

## **EQUIPMENT – PROJECT 2 – PRECLINICAL MODEL FOR ANTIEPILEPTOGENIC THERAPY SCREENING IN POST-TRAUMATIC EPILEPSY**

### **1. Laboratory Of Developmental Epilepsy (Directors Aristeia S. Galanopoulou, Solomon L. Moshé):**

Deep-freezing box (REVCO; -80°C), a liquid nitrogen tank for storage of frozen cell cultures, Nikon E1000M microscope with epifluorescence, on-line CCD or digital cameras are also available to the PI. One cryostat (Microm) and a Leica vibratome, a Hybaid PCR thermocycler, a StepOne real time PCR system (Applied Biosystems), a UV transilluminator, a refrigerated centrifuge, two spectrophotometers including a Nanodrop for small volume samples, vacuum evaporator, microcentrifuges, homogenizers, a roller incubator for membrane hybridizations, electrophoresis apparatus for nucleic acid and protein electrophoresis (DNA electrophoresis, Northern and Western blot studies), an iBlot gel transfer system, a water bath incubator, a shaker incubator are also available. For behavioral recordings, four Sony MiniDV/Sony CCD videocameras are available, as well as two CCD video cameras with infrared capability, a monitor for playback and frame-by-frame analysis of seizures, a neonatal rat EEG monitoring setup based on Pinnacle technology as well as Stellate-based and XLTEK-based EEG recording systems and software that are capable of recording video-EEGs from the neonatal ages till adulthood are available. Two in vitro electrophysiology setups with capability for patch clamps are available with Nikon microscopes, and one setup for extracellular recordings, Narishige micromanipulators, pClamp, Axopatch, and Axon A/D data acquisition. The first is outfitted with: Axopatch 200B, TDS224 oscilloscope, Digidata 1322A, LAMBDA DG-4, E600FN Nikon microscope, Coolsnap HQ CCD camera. In vitro live imaging system for fluorescent indicators (i.e. fura2AM) is available with a Lambda DG4 wavelength switcher, a Photometrix HQ2 digital camera and the NIS-Elements Advanced research Software coupled to one of the electrophysiology setups. The second is set up with a Digidata 1440A, Tektronix TDS 2004B oscilloscope, Multiclamp 700B, Master 8 AMPI, QImaging CCD, Nikon microscope, Nikon Intensilight EHGFIE. A setup for extracellular recordings is available with Digidata 1322A, Axoclamp-2B, two A-M Systems Isolated Pulse Stimulators (2100 model). For the in vivo single unit recordings a complete setup with 32-channel recording capability and optogenetics capacity exists (Neuralynx, Spectralynx) with Cheetah, Neuraview, and Pulse software. Also, an A-M systems amplifier (16 channel), 16-channel swivel (Dragonfly) and DataWave Recording/Analysis software are available. A separate surgical stereotaxic setup for adult rodents (Heinrich Kopf) is also available. Isoflurane anesthesia setup is available as well as a Benchmark AngleOne stereotaxic surgery instrument for neonatal rats. Equipment for Barnes maze testing, and a social chamber are available.

Available through the Core facilities: A Tissue Engineering and Cellular Reprogramming Core Facility is also available at Kennedy Center, in which the PIs have access to use. It includes four tissue culture hoods, several incubators for culture maintenance, light and epifluorescence equipped microscopes, capability for viral vector cellular manipulations, FACS, centrifuges and liquid nitrogen tanks for preservation of frozen cells. For more information please see: <http://www.einstein.yu.edu/home/SharedFacilities/ViewFacility.asp?ID=48>

A dark room equipped with Konica Minolta SRX-101A and Kodak Imaging 2000R system for film developing and digital imaging is available as part of the Kennedy Core facilities.

The College maintains shared scientific and core facilities for transgenic animals, DNA plasmid sequencing, proteomics, mass spectroscopy, micro-arrays, deep sequencing and Li-Cor system for Western blots. For more information see <http://www.aecom.yu.edu/home/shared.asp>.

There is a Biostatistics Shared Resource available to the investigators at Kennedy Center for consulting with the design, conduct, and analysis of biomedical research (Director Dr. Mimi Kim):

[http://eph.aecom.yu.edu/web/division\\_details.aspx?id=10](http://eph.aecom.yu.edu/web/division_details.aspx?id=10)

There is a Neurogenomics Core Facility (Director John Greally MB PhD) for consultation on data analysis for the microarray and genomics data:

[http://www.einstein.yu.edu/centers/iddrc/iddrc.aspx?id=30134&ekmense=15074e5e\\_4332\\_4335\\_btnlinkHuman](http://www.einstein.yu.edu/centers/iddrc/iddrc.aspx?id=30134&ekmense=15074e5e_4332_4335_btnlinkHuman)

There is a Cell and Molecular Imaging Core Facility at Kennedy (Director Dr. Kostantin Dobrenis) with the following capabilities:

Ultrastructural Analysis Facility: A Philips CM10 electron microscope, two Sorvall model ultramicrotomes on antivibration tables are available for thick and thin tissue sectioning.

Confocal Bio-Imaging: (1) An Olympus Fluoview 500 Confocal Microscope built around a BX50WI upright microscope, equipped with 4 lasers, five channel acquisition and capable of 2-photon analysis. (2) A Nikon RCM8000 Real Time scanning fluorescence confocal system equipped with an inverted Diaphot microscope with incubation chamber, Argon Ion and green Hene lasers, 2 PMTs, on-line ratio imaging, a Sun, Silicon Graphics and PC computer, and sophisticated Nikon and Metamorph (UIC) quantitative image analysis software tools. Neurolucida/Stereoinvestigator and electron microscope.

[http://www.einstein.yu.edu/centers/iddrc/iddrc.aspx?id=30135&ekmense=15074e5e\\_4332\\_4335\\_btnlinkHuman](http://www.einstein.yu.edu/centers/iddrc/iddrc.aspx?id=30135&ekmense=15074e5e_4332_4335_btnlinkHuman)

A Rodent Behavioral Evaluation Core Facility (Director Dr. Maria Guinello) is available to the investigators at Kennedy that provides the possibility to perform a variety of behavioral and functional assays on rodents.

<http://einstein.yu.edu/research/shared-facilities/cores/49/rodent-behavioral-evaluation/>

<http://www.kennedy.aecom.yu.edu/core/behavioral/>

### **Gruss MRRC Equipment**

MRI laboratories (floor 1): A magnetic/RF shielded room housing the 9.4 T, 21 cm Varian Direct Drive MRI/MRS system. Adjacent to the MRI laboratories are the following supporting areas; a machine shop, electronics and coil fabrication laboratory, a wet laboratory, a physiology instrumentation laboratory and an animal physiological laboratory including housing for barrier and non-barrier animals.

#### **Animal MRI (9.4 Tesla laboratory):**

A 9.4 T 21 cm ID Agilent Direct Drive MRI, running VNMRJ 3.2. This system is equipped with 2 channel Transmit / 4 channel Receive capability. A 12 cm ID gradient set (60 G/cm, 180 us rise-time) with integral 14 channel shim set is installed, and driven by a Copley 266 gradient amplifier (Analogic, MA) and a 14 channel, 10 amp / channel shim power supply (Resonance Research Inc., Billerica, MA). *RF Coils:* Three double tuned transmit body coils (tuned to proton and either 19-F, 13-C or 31-P, M2M Imaging Corp., Cleveland, OH) and receive only surface coils for 1-H, P-31, 19-F and 13-C (Doty Scientific, Columbia, SC) are available for brain and body rodent applications along with mouse and rat brain 4-channel array coils for SENSE imaging. All coils are actively decoupled from surface coils using an M2M coil control unit and fiber optic communications.

Adjacent to the 9.4 Tesla MRI laboratory is a 14 m<sup>2</sup> fully equipped surgical facility, general surgery tools, an operating light and autoclave. Physiological monitoring in the 9.4 Tesla MRI is achieved using either a BioPac (Goleta, CA) Systems MP150 Data acquisition system or an SA Instruments (Bayshore, NY) model 1025 monitoring and gating system.

### **Computer Resources**

*Imaging Analysis Hardware:* All MRRC data processing is done transparently through the Einstein-Yeshiva High-Performance Supercomputing Cluster (HPSC or "the Cluster"). The Cluster consists of 120 computing "nodes" for a grand total of over 2,000 processor cores supported by 8 gigabytes and 4 terabytes of RAM memory per node and a total 800 terabytes of raw storage. *General Image Analysis/Scientific Software:* Managed software access is provided to all users. Supported software includes AFNI, FSL, SPM, AEDS, ImageJ, FreeSurfer, MRICro/n, and several MATLAB toolboxes for functional MRI analyses; LCModel, MRUI, and LUIS for spectroscopy and ASL; and MedINRIA, Slicer, TrackVis, CATNAP, and CAMINO for diffusion and tractography analyses. Other computing and statistical software is available through the University including SPSS. *Specialized Image Analysis Software:* Image analysis software is also developed in-house for a variety of purposes. The code is written in C++, MATLAB, or IDL.

## **2. 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 sqm) 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 Tesla Bruker BioSpin magnet upgraded to the *Avance III<sup>TM</sup>* 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).

### 3. 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 -70°C 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 is used for gross histological sections and the second is 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 PCO<sub>2</sub> and non-invasive blood pressure. The instrument is installed in a space occupying 50m<sup>2</sup> 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.

#### 4. University of Eastern Finland (Asla Pitkanen, Olli Grohn)

**Functional Neuroanatomy Laboratory (Pitkänen lab):** Fully equipped histology and molecular biology laboratory (40 m<sup>2</sup>) with microtomes and cryostats. Three microscopy rooms (each 12 m<sup>2</sup>), including microscopes with brightfield, darkfield and fluorescence optics and analysis software; Zeiss microscope equipped with apotome. 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 m<sup>2</sup>), 2) video-EEG monitoring (two rooms, each 12 m<sup>2</sup>), including 5 video-EEG monitoring units (32 channels each), 4) behavioral testing (2 rooms, 12 m<sup>2</sup>), and 5) perfusion (8 m<sup>2</sup>). All these are located in the UEF Animal Center. **Induction of TBI animal model, video-EEG analysis (Pitkänen lab):** Lateral-fluid percussion injury device, 5 video-EEG monitoring units (each can monitor 6 rats with 4 channels at a time), five licences for Nervus 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, capnogram, 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 Nervus EEG-analysis software. In Gröhn lab more than 15 computers equipped with MRI analysis softwares (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 on 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)

## **5. University of Minnesota, Center for orphan drug research (CODR) (James Cloyd, Lisa Coles)**

Major equipment in the analytical laboratory includes a Hewlett Packard series 1100 LC/MSD Mass Selective Detector system, Perkin Elmer Series 200 HPLC system with UV/Visible detector, Agilent 1100 Series HPLC system with variable wavelength detector (190-600 nm), and equipped with an 80-sample automatic injector, API-electrospray source, quaternary pump, column heater, micro flow cell, and nitrogen gas generator. The laboratory also has a state of the art ultra-sensitive Agilent 1260 Infinity HPLC system with fraction collector and three detectors; fluorescence, diode array, and UV/Visible detectors. Also available in the laboratory are a Barnstead Nanopure Diamond water purification system, Scientech SM124D analytical balance, Orion Cahn C-35 microbalance that can weigh a 250 mg sample to 1 µg or a 25 mg sample to 0.1 µg, Caliper TurboVap LV Concentration Evaporator Workstation, Branson B8510 and 1510 sonicators, Lab Companion BS-06 Waterbath, Marvel division explosion-proof refrigerator, Hettich Rotanta 460RS temperature control centrifuge, Accumet AB15 pH meter, Incubator with maximum temp for 60°C, -20°C and a -80°C freezer, refrigerator/freezer, a microcentrifuge, automatic pipettes, vortex mixers, Sentry safe to secure controlled substances and glassware.

Dr.. Cloyd's laboratory have a 9 m<sup>2</sup> cell culture facility with a Forma CO<sub>2</sub> cell culture Incubator and a high performance state-of-the-art Baker SterilGARD® III Advance Class II Type A/B3 Biosafety Cabinet and Forma Scientific Cryomed tank for storage with liquid nitrogen. The molecular biology lab is equipped with a -20°C and a -80°C freezer, refrigerator/ freezer, BioTek Synergy 2 Multi-Detection Microplate Reader with Luminescence and UV-Visible Absorbance, Gen5™ Data Analysis Software, and a Take 3 Multi-Volume Plate for nucleic acid quantitation, tabletop refrigerated centrifuge (Eppendorf 5430R) air-cooled centrifuge, max rcf = 30,130 x g (17,500 rpm), with 3 rotors; 24 x 1.5/2.0, MTP for two multi-well plates, 6 x 15 mL/50 mL, New Brunswick Innova 4230 refrigerated incubator shaker: temperature range from 20°C below ambient to 80°C, Agitation = 25 - 400 rpm, Orbit - 3/4" or 1" (1.9 or 2.54 cm) diameter orbits, Nunc plate washer, Biorad electrophoresis apparatus with dual access power source, orbital plate stirrers, automatic pipettors and chemical/fume hood.

## **6. University of British Columbia (Terrance Snutch)**

The main lab space consists of five twin wet benches with a desk at each for trainees to work on their notes, computer, etc. Other shared benches include equipment for qRT-PCR (two instruments), DNA sequencing (Illumina), and protein and molecular biology manipulations. There are three chemical fume hoods including a 1.5-person glovebox equipped with a parallel synthesizer. Other chemical synthesis resources include an Agilent 6100 series of Single Quadrupole LC/MS system, a Beckman Coulter P/ACE MDQ Capillary Electrophoresis instrument, high-pressure reactions vessels, and a Biotage Isolera One automated flash column chromatography instrument.

**Shared and Fee-for Service Analytical Facilities:** The Department of Chemistry offers a number of analytical services for compound purity and structural characterizations. These include: Capillary electrophoresis chromatograph, Circular dichroism/optical rotary dispersion (CD/ORD) spectrophotometer, Fluorometer, Fourier transform infrared spectrometer (FTIR) with attenuated total reflectance (ATR), Gas chromatograph (GC) with flame ionization detector (FID) and thermal conductivity detector (TCD), Gas chromatograph-mass spectrometer (GC-MS), Laser-induced resonance-enhanced multiphoton ionization-TOF-mass spectrometer (REMPI-TOF-MS), Liquid chromatograph-mass spectrometer (LC-MS), Nuclear magnetic resonance spectrometer, Polarimeter, Thermogravimetric analyzer/differential scanning calorimeter (TGA/ DSC), Ultra-high-performance liquid chromatograph (uHPLC), and three UV/Vis spectrophotometers – one with NIR capabilities.