FACILITIES & OTHER RESOURCES – PROJECT 2 – PRECLINICAL MODEL FOR ANTIEPILEPTOGENIC THERAPY SCREENING IN POST-TRAUMATIC EPILEPSY

1. Albert Einstein College of Medicine (Einstein)

Scientific Environment: The research team of the Laboratory of Developmental Epilepsy, led by Drs. Galanopoulou and Moshé, is fully qualified to successfully conduct the proposed research. Their research group has developed and characterized models of adult and early life epilepsies and have extensively utilized chronic models of epilepsies for the preclinical screening of new therapeutics. Drs. Galanopoulou and Moshé are Board Certified clinical epileptologists as well as basic science researchers and are qualified to design, perform, and interpret findings with an ultimate goal to translate them eventually to the clinical arena. Dr Galanopoulou was a co-organizer of an international effort (ILAE, AES supported; in collaboration with NINDS) to optimize preclinical epilepsy research so that it is more predictive of clinically relevant discoveries. The research team led by Drs. Galanopoulou and Moshé consists of three post-doctoral research fellows, one graduate student and 2 research technicians who are fully qualified to conduct the proposed experiments. The PIs' laboratory is fully equipped to carry on all aspects of this study, including stereotactic surgery, drug administration, seizure and video EEG monitoring of immature and adult rodents, behavioral studies, histology, molecular biology, in vitro and in vivo electrophysiology, and statistical analysis.

Dr. Wenzhu Mowrey is an Instructor at the Department of Biostatistics at the Albert Einstein College of Medicine and has been collaborating with Drs. Moshé and Galanopoulou on projects related to identifying new treatments for infantile spasms, funded by NINDS, the Department of Defense, and CURE. Dr. Mowrey has all the expertise to address the statistical aspects of the data analysis in the current project.

Dr. Craig Branch is Associate Professor at the Department of Physiology & Biophysics, Albert Einstein College of Medicine, and Director of the Integrated Imaging Program, Gruss MRI Imaging Center, and Blaufox PET/CT/SPECT Imaging Facility, Einstein COM. Dr. Branch has experience with MRI methodology, including rodent MRI and will offer his expertise and leadership at the Gruss MRI center for the purpose of this project.

Laboratory: The laboratory available to the PIs and their associates is in the modern Rose F. Kennedy Center and consists of nine laboratory and seven office space rooms (totaling 167 m^2.) completely equipped for stereotactic surgeries, electrographic recordings (in vivo and in vitro), seizure and behavioral testing, histology and histochemistry, molecular biology and has four fume hoods.

Clinical: N/A

Animal: Rose F. Kennedy Center contains animal housing colony rooms (for rats and mice), fully equipped with a supporting staff, including three full-time institutional veterinarians. These facilities and staff are 100% available to the PI.

http://einstein.yu.edu/research/shared-facilities/cores/52/animal-housing-and-studies/

Computer: Office computers (Windows or Mac OsX) with appropriate software (MS Office, Adobe Acrobat, Photoshop, Kaleidagraph, SigmaPlot, MATLAB, Endnote, SAS, Statview, geNorm, ABI 7000 sds v1.2.3, StepOne software, Image J, JMP10 statistical software; Stellate, Sirenia and XLTEK software for EEG recording and analysis; Cheetah software for in vivo recordings) and a scanner are available for every member of the team. Computers with digital cameras or A/D converters are available for electrophysiology and histology. Internet access is available to all laboratory members.

Office: Office space is available for the PIs and each of their associates, including post-doctoral research associates.

Other: A Core facility on Cellular and Molecular Neuroimaging (Director Dr. K. Dobrenis) is available to all investigators at the Kennedy Center for imaging of biological specimens, including confocal imaging, Neurolucida/Stereoinvestigator microscope for cell counts and neurite tracing, electron microscope, and state of the art image processing and analysis software.

http://einstein.yu.edu/research/shared-facilities/cores/47/cellular-and-molecular-neuroimaging/

A Rodent Behavioral Evaluation Core Facility (Director Dr. Maria Guilinello) 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/

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^2 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 Systems (Goleta, CA) 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, AEDES, 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. The University of Melbourne (UM)

The University of Melbourne is the #1 ranked Australian institution on the Times Higher Education Ranking (33rd worldwide) and the Shanghai Jiao Tong rankings (43rd worldwide). The University of Melbourne Parkville Predict is one of the largest and most productive neuroscience hubs worldwide. The O'Brien's 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 in-vivo neuroimaging, behavioural testing, EEG, immunohistochemical, and molecular facilities.

Epilepsy and Neuropharmacology Laboratory: This laboratory (~100 m^2) has successfully developed and optimized a facility for studying a variety of rodent models of epilepsy (e.g. amygdala kindling, GAERS, post-kainic acid status epilepticus, post-traumatic, post-stroke, cerebral tumour associated epilepsy) and traumatic brain injury (fluid percussion injury, cortical contusion, weigh drop and repeated concussions). We have extensive published experience in discovery and drug development research using these models. We have the capacity for prolonged video-EEG monitoring 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 regimens. We also have electrophysiology rigs for in-vivo and ex-vivo single cellular and optical studies, a full range of histological and immunohisochemistry facilities, stereological microscopy, confocal microscopy and molecular biology including rtPCR, Western Blotting, in-situ hybridization.

The Neurobehavioral Testing Facility: The Neurobehavioral Testing Facility ((~40 m^2) in the Department of Medicine, Royal Melbourne Hospital, University of Melbourne is fully equipped for the study of small animal behavior, such as that proposed in the current application. With a focus on cognition and anxiety- and depression-like behaviors. The laboratory head, Dr. Jones, has 10 years of experience in the measurement of animal behavior, and his primary expertise is in assessment of interventions on behavioral deficits. The

Neurobehavioral Testing Facility is in the immediate vicinity of the Epilepsy and Neuropharmacology laboratories in the Department of Medicine - ideally located to conduct the described behavioral studies.

The Small Animal MR Facility: 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*^{TM} 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 Paravison analysis tools, and most commonly used open research based software packages (eg. Mricro, FSL, SPM, MRtrix, MRStudio).

3. University of California, Los Angeles (UCLA)

Seizure Disorder Center Animal Laboratories: Animal study areas are located in RNRC rooms 2253 (12 m^2), 2253A (10 m^2), and 2253B (17 m^2) that form a three room suite with an adjoining interior door to room 2243 (19 m^2) with an adjacent storage room (5 m^2). Hallway access is limited to rooms 2253 and 2243 so that experimental isolation is available for the two interior recording rooms (2253A & B). Room 2253 contains a digital video review station and EEG data analysis and is the office to Mr. Joyel Almajano, Staff Research Associate III. In addition, room 2136 (28 m^2) is used for both chronic and acute in vivo experiments. Room 2243 is used as a surgery room and contains facilities for animal care, as well as a workbench with dissecting scope for microelectrode preparation. We currently have seven setups each with 16-channel capacity for chronic electrophysiological experiments, one dedicated setup for intracellular recordings on anesthetized rodents with ability to label recorded cell with neurobiotin, and another setup for whole cell intracellular recordings in living tissue slices. Animals are maintained in the vivarium on the 6th Floor of the Brain Research Institute where there is a second 24-hour infrared video monitoring system. Here we are currently capable of monitoring 16 rats housed in individual cages using a 4-channel, high-storage capacity DVR.

Offices: Key personnel directly involved with this research are located in RNRC as follows: Richard Staba, PhD in room 2155, Anatol Bragin, PhD in room 2147, and Jerome Engel, Jr., MD, PhD in room 1250. Offices for postdoctoral students are located in 2144 directly across the hall from Drs.. Staba's and Bragin's offices.

UCLA Brain Mapping Center: Located in the David Geffen School of Medicine at UCLA the Brain Mapping Center houses a Bruker Biospin a 7.0 Tesla magnetic resonance imaging/spectroscopy instrument with a clear bore diameter of 30 cm and is staffed by a full time lab manager. 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. A range of radiofrequency receive and transmit volume quadrature coils and receive-only or receive-transmit surface coils are available for use with these systems. A mouse 4-channel phased array coil is also available. These options support the full spectrum of modern neuroimaging techniques including structural MRI, functional MRI, perfusion MRI, diffusion tensor MRI, and multinuclear MR spectroscopy for imaging rats and mice. The system runs off a Linux-based consoles running Paravision v6.0 or v5.3. While the instrument is optimized for neuroimaging studies it is also be capable of imaging other body areas in rodents, including the heart and visceral organs. 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 50 m^2 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. Offline image processing is achieved on a dedicated Linux workstation with 0.75Tb of raid 5 storage space and 2GB of RAM and on Macintosh computers within the Brain mapping Center. The MR unit is available for use by UCLA-affiliated investigators who have research studies that require imaging of animals.

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 a well as a grid-engine, job submission queue for parallel, high through-put analysis. Supercomputing resources are also available through the UCLA Hoffman Cluster which has 256 nodes for general campus computing but many more nodes available for special jobs.

Harris Laboratory: Dr. Harris shares laboratory space within the UCLA Brain Injury Research Center (BIRC), which has developed a core basic laboratory facility for use by all BIRC investigators (Drs.. Giza, Glenn, Harris, Hovda, Prins, Sutton). The BIRC laboratories are within the Department of Neurosurgery and consists of multiple rooms totaling 279 m^2, including separate lab space for the following procedures: Animal surgery with two surgical areas, each equipped for isofluorane anesthesia and a surgical microscope;

Necropsy/perfusions with equipment for tissue harvesting, a perfusion hood and two cryostats; A wet lab equipped for studies on molecular biology (protein and RNA anlaysis, 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 MicroBrightfiled Stereology Software System) and one containing a Zeiss confocal microscope; Two rooms for behavioral testing with tracking software; A darkroom, with developer for X-ray films.

4. University of Eastern Finland (UEF)

The Pitkänen and Gröhn laboratories are located in the same building (3rd and 4th floor) at the A.I.Virtanen Institute for Molecular Sciences (AIVI) in the University of Eastern Finland, the 3rd largest University in Finland with neurosciences as a strategic focus area for research. AIVI is one of the five highly research-oriented biocenters in Finland, with teaching focus on post-graduate training. MRI facility is in the 1st floor of the same building as laboratories, and the underground tunnel connects the MRI unit with the animal facility where EEG monitoring laboratories are located. All facilities described are within 600 feet radius from each other.

Functional Neuroanatomy Laboratory (Pitkänen lab): Fully equipped histology and molecular biology laboratory (40 m^2) with microtomes and cryostats. Three microscopy rooms (each 12 m^2), 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^2), 2) video-EEG monitoring (two rooms, each 12 m^2), including 5 video-EEG monitoring units (32 channels each), 4) behavioral testing (2 rooms, 12 m^2), and 5) perfusion (8 m^2). All these are located in the UEF Animal Center. Importantly, animals can be transferred from the animal center to imaging unit and back via underground tunnel, which is critical for chronic imaging follow-up studies to maintain the best possible animal well-being. Animals are taken care by the staff of 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): MRI systems are located in the Biomedical Imaging Unit (~60 m^2) of the A.I.Virtanen Institute, which also serves as National Core Facility for experimental MRI (Grohn is the director of the facility). Imaging facility is part of the Animal Center which enables long term follow-up studies of experimental animals. There is a dedicated space for micro surgery of the rodents.

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-chanel 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.

The facility is equipped with microsurgery unit and extensive MRI compatible physiological monitoring instruments including respiratory and cardiac gating unit, blood pressure monitoring, pulse oximeter, capnograph, blood gas analyzer 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 the 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.

Office: In Pitkänen lab 6 office rooms (four 16 sm^2, two 20 m^2). In Gröhn lab: 5 office rooms (16-20 m^2each).

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

	Project 2. *MRI Instrumentation*			
	UEF	***U Melbourne***	***UCLA***	***Einstein***
Magnet	*7T/16cm*	*4.7T/33cm*	*7T/30cm*	*9.4 T 31 cm*
Console	Bruker Pharmascan	*Bruker Biospec*	*Bruker Biospec*	*Agilent Direct Drive*
Gradients	*600 mT/m risetime 150 us*	*440mT/m risetime 150 us*	*400 mT/m rise time~110us*	*600 mT/m, 180 us risetime*
Head Rf-coils	*Actively decoupled volume transmitter and quad. surface coil receivers (Bruker)*	*Actively decoupled volume transmit and 4-channel surface coil receiver (Bruker)*	*Actively decoupled volume transmitter and single channel surface coil receivers*	*Actively decoupled volume transmitters (M2M), 4 channel array coils (M2M)*

5. University of Minnesota, Center for Orphan Drug Research (CODR)

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 60^0C, -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 has a 9 m^2 cell culture facility with a Forma CO_2 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 20ºC below ambient to 80ºC, 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 (UBC)

Resources and Environment

The lab environment consists of ~ 3,500 sq.ft of wet lab space including separate rooms for tissue culture (2 hoods), whole cell and brain slice electrophysiology (4 rigs) and light microscopy. The main lab space consists of 5 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 (2 instruments), DNA sequencing (Illumina), and protein and molecular biology manipulations. There are 3 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. Current personnel include three Research Associates each with between 6 to 10 years of experience after their PhDs, two lab technicians, three graduate students, one postdoctoral fellow and three undergraduate/coop students.

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.