

EQUIPMENT – PROJECT 1- BIOMARKERS OF EPILEPTOGENESIS AFTER TRAUMATIC BRAIN INJURY

University of Eastern Finland (Asla Pitkanen, Olli Grohn)

Functional Neuroanatomy Laboratory (Pitkänen lab): Fully equipped histology and molecular biology laboratory (40 sq meters) with microtomes and cryostats. Three microscopy rooms (each 12 m²), including microscopes with brightfield, darkfield and fluorescence optics and analysis software; Zeiss microscope equipped with apotome. Electron microscopy, confocal and multiphoton microscopes are available on-need basis. All facilities are available for the project full time.

Animal Facility: Separate laboratories for 1) surgical operations (8 sq meters), 2) video-EEG monitoring (two rooms, each 12 sq meters), including 5 video-EEG monitoring units (32 channels each), 4) behavioral testing (2 rooms, 12 sq meters), and 5) perfusion (8 sq meters). All these are located in the UEF Animal Center. Induction of TBI animal model, video-EEG analysis (Pitkänen lab): Lateral-fluid percussion injury device, Leica CCI device, 5 video-EEG monitoring units (each can monitor 6 rats with 4 channels at a time), five licences for nervous EEG analysis software (installed on computers).

MRI facility (Gröhn lab): Three experimental MRI systems are located in the Biomedical Imaging Unit). Large bore horizontal 9.47/31 cm magnet is currently interfaced to two different consoles: Bruker Biospec and Agilent DirectDrive and is equipped with two gradient sets, 4 receiver channels and large number of RF-coils including actively decoupled 4-channel receiver coil optimized for rat head. In addition we have horizontal 7T/16cm Bruker PharmaScan MRI system and a vertical 9.4 T/89 mm Varian DirectDrive micro-imaging system. All the pulse sequences and analysis software described in research plan have been implemented and either used in our previous studies or thoroughly tested. Facility is equipped with microsurgery unit and extensive MRI compatible physiological monitoring instruments including respiratory and cardiac gating unit, blood pressure monitoring, pulse oximeter, capnographm, blood gas analyser and MRI compatible EEG (Brainamp).

Computer: In Pitkänen lab 16 computers for routine office work with all necessary software for word processing, image analysis and processing, preparation of presentations, and statistics (all bought within past 3 years). Five licences for nervous EEG-analysis software. In Gröhn lab more than 15 computers equipped with MRI analysis software (SPM, FSL, LC model, MATLAB), including a Linux server with 2 processors (6 cores/processor, 2 threads/core), with 96GB of memory. Access to several servers in national supercomputing facility at Center for Scientific Computing (CSC) if large datasets or excessive computing recourses are needed.

Data storage: A secure private cloud will be installed and used as a temporary repository for all data acquisition. Six systems gathering EEG data in Lab Animal Centre will be connected to University of Eastern Finland (UEF) private cloud. All research group members will be able to store and access the data via UEF local area network (LAN) connection and perform the analysis on their personal computers. The data will remain in the private cloud as long as analysis is completed, after which the data will be moved into a long-term storage to CSC IDA service. Additionally, the data will be backed-up to magnetic tape cartridges to ensure data security. MRI data will be stored in an existing file server (Synology DS509+ NAS server with 10TB of space, 5 disks in a stack, RAID 6) that can be accessed via LAN connection from magnet consoles, computing servers, and personal computers. The data will be backed-up into magnetic tapes as a means of long-term storage after the project completion.

Other: University Computer Center (on need basis). Bioinformatics Center (on need basis). Library with Electronic Journal Collection (fully available)

University of Melbourne (Terence O'Brien, Nigel Jones, Sandy Shultz)

Epilepsy and Neuropharmacology Laboratory: This laboratory is located at the University of Melbourne's new state-of-the-art Neuroscience building, the Melbourne Brain Centre. The research environment at the Melbourne Brain Centre is both ideal and necessary for this project as it offers full access to advanced imaging (MRI and PET), behavioural testing, EEG, immunohistochemical, and molecular facilities.

This laboratory (~100 sqm) has successfully developed and optimized a facility for studying a variety of rat and mouse models of seizures/epilepsy (e.g. amygdala kindling, 6Hz psychomotor test, acute PTZ challenge, GAERS, post-kainic acid status epilepticus, post-traumatic, post-stroke, cerebral tumour associated epilepsy).

We have extensive published experience in discovery and drug development research using these models. We have the capacity for prolonged video-EEG monitoring (Compumedics, Graef HD amplifiers with recording bandwidth up to 2000 Hz) of up to 60 rats/mice simultaneously as well for chronic drug administration via a variety of routes (icv/iv/sc) including capacity for continuous or intermittent dosing regimes. We also have electrophysiology rigs for in-vivo and ex-vivo single cellular and optical studies, a full range of histological and immunohistochemistry facilities, stereological microscopy, confocal microscopy and molecular biology including q-rtPCR, Western Blotting, in-situ hybridization. A custom-made Fluid Percussion pneumatic device (impact: 22 msec, 2.5-3 atm) (Thompson et al., J Neurotrauma 2005). Analysis software including Spike2 8.07, LabChart v7, Matlab.

The Neurobehavioral Testing Facility (N. Jones): The Neurobehavioural Testing Facility (~40 m²) in the Department of Medicine, Royal Melbourne Hospital, University of Melbourne is fully equipped for the study of small animal behavior, with a focus on cognition and anxiety- and depression-like behaviors. The laboratory head, A/Prof. Jones, has 17 years of experience in the measurement of animal behavior, and his primary expertise is in assessment of interventions on behavioral deficits. The Neurobehavioural Testing Facility is equipped with a variety of equipment, including mazes (Y, T, zero, elevated plus, Morris, Barnes), and is located in the immediate vicinity of the Epilepsy and Neuropharmacology laboratories in the Department of Medicine.

The Small Animal MR Facility (L. Johnston): The small animal MR facility in the Florey Neuroscience Institutes, University of Melbourne is equipped with a 4.7 Telsa Bruker BioSpin magnet upgraded to the *Avance IIITM* platform. The system consists of two broadband transmitter channels and eight broadband receiver channels for multi-coil phased array coil imaging applications. In addition to volume and single surface coils for rat brain imaging, a 4-channel phased array receive-only coil with geometrical shape adapted to the rat head is available for an optimal homogeneity and sensitivity of the radiofrequency excitation field. The system is configured with Paravision 5 software including packages for parallel imaging, spiral imaging, echo planar diffusion imaging, and susceptibility weighted imaging. Physiological monitoring of animals is carried out using a PowerLab 8-channel recording system to measure ECG, arterial blood parameters, and temperature. Rat models can be ventilated using a Columbus CIV-101 ventilator and anaesthesia can be delivered via inspired gases, intravenous, intramuscular or intra-peritoneal routes. Rat models are monitored continuously throughout experiments with core temperature maintained using a heated water pad placed under the animal. Comprehensive image analysis software is available for post-processing including Matlab and Paravision analysis tools, and most commonly used open research based software packages (eg. Mricro, FSL, SPM, MRtrix, MRStudio).

UCLA (Richard Staba)

Seizure Disorder Center Animal Laboratories: Our animal electrophysiological and behavior laboratory currently contains eleven 16-channel setups to record scalp and/or intracranial EEG under freely moving conditions. Four of the setups located in RNRC 2136 can record wide bandwidth EEG (0.1 Hz to 6 kHz) sampled up to 28 kHz per channel Cheetah System (Neuralynx, Inc. Bozeman, MT). This system can be configured to record from a single rat or up to eight rats simultaneously. The 7 remaining setups in RNRC 2253A&B can sample up to 20 kHz per channel (band width 0.1 Hz to 6 kHz). A mobile 64-channel BMSI EEG/video monitoring system can be placed in either recording room for behavioral and electrographic seizure monitoring and spike detection. There is a dedicated setup for intracellular and extracellular electrophysiological experiments with juxtacellular labeling of recorded neurons (RNRC 2144), while a separate workstation is setup for in vitro electrophysiological studies (RNRC 2136). Our three study areas (RNRC 2136, 2253A&B, CHS 63-399 vivarium) each contain a 4-channel networked DVR system (500GB storage capacity each) with 4 infrared cameras (model SYRF04, Supercircuits, Austin, TX) using illumination above 760nm, which is not visible to rodents. Video can be review on site or remotely via Internet. With our current video recording equipment we can record animal behavior for the occurrence of spontaneous seizures from 24 rats 24 hours/day. Of these rats, simultaneous video-EEG recordings can be performed on 11 rats.

Tissue histology: Our histological laboratory (RNRC 2144) contains a Cryostat (HistoStat, AO), vibrating microtome (Leica, Heidelberg, Germany), two regular refrigerators, oven for in situ hybridization, shakers, etc. A -70oC freezer is in RNRC room 2132. We also have a Zeiss microscope configured with digital camera and computer for image analysis, and 2 stereoscopes -- one used for gross histological sections and the second attached to the micromanipulator used primarily for microelectrode fabrication.

UCLA Brain Mapping Center: Bruker Biospin a 7.0 Tesla magnetic resonance imaging/spectroscopy instrument with a clear bore diameter of 30. Three gradient systems are included: (1) BGA-20: 200 mm inner diameter with a maximum gradient strength of 200 mT/m. (2) BGA-12: 116 mm inner diameter with a maximum gradient strength of 400 mT/m (3) BGA-6: 60 mm inner diameter with a maximum gradient strength of 950 mT/m. Full physiological monitoring is possible including core temperature control, heart and ventilation rate, end-tidal PCO₂ and non-invasive blood pressure. The instrument is installed in a space occupying 535 ft² located in the Brain Mapping Center. A full surgical suite is available in the adjoining room with a surgical microscope and downdraft air exhaust table. Both surgery and magnet rooms are equipped with isoflurane gas anesthesia equipment.

Computers: A 12-core/24-CPU linux workstation with 48G of RAM and 12TB disk memory and 30TB backup is used for all neuroimaging analysis and is administered by Dr Harris. It is equipped with all standard imaging and statistics software (FSL, SPM, AFNI, MATLAB, R) as well as a grid-engine, job submission queue for parallel, high through-put analysis. Supercomputing resources are also available through the UCLA Hoffman2 Cluster which has 256 nodes for general campus computing but many more nodes available for special jobs.

Harris Laboratory: Animal surgery with two surgical areas, each equipped for isoflurane anesthesia and a surgical microscope; necropsy/perfusions with equipment for tissue harvesting, a perfusion hood and two cryostats; wet lab equipped for studies on molecular biology (protein and RNA analysis, ELISA or spectroscopy); two histology rooms, one with a fume hood; two rooms for microscopy/image analysis, one containing a Leica upright vertical and Zeiss M2; axioimager (both interfaced with MicroBrightfield Stereology Software System) and one containing a Zeiss confocal microscope.