**Section 1: Project Details**

**AEC Project Number (Assigned by the AEC)**

**Project Title: Test Experimental AEC Protocol**

**Application Status: New Project**

**Dates:**

**Submission Date:**

**Commencement Date:**

**Completion Date:**

**BAW Purpose and Benefit Codes:**

**Overall Purpose of the project:** 02 – The maintenance and improvement of human or animal health and welfare.

**BAW Benefit Code:** 02 – Diseases-human

**Investigators:**

**Type: Name: Phone: Email Address: Signature: Date**

Principal Mark Cook 0411099000 [mark@neurology.net.au](mailto:mark@neurology.net.au) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_

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Associate Richard Balson 0415893010 rbalson@bionicsinstitute.org \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_

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Associate Karin Mclean \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_

**These investigators have signed this proposal to acknowledge they will:**

**a) comply with the requirements of the NHMRC Australian code of practice for the care and use of animals for scientific purposes 7th edition 2004**

**and**

**b) accept responsibility for the conduct of the experimental procedures detailed in this proposal.**

**Experimental procedures must be conducted in accordance with the Code and The Guidelines for the Care and Use of Animals (February 2003) prepated by the Animal Ethics Committee. A copy of these**

**two documents can be downloaded from the WEHI Intranet. Non-complicance may lead to approval being revoked.**

**Section 2: Lay Explanation of the Project**

**2a) Provide a lay summary of the proposed project. This statement must be clearly comprehensible to a lay person.**

With approximately 1% of the Earth’s populace affected by epilepsy the need to treat the disease is vital. With current anti-epileptic drugs not providing adequate seizure control for one third of people with epilepsy the need to develop an alternate treatment is critical to improve the lives of these people. Temporal lobe epilepsy is an example of a type of epilepsy that is often poorly controlled by anti-epileptic drugs. When this is the case the only other treatment option for these patients is to resect a part of the brain. This procedure is highly invasive, non-reversible and carries significant risks for the patient, such as disruption of memory or motor skills.

There is evidence in literature that electrical stimulation of the brain can help reduce seizure frequency or severity, and in rare cases stop seizures completely. However the effectiveness of any given stimulation paradigm can vary from patient to patient for reasons that are not well understood. The current proposal is to develop a method of altering the stimulation parameters based on responses from the brain, in order to tailor stimulation to individual patients. We will use animal models of epilepsy to address this question. These animals will undergo a surgical procedure where electrodes will be implanted on the surface of or in the brain so that signals can be recorded and electrical stimulations can be delivered to the brain. After a small electrical stimulus is applied, the brain’s response will be measured and then used to estimate parameters such as excitability. We believe that such parameters are linked to seizure generation. From these results, we will attempt to identify trends that may help to determine when and how therapeutic stimulation could be delivered. How these trends change with the delivery of therapeutic electrical stimulation will also be measured. In so doing the effect of electrical stimulation on the brain with regard to these parameters can be determined. This may show whether or not a specific stimulation therapy is effective for reducing the likelihood of seizures, or possibly abating seizures indefinitely.

**2b) Provide justification for the use of animals in this research project and indicate why the scientific objectives cannot be achieved without the use of live animals. Also describe the potential benefits of the**

**research.**

There are devices that are currently being developed in order to treat epilepsy in humans. These devices directly electrically stimulate the brain. The most recently published outcomes of these clinical trials show that these devices can provide some benefit to some patients, but they are far from totally effective across all patients. In order to determine how to more effectively use such devices in humans further research is required to understand the interaction between the stimulation that these devices deliver and the actual neuronal processes that cause seizures. Ideally this research would be conducted in using other techniques but there are a number of reasons that preclude this.

* The complexity of both the brain and of the epilepsy syndrome, make computer modeling of these interactions very difficult. It is our hope that through these controlled experiments in rats, we will be able to refine the specific parameters within the computer models of the epileptic brain. Once these models are more realistic, it will be possible to conduct preliminary investigations using these models rather than in animals.
* During the monitoring period for patients within the hospital, the frequency with which seizures occur is very low. For this research to be reliable, it is important to have a high degree of confidence in our understanding of how particular stimuli affect the brain. This confidence can only be built from highly repeatable and unchanging seizures, which is the defining characteristic of these well accepted models.
* Similarly, the patients that present to hospital for resective surgery have many and varied causes of epilepsy. This variability also leads to limitations in our ability to make significant inferences from our findings across these patients.

The combined effect of these reasons is that the use of animal models in this experiment is justified in order for researchers to be able to better understand and build upon the promising results that have already been obtained from previous studies.

Due to the varying results obtained from studies and the uniqueness of each individual’s brain it is vital to understand the effect of electrical stimulation, and how to alter the stimulation in order to reduce seizures. This trial will demonstrate how electrical stimulation affects parameters that can lead to seizures and in so doing provide the opportunity of making use of the electrical stimulation paradigm results in human cases.

**Please detail any efforts made to replace animals in experiments with alternative methods:**

Part of the study involves measuring and tracking the changes in certain parameters that may change as seizures approach. From research conducted under previous applications (AEC 51-06) much data has already been collected that details the pre-seizure state within this model. Results from these previous studies can be used to estimate some parameter changes, and help with the selection of parameters to be used in this study. Within this study, we are also proposing the use of small electrical stimuli as a way of ‘probing’ the brain to measure these parameters. This approach has not previously been trialed in animals and as such, some new experiments are required. Overall though, the use of pre-existing data wherever possible will result in significantly lower numbers of animals being required over the project lifetime.

As mentioned in section 2b, the project also proposes the development of computer models to enable the simulation of these experiments in the future. If sufficient data is collected through these experiments such that the models can be shown to be acceptably accurate, then the future use of animals within this and other projects should also be significantly decreased.

**Please detail any efforts made to reduce the number of animals needed for experimentation:**

By building upon techniques that have been refined within previous projects, aspects such as surgical techniques will not need to be further refined within this protocol. This will mean that from the outset, the data obtained from every animal will be maximally useful, thereby reducing total animal numbers. By making use of electrodes that are similar to those in human patients, in terms of size relative to the brain, the results obtained will be easily translatable to the results obtained from human patients. By doing so the number of animals required will be reduced, due to the fact that the measurements recorded will be similar to that of human recordings. No assumptions will need to be made about whether the recording will be similar to that in human experiments. And fitting of data from experiments done on other animals will be required. The amount of data obtained from the animals is dependant on the duration during which seizures occur, which can vary from 3-6 months. Therefore the animals will be monitored until their seizure frequency reduces beyond a useful level. This will ensure that maximal experimental usefulness is extracted from each animal in order to ensure that the number of animals required is reduced, and that the results are relevant.

By making use of brain stimulation targets and stimulation paradigms that have been shown to be successful in previous studies the number of possibilities for stimulation strategy will be reduced. This will reduce the total number of rats required as less animal groups will be required.

**Please detail any technique refinements which may reduce the adverse impact(s) on animal welfare:**

An analgesic will be administered at the beginning of surgery to reduce pain and distress.

Post-operatively the animal is allowed to recover on a heating pad.

Rats are housed in 24 hour cages as described in SOP #47. If seizure frequency is severe enough to possibly impede animals normal feeding habits, as per SOP #47, their food will be made into a paste that will be easily accessible for the animal.

Baytril will be administered as per SOP #34 in order to reduce the chance of infection. It is worthwhile noting that Baytril is a proconvulsant, and thus can potentially bias experimental results by leading to an increase in the number of seizures due to the drug, therefore a five day waiting period before recordings commence is necessary. The extra days required are necessary to ensure that the animals are healthy and that the head mount is in a condition suitable for attachment to the recording cables.

**2c) If this proposal is to request the continuation of an existing project, please provide a paragraph summarizing the results from the previous project, including scientific results and any other information**

**that the Committee should be aware of.**

N/A (This is a new project)

**Section 3: Experimental Procedures**

**3a) Give a clear step-by-step description of all procedures to be carried out on each animal/group of animals, including controls. If Standard Operating Procedures (SOPs) are referred to in the application,**

**please attach a copy to the application. List all possible sources of pain and distress to the animals during the experiment and procedures proposed in Section 3a. For example, a) slight pain/distress is**

**caused by inoculation and blood collection; b) moderate pain/distress is caused by minor surgery, use of CFA for immunization and the induction of diabetes; and c) severe pain/distress is caused by major**

**surgery, irradiation and the induction of arthritis. All submissions must be stand alone documents. Use the BAW Code List to determine the Impact and Particular Procedure for each cohort. Please note that**

**the highest Impact applicable should be chosen for each cohort.**

Summary of Procedure #1: Electrode Implantation and tetanus toxin injection

# Animals: 120

Species: Rat

Level of Discomfort: Severe

Impact: 5, PP: 1

Description of Procedure 1:

In this procedure the animals will be implanted with electrodes in order to record brain activity, these electrodes will also be used to apply therapeutic stimulation to the rats’ brain. Tetanus toxin is injected into the brain of the animals in order to cause the animal to have seizures.

The pre-surgery, surgery and post-surgery procedures will be done in accordance to SOP #34. There is the possibility that micro-array, twisted wire or screw electrodes will be used. If this is required these electrodes will be implanted in a similar way to the electrode implantation described in SOP #34.

Clinical Signs for Procedure #1:

Severe discomfort will occur after this procedure. Signs of discomfort will be monitored via SOP #47 and SOP #34.

Welfare & Monitoring for Procedure #1: Animals will be monitored throughout all aspects of this protocol. This will occur as per SOP #40.

Summary of Procedure #2: Therapeutic Stimulation

# Animals: 45

Species: Rat

Level of Discomfort: Slight

Impact: 1, PP: 2

Description of Procedure 2:

This protocol aims to determine a method to measure the effect of different electrical stimulations. The stimulation that will be used will be within specified safety limits. In order to ensure that the varying effects of electrical stimulation on seizure reduction can be determined, the stimulation protocol will be specified in ranges, rather than specific values. The specifications are in the list below:

* Frequency 0-10kHz.
* Charge balanced waveforms.
* Aperiodic or periodic stimulation.
* Synchronous or asynchronous stimulation.
* Current intensity below recommended safety limits.

By doing so it may be possible to determine how the effect of stimulation alters as the animal is approaching a seizure, and how different stimulation strategies affect this.

This procedure will involve usage of the 24 hours recording facilities as detailed in SOP #47. We will examine two different groups and one control group that is not stimulated.

Clinical Signs for Procedure #2:

Slight discomfort will occur during this procedure. Signs of discomfort will be monitored via SOP #47.

Welfare & Monitoring for Procedure #2: Animals will be monitored throughout all aspects of this protocol. This will occur as per SOP #40.

Summary of Procedure #3: Therapeutic Stimulation

# Animals: 75

Species: Rat

Level of Discomfort: Slight

Impact: 1, PP: 2

Description of Procedure 3:

This protocol aims to determine whether systematic altering of parameters of electrical stimulation is better than standard electrical stimulation. The parameters that could possibly be altered are frequency, current intensity, pulse width etc. All parameters will be altered in order to remain within current recommended limits for safe electrical stimulation of the brain.

This procedure will involve usage of the 24 hours recording facilities as detailed in SOP #47. We will examine five different groups.

Clinical Signs for Procedure #3:

Slight discomfort will occur during this procedure. Signs of discomfort will be monitored via SOP #47.

Welfare & Monitoring for Procedure #3: Animals will be monitored throughout all aspects of this protocol. This will occur as per SOP #40.

**3b) Do you anticipate out-of-hours monitoring is necessary? For example, weekends, public holidays and/or overnight?**

Yes

**If yes, who will perform the out of hours monitoring? NB: The legal responsibility for ensuring an appropriate regiment of monitoring lies with the Principal Investigator.**

Richard Balson

**3c) Other than genotyping, will animals undergo more than one procedure?**

Yes

**If yes, provide details below:**

Surgery will be performed on the rats as described in procedure 1 and then recording/stimulating will be done in order to determine the efficacy of stimulation.

Only one surgery will be performed and the procedures are complimentary i.e. the surgery is performed in order to allow for electrical stimulation. The reason for separating them is for ease of understanding.

**3d) Will anaesthetics/drugs be administered for any of the procedures listed in section 3a?** Yes

**If Yes, provide details below:**

Agent: Route: Dose: Duration:

Ketamine i.p 70 mg/Kg 1 hour

Xylazine i.p. 10 mg/kg 1 hour

Carprofen s.c. 5mg/Kg 1-3 hours

Baytril oral 3.5mL/L 1-7 days

Isoflurane Inhalation 2-3% in Oxygen 5 minutes

Isoflurane Inhalation 1-3% in Oxygen 1-2 Hours

**3e) Will analgesia be administered for any of the procedures listed in section 3a?** Yes

**If Yes, provide details below, If no analgesic agent is administered, please justify:**

Agent: Route: Dose:

Carprofen s.c. 5mg/Kg

**3f) Animals requested. Provide a summary of the clinical observations made for any mutant, transgenic or knockout animals being used. If the phenotype is unknown, please describe the expected**

**phenotype. Please inform the Committee when details of the phenotype are available.**

Proc#: Species: Strain: Number: Housing: Source:

0 Rat Sprague Dawley 120 EMSU Western Australia

Total (All animals):120

**3g) Please explain how the figure of total animal use was established, bearing in mind that the project should be designed to achieve statistical significance while using the minimum number of animals.**

**Justify the number of animals requested in terms of statistical considerations and/or other considerations in the experimental design with regard to prior experience.**

From previous studies done by the Jeffreys group, the number of animals using in groups for the tetanus toxin model is ten. These studies have been done in order to characterise the seizures that are experienced by the animals. This protocol will be determining the effect of electrical stimulation on the model, therefore it would make sense to increase this number to ensure significance of results. Therefore groups of 15 will be used, 5 more than the Jeffreys group due to relative inexperience when compared with the Jeffreys group and different experimental reasons, by doing so it is ensured that results will be significant. This number is in line with the number previously used by our group as in the approved ethics AEC51-06. Seven of these groups will be required to ensure that the effects of different stimulation protocols can be determined. A control group is also necessary, therefore there will be 8 groups of 15, which amounts to a total of 8x15 animals, which amounts to 120.

**Section 4: Phenotype**

**4a) Provide a summary of the clinical observations made for any mutant, transgenic or knockout animals being used. Please include a clinical description of such animals and any special husbandry**

**requirements.**

N/A

**4b) If the phenotype is unknown, please describe the expected phenotype. Please inform the Committee when details of the phenotype are available.**

N/A

**Section 5: Endpoint of the Experiment**

**5a) In experiments in which illness occurs (whether associated or not with the experimental procedures), what criteria will be used to determine the end point of the experiment?**

Rat health monitoring will be done according to SOP #40.

**5b) Will the animal be killed immediately after the experiment? Yes**

**If yes, provide details of the method of euthanasia to be used, if no, provide details of what is to happen to the animal**

Rats will be euthanized according to SOP #26. The brain will be dissected and prepared for further analysis, and the bodies will be taken to the EMSU cold room for disposal.

**Section 6: Staff Roles and Experience**

**6a) Complete the table below for all investigators who will be handling the animals, including the principal investigator.**

Investigator: Mark Cook

Qualifications: MBBS

Experience: I will not be performing animal experiments

Investigator: Tim Nelson

Qualifications: BEng(Hons), BSc

Experience: Electrophysiological recording, neural implants, perfusion and euthanasia.

Investigator: Richard Balson

Qualifications: BEng (Hons)

Experience: N/A

Investigator: Nicola Beattie

Qualifications:

Experience:

Investigator: Dean Freestone

Qualifications:

Experience:

Investigator: Karin Mclean

Qualifications:

Experience:

**6b) Provide details of any training required, including details of the person(s) responsible for performing and assessing the training.**

Training on surgical implantation will need to be done, this will be done by Tim Nelson who has experience with such procedures.

**6c) Bioservices staff under the supervision of Ms Sue McKay will provide technical assistance for this project.**

**Senior Animal Technician Declaration:**

**I am satisfied that the housing location listed in Section 3e can accommodate the requirements of this project.**

**Name: Sue McKay**

**Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Section 7: Health Risks**

**7a) Does this project pose any health risks?** No

**If yes, please establish and give details of preventative measures taken.**

**Section 8: Certification by Principal Investigator**

**8) I certify that all personnel involved in this project are appropriately qualified and experienced, or will undergo the appropriate training, to perform the procedures required of them and the attached**

**standard procedures for containment at PC2 level shall be observed at all times, if appropriate.**

**Name: Mark Cook Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**