CONFIDENTIAL MATERIAL

THE UNIVERSITY OF BIRMINGHAM

BIOMEDICAL ETHICAL REVIEW SUB-COMMITTEE (BERSC)

4th June 2015

MINUTES

.

15/06-02 Minutes

The minutes of the meeting held on 23rd April 2015 were considered by the Committee and were approved subject to minor amendments.

15/06-03 Matters Arising

Minute 15/04-05 – Recent news article on animal experimentation at Birmingham

Following the recent news article on animal experimentation at the University of Birmingham, there has since been a very balanced follow up article in a student newspaper.

Minute 15/06-08 – Educational remit of BERSC

This will be discussed further, hopefully at the July 2015 meeting.

Minute 15/06-08 – Tracking translational benefits of animal research

A summary report on interim reviews will be circulated to the Committee for discussion.

Minute 15/06-08 – Charles River visit to BMSU

The presentation given during Charles River's visit to BMSU was very well received; however, it was noted that relatively few researchers from the University of Birmingham attended.

15/06-04 Chairperson's Items

No Chairperson's items were reported.

15/06-05 Verbal Reports from the Director of BMSU and Named Persons

Report from Director of BMSU:

Key issues reported were:

- BMSU sent three attendees to Understanding Animal Research (UAR)'s recent 'Openness in Animal Research' meeting. The outcomes of the meeting will be circulated; it was considered very useful, and included discussion of how other institutions are meeting their commitment to the Concordat on Openness in Animal Research.

- The University has agreed to pay the recent increase in UAR subscription costs. It was explained that the cost increase is associated with an increase over time in the amount of animal research carried out within universities; this has necessitated a change in cost structure to allow UAR to continue to provide appropriate support.

- A member of BERSC will present a poster at the forthcoming NC3Rs/Society of Biology meeting.

- A European Citizen's Initiative has resulted in over a million signatures supporting a proposed rewrite/repeal of Directive 2010/63/EU on the protection of animals used for scientific purposes. However, the Commission considered it premature to repeal the current Directive, as the signatories of the initiative had requested, so no further action will be taken.

- Activities within BMSU are very much 'business as usual' at the current time.

- The Home Officer Inspector recently visited the outlying rooms used for animal experimentation at the University of Birmingham. While everything was generally found to be satisfactory, efforts should be made to improve asepsis.

Report from the NVS:

- It was reported that BMSU's new supply of captive-bred xenopus frogs are settling in well.

Report from the NACWOs:

- No issues were reported.

15/06-06 Report from the Fast Track Procedure

The fast track procedure is up-to-date, and there are no outstanding issues.

15/06-07 Project Licence Proposals

15/06-07-1 Application Ref TBA – Protecting and repairing injured retinal cells in Glaucoma

The aim of this project is to determine changes that occur after induction of glaucoma, which causes a rise in pressure in the eye that leads to neuronal death.

The PI gave a presentation explaining the application to the Committee.

The Committee asked for an indication of the typical number of instances of recovery anaesthesia which any one animal will experience. It was explained that over a 30 day period, there may be up to 8 such episodes, with the animal typically being unconscious for a few minutes each time. The protocol may exceed 30 days on occasion. The application will be amended to limit the number of recovery anaesthesias to 8, with more than 8 requiring an amendment.

It was queried whether it will be necessary to give the injections twice a week, and the PI explained that this has been found to be the best way to elevate intra ocular pressure (IOP) as required.

The abbreviation IOP should be written in full in the lay summary. It may be helpful to amend the lay summary to clarify what will be experienced by the animals included in the study.

In order to record visual evoked potentials, a small screw will be implanted into a hole in the animal's skull. This provides a metal node, to which a clip can be attached. This should be explained in the lay summary.

The Committee noted that as the application is currently written, all of the possible experimental procedures could happen to the same animal. The application should be amended to make steps 'either/or', where appropriate. It was suggested that tomography and fundoscopy should be combined together into one 'imaging' step.

The PI was asked whether anything relevant to this work could be learned from research into how fish regenerate their optic neurons. It was explained that stem cells in fish behave in a fundamentally different way, but this is an area of interest.

The Committee queried the causes of IOP in humans, and the PI explained that it is related to resistance to outflow, and an imbalance between production and outflow, usually with deposits reducing outflow.

In relation to the 'reduction' aspect of the 3Rs, it could be argued that rats depend little upon their sight, instead relying on their other senses, and therefore that it is preferable from a welfare perspective to use a lower number of rats with bilateral (rather than unilateral) blindness. It was also noted that the anticipated death of 30% of the RGCs in the rats studied is a considerably lower percentage than the 50% RGC death which is the threshold for human beings to experience problems with visual field depth, so it is anticipated that the rats will be minimally affected.

It was suggested that the application should be amended to specify the amount of funding and staff resource available.

As microinjections are considered a refinement, the Committee therefore queried why they will not be used in all experiments. The PI clarified that no one model is perfect, and that it is important to use both proposed models of glaucoma in order to maximise translational potential.

It was explained that both rats and mice will be used; using mice allows for the use of genetically altered models, but their relative size makes the work of greater technical difficulty, whereas rats are of higher sentience, but are easier to operate on and provide a greater amount of tissue (thus reducing the number of animals required).

As intramuscular injections can be painful, if they are not absolutely necessary they should be removed from the study.

There was some discussion about the measurement of IOP, and it was explained that the measuring device is calibrated for rats and mice. The measurements (in mmHg) obtained are not necessarily accurate, but are consistent/reproducible and they are effective in illustrating the change in IOP.

It was felt that the maximum IOP allowed by this application should be reduced to 40, which is the threshold in humans beyond which pain may be experienced. The PI clarified that the IOP experienced by the animals will be in the region of 14-17, but the laser model could take this up to 20-30. Signs that an animal is experiencing pain related to IOP would be the white of the eye becoming red, and/or clouding to

the cornea. The NACWOs will need appropriate training to pick up such symptoms.

After the PI left the meeting, the Committee continued its discussions.

The application requires careful checking to ensure that anaesthesia is stated where it will be necessary.

The lay summary should be amended to provide information about the proposed surgery.

To date, the researchers have no direct experience with the laser model and the PI will have to prove competency in the proposed techniques to the Training and Competency Officer before the work takes place.

The necessary equipment has been purchased, but is not yet installed within BMSU.

Resolved that:

The revisions discussed above will be made. Once the Chair is happy with the changes, a recommendation will be made that the Establishment Licence Holder submits the application to the Home Office.

15/06-07-2 Application Ref TBA – Vitamin D and immune function

The aim of this project is to determine how vitamin D contributes to normal immune function and then to explore how this is disrupted under conditions of vitamin D deficiency or loss of the vitamin D in target tissues. This application is to extend upon work previously carried out in the United States.

The PI gave a presentation explaining the application to the Committee.

It was agreed that a pilot study will be needed to establish the appropriate dosages of LPS and DSS, i.e. the minimum dosages to achieve the required result. These pilot studies should be mentioned in the 3Rs section of the application.

The lay summary should be edited to correct typographical errors, and it should be clarified that one of the protocols is of moderate severity.

The proposed numbers of animals are high, due to the required breeding activity and the need to generate timed matings. For timed pregnancies, multiple possible females are required; non-pregnant females cannot be used as non-pregnant controls, as this would be classed as reuse. On consideration, it was felt that the stated numbers

may actually be too low and may limit the proposed work; consideration should be given to raising the numbers for breeding.

Litters taken to term will be euthanized within 7 days.

The Committee queried the funding/resources available for the proposed work. The PI clarified that there will be 2 postdoctoral researchers, and there are two US funding streams associated with the work. Efforts are underway to secure UK funding, and this should be explained in the application.

Cardiac bleeding should be included as an optional endpoint.

Given the effects of vitamin D deficiency upon autoimmunity, it was queried whether foetal rejection may be a problem. The PI explained that in heterozygous cases this is not an issue; in homozygous cases, it has not so far been a problem. The PI will clarify this.

It was felt that as currently stated, the scientific background 'jumps around' and that whilst the researcher is hoping for translational benefits, this is fundamentally a basic science project and must be achievable with the available resources. It was suggested that the objectives may currently be too broad, and the PI should make them more specific.

Pregnancy provides a complex model of autoimmune challenge, with a relatively high cost to the animals, and the Committee queried whether there are other, more straightforward models available to look at the effect of vitamin D deficiency on immune function. The PI explained that vitamin D deficiency is more common in pregnant women and is linked to miscarriage. This animal model is therefore important as the work cannot practically be carried out with pregnant humans. This justification for the pregnancy model should be fully explained in the application.

The size of the proposed groups of animals will be determined on the basis of preliminary studies.

The PI clarified that blood pressure will not be measured.

Regarding vitamin D supplementation, the levels to be given to the animals are within the equivalent range given to human subjects. A five-fold increase in dietary vitamin D is needed to effect a doubling of the vitamin D in the animals' circulation.

After the PI left the meeting, the Committee continued its discussions.

Concerns were expressed that as the application currently stands, it is not sufficiently clear. The objectives need to be clarified and made more specific, and need to be achievable with the available resources.

Further work is needed to explain why the pregnancy model is being used, and the scientific background should be strengthened. The lay summary needs considerable revision.

It was also noted that previous work in BMSU involving dietary manipulation of vitamin D (albeit using a different model) has resulted in significant systemic effects. The PI has no previous experience with immunological challenge models.

The PI and the Director of BMSU will discuss the application further with the Home Office Inspector. Given the Committee's concerns, it was felt that a revised version of the application should be resubmitted to the Committee for consideration.

Resolved that:

The revisions discussed above will be made, and the revised version of the application will be recirculated to the Committee. Once the Committee is happy with the changes, the Chair will recommend that the Establishment Licence Holder submits the application to the Home Office.

15/06-07-3 Application Ref TBA – Repairing the damaged spinal cord

The aim of this project is to determine changes that occur after injury to the spinal cord that provides the information highway from the entire body to the brain and back, with particular interest in how neurons deal with injury that makes them vulnerable to death, the scar tissue that forms after injury and the lack of nerve regrowth that follows nerve injuries of this type.

The PI gave a presentation explaining the application to the Committee.

The proposed work will be classed as severe. The PI has consulted extensively with the Home Office Inspector whilst preparing the application, and has considerable experience in this area of research.

The PI explained that the animals will need no additional care after the dorsal column injury, apart from analgesia and observation. However, after the contusion injury, significant additional care will be required. Bladder function should be regained over the two weeks following the procedure, and until it is, manual expression of the bladder will be necessary three times per day. Once an animal has been able to clear its bladder for three consecutive days, manual expression will cease; if bladder function does not return after two weeks, the animal will be

culled. An additional problem is that after the contusion injury the animals will be unable to feel their toes and so may bite them; more than minor nibbling will mean that the animals have to be culled, as in extreme cases, phalanges may be bitten off. During the first two weeks after the procedure, the animals will have to be constantly observed and will be housed in groups of at least three, with the symptomatic animals grouped together.

In relation to the biting off of phalanges, it was noted that there is inconsistency between the endpoints stated in protocols 1 and 2. In protocol 1, an animal will be culled if 1 phalange is lost; in protocol 2, the loss of 2 phalanges will prompt culling. The PI explained that in protocol 1, the animals are highly unlikely to bite off phalanges. In protocol 2, biting is more possible as the animals' feet will lack sensation for longer. The Committee discussed this, and it was suggested that the pain may be minimal if healing has taken placed by the time that sensation returns. It may be possible to apply some form of 'anti-chewing' substance to the animals' feet, although this could make the problem worse. The NVS expressed concerns about the welfare implications of this aspect of the application, and suggested that it may be necessary to euthanize any animals exhibiting such biting behaviour, subject to consideration of degree, location, etc.

As well as the dorsal column model (which is of moderate severity), the application includes two more severe models which have more direct relevance to human injury (one of compression and one of impact). The dorsal column model is particularly concerned with regeneration, and will be the first model used; the PI will then only progress on to the more severe models if the findings of the first indicate that this is appropriate. It was felt that this 'progression' should be explained more clearly in the application.

The PI explained that injury to a peripheral (sciatic) nerve a week prior to spinal injury 'primes' the relevant neurons and provides a growth stimulus for regeneration. However, it is felt that the focus needs to move away from this finding as it has no translational potential, and this should be mentioned in the 3Rs section of the application.

Considering one of the diagrams shown during the presentation, the Committee queried whether breathing difficulties are possible if injury occurs at thoracic level. The PI explained that the procedures will be carried out at T8, well below the level at which breathing difficulties might be caused.

In relation to the 'replacement' element of the 3Rs, more information should be provided about the limitations of in vitro work for research in this area.

Advice will be sought from an institution already carrying out these types of protocol on an appropriate plan for administering analgesia.

The contusion model is likely to cause pain for the first 48 hours after injury, and the animals will require opiates at least every 8 hours – this will be revised in the application.

The adverse effects listed for the proposed behavioural testing should be amended to reflect the fact that not all such experiments will be reward based, as some will involve negative stimuli (e.g. heat).

After the PI left the meeting, the Committee continued its discussions.

It was emphasised that the justification for the proposed work should be more clearly explained in the application.

There will be further discussions with the Home Office Inspector about the previously discussed concerns around toe biting.

Resolved that:

The revisions discussed above will be made. Once the Chair is happy with the changes, a recommendation will be made that the Establishment Licence Holder submits the application to the Home Office.

15/06-08 Any Other Business

No further items were raised.

15/06-09 Date of Next Meeting

The date of the next meeting is 9th July 2015.

GLOSSARY

3Rs	Reduction, Refinement and Replacement
AWERB	Animal Welfare and Ethical Review Body
BERSC	Biomedical Ethical Review Sub-Committee
BMSU	Biomedical Services Unit
DSS	Dextran Sodium Sulphate
IOP	Intra Ocular Pressure
LPS	Lipopolysaccharide
mmHg	Millimetre of mercury
NACWO	Named Animal Care and Welfare Officer
NC3Rs	National Centre for the Replacement, Refinement and Reduction of Animals in Research
NVS	Named Veterinary Surgeon
PI	Principal Investigator
TBA	To Be Announced
UAR	Understanding Animal Research
RGC	Retinal Ganglion Cell