplied.

S18(b) – Details of how the MAM treatment will be performed and analysed are given here. In particular with respect to the achievement of Objective 4 of the PPL as a whole.

S19(b) 1 – This protocol is amended to allow the use of MAM treated animals. This includes a description of the source of the animals and likely adverse effects that may occur.

Le Pen, G., Gourevitch, R., Hazane, F., Hoareau, C., Jay, T. M., & Krebs, M. O. (2006). Peri-pubertal maturation after developmental disturbance: A model for psychosis onset in the rat. *Neuroscience, 143*(2), 395-405.

Amendment 2 The genetic control of cancer

Lay Summary: What the changes are:

Change to protocol 9 to allow genetic modifications targeted to the ovary surface epithelium (OSE) using an injectable, replication deficient virus. Mice will be placed under anaesthetic and using this injectable virus, tumour suppressor genes will be deleted specifically in the OSE, which may lead to tumourigensis.

Why the changes are needed:

Neoplasm's arising from the OSE is reported to account for 70% of all human ovarian tumours. Ovarian cancer is one of the highest causes of cancer related deaths amongst women, but one of the least understood. Recent methods using viral vectors to alter gene expression of OSE in mouse models have been published. We wish to use these methods to test our models of tumorigenesis within the OSE to increase understanding of the causes of ovarian cancer. We will specifically address the function of known tumour suppressors' involvement in the development of ovary tumours.

Species and number to be used

- Ovary sac injection of replication deficient virus to be applied to up to 50 mice a year as continued use from existing protocols.

Effects on the animals:

The individual effect on the animal will be the single administration of a general anaesthetic and surgery, followed by genetic changes which may lead to tumourigensis. Surgery will involve opening of the abdominal wall, injection into the sac surrounding the ovary, and wound repair by suturing. All animals will be closely monitored for three months following surgery to detect signs of tumourgenesis and any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health deteriorates, will be humanely killed. The degree of tumorigenesis will be detected by distortion of body shape and/or palpitation of the abdomen to detect abnormal growth of the ovary. Assuming good health throughout, the experiment will promptly end after 3 months (post-injection) using a schedule 1 method to cull the animals.

Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

A maximum of 50 mice each year will experience general anaesthetic and undergo a single surgery to access the ovarian bursula for a single unilateral viral injection. The cost to any given mouse will therefore be anaesthetics, surgery and potential tumourigensis. Tumourigensis may affect the growth of the mouse's ovary and/or fertility however none of the mice will be bread following surgery and any pain noticed from growth of the ovaries will lead to the animal being culled.

b) How the benefits outweigh the costs:

The development of a mouse which develops ovarian cancer arising from the OSE is a major step forward in the pre-clinical analysis of this disease. It should compliment previously limited models in understanding, studying cancer progression and response to drugs.

As well as enhancing our scientific understanding of the disease and therapies for it, unilateral injection allows for the reduction of mice numbers as the opposite ovary serves as a biological control. Overall therefore, this modification should help in our quest to generate successful therapies for cancer in humans, but also reduce the numbers of mice required to achieve this and reduce the distress placed on each experimental mouse.

As in all our work, the well being of the mouse is put at a premium. All anaesthetic and surgery will be carried out by highly trained staff already

familiar with a procedure which is very similar, embryo transfer, which is routinely carried out in our lab.

Amendment 3 Myeloid cells in inflammation and immunity

Lay Summary: What the changes are:

Amendment of an appendix (Appendix 5, 'Immunomodulators'). The appendix already contains 'pathogen derived agents' as immunomodulators and some examples are given 'lipopolysaccharide' and 'peptidoglycan'. We would like to add 'lentiviral vectors'. Whilst a lentiviral vector is a 'pathogen derived agent' and probably covered by this definition and hence already licensed, we felt that some people may think this a liberal interpretation given that the other examples were purified components. We already have GM safety approval for these studies that shows this was always the intent.

Why the changes are needed:

The use of lentiviral vectors provides a method to immunomodulate and ask similar to questions to those we are already addressing. Whilst we felt the licence covers this we are worried about interpretational ambiguity and wanted it clarified in the text.

Species and number to be used

There will be no additional use of animals (exclusively mice) as a consequence of this, in fact it may actually reduce numbers – see below. We will simply be using these 'immunomodulators' as an alternative to other experiments.

Effects on the animals:

The procedures are not being changed. We anticipate that the injection of lentiviral vectors may cause some inflammation (which we are well placed to characterise since this is our area of interest), but we do not expect anything more than mild severity. The induction of some inflammation is consistent with many immunomodulators we currently use.

Brief summary of the cost-benefit ratio of the proposal:

Costs to the animals:

The animal will suffer the transient discomfort of the administration as with any other immunomodulator administration. We also anticipate some sub-clinical inflammation. The viral vectors are non-productive infections so there will be no established 'active infection' leading to pathology. We do not anticipate additional adverse effects.

Potential benefits:

We will have (legal) confidence in the use of another immunomodulator. Many of the lentiviral vectors used will 'tag' the cells they infect, so by comparing tagged and untagged cells from the same animals (paired statistics) we may actually reduce animal usage compared to alternative immunomodulators that require clear distinct control groups with increased biological variability and reduced *power*.

How the benefits outweigh the costs:

The justification is the same as for the whole PPL. Our objective is to identify novel mechanisms to manipulate cells for therapeutic gain. This approach will be another mechanism to achieve this. We only anticipate mild adverse effects, no more serious than those already experienced during the administration of an immunomodulator.

By performing these experiments in vivo and by tagging manipulated and non-manipulated cells within the same animal we will have more powerful statistical design that will improve our ability to discover meaningful differences and potentially reduce animal usage. As we specialise in studying inflammation we will observe inflammation resulting from the administration and will objective evaluation of the extent of this inflammation even in the absence of clinical alterations in animal wellbeing.

<u>Amendment 4</u> Modelling neuroendocrine and metabolic function

Lay Summary: What the changes are:

I have made the following changes:

c) Incorporated the annotations made in pen by s40 HOI) on the originally issued licence (04/07/07)

d) Renamed Objective 2c to the endocrine control of reproduction

e) Introduced a new Objective 2d

f) Introduced 2 amendment paragraphs at the end of section 17 (with accompanying references

g) Introduced sentences in section 18 to incorporate the new work on the novel model of ghrelin-null Prader-Willi syndrome

h) Modified the procedure flow chart (Appendix 18.1) to include both the mild and moderate breeding protocols (NM05A & B) and incorporate excitotoxic lesions in Protocols NM06, MN08 and NM10

i) Incorporated the new wording (supplied from s40 Personal Data at the end of section 18 regarding transfer of GA animals

j) Introduced a new Protocol (NM05B) for breeding and maintenance of GA animals with a moderate severity limit

k) Incorporated new wording on sources of animals for continued use in protocols NM05A, NM05B, NM06, NM07, NM08, NM09, NM10, NM11 and NM12

l) Clarified the phrasing of protocol NM06 in relation to altered feeding schedules

m) Introduced a description of the excitotoxic lesion procedure, together with potential adverse effects and approaches for amelioration in protocols NM06, NM08 and NM10

NOTE: Despite these changes the overall severity limit of the licence is NOT increased and remains at moderate.

1. Why the changes are needed:

The two major amendments arise from novel actions of ghrelin, a hormone for which s40 Personal Data is a recognized international expert.

Amendment 1: Feeding and ghrelin patterns in the regulation of neurodegeneration and neurogenesis (May 2011)

It has recently come to light that the rate of neuronal loss in Parkinson's Disease (PD) is influenced by metabolic dysfunction, with disturbances in ghrelin secretion emerging as a likely mechanistic cause. Among the many actions of this gut hormone in the brain, ghrelin has been shown to modify the activity and organization of dopamine neurones and ameliorate chemically induced neurotoxicity in a rodent model of PD. However, we have shown that these effects may be dependent upon the pattern of exposure, because continuous ghrelin infusion elevates expression of an inflammatory cytokine associated with nigral dopamine cell loss in PD. Thus continuous ghrelin may be neurodegenerative, whilst intermittent ghrelin may be neuroprotective. Since ghrelin secretion is regulated by the pattern of feeding, we have attracted funding to establish whether patterned exposure to ghrelin and, by extension, patterned access to food, regulates cell survival in a rodent model of PD. Whilst has considerable experience in manipulating ghrelin patterns either by infusion or by modifying dietary patterns), we need to introduce excitotoxic lesioning (Objective 2d) to determine the importance of these signals in the progression of PD.

Amendment 2: The significance of elevated ghrelin in Prader-Willi syndrome. (May 2011)

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder caused by disruption of an imprinted gene cluster on human chromosome 15q11-q13 and is characterized by behavioural and metabolic impairment. PWS individuals display severe hypotonia at birth and a failure to thrive in infancy, but on emerging from infancy, reduced growth hormone secretion leads to skeletal growth retardation, whilst an insatiable appetite and overeating result in obesity. We have now shown that the majority of these characteristics are replicated in a novel imprinting centre-deletion model of PWS, the PWS-IC mouse. In both human PWS and the PWS-IC model, these metabolic disturbances are accompanied by a marked elevation in circulating ghrelin. Since chronic elevation of circulating ghrelin may result in many of the metabolic and behavioural characteristics of PWS, we have attracted funding to generate a novel mouse model of ghrelin-null PWS. Whilst analysis of these mice has been subsumed under the previously accepted objectives (Objectives 2a-c – with some minor modifications), the breeding of PWS-IC mice is considered be of moderate severity. Thus I have introduced a new protocol (NM05B) to cover the breeding of animals with a moderate severity limit and modified other protocols to receive animals from NM05B (including increasing the severity limit of NM11 (venous injection and sampling) to receive animals from NM05B with this severity limit.

In addition, I have:

n) Renamed Objective 2c to the endocrine control of reproduction to enable a broader exploration of the role of ghrelin in reproductive function, including in PWS-IC mice.

o) Clarified the phrasing of protocol NM06 in relation to the examples of altered feeding schedules to better reflect the types of feeding pattern manipulations that will be used.

2. Species and number to be used

Amendment 1.

The introduction of excitotoxic lesions will NOT increase the number of RATS and MICE currently permitted in protocols NM06, MN08 and MN10.

Amendment 2.

The additional MODERATE SEVERITY breeding protocol (NM05B) will permit the use of up to 500 RATS and 1000 MICE per annum for the REMAINING YEAR of this licence.

3. Effects on the animals:

Amendment 1.

As described in 19b Protocols NM06, NM08 and NM10, excitotoxic lesions will be performed on animals that have been the subject of altered feeding regimes, patterned iv hormone infusions or subcutaneous hormone treatment. This additional manipulation will have the expected neurodegenerative consequences in rats and mice so treated, and may result in the potential adverse effects listed in these protocols. Monitoring and approaches to minimise pain, suffering, distress and lasting harm are also outlined in protocols NM06, NM08 and NM10.

Amendment 2.

As described in 19b Protocol NM05B, the breeding of PWS-IC mice is considered to be of moderate severity. PWS is only manifested if inherited from the father. Therefore, for routine preservation of the colony the health of the mice is maintained by breeding females with the imprinting centre deletion with wild-type males. The resultant offspring have no phenotypic abnormalities. Similarly, two steps are taken to improve the survival of pups for experimentation. Firstly, males bearing the imprinting centre deletion are crossed with CD1 females (amore robust strain) to give more virulent offspring. Secondly, after the initial genotype screen, litter sizes are reduced by culling unwanted wild-type animals.

Crossing the PWS-IC line with mice with a deleted ghrelin gene is expected to alleviate some of the consequences of loss of paternal expression from the PWS gene cluster. These mice will be expected to have improved skeletal growth, reduced overeating, improved reproductive function and possibly restored metabolic activity in brown and white fat.

4. Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

Amendment 1.

The introduction of excitotoxic lesions will NOT increase the number or severity of RATS and MICE currently permitted in protocols NM06, MN08 and MN10.

Amendment 2.

The additional MODERATE SEVERITY breeding protocol (NM05B) will permit the use of up to 500 RATS and 1000 MICE per annum for the REMAINING YEAR of this licence.

b) Potential benefits:

Amendment 1.

This work will have TWO benefits for those suffering with PD:

a) Predict the potential therapeutic value of blocking the ghrelin receptor in reducing the progression of PD-associated neurological decline

b) Indicate whether modifying feeding patterns may have a significant beneficial influence in slowing the progression of this disease in humans

Amendment 2.

The generation and characterisation of a novel model of ghrelin-null PWS will have THREE benefits:

a) Determine which of the characteristics of PWS (including disrupted feeding patterns and associated behaviour, impaired skeletal growth, profoundly altered white and brown adipose tissue function and reduced reproductive performance) are due to elevated ghrelin

b) Establish the potential therapeutic value of blocking the ghrelin receptor in alleviating the symptoms of this condition

c) Describe novel actions of ghrelin

c) How the benefits outweigh the costs:

As a highly experienced in vivo scientist, s40 Personal will work in close consultation with s40 Personal Data and the named veterinary surgeon to maintain the professional standard of animal care for which he has an unblemished reputation. The new procedures will be introduced in consultation with highly experienced PPL holders with whom s40 Personal has established collaborative programmes – excitotoxic lesions; – breeding of PWS-IC mice, with pre- and post-operative monitoring (the details of which are given the 19b protocols) closely controlled.

Whilst the introduction amendment 1 will increase the impairment of brain function in rats and mice given excitotoxic lesions, the knowledge gained may have a profound benefit in indicating therapeutic and non-therapeutic approaches to reducing the rate of neuronal loss in patients with PD. These interventions may prolong an active lifestyle in those suffering from this debilitating disease.

Similarly, whilst the generation of a novel model of ghrelin-null PWS will increase the number of mice bred under a moderate severity protocol, understanding the importance of elevated ghrelin in PWS will significantly enhance the lives of the 1:15,000 children born with this condition. Indeed, the combination of genetic, behavioural and endocrine expertise may lead to the discovery of novel aspects of ghrelin action with additional significance in the treatment of obesity and other childhood developmental disorders.

<u>Amendment 5</u> Comparison of adenovirus subtypes for vaccination

Lay Summary: What the changes are:We would like to amend our injection regime. Firstly, we would like to be able to inject our vaccine under or into the skin. Secondly, we would like to be able to give the vaccine more than once, up to a maximum of three times.

We would also like to challenge with cell lines expressing proteins derived from a pathogen rather than the pathogens themselves.

Why the changes are needed:

While we are ultimately aiming to give our vaccine as a tablet or nasal spray, similar vaccines are currently used under the skin. Using this method will allow us to compare our work with other vaccine candidates in other laboratories. We would also like to inject into the skin (intradermal) to look at the migration of the virally-transduced cells into the local lymph node, where the vaccine is expected to act. This can be most reliably done in the ear. Given the mouse ear is very thin, the injections at this site are intradermal.

We already have in our protocol the ability to use peptides to vaccinate up to three times, but we can only inject the vaccine once. We would like to correct this oversight. The use of vaccines more than once (prime-boosting) is commonly used to increase immune responses and has recently been shown to be very successful for constructs similar to our own. It is also a technique regularly used in people, eg the booster injections given to children before they begin school. It is very likely that by prime-boosting we will be able to enhance the effectiveness of the vaccine. If it does not, this is an equally important finding.

The use of cell lines expressing relevant proteins from pathogens to challenge mice would allow us to check immunity without using pathogens. The cell lines have been used previously and do not result in any observable symptoms in the mice. We would like to incorporate this change to improve animal welfare.

Species and number to be used

No additional mice are to be used. It is estimated 250 mice would be affected by the change in injection regime, and up to 150 would be affected by the ability to inject cell lines.

Effects on the animals:

For skin injections, there may be transient discomfort at the injection site.

Injection into the ear is done under anaesthetic. However there may be some discomfort at the site of injection on waking.

The increase in injections from 1 to up to 3 will result in transient discomfort during injections. It should be noted that we are not asking that all mice should be given three injections; this is a maximum and it is envisaged that the majority of mice will still be given only one injection.

The use of cell lines will be a positive welfare move, as they will be used where possible in the place of pathogens.

As with all of our experiments, there should be no lasting harm from any of the changes proposed.

Brief summary of the cost-benefit ratio of the proposal:

Costs to the animals:

250 mice will experience transient discomfort on injection under the skin or booster injections.

150 mice will have a decrease in costs as the use of cell lines will replace pathogens.

Potential benefits:

Our aim is to compare a new vaccination vector with a current vector in the lab and similar vectors published by other laboratories. By extending our protocols we should be able to more effectively compare our work with others and explore the best way to apply the vaccine with the minimal impact on the mice.

How the benefits outweigh the costs:

Our study is examining a potential vaccine vector which it is expected will allow rapid development of vaccines that will ultimately be taken orally or via a nasal spray. The benefits of such a vaccine vector, particularly for the third world where there may be restricted numbers of medical personnel and equipment available for injectable vaccination programmes, are clear.

There are no changes to the numbers of mice in our protocol, or to the severity of the procedures. Thus the most significant adverse effect is that the mice may be injected with the vaccine vector up to three times, as is currently allowed with the comparison protein vaccines. The changes will allow us to compare the vector with other laboratories and more fully explore whether vaccination can be improved by boosting the immune response or changing the route of vaccination to one tailored towards the pathogen of interest. This should allow us to more rapidly assess the vaccine candidates and so reduce the time required for the study and the overall number of animals involved.

Amendment 6 Immune cell migration in health and disease

Lay Summary: What the changes are:

A new objective 4 and changes to protocols 19b, 1 and 2 to analyse infiltrating immune cells within (a) growing tumours and (b) ovaries and to quantitate (c) cell proliferation and (d) tumour growth in experimental animals. A refinement to protocol 19b, 2 is requested for intravital imaging of infiltrating immune cells (e). A further refinement to protocol 19, 3 will permit the interbreeding of motheaten and CD62L transgenic mice.

Why the changes are needed:

(a) The treatment of cancer patients with their own (autologous) T lymphocytes to induce tumour regression is being trialled in the clinic. Current approaches include pre-selecting T cells that express high affinity receptors for tumour antigens and transferring tumour-specific T cells back to patients. We have found that the rate of tumour growth is significantly reduced in genetically altered mice expressing a metalloproteinase-resistant form of the lymph node homing receptor CD62L/L-selectin. In addition, adoptive transfer of antigen-specific T cells expressing metalloproteinase-resistant CD62L is better able to eliminate antigen bearing cells than wildtype T cells. In a separate study, we have also shown that adoptive

transfer of SHP-1 deficient T cells prior to administration of tumour cells results in enhanced tumour protection. We therefore now wish to examine whether a combined loss of SHP-1 expression together with maintained expression of L-selectin may lead to enhanced regression of tumours. Hence in a new Objective 4 we now want to capitalise on our new findings and determine whether expression of metalloproteinase-resistant CD62L and/or deficiency in SHP-1 on adoptively transferred T cells accelerates the regression of established tumours. In a new Objective 4 we want to establish growing tumours in mice and monitor tumour infiltrating immune cells and tumour regression following adoptive transfer of tumour specific $CD8^+$ T cells. This programme of work has been fully funded by the Wellcome Trust.

Since the PPL was awarded, I have been trained by s40 Personal Data in intravital imaging of mouse lymph nodes and I am in the process of establishing this procedure at s38 Health and Safety. This procedure will be used to directly image infiltrating immune cells within tumours and involved lymph nodes and tumours are included in an amendment to protocol 19b, 2.

(b) Ongoing studies to analyse the homing of virus-specific CD8+ T cells uses recombinant vaccinia virus expressing defined peptide antigens. Vaccinia virus replicates to high titres in ovaries of female mice. Since basal trafficking of immune cells is very low in ovaries, the increased immune cell trafficking in infected mice is readily detectable and can be correlated with viral clearance. has generated a recombinant GFP vaccinia virus which enables $CD8^+$ cytotoxic cell interactions with virally infected stromal cells to be visualised. The anatomic location and ability to exteriorise the ovary makes this organ accessible for direct imaging using intravital microscopy and the ovary has been included in an amendment to procotol 19b, 2.

(c) Important components of the immune response are increased proliferation and altered survival of immune cells. Cell proliferation and lifespan can be measured in experimental animals by timed administration of 5-bromo-2'-deoxyuridine (BrdU), a homologue of thymidine, which is incorporated into replicating DNA. BrdU-labelled cells are identified by immunolocalisation in tissues or following isolated from tissues. This technique generates additional information to that gained by ex-vivo staining using replication associated antigens such as Ki67, since timed administration of BrdU allows conclusions about cell life-span to be monitored in addition to cell division. Administration of BrdU is included in an amendment to 19b, protocol 1.

(d) The ability to accurately measure tumour size and monitor tumour growth in a temporal manner will generate more data per animal in the new objective 4. Two imaging techniques will be used, bioluminescence and positron emission tomography (PET) and these have been included as amendments to 19b, Protocols 1 and 2.

In the first, genetically marked tumour cells expressing luciferase will be used. Mice will be anaesthetised at regular intervals and injected intravenously with luciferin and bioluminescence imaged in an IVIS 200 in vivo biophotonic imaging system (Xenogen). Tumour growth will be quantitated using Living Image 3D Software (Xenogen) (Fig. 1) and compared directly with that measured by palpation. Preliminary data indicates that tumour growth is detectable before tumours are palpable .

In collaboration with the s38 Health and Safety, s40 Personal Data s38 Health and Safety, s40 Personal Data we will administer ^{18}F-fluorodeoxyglucose to monitor tumour metabolism and develop novel tracers based upon proteins and peptides key to immune cell homing to better characterise the

role of immune cell migration in controlling tumour growth and to test the efficacy of therapeutic agents.

(e) For intravital imaging of tissue infiltrating immune cells, fluorescently labelled immune cells are administered intravenously to surgically prepared mice under terminal anaesthesia. The surgical preparation involves cannulating a peripheral vein or artery and exteriorisation of lymph node or organ to be imaged. If the cannula fails during the surgical preparation or period of imaging, labelled immune cells will be administered via the retroorbital plexus to avoid premature termination of the experiment. This is included in an amendment to 19b, protocol 2.

Species and number to be used

Wildtype and genetically altered mice:

300 wildtype and 250 genetically altered mice will be used for adoptive T cell therapy of tumours (new Objective 4) over the remaining 3.5 years of the licence. I am not requesting additional animals since the tumour studies have arisen directly from our observations on T cell trafficking in viral infection for which all animals are already included in the licence. The additional number of 3000 mice requested in protocol 19, 3 covers the additional numbers of breeding mice required to generate motheaten mutants. The figures for estimated annual numbers of mice reflect the recognised poor breeding efficiencies of the C57BL/6J strain and the increased morbidity of SHP-1 mutant mice before reaching the age at which they can be used experimentally. Furthermore, a transgenic TCR has been introduced into the SHP-1 deficient genetic background and all mice expressing the transgene have to be recorded. Hence, the overall estimates of mice required for the SHP-1deficient breeding colonies reflect not just the total number of SHP-1 deficient births but also the need to genotype and record the total numbers of transgenic mice generated. All other modifications to the licence allow improved or additional data to be collected from ongoing studies.

Effects on the animals:

Transplantable tumour cell lines, such as the B16F10 melanoma, will be used to monitor the ability of CD8+ T cells to induce the regression of established tumours. B16F10 melanoma subline was isolated in the early 1970s. It has been maintained in culture and used extensively for studies of tumour cell biology and tumour immunity in vitro and in vivo. Tumour cells will be injected subcutaneously into upper right thigh and tumour growth monitored according to UKCCCR guidelines. In cases where mice are inoculated intravenously or intraperitoneally with tumour cells, no adverse reactions are expected to occur.

The motheaten mouse suffers from a progressive, systemic auto-reactive disease that includes skin abscesses, anaemia, pneumonitis and necrosis of the extremities. The skin abscesses are the earliest phenotypic manifestation of the motheaten disease process and facilitate identification of mutants prior to the onset of more severe disease problems such as pneumonitis. All the studies proposed above would involve the use of motheaten and control mice that will be killed by schedule 1 methods. The motheaten mice will be killed prior to the onset of severe disease problems regardless of whether they are to be used experimentally.

Brief summary of the cost-benefit ratio of the proposal:

a) Costs to the animals:

A maximum of 550 mice will experience transient discomfort from being restrained for intravenous, intraperitoneal or subcutaneous injection and general anaesthetic on no more than five occasions routinely and up to 15 times if the kinetics of tumour regression is prolonged. Some mice will receive small implants of tumour under the skin. Adverse effects experienced by the animals under this procedure will be minimised by general anaesthesia throughout the procedure and the use of analgesic drugs to control any post-operative discomfort. No adverse effects from the administration of luciferase substrates or PET tracers and from serial imaging of mice are anticipated.

b) Potential benefits:

The ability to accurately measure tumour size and monitor tumour growth in a temporal manner allow more data to be obtained per mice in the new objective 4. The ability to group mice according to tumour size before therapy commences will allow the efficacy of therapy to be related to tumour size which will allow fewer mice to be used to gain statistically significant comparisons. The modifications to protocols 1 and 2 will provide additional data to address Objectives 1, 2 and 3 of the PPL thus providing a valuable contribution to our knowledge of how immune cells move around the body in mice and its potential usefulness to model infection and immune responses in humans.

c) How the benefits outweigh the costs:

The activation of T cells is critical to the efficient clearance of tumours. Hence, an understanding of the molecular mechanisms regulating T cell responsiveness has potential to impact upon the development of new treatments for cancer. Hence, an understanding of the influence of SHP-1 on T cell responsiveness in the context of the proposed tumour models may have important implications for future cancer immunotherapy. The modulation of SHP-1 activity may potentially be integrated into T cell immunotherapy strategies if it can be demonstrated that SHP-1 has the capacity to alter the in vivo expansion, trafficking and killing properties of T cells.

The new information that will be gained from this study about the ability to induce the regression of established solid tumours by using tumour reactive T lymphocytes is important for the therapy of clinical disease. The study will provide basic information about how immune cells move around the body and whether it is possible to target these cells to specific organs in order to improve their function. The experimental procedures have been used in Cardiff by my colleagues for a number of years and I will seek training and advice from them for the tumour studies. Adverse effects on mice will be minimised by close monitoring of tumour growth and by seeking advice from the Named Veterinary Surgeon or Named Animal Care and Welfare Officer (NACWO) in the event of any unexpected adverse effects. Should the individual fail to respond to any treatment prescribed or if any signs of suffering and distress cannot be alleviated, the animal will be killed by a Schedule 1 method.

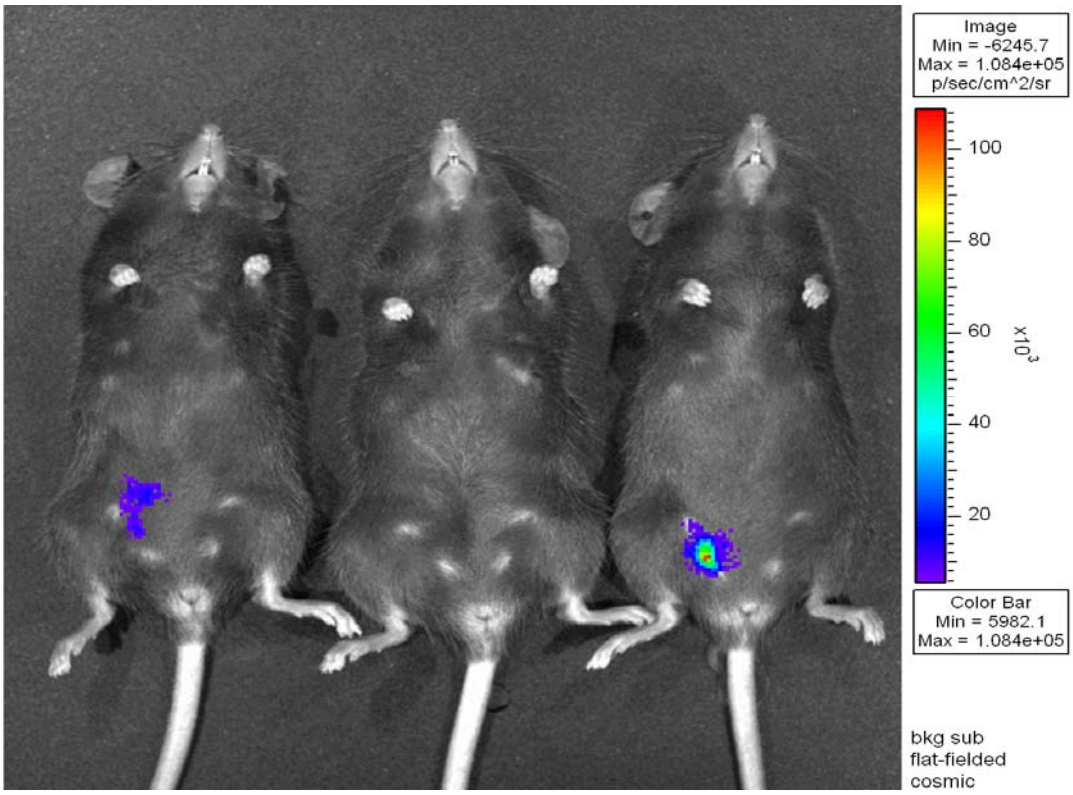

Fig. 1 Bioluminescent imaging of subcutaneous B16F10 melanomas x 10^4 luciferase expressing B16F10 melanoma cells were injected s.c. into the upper right thigh of two C57BL/6 mice and one mou se (middle) was left uninjected. At intervals of 2 days, luciferin was administered i.p. and images collected from anaesthesised mice within 30 min using an IVIS 200 biophotonic imaging system (Xenogen). Luminescence was detectable in inoculated mice before tumours were palpable. Image above was taken 10 days after tumour inoculation showing detectable bioluminescence only in tumour inoculated mice