FACILITIES & OTHER RESOURCES – PROJECT 1 – BIOMARKERS OF EPILEPTOGENESIS AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

University of Eastern Finland (UEF)

The Pitkänen and Gröhn laboratories are located in the same building (third and fourth floors) at the A.I. Virtanen Institute for Molecular Sciences (AIVI) in the University of Eastern Finland, which as the third-largest university in Finland has neurosciences as a strategic research focus area. AIVI is one of the five highly research-oriented biocenters in Finland, and has a teaching focus on post-graduate training. Its MRI facility is in the same building as laboratories (first floor), and an underground tunnel connects the MRI unit with the animal facility where EEG monitoring laboratories are located. All facilities described are within a 200m radius.

Functional Neuroanatomy Laboratory (Pitkänen lab): Fully equipped histology and molecular biology laboratory (40 sq meters) 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 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 five video-EEG monitoring units (32 channels each); (3) behavioral testing (two rooms, 12 m^2), and (4) perfusion (eight 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 wellbeing. 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 six rats with 4 channels at a time), five licenses 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 (Gröhn is the director of the facility). The imaging facility is part of the Animal Center which enables long-term follow-up studies of experimental animals. There is a dedicated space for microsurgery of the rodents.

Three experimental MRI systems are located in the Biomedical Imaging Unit. Large bore horizontal 9.4T/31-cm magnet is currently interfaced to two different consoles: Bruker Biospec and Agilent DirectDrive, and is equipped with two gradient sets, four receiver channels and large number of RF-coils including actively decoupled 4-channel receiver coil optimized for the rat head. In addition, the facility has a horizontal 7T/16-cm Bruker PharmaScan MRI system and a vertical 9.4T/89-mm Varian Direct Drive micro-imaging system. All the pulse sequences and analysis software described in the research plan have been implemented and either used in our previous studies or thoroughly tested.

The facility is equipped with a 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 the 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 three years); Five licenses for Nnervus 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 two processors (6 cores per /processor, 2 threads per /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 resourses 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 until 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: six office rooms (four 16 m^2, two 20 m^2); In Gröhn lab: five office rooms (16-20 m^2 each).

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

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 (27 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. There are seven setups 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 sixth Floor of the Brain Research Institute where there is a second 24-hour infra-red video monitoring system, 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 and (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 on Linux-based consoles running Paravision v6.0 or v5.3. While the instrument is optimized for neuroimaging studies it is also 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 throughput 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 278 m^2, including separate lab space for the following procedures: 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; A 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 MicroBrightfiled Stereology Software System) and one containing a Zeiss confocal microscope; Two rooms for behavioral testing with tracking software; A dark room, with developer for X-ray films.

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 Precinct 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, behavioral testing, EEG, immunohistochemical, and molecular facilities.

Epilepsy and Neuropharmacology Laboratory: This laboratory (~100 sqm) 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 tumor-associated epilepsy) and traumatic brain injury (fluid percussion injury, cortical contusion, weigh drop and repeated concussions). The laboratory has extensive published experience in discovery and drug development research using these models. The laboratory hasthe 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. There are also 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 rtPCR, Western Blotting, in-situ hybridization.

The Small Animal MR Facility: 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 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 6 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 anesthesia 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).

Project 1 - Inter-site harmonization of the make, type, and vendor of hardware and software for induction of TBI and wide band and video-EEG monitoring.			
	UEF	**UCLA**	**Melbourne**
TBI INJURY DEVICE AND IMPACT			
	AmScien Instruments, Richmond, VA, USA Anesthesia: isoflurane Impact:20 msec, 3.2 atm	Compressed Air Apparatus, Los Angeles, CA, USA Anesthesia: Isoflurane Impact 40 msec, 3.4 atm	custom-made (Thompson et al., J Neurotrauma 2005). Anaesthesia: isoflurane Impact: 22 msec, 2.5-3 atm
WIDE BAND EEG			
Amplifier type	**To be purchased:** **RHD2000 digital system** equipped with 4X RHD2216 16-channels amplifier Part Numbers: C3100 (USB interface) C3334 (16-ch monopolar amplifier) (Intan Technologies – Los Angeles, CA, USA)	RHD2000 digital system equipped with 4X RHD2216 16-channels amplifier Part Numbers: C3100 (USB interface) C3334 (16-ch monopolar amplifier) (Intan Technologies – Los Angeles, CA, USA)	**To be purchased:** **RHD2000 digital system** equipped with 4X RHD2216 16-channels amplifier Part Numbers: C3100 (USB interface) C3334 (16-ch monopolar amplifier) (Intan Technologies – Los Angeles, CA, USA)
Amplifier features	**To be purchased:** bandwidth range: 0.1 - 20 KHz Sampling rate max: 30 ksamples/s. High-impedance electrodes compatible	bandwidth range: 0.1 - 20 KHz Sampling rate max: 30 ksamples/s. High-impedance electrodes compatible	**To be purchased:** bandwidth range: 0.1 - 20 KHz Sampling rate max: 30 ksamples/s. High-impedance electrodes compatible
Commutator	**To be purchased:** **12-channel** commutator Part Number: SRC 012-12-AB (Servotecnica – Nova Milanese, MB, ITA)	24-channel commutator Part Number: AC6023-24 (Moog, Inc - East Aurora, NY, USA)	**To be purchased:** **12-channel** commutator Part Number: SRC 012-12-AB (Servotecnica – Nova Milanese, MB, ITA)
Cables	**To be purchased:** Standard and ultra-thin SPI interface cables Part Numbers: C3206 (standard cable) C3213 (ultra-thin cable) C3430 (Cable-commutator adapter) (Intan Technologies – Los Angeles, CA, USA)	Animal: Served Litz Wire Part Number 50/34 SPSN (MWS Wire, CA, USA) Braid Cable 1/8" Width Part Number 1229-SIL (Alpha Wire Co, NJ, USA) Amplifier: Standard and ultra-thin SPI interface cables Part Numbers: C3203 (Intan Technologies – Los Angeles, CA, USA)	**To be purchased:** Standard and ultra-thin SPI interface cables Part Numbers: C3206 (standard cable) C3213 (ultra-thin cable) C3430 (Cable-commutator adapter) (Intan Technologies – Los Angeles, CA, USA)

Connectors	**To be purchased:** NSD-18-DD-GS Nano miniature connectors Part number: A79039-001) (Omnetics, Minneapolis, MN, USA)	Female Connectors Part # GM-4 (Microtech, Inc – PA,USA)	**To be purchased:** NSD-18-DD-GS Nano miniature connectors Part number: A79039-001) (Omnetics, Minneapolis, MN, USA)
STANDARD EEG			
Amplifier type	**Nervus Magnus -32/8** 32 monopolar channels + 8 bipolar channels **NicoletOne V32** 23-monopolar channels + 9 bipolar channels (Natus Neurology – Middleton, CA, USA)	N/A	GRAEL HD EEG amplifier – 32 monopolar + 8 bipolar inputs (Compumedics, Melbourne Australia)
Amplifier features	bandwidth range: **0.053 (0.16) - 500 Hz** (digital downsample: 128 Hz) Sampling rate max: 2 Ksamples/s, 22 bits	N/A	data sampled at 16384 samples/sec (max digitized output: 2048 samples/sec
Commutator	6-channels commutator Part Number: SL6C/SB (PlasticsOne - Roanoke, VA, USA	N/A	6-channels commutator Part Number: SL6C/SB (PlasticsOne - Roanoke, VA, USA
Cables	Six Channel spring covered cable Part Number: 363-363/WS Six Channel cable Part NumberS: 363-441/6/WO (TOUCHPROOF) 363-000/WO (OPEN-END) (PlasticsOne - Roanoke, VA, USA)	N/A	Six Channel spring covered cable Part Number: 363-363/WS (PlasticsOne - Roanoke, VA, USA)
Connectors	Six Channel Electrode Pedestal Part Number: MS363 (PlasticsOne - Roanoke, VA, USA)	N/A	Six Channel Electrode Pedestal Part Number: MS363 (PlasticsOne - Roanoke, VA, USA)
ELECTRODES			
Screw electrodes	0-80 1/16 S-steel screw electrode Part Number: E363/20/1.6/SP (PlasticsOne - Roanoke, VA, USA)	000 x 3/32 Flat Self Tap Part Number F000CE094 (Morris Co – Maine, USA) 34 AWG Magnet Wire Belden 8057 (Indiana, USA)	0-80 1/16 S-steel screw electrode Part Number: E363/20/1.6/SP (PlasticsOne - Roanoke, VA, USA)

Bipolar electrodes	2 x 0.125 mm twisted S-steel intracranial electrodes Part Number: E363/3-2TW/SPC (PlasticsOne - Roanoke, VA, USA)	Circuit Pin, Swage mount #3114-2-00-21-00-00-08-0 (Mill Max –New York, USA)	2 x 0.125 mm twisted S-steel intracranial electrodes Part Number: E363/3-2TW/SPC (PlasticsOne - Roanoke, VA, USA)
ANALYSIS SOFTWARES			
EEG Reader	NicoletOne EEG reader v5.7 (Natus Neurology – Middleton, CA, USA) File format: ".e" *(property)*; ".edf"	Stellate & Nihon Kohden signal browsers File format: ".rec" (proprietary); ".edf"	Profusion EEG recording v.4 (Compumedics, Australia)
Spike2	Spike2 8.07 x64 Unicode (CED-Cambridge Electronic Design – Cambridge, UK) File format: ".csf" *(property)*; ".edf"; ".txt"; ".bin"; ".mat"; ".abf"; ".pxp"; ".wav"; …	DataPac 2K2 (Run Technologies, Inc. San Diego, CA) File format: ".per" (proprietary); ".edf"; ".txt"; ".ncs"	Spike2 8.07 x64 Unicode (CED-Cambridge Electronic Design – Cambridge, UK) File format: ".csf" *(property)*; ".edf"; ".txt"; ".bin"; ".mat"; ".abf"; ".pxp"; ".wav"; …
LabChart	LabChart v7 (ADInstruments – Oxford, UK) File format: ".adicht" *(property)*; ".edf"; ".txt"; ".bin"; ".mat"; ".abf"; ".pxp"; ".wav"		LabChart v7 (ADInstruments – Oxford, UK) File format: ".adicht" *(property)*; ".edf"; ".txt"; ".bin"; ".mat"; ".abf"; ".pxp"; ".wav"
Matlab	R2014a/R2015a x64-bit (MatWorks – Kista, SE) File format: ".mat" *(property)*; ".txt"; ".bin"; *converters available for other file formats*	R2011b/R2015a x64-bit (Mathworks, Inc., Natick, MA) Files format: ".mat"; ".edf"; ".txt"; multiple other formats	R2014a/R2015a x64-bit (MatWorks – Kista, SE) File format: ".mat" *(property)*; ".txt"; ".bin"; *converters available for other file formats*

Project 1 - Inter-site harmonization of MRI.			
	UEF	**UCLA**	**U Melbourne**
Magnet	7T/16cm	7T/30cm	4.7T/33cm
Console	Bruker Pharmascan	Bruker Biospec	Bruker Biospec
Gradients	600 mT/m risetime 150 us	400 mT/m rise time~110us	440mT/m risetime 150 us
Head Rf-coils	Actively decoupled volume transmitter and quad. surface coil receivers (Bruker)	Actively decoupled volume transmitter and single channel surface coil receivers	Actively decoupled volume transmit and 4-channel surface coil receiver (Bruker)