

VERTEBRATE ANIMALS – PROJECT 1 – BIOMARKERS OF EPILEPTOGENESIS AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

Description of animals and how they will be used [University of Kuopio (UEF), University of Melbourne (UM), University of California at LA (UCLA)]

Rats will be used to identify biomarkers for the development of epilepsy after traumatic brain injury (TBI). Animals will undergo TBI with lateral fluid-percussion injury (FPI). Thereafter they will be examined in a clinically translatable way by collecting blood samples, performing magnetic resonance imaging (MRI), and video-electroencephalogram (EEG) monitoring.

Animals are anesthetized (with 2% isoflurane) during the induction of TBI, during MRI, and during collection of blood from the lateral tail vein according to 3R recommendations (<http://www.nc3rs.org.uk/bloodsamplingmicrosite/page.asp?id=420>). Xylocain cream will be used for local pain-relief around the wound. If any sign of infection is seen, wound will be treated with antibiotics (Basibact). During the first days after TBI, rats will receive subcutaneous injections of saline and glucose. Animals are allowed to recover fully from the effects of general anesthesia in a normothermic chamber before being returned to the animal care facility.

Adult (11 weeks old in the beginning of study, 325 ± 25 g) male Sprague-Dawley rats (UEF - Harlan, The Netherlands; UM –aAnimal Resources Centre, Canning Vale, WA, Australia; UCLA –nHarlan, Livermore, CA, USA) will be used.

Power analysis shows that we need 21 TBI rats with epilepsy, 63 TBI rats without epilepsy, and to control the experiment, we need 21 sham-operated controls per cohort (total two cohorts). Consequently, at each of the three study sites (UEF, UM, UCLA) total of 65 rats (55 TBI, ten sham) will be included in each of the two cohorts (SA1 1st cohort, SA2 2nd cohort) as we expect acute (<48 h) post-TBI mortality to be about 30 % (17 rats) and follow-up mortality 25% (ten rats; due to injury, anesthesia for MRI; loss of electrode headset leading to euthanasia) resulting in 28 TBI rats (seven with epilepsy, 21 without epilepsy) and seven sham-operated animals to be analysed per site per SA. To achieve statistical power, data from the three study sites (UEF, UM, UCLA) will be combined. All experiments have been designed to minimize the number of animals, but not sacrificing the rigorous experimental designs for robust and unbiased results (power analysis for plasma molecules, video-EEG, MRI) using 3R principles.

Justification of use of animals [University of Kuopio (UEF), University of Melbourne (UM), University of California at LA (UCLA)]

Rats will be used for the following reasons: (1) These studies attempt to examine the mechanistic factors that signal about pathologic changes which gradually develop after TBI, such as neurodegeneration, neuroinflammation, axonal/dendritic damage/growth, and metabolic changes. Since these relationships are unknown in the living brain, they cannot be adequately simulated using computer models, and the complex interplay of brain activity and the anatomical substrate(s) of neuroplasticity after injury cannot be modeled in cell cultures. (2) The assessment of epileptogenesis requires a live animal that is capable of generating spontaneous recurrent seizures, and therefore cannot be achieved with in vitro or ex vivo methods. (3) Studies presented here, including long-term video-EEG follow-up of epileptogenesis and combined assessment of histology with imaging are not possible in humans. (4) TBI in rats recapitulates many of the key features of human TBI including similar histopathology, motor impairment, memory decline and epileptogenesis, making the model proposed feasible for studies that are relevant for the aftermath of human TBI. (5) Exploration of promising novel treatments requires preclinical testing of drugs in animal models, which is most often done in rat models. This justifies the use of rats in the present experiment as biomarkers identified would not only be useful for clinical studies, but also for stratification of animals for preclinical studies of investigational new treatments. (6) Spontaneous seizures are the gold-standard outcome measure to classify animals to epilepsy vs. no-epilepsy groups, and are needed for assessment of biomarker sensitivity and specificity. Current video-EEG systems head pieces are still relatively heavy and would cause extra discomfort and restriction of mobility if, for example, mice would be used instead of rats. (7) The relatively large size of the rat brain (e.g., as compared to the mouse brain) makes it significantly easier to obtain good signal-to-noise MRI data. Furthermore, the rat brain poses fewer problems regarding magnetic field inhomogeneities caused by different magnetic susceptibilities in tissue interfaces than does the smaller mouse brain, which is a critical factor for data quality in EPI-based DTI approaches.

Veterinary care [University of Kuopio (UEF), University of Melbourne (UM), University of California at LA (UCLA)]

The animals will be housed in an approved animal facility, which is maintained under supervision of the vivarium staff. Veterinary expertise is continuously available. Animals are single-housed together in a clear plastic bin (20x10x10 inches) that is lined with bedding material. Food and water are available *ad libitum*. Cages are cleaned twice weekly and food and water are checked daily. Daily monitoring of rats includes (a) general well-being of animals using the standardized form provided by Animal Center, (b) weight (once per week), (c) rectal temperature (once per week). Each animal is checked daily for loss of stereospecific behavior and coat color – eany wound incisions/sutures are also examined daily. If any major health problems are noticed (over 15% weight loss, reduced grooming, signs of reduced well-being beyond what is expected to be TBI-related), the animal will be euthanized, and excluded from the study.

Provisions to minimize discomfort, distress, pain and injury [University of Kuopio (UEF), University of Melbourne (UM), University of California at LA (UCLA)]

All surgical procedures (e.g., induction of craniotomies, collection of blood via tail vein) are conducted under deep anesthesia. After completion of surgery for induction of brain injury, bupivacaine (0.25%) is locally infiltrated into all wound margins and topical antibiotic ointment is applied.

Euthanasia [University of Kuopio (UEF), University of Melbourne (UM), University of California at LA (UCLA)]

Euthanasia of anesthetized rats will be performed by terminal intravenous or intraperitoneal injection of an overdose of sodium pentobarbital (75 mg/kg, i.v.). This method has been selected because it is painless and rapid; the method is consistent with the recommendations of:

UEF: The Committee for the Welfare of Laboratory animals of the University of Kuopio and by the Provincial Government of Kuopio (ESAVI/5146/04.10.07/2014) and European Community Council Directive 2010/63/EU.

UM: The University of Melbourne/Florey Neurosciences Institute Animal Ethics Committee and the Australian and New Zealand Council for the Care of Animals in Research and Teaching.

UCLA: The University of California, Los Angeles, (UCLA) Chancellor's Animal Research Committee and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.