

BUDGET JUSTIFICATION – PROJECT 1 – BIOMARKERS OF EPILEPTOGENESIS AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

Senior personnel

Asla Pitkänen, M.D., Ph.D., D.Sci. (Project Lead; 2.4 calendar months) - UEF. Dr. Pitkänen will be responsible of coordination of Project 1, implementing collaborative arrangements with the three study sites, and overseeing all aspects of integration of research in the three study sites in Project 1. She will oversee the integration of Project 1 with the EpiBios consortium. She will be primarily responsible for production of animal model in UEF, blood sampling in UEF, and video-EEG monitoring in UEF. She will oversee that Vertebrate Animal Regulation standards are met. Dr Pitkänen also will be involved in data analysis and in decisions regarding changes in experimental design, and will participate in data collection.

Olli Gröhn, Ph.D. (Co-investigator; 1.2 calendar months) – UEF Dr Gröhn will be responsible of coordination and harmonization of MRI experiments in all three study sites. He will be responsible for organizing MRI experiments and data analysis in UEF, and will also participate in data collection. He will also be intimately involved in data analysis, decisions concerning changes in experimental design and protocols in MRI.

Terrence O'Brien, M.D., Ph.D. (Co-investigator; 1.2 calendar months) – UM Dr. O'Brien will overall be responsible for the live animal studies at the University of Melbourne, including acquisition of the MRI and video-EEG data, blood sampling, as well as the histological studies. He will also be responsible for coordinating and synergizing the procedures at the UM with those at the other three sites. He will also contribute to overall experimental design, data management and analysis, and publication of the study results.

Richard Staba, Ph.D. (Co-investigator; 1.2 calendar months) – UCLA Dr. Staba will be involved with all aspects of the experiments, including coordination and scheduling the TBI and sham injury, organizing with Mr. Almajano the surgical implantation of electrodes. and supervising the T.B.N. postdoctoral fellow the video-EEG data collection and analysis. Dr. Staba will also carry out data collection, including blood samples, manual review of EEG for occurrence of seizures, and quantitative analysis of pHFOs, rHFOs, and other electrophysiological disturbances in TBI rats with epilepsy and those without epilepsy. He will work closely with Dr. Harris in coordinating the in vivo and ex vivo MRI for all rats. Fringe benefits rate: 50.5%.

Neil Harris, Ph.D. (Co-investigator; 2.4 calendar months) – UCLA Dr. Harris will be responsible for all in vivo and ex vivo MRI experiments, including data acquisition and analysis at UCLA and work collaboratively with the other site coordinators in MRI acquisition and analysis. He will also supervise the T.B.N. postdoctoral fellow during MRI experiments. Fringe benefits rate: 40.0%.

UCLA Fringe Benefits – UCLA Fringe benefits provide the employee's sick leave, holidays, payroll taxes, etc. UCLA fringe benefit rates vary due to actual benefit rates being used for current employees. UCLA fringe benefit rates are calculated with a 2% escalation annually in accordance with the University of California Office of the President.

Other Personnel

T.B.N. – UEF, Pitkänen lab (postdoctoral fellow; 12 calendar months). Dr. T.B.N. will have a PhD in neurosciences and will be responsible for organization of monthly inter-site teleconferences to follow-up details of research progress, daily coordination of projects in UEF, helping technician during weekends in video-EEG analysis and animal monitoring, coordination of EEG analysis with Informatics and Analytics Core, statistical analysis of all data, and reporting. Fringe benefits rate: 25% (included in \$68 875; UEF Pitkänen budget) T.B.N. – UEF, Pitkänen lab (postdoctoral fellow; 5 months year 3, 7 months year 5). Dr. T.B.N. will have a PhD in molecular neurosciences and will be responsible for bioinformatics analysis of miR-seq data, RT-qPCR/ddPCR validation of the most interesting miRnas, statistical analysis of data, and reporting. Fringe benefits rate: 25% (included in \$68 875; UEF Pitkänen budget).

T.B.N. – UEF, Gröhn lab (postdoctoral fellow; 12 calendar months). Dr. T.B.N. will have a PhD and experience in experimental MRI, physiological monitoring of the animals and data analysis. He will perform data collection and data analysis for structural MRI at UEF. He is also responsible of data transfer and general communication with data analysis core regarding MRI data acquired at UEF. Fringe benefits rate: 25% (included in \$68 8; UEF Grohn budget).

T.B.N. – UM, O'Brien lab (postdoctoral fellow; 12 calendar months). Dr. T.B.N. will have a PhD in a relevant area of neuroscience and will be responsible for the day-to-day study coordination, animal experiments, including injuries, surgeries, video-EEG, and MRI acquisitions and analyses, statistical analyses, reporting of the results of the studies. Fringe benefits rate: 23% (included in \$87 547; UM O'Brien budget).

Nigel Jones, PhD – UM (Key personnel; Co-investigator; 0.6 calendar months). Dr. Jones is a behavioral neuroscientist with internationally recognized expertise in animal models of the psychiatric co-morbidities of epilepsy. He will work with Dr. O'Brien to implement the behavioral testing at the UM, as well have a role in oversight and synergy of the behavioral testing across the three sites. He will also work with Dr. O'Brien on the video-EEG monitoring and EEG analysis. He will also contribute to overall experimental design, data management and analysis, and publication of the study results.

Sandy Shultz, PhD – UM (Key personnel; Co-investigator; 0.6 calendar months). Dr. Shultz is a behavioral neuroscientist with internationally recognized expertise in animal models of neurotrauma, and post-traumatic epilepsy. He will work with Dr. O'Brien to implement the neurotrauma procedures testing at the UM. He will also work with Dr. O'Brien on the MRI acquisitions and analysis, in particular DTI. He will also contribute to overall experimental design, data management and analysis, and publication of the study results.

Leigh Johnston, BSci, BE, PhD – UM (Key personnel; Co-investigator; 0.6 calendar months). Dr. Johnston is an academic biomedical engineer who has particular expertise in Magnetic Resonance Imaging acquisition and analysis techniques, with a particular focus on neuroscience applications in pre-clinical research. She will oversee and advice regarding the MRI acquisitions at UM. She will also contribute to overall experimental design, data management and analysis, and publication of the study results.

T.B.N. - UCLA (Postdoctoral Fellow, 12.00 Calendar Months) The T.B.N. postdoctoral fellow will carry out the continuous EEG-video recordings in rats, review and detect spontaneous posttraumatic seizures from video-EEG data using manual and semi-automated computer algorithms, and perform advance signal analysis using MATLAB to identify recording sites where seizure begin and severity of seizures. As described in the experimental design, this will be an extraordinary amount of data, and will require careful review of all these data and accurate detection of seizures in order to successfully complete study. S/he will also perform MRI under supervision of Dr. Harris and help with collection of blood samples. To reduce some of labor-intensive aspects s/he will have the support from Mr. Almajano on data collection and analysis related to this Project. Fringe benefit rate: 19%

Joel Almajano, MS – UCLA (Staff Research Associate III; 9.6 calendar months). Mr. Almajano has over 13 years of experience in our animal laboratory and has been directly involved in a wide range of in vivo animal research. He will be responsible for preparing and performing each rat surgical procedures as described in the experimental design. Mr. Almajano will be responsible for all acute animal care and treatment following each surgical procedure, which is especially critical to mitigate animal death following TBI, and will help prepare blood samples for shipping. He is also trained in euthanasia procedures and will carry out the appropriate procedures on distressed animals using a protocol approved by the UCLA Animal Research Committee. In addition, Mr. Almajano will perform animal perfusion that will be needed to obtain tissue for histologic analysis. Fringe benefits rate: 41.1%.

Jarmo Hartikainen – UEF, Pitkänen lab (Senior Technician; 12 calendar months). Mr. Hartikainen has 20-y experience in induction of various types of animal models of seizures and epilepsy, including

lateral fluid-percussion model, and performing other methodologies required in project 1. He will be responsible for performing lateral fluid-percussion injuries, daily animal follow-up, video-EEG monitoring and screening of spontaneous seizures in video-EEGs, blood sampling, processing of tissue for *ex vivo* MRI and brain biobank. Fringe benefits rate: 25% (included in \$56 684; UEF Pitkänen budget).

T.B.N. – UM, O'Brien lab (Technician; 10 calendar months). Mrs.T.B.N. will assist the post-doctoral fellow in the large animal procedures, surgeries, video-EEG monitoring, behavioral tests, EEG, and MRI acquisitions and histology. Fringe benefits rate: 23% (included in \$69 659; UM O'Brien budget).

Unpaid Consultants

Anatol Bragin, Ph.D. - UCLA Dr. Bragin is an expert in animal studies of epileptogenesis and ictogenesis using long-term video monitoring and electrophysiological recordings. He will chiefly assist Dr. Staba to schedule induction of TBI and implantation of recording electrodes. He will primarily be involved with video-EEG recordings and data analysis.

Istvan Mody – UCLA. Prof. Mody is a world leader in understanding the electrophysiology of epileptogenesis and ictogenesis. He will provide advice for Project 1 in electrophysiology.

Jerome Engel Jr – UCLA. Dr. Engel is an expert in clinical epilepsy and experimental studies of epileptogenesis and PI of the EpiBioS4Rx Center Without Walls (CWOW). He will assist Drs. Staba, Harris, and Bragin in all scientific aspects of this project, including interpretation of epileptiform EEG events in animals. Dr. Engle will also be involved with integrating this project's results into the entire CWOW.

David Henshall – Royal College of Surgeons in Ireland. Prof. Henshall is the leading investigator of microRNAs in epilepsy. He will advise Project 1 in all aspects of miRNA analysis and data interpretation. Jussi Paananen – University of Eastern Finland (Kuopio, Finland). Asst. Prof. Paananen has extensive knowledge in bioinformatics analysis of large molecular datasets. He will provide advice in all aspects of bioinformatics analysis of miRNA data.

Materials, and analysis costs – Project 1 EpiBioS4Rx

Specification of costs in individual cost categories. Distribution of costs over the 3-year period is shown in budget sheet.				
Cost	Pitkänen (Project Lead) (UEF Kuopio)	Gröhn (Co-investigator) (UEF Kuopio)	Staba (Co-investigator) (UCLA)	O'Brien (Co-investigator) (UMelbourne)
Animals and per diem	\$20 006 (both years 1-2: TOTAL \$40 012) 65 rats (a \$107) 194-394 d/rat (\$1.17/d)		\$29 458 (Total yrs. 1-2, & 3 per diem = \$58 916) 5 rats (a \$40) 194-394 d/rat (\$1.90/d)	\$ 11 775 65 rats (\$80AUD/rat = 3600 USD), housing \$11.80AUD/week
Laboratory supplies (drugs, tubes, instruments, gloves, storage materials, dental cement)	\$1 800		\$1 800	\$4,000 anesthetic, sutures, analgesia, surgical equipment, cannulae, dental cement, needles, euthanasia, syringes

Brain bank materials	\$980 70 brains (a \$14): fixation, cryoprotection, storage boxes		\$1 000	\$1 000
Blood collection, Materials	\$720 TOTAL \$1 170 130 rats, 5 samples/rat, 10 tubes (aliquots)/sample, 6 500 RNA-free tubes (a \$0.33)		\$720	\$720
Plasma miRNA-seq Exicon	9 d samples 10 sham + 10 TBI-no epilepsy + 10 TBI-epilepsy \$23 218 (Exiqon offer Jan 2016) (Year 3)			
Wet-lab validation of miR findings 21+63 samples	\$5 544 3 best miRNA candidates+ 1 house-keeping gene: 84 samples (21 epilepsy + 63 no epilepsy) x 4 miRNAs =252 assays (\$22 per sample in 3 replicates; includes RNA isolation) (Year 3)			
wideband-EEG, video-EEG materials; surgical supplies	\$63 349 (1st year) wide-band motherboard/amplifiers /cables/commutators for 20 animals (\$56 689) Year 1 electrode wire (\$3 000), vEEG screws (\$2 700), surgical supplies (\$960) Total/y \$6 660		\$42 600 (1st year) InTan Tech. EEG equipment for 12 animals (\$33 950) Year 1 Electrode supplies, including wires, screws, connectors, & surgical supplies (\$8 650) Total/y \$8 650	\$10 000 cables, electrodes, surgical supplies Amplifier charged in project 2
MRI costs		\$25 107 (per year for the 1st 3 years) <u><i>in vivo</i></u> 16 rats 4 time points /rat 2 h/rat/time point \$130/h <u><i>ex vivo</i></u> 16 rats 1 h/rat \$130/h Anesthetics \$1 000/y	\$38 600 (per year for the 1st 3 years) <u><i>in vivo</i></u> 16 rats 4 time points /rat 2 h/rat/time point \$200/h <u><i>ex vivo</i></u> 16 rats 1 h/rat \$200/h Anesthetics \$1 000/y	\$23 650 (per year for the 1st 3 years) <u><i>in vivo</i></u> 16 rats 4 time points /rat 2 h/rat/time point \$140/h <u><i>ex vivo</i></u> 16 rats 1 h/rat \$200/h Anesthetics \$1 000/y
On-site data storage and backups	\$3 500		\$16 400 (Yr. 1 only) Equipment 90TB RAID w/ backup Year 1 only	\$4000 Years 1, 2, 3

Shipping of tissue to IAC/Kuopio	\$2 000		\$2 000	\$2 000
Publication costs	\$2 000		\$2 000	\$2 000
Travel	\$2 000	\$2 000	\$2 000	\$2 000
Indirect costs	10%	10%	54%	10%