BUDGET JUSTIFICATION - PROJECT 2 – PRECLINICAL MODEL FOR ANTIEPILEPTOGENIC THERAPY SCREENING IN POST-TRAUMATIC EPILEPSY

1. ALBERT EINSTEIN COLLEGE OF MEDICINE

We request a total of $1,604395 total direct cost for 5 years, to be distributed as follows:
Year 1 =$ 333,738
Year 2=$ 302,531
Year 3=$ 282,929
Year 4= $ 374,767
Year 5= $ 310,430

Key Personnel

Aristea Galanopoulou MD PhD (contact PI): We request 2.4 calendar months effort support during Years 1-5 ($46,294 annually including fringe). Dr. Galanopoulou will be the project leader and contact PI for project 2. Dr. Galanopoulou will contribute to the following: design of experiments, coordination of work among the 6 sites involved in Project 2, supervision and training of post-doctoral fellows, supervision and training of personnel in survival surgeries, minipump placement, seizure induction, supervision of video-EEG studies and analysis, histology studies, animal handling and behavioral studies, qRT-PCR and histological studies planned here; data discussion and analysis and presentation in manuscripts and conferences. Dr. Galanopoulou will also be responsible for the blinding of the experiments at Einstein. Dr. Galanopoulou will also participate in the analysis and interpretation of rodent video-EEG studies planned in this proposal. No annual increases are planned in this requested amount. Fringe benefits at Einstein are 34.5%.

Solomon L. Moshé MD (PI): We request 1.2 calendar months effort support during Years 1-5 ($24,896 annually including fringe). Dr. Moshé will be contributing to the following aspects of the work. Dr. Moshé will contribute to the following: design of experiments, supervision and training of post-doctoral fellows, data discussion and analysis and presentation in manuscripts and conferences. Dr. Moshé will also participate in the analysis and interpretation of rodent video-EEG studies planned in this proposal. Dr. Moshé will also be responsible for the blinding of the experiments at Einstein. No annual increases are planned in this requested amount. Fringe benefits at Einstein are 34.5%.

Wenzhu Mowrey PhD (co-I): We request 0.6 calendar months effort support during Year 1 ($6,188 annually including fringe), and 1.08 months during Year 2, 3, and 5 ($11,139 including fringe) and .36 months during Year 4 ($3,714 including fringe). Dr. Mowrey will contribute to the statistical analysis included in this grant proposal. No annual increases are planned in this requested amount. Fringe benefits at Einstein are 34.5%.

Craig Branch, PhD (co-I): We request 0.6 calendar months effort support during Year 4 and 5 ($12,448 annually, including fringe). Dr. Branch will oversee the MRI studies, including technical part, data analysis and training of personnel. No annual increases are planned in this requested amount. Fringe benefits at Einstein are 34.5%.

Oleksii Shandra, MD PhD (post-doctoral fellow): We request 12 calendar months effort support during Years 1-5 (Year 1: $66,109; Year 2: $68,756; Years 3 - 5: $71,500 per annum, including fringe). Dr. Shandra will be performing the survival surgeries, EEG placement surgeries and minipump placement, video-EEG analysis, seizure scoring, behavioral tests, animal handling, qRT-PCR, and seizure induction. Fringe benefits at Einstein are 34.5%.

Qianyun Li (Research Technician): We request 12 calendar months effort support during Years 1 – 5 ($71,495 annually including fringe). Mrs Li will perform the following: animal handling, transcardiac perfusions, histological procedures (cryosections, staining, signal analysis of stained sections), Western blots, video monitoring of pups. No annual increases are planned in this requested amount. Fringe benefits at Einstein are 34.5%.

Equipment

In Year 1:

We request a Fluid percussion device FP 302 ($11,000) for induction of the LFP model.
We request 3 Intan wide band EEGs ($27,531) for the video-EEG monitoring of 12 rats.

In Year 2:

We request 2 Intan wide band EEGs ($18,354) for the video-EEG monitoring of 8 rats.

In Year 3:

We request funds for a surface coil for MRI ($3,200).

Animals

We request funds towards the purchase of 213 adult male rats in Year 1 ($45,175 for purchase and maintenance up to 8weeks); 95 adult male rat in Years 2 and in Year 3, maintained for 17 days each, (Year 2: $19,805 , Year 3: $19,805 for purchase and maintenance); and 92 adult rats in Year 4 maintained for up to 365 days each, ($ 36,536 for purchase and maintenance). In Year 5, we request $11,158 for maintenance of rats. Per diem costs are $0.77 per rat, cost of purchasing the rats is $120/rat (including shipping, and IAS charge of 15%).

Lab Supplies

In Year 1: We request funds for 3 computers, 3 monitors, and 3 camcorders to support the wide band EEG systems, in the total sum of $4,650.

We request lab supplies in the total sum of $ 25,400, that includes expenses for surgeries (anesthesia, oxygen, sterile instruments and supplies), chemicals, drugs, histology and Western blots (staining kits, antibodies for immunochemistry and Western blots, slides and coverglasses, tubes), qRT-PCR and blood collection. Supplies will include surgery supplies isoflurane anesthesia ($25/100ml), sterile tools and gloves, oxygen ($3/tank/surgery)), gloves, tubes for blood collection, pipette tips, chemicals (Kineret ~$770 per 500mg, Levetiracetam $1500 / 50mg; sodium selenate $183/ 100g; deferiprone $227/25g) and FEDEX shipments of biosamples to the University of Minnesota for pharmacokinetics ($ 80/shipment). Other expenses will include slides ($766/case) and coverslips ($294/pack), chemicals for immunochemistries, Western blots and qRT-PCR (oligos, enzymes, etc).

In Year 2: We request funds for 2 computers, 2 monitors, and 2 camcorders to support the wide band EEG systems, in the total sum of $3,100.

We request lab supplies in the total sum of $ 34,692. The justification is as in Year 1, and in addition, we will need osmotic minipumps ALZET ($27.15/pump, for 95 rats), data storage expenses ($2,000), and electrodes and cables for the video-EE recordings ($8,000).

In Year 3: We request lab supplies in the total sum of $ 30,600. The justification is as in Year 1, and in addition, we will need osmotic minipumps ALZET ($27.15/pump, 95 rats), data storage expenses ($2,000), and electrodes and cables for the video-EE recordings ($8,000). We also include FEDEX shipments of biosamples to the University of Minnesota for drug levels ($ 80/shipment).

In Year 4: We request lab supplies in the total sum of $ 16,909. The justification is as in Year 1, and in addition, storage and backup of data is $1000. We request funds for the rat MRI studies ($87,975) for $225/hr. Each will need 4hours for in vivo and 1hr for ex vivo MRI, 92 rats, with the cost split between Year 4 and 5.

In Year 5: We request lab supplies in the total sum of $ 14,100. The justification is as in Year 1, and in addition, storage and backup of data is $2,000. We request funds for the rat MRI studies ($41,400) for $225/hr. Each will need 4hours for in vivo and 1hr for ex vivo MRI, 92 rats, with the cost split between Year 4 and 5.

Travel

In Year 1: We request $5,000 funds for travel to (a) UCLA (Dr. Staba's and Dr. Bragin's lab) to harmonize practices for placement of microelectrodes and recording and analysis of pHFOs and rHFOSs and (b) to UEF, Finland (Dr. Pitkänen's lab) to harmonize practices for the LFP induction model.
In Year 2: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.
In Year 3: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.
In Year 4: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.
In Year 5: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.

Publication costs

We request $2000 in Year 5 for publication costs of our manuscripts.

Indirect costs are 67%.

2. THE UNIVERSITY OF MELBOURNE

We request a total of $1,379,593 in total direct cost for 5 years, to be distributed as follows:
Year 1 = $301,548
Year 2=$230,230
Year 3=$230,230
Year 4= $328,779
Year 5= $228,806

Key Personnel

Terence O'Brien MD (PI): We request 1.2 calendar months effort support during Years 1-5 ($17,467 annually including fringe). Dr. O'Brien will be the project leader for Project 2 at the University of Melbourne. Dr. O'Brien will contribute to the following: design of experiments, MRI acquisitions and analysis, supervision and training of post-doctoral fellows and Research Assistants, supervision and training of personnel in surgeries, drug administrations, video-EEG studies and analysis, histology studies, animal handling and behavioral studies, blood sampling, molecular and histological studies at the University of Melbourne. He will also contribute to data discussion and analysis and presentation in manuscripts and conferences. Dr. O'Brien will also be responsible for the blinding of the experiments at the University of Melbourne. No annual increases are planned in this requested amount. Fringe benefits at The University of Melbourne are 31.19%.

Nigel Jones PhD (co-I): We request 0.6 calendar months effort support during Years 1-5 ($6,398 annually including fringe). Dr. Jones will work with Dr.. O'Brien to contribute to the following aspects of the work at the University of Melbourne. Dr. O'Brien will contribute to the following: design of experiments, supervision and training of post-doctoral fellows and Research Assistants, supervision and training of personnel in surgeries, drug administrations, video-EEG studies and analysis, MRI acquisitions and analysis, histology studies, animal handling and behavioral studies, blood sampling, molecular and histological studies at the University of Melbourne. Dr.. Jones has particular expertise in behavioral testing in rodents, and will take primary responsibility for supervising these. He will also contribute to data discussion and analysis and presentation in manuscripts and conferences. No annual increases are planned in this requested amount. Fringe benefits at The University of Melbourne are 31.19%.

Sandy Shultz PhD (co-I): We request 0.6 calendar months effort support during Year 1-5 ($5,131 annually including fringe). Dr. Shultz will work with Dr.. O'Brien to contribute to the following aspects of the work at the University of Melbourne. Dr. O'Brien will contribute to the following: design of experiments, supervision and training of post-doctoral fellows and Research Assistants, supervision and training of personnel in surgeries, drug administrations, video-EEG studies and analysis, MRI acquisitions and analysis, histology studies, animal handling and behavioral studies, blood sampling, molecular and histological studies at the University of Melbourne. Dr.. Shultz has particular expertise in the FPI rat model, and will take primary responsibility for supervising these. He will also contribute to data discussion and analysis and presentation in manuscripts and conferences. No annual increases are planned in this requested amount. Fringe benefits at The University of Melbourne are 31.19%.

To be appointed, PhD (post-doctoral fellow): We request 12 calendar months effort support during Years 1-5 ($87,547 annually). The post-doctoral fellow will, with the assistance of the Research Assistant/Technician, perform the live animal studies at the University of Melbourne, including surgeries, drug administrations, video-EEG studies and analysis, MRI acquisitions and analysis, histology studies, animal handling and behavioral studies, blood sampling, molecular and histological studies, Fringe benefits at The University of Melbourne are 31.19%.

To be appointed, BSc (Research Technician/Technician): We request 12 calendar months effort support during Years 1 – 5 ($69,659 annually including fringe). The Research Assistance will assist the post-doctoral fellow in the live animal studies at the University of Melbourne the following: animal handling, video-EEG recording, MRI acquisitions, transcardiac perfusions, histological procedures (cryosections, staining, signal analysis of stained sections), Western blots, No annual increases are planned in this requested amount. Fringe benefits at The University of Melbourne are 31.19%.

Equipment

In Year 1: We request five new amplifiers with broad band capacity for the recording of pHFOs on the EEG, including PCs – $57000.

Animals

We request funds towards the purchase of 213 adult male rats in Year 1 ($24,896 for purchase and maintenance up to 8 weeks); 95 adult male rat in Years 2 and in Year 3, maintained for 17 days each, (Year 2: $10,578, Year 3: $10,578 for purchase and maintenance); and 92 adult rats in Year 4 maintained for up to 365 days each, ($36,841 for purchase and maintenance). In Year 5, we request $17,098 for maintenance of rats. Housing costs are $8.51 per rat per week, cost of purchasing the rats is $57.60/rat.

Lab Supplies
In Year 1: We request lab supplies in the total sum of $54,346 that includes expenses for surgeries (anesthesia, oxygen, sterile instruments and supplies - $10,000), electrodes and video-EEG equipment ($6,000), chemicals and drugs ($1350), histology and Western blots (staining kits, antibodies for immunochemistry and Western blots, slides and coverglasses, tubes - $6500), ALZET osmotic pumps ($2900), data storage and backup ($2000), blood collection ($400) and shipment ($300).
In Year 2: We request lab supplies in the total sum of $54,346 that includes expenses for surgeries (anesthesia, oxygen, sterile instruments and supplies - $10,000), electrodes and video-EEG equipment ($6,000), chemicals and drugs ($1350), histology and Western blots (staining kits, antibodies for immunochemistry and Western blots, slides and coverglasses, tubes - $6500), ALZET osmotic pumps ($2900), data storage and backup ($2000), blood collection ($400) and shipment ($300).
In Year 3: We request lab supplies in the total sum of $54,346 that includes expenses for surgeries (anesthesia, oxygen, sterile instruments and supplies - $10,000), electrodes and video-EEG equipment ($6,000), chemicals and drugs ($1350), histology and Western blots (staining kits, antibodies for immunochemistry and Western blots, slides and coverglasses, tubes - $6500), ALZET osmotic pumps ($2900), data storage and backup ($2000), blood collection ($400) and shipment ($300).
In Year 4: We request lab supplies in the total sum of $76,182. This includes expenses for surgeries (anesthesia, oxygen, sterile instruments and supplies - $7,500), electrodes and video-EEG equipment ($4,000), chemicals and drugs ($2000), ALZET osmotic pumps ($2442), data storage and backup ($1500), blood collection ($500) and shipment (blood biomarkers $300, miRNA $1500, blood levels $1500). We also request funds for the rat MRI studies ($54,740) for $112/hr. Each will need 4hours for in vivo and 1hr for ex vivo MRI, 92 rats, with the cost split between Year 4 and 5. In Year 5: We request lab supplies in the total sum of $51,952. This includes expenses for surgeries (anesthesia, oxygen, sterile instruments and supplies - $10,000), electrodes and video-EEG equipment ($6,000), chemicals and drugs ($2000), ALZET osmotic pumps ($2442), data storage and backup ($1500), blood collection ($500) and shipment (blood biomarkers $750, miRNA $1500, blood levels $1500). We also request funds for the rat MRI studies ($25,760) for $112/hr. Each will need 4hours for in vivo and 1hr for ex vivo MRI, 92 rats, with the cost split between Year 4 and 5.

Travel
In Year 1: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.
In Year 2: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.
In Year 3: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.
In Year 4: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.
In Year 5: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.

Publication costs
In Year 1: We request $2000 for publication costs of our manuscripts and posters.
In Year 2: We request $2000 for publication costs of our manuscripts and posters.
In Year 3: We request $2000 for publication costs of our manuscripts and posters.
In Year 4: We request $2000 for publication costs of our manuscripts and posters.
In Year 5: We request $2000 for publication costs of our manuscripts and posters.

Indirect costs are 10%.

3. UNIVERSITY OF CALIFORNIA, LOS ANGELES
Key Personnel
Richard Staba, Ph.D., Project Lead, 1.20 Calendar Months (Years 2-5), no salary requested in Years 2 & 3.
Dr.. Staba will be responsible for the coordination and scheduling the TBI and sham injury, organizing with Mr. Almajano the surgical implantation of electrodes and with TBN postdoctoral fellow the video-EEG data

collection and analysis. Dr.. Staba will also carry out data collection, including blood samples and drug trial studies, manual review of EEG for occurrence of seizures, and quantitative analysis of pHFOs, rHFOSs, 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 MRI for all rats.
Neil Harris, Ph.D., Co-Investigator, 1.20 Calendar Months in Years 4 & 5. Dr.. Harris will be responsible for all MRI experiments, including data acquisition and analysis at UCLA and work collaboratively with the other site coordinators in MRI acquisition. He will also supervise the TBN postdoctoral fellow during MRI experiments.
Jerome Engel, Jr., M.D., Ph.D., Co-Investigator, 0.60 Calendar Months, no salary requested (Years 2-5). Dr.. Engel is an expert in 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 integrating this project into the entire CWOW.

Other Personnel
TBN, Postdoctoral Fellow, 12.00 Calendar Months. This TBN 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, help with collection of blood samples and drug trial studies. To reduce some of labor-intensive aspects s/he will have the support from the SRA on data collection and analysis related to this project.
TBN, Staff Research Associate I, 12.00 (Years 2 & 3) & 9.0 Calendar Months (Years 4 & 5). This TBN SRA will receive training from Mr. Almajano (SRA III Project 1) and will be responsible for preparing and performing each rat surgical procedures as described in the experimental design. S/he 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. S/he will also perform euthanasia procedures and will carry out the appropriate procedures on distressed animals using a protocol approved by the UCLA Animal Research Committee. In addition, s/he will perform animal perfusion that will be needed to obtain tissue for histologic analysis.

Equipment
Electrophysiological Recording Setups - $15,000. Intan Technologies USB Interface Boards, SPI Interface Cables, Small Amplifier Boards with computer and monitor for scalp and depth electrode EEG recordings in 8 rats simultaneously.
Travel - $2,500 in Years 2 & 3. Includes travel for PI and TBN postdoctoral student for project training and standardization of experimental procedures across sites.

Other Expenses
EEG recording supplies - $6,628 in Year 2; $5,528 in Year 3; $2,100 in Years 4 & 5. Include screws, wires, and solder for electrodes, dental acrylic, glue, connectors, commutators, and cabling.
Laboratory consumables - $2,500 in Years 2 & 3; $2,000 in Years 4 & 5. Includes supplies for surgery and instrument sterilization.
Blood collection supplies - $600/year in Years 2 & 3, Includes butterfly catheters, syringes, collection vials.
ALZET 2006 Osmotic Pumps - $4,200/year in Years 2 & 3; $9,310 in Year 4. Drug delivery studies.
Animals and per diem - $9,334 in Years 2 & 3; $51,865 in Year 4; $27,530 in Year 5. Years 2 & 3: 154 rats at $40/rat and per diem for 119 rats for 14 days/rat at $1.90/day. Years 4 & 5: 150 rats at $40/rat and per diem for 115 rats for 210 days/rat (Year 4) and 126 days/rat (Year 5) at $1.90/day.
Animal imaging - $78,200 in Year 4; in $37,800 in Year 5. 115 rats/year, 2hr/rat/time, 1.7 (Year 4) and 0.8 (Year 5) time points/rat, $200/hr. Anesthesia - $1,000.
Shipping of blood sample & miRNA - $200/year in Years 2 & 3; $1,550/year in Years 4 & 5.
Technology infrastructure service (TIF): is a consistently-applied direct charge that is assessed to each and every campus activity unit, regardless of funding source, including units identified as individual grant and contract awards. The TIF pays for campus communication services on the basis of a monthly accounting of actual usage data. These costs are charged as direct costs and are not recovered as indirect costs. This is

calculated at $33.28 per FTE per month, which is mandated by the university to be part of the direct costs of grants.

Facilities and Administrative (F&A): The University of California and the United States Department of Health and Human Services (the responsible Federal audit agency) negotiated facilities and administrative cost rate agreement for the Los Angeles campus. The on-campus Research rate currently in effect is 54%.

4. UNIVERSITY OF EASTERN FINLAND

Key personnel

Asla Pitkänen, M.D., Ph.D., D.Sci. (PI; 2.4 calendar months; No salary request) - UEF. Dr.. Pitkänen will be responsible of coordination of Project 2 in UEF, implementing collaborative arrangements with the other three study sites in Kuopio in Project 2. She will be primarily responsible for production of animal model in UEF, treatment trial 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; No salary request) – UEF Dr. Gröhn will be responsible of coordination and harmonization of MRI experiments in UEF. 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.

Other Personnel

T.B.N. – UEF, Pitkänen lab (postdoctoral fellow; 12 calendar months years 4 and 5). Dr. T.B.N. will have a PhD in neurosciences and will be responsible for organization of the practicalities related to antiepileptogenesis treatment trial, daily coordination of projects in UEF, helping technician during weekends in video-EEG analysis and animal monitoring, pump changes, 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 budget).

T.B.N. – UEF, Pitkänen lab (postdoctoral fellow; 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 $40 166; UEF budget).

T.B.N. – UEF, Gröhn lab (postdoctoral fellow; 12 calendar months years 4 and 5). 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 $68 875; UEF budget).

Jarmo Hartikainen – UEF, Pitkänen lab (Senior Technician; 12 calendar months years 4 and 5). Mr. Hartikainen has 20-y experience in induction of various types of animal models of seizures and epilepsy, including lateral fluid-percussion model, and antiepileptic and antiepileptogenic trials, and performing other methodologies required in Project 2. He will be responsible for performing lateral fluid-percussion injuries, daily animal follow-up during treatment, pump implantations and changes, 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).

Equipment

N/A

Animals

Years 4 and 5: We request funds towards the purchase of 107 adult rats in Year 4-5 maintained for up to 365 days each, ($22 898 for purchase; and maintenance $59 816. Total divided: year 4 $55 000 and year 5 $27 714). Housing costs are $1.17 per day; purchasing the rats is $107,00 per rat.

Lab Supplies

In Year 4: We request lab supplies in the total sum of $33, 398. This includes laboratory supplies ($5 400), ALZET osmotic pumps ($11 610), material for brain banking ($1 529), blood collection tubes ($1 076), EEG recording supplies ($8 283), on-site data storage expenses ($3 500), shipping of samples to IAC ($2 000). We also request funds for the rat MRI studies ($48 850) for $150/hr. Each will need 4 hours for in vivo and 1hr for ex vivo MRI (total number of rats for years 4-5 is 150).

In Year 5: We request lab supplies in the total sum of $62, 268. This includes laboratory supplies ($5 400), material for brain banking ($1 529), blood collection tubes ($1 076), EEG recording supplies ($8 283), on-site data storage expenses ($3 500), shipping of samples to IAC ($2 000), plasma miRNA analysis materials and reagents ($40 480). We also request funds for the rat MRI studies ($48 850) for $150/hr. Each will need 4 hours for in vivo and 1hr for ex vivo MRI (total number of rats for years 4-5 is 150).

Travel
In Year 4: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.
In Year 5: We request $2,000 funds for travel to the annual consortium meeting to discuss progress.

Publication costs
In Year 4: We request $2000 for publication costs of our manuscripts and posters.
In Year 5: We request $2000 for publication costs of our manuscripts and posters.

Indirect costs are 10%.

5. UNIVERSITY OF MINNESOTA, CENTER FOR ORPHAN DRUG RESEARCH (CODR)
Key Personnel
Jim Cloyd, Pharm.D. (site PI): We request 0.48 calendar months for Dr. Cloyd in Year 1-2 ($9,900 including fringe) and 0.24 calendar months in Year 3 ($4,950 with fringe), and 0.24 calendar months in Year 4 and 5 ($4,950 /year with fringe).

Dr.. Cloyd is the Director of the Center for Orphan Drug Research (CODR), the Lawrence C. Weaver Endowed Chair in Orphan Drug Development, and Professor of Neurology and Experimental and Clinical Pharmacology at the University of Minnesota. Dr.. Cloyd will assist Dr.. Coles in overall management of the study and serve as a mentor for her. He will also ensure that CODR laboratory facilities and personnel are available to perform the drug assays. His responsibilities will include oversight of the project at the University of Minnesota; serve as liaison with other project investigators; chair periodic meetings to address pharmacokinetic/pharmacodynamic issues; and prepare and review abstracts, manuscripts, and reports.

Lisa Coles, M.S., Ph.D. (site co-PI): We request 1.2 calendar months for Dr. Coles in Year 1-2 ($8,704 including fringe) and 0.6 calendar months in Years 3-5 ($4,352 with fringe).

Dr.. Coles is a Research Assistant Professor in The Department of Experimental and Clinical Pharmacology at the University of Minnesota. Dr.. Coles will act as the pharmacokinetic/pharmacodynamic project coordinator. Her responsibilities will include design of the PK studies; coordination of sample analysis; pharmacokinetic and pharmacodynamic analysis; data interpretation; preparation of site reports; and participate in abstract and manuscript preparation.

Other personnel
Usha Mishra, M.S. (Laboratory Analytical Scientist): We request 2.16 calendar months for Mrs Mishra in Year 1-2 ($12,760 including fringe) and 0.72 calendar months in Year 3 ($3,840 with fringe) and 1.92 calendar months in Years 4 and 5 ($12,992 with fringe).
Usha Mishra is a Senior Scientist in The Center for Orphan Drug Research at the University of Minnesota. She will provide technical expertise in the performance of HPLC-mass spectrometry. Her responsibilities will include assay development and validation; sample analysis to quantify drug concentration in plasma and brain samples; preparation of site report; and participation in manuscript preparation.

Supplies
We request $9,240 in Year 1 and Year 2 and #3360 in Year 3 and $15,120/year in years 4-5. These costs include supplies and instrument costs for the bioanalytical method development and validation; and bioanalysis

of samples collected from the proposed studies. These costs are for the measurement of drug concentrations in ~20 brain and ~20 plasma samples per drug per dose level.

Travel
These costs include travel and housing ($1,500 per annum, in Years 1-3) to attend 3 scientific meetings over the course of the grant to present the pharmacokinetic and pharmacodynamic study results.

Indirect costs are 52% till 6/30/2017, 53% till 6/30/2018, 54% thereafter.

6. UNIVERSITY OF BRITISH COLUMBIA
Key Personnel
Terrance Snutch (site PI): We request effort towards 0.12 calendar months in Years 1-3 and 0.6 calendar months in Year 4 ($1,875 per annum in Years 1-3, $9,375 in Year 4, no fringe benefits). Dr. Snutch will be the site PI and contact person for this project and will supervise the synthesis, purification and quality control of the Z944 synthesis that will be provided to the Project 2 investigators. He will also participate in the relevant manuscripts.

Other Personnel
Synthetic Chemist TBD (post-doctoral associate): We request 0.25 calendar months in Years 1-3 and 1.25 calendar month salary support ($3,000 per annum in Years 1-3, $15,000 in Year 4, including 20% fringe benefits. This person will be responsible for the synthesis, purification and quality control of Z944 that will be provided to Project 2 investigators. The candidate will have organic synthesis skills and experience especially as these relate to substituted N-heterocycles and substituted piperazines. Additional criteria will include experience with molecular spectroscopy (UV, IR), mass-spectrometry, spectroscopy NMR, TLC, HPLC, GCMS, CE, column chromatography.

Supplies
We request lab supplies for starting materials and chemicals ($4,375 in Years 1-3 per annum; $21,875 in Year 4) and expenses for the purification and product characterization ($1,125 per annum in Years 1-3, $5,625 in Year 4). Starting materials include: 3-Chloro-5-fluorobenzic acid, oxalyl chloride, DMF, methylene chloride, 3-chloro-5-fluorobenzoyl chloride, *tert*-Butyl-4-(aminomethyl) piperidine-1-carboxylate, N,N-Di-isopropylethylamine, ethyl acetate, sodium bicarbonate, sodium sulphate, sodium hydroxide, 3-Chloro-5-fluoro-N-(piperidin-4-ylmethyl) benzamide, N-(*tert*-Butyl)-2-chloroacetamide, potassium carbonate, acetone, di-isopropylether. Purification and product characterization will cover internal fee-for-service concerning analytical analyses across four synthetic batches: infrared spectroscopy, differential thermal analysis, HPLC, mass spectroscopy, infrared spectroscopy.

Indirect costs are 10%.

F. CONSULTANTS (No effort)
Dr. Anatol Brain (UCLA) will be a consultant on the methods of EEG analysis for the detection of pHFOs and rHFOSs.
Dr. Istvan Mody (UCLA) will be a consultant for the characterization and study of epileptogenesis related biomarkers and electrophysiological patterns.
Dr. Ashley Bush (Melbourne) will be a consultant on the use and monitoring of efficacy of deferiprone. Dr. Chris Hovens (Melbourne) will act as consultant on the use and monitoring of efficacy of sodium selenate.