OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
MH119880-01

b. Agency Routing Number

2. DATE SUBMITTED	**Application Identifier**	**c. Previous Grants.gov Tracking Number**
2019-04-05		GRANT12704511

5. APPLICANT INFORMATION **Organizational DUNS*:** 0468381840000

Legal Name*: OPEN SOURCE INSTRUMENTS, INC.
Department:
Division:
Street1*: OPEN SOURCE INSTRUMENTS, INC.
Street2: 130 MOUNT AUBURN ST
City*: WATERTOWN
County: Massachusetts
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 024531234

Person to be contacted on matters involving this application
Prefix: First Name*: Kirsten Middle Name: Last Name*: Hashemi Suffix:
Position/Title: Operations Manager
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: Waltham
County: Massachusetts
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 024723932
Phone Number*: 617-733-1553 Fax Number: Email: kirsten@opensourceinstruments.com

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 141911312

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*
❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
An optogenetic brain implant with EEG monitoring and response for mice

12. PROPOSED PROJECT		**13. CONGRESSIONAL DISTRICTS OF APPLICANT**
Start Date*	Ending Date*	MA-005
09/01/2019	08/30/2020	

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Kevan Middle Name: Last Name*: Hashemi Suffix:
Position/Title: President and Founder
Organization Name*: Open Source Instruments, Inc.
Department:
Division:
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: WATERTOWN
County: Massachusetts
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 024723932
Phone Number*: 6173353472 Fax Number: Email*: hashemi@opensourceinstruments.com

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$245,761.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$245,761.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Kirsten Middle Name: Last Name*: Hashemi Suffix:
Position/Title*: Operations Manager
Organization Name*: Open Source Instruments, Inc.
Department:
Division:
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: BELMONT
County: Massachusetts
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 024723932
Phone Number*: 617-733-1553 Fax Number: Email*: kirsten@opensourceinstruments.com

Signature of Authorized Representative*
Kirsten Hashemi

Date Signed*
04/05/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:89743-Schaffer-Prop-P1-cover_letter.pdf

424 R&R and PHS-398 Specific
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OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	OPEN SOURCE INSTRUMENTS, INC.
Duns Number:	0468381840000
Street1*:	OPEN SOURCE INSTRUMENTS, INC.
Street2:	130 MOUNT AUBURN ST
City*:	WATERTOWN
County:	Massachusetts
State*:	MA: Massachusetts
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	024723932
Project/Performance Site Congressional District*:	MA-005

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Cornell University
DUNS Number:	8726124450000
Street1*:	School of Biomedical Engineering, 237 Tower Road
Street2:	B57 Weill Hall
City*:	Ithaca
County:	Tompkins
State*:	NY: New York
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	148537202
Project/Performance Site Congressional District*:	NY-023

Additional Location(s) File Name:

Funding Opportunity Number: PA-18-871. Received Date: 2019-04-05T17:35:27.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ❍ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ● No

IACUC Approval Date: 04-13-2018

Animal Welfare Assurance Number A3347-01

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Project-Summary-Abstract-20180905-0948.pdf
8. Project Narrative*	Narrative-20180905-0946.pdf
9. Bibliography & References Cited	References-20190405-1630.pdf
10.Facilities & Other Resources	Facilities_OSI_Cornell.pdf
11.Equipment	Equipment_OSI_Cornell.pdf
12. Other Attachments	SBC_000814955_(1).pdf

Funding Opportunity Number: PA-18-871. Received Date: 2019-04-05T17:35:27.000-04:00

Abstract: Optogenetics can be used to selectively stimulate or suppress the firing of genetically targeted and spatially targeted mammalian neurons. It is used to study neuropsychiatric diseases *in vivo* with mouse models of conditions including epilepsy, schizophrenia, and Parkinson's. Optogenetics may be used as functional neurosurgical intervention for correcting disease states in the brain. It has been previously shown that seizures have the ability to be halted or reduced by optogenetic activation of inhibitory neurons with the use of Channelrhodopsin-2. It has also been shown that expressing Halorhodopsin (HR) in cortical pyramidal neurons can also reduce seizure propagation. By monitoring EEG data in real time, seizures can be identified at their onset and correcting pulses of optogenetic stimulation may be applied. This line of research is currently limited by the lack of suitable instruments. This project proposes the development of a **fully implantable, wireless EEG monitor capable of autonomously detecting EEG events in real-time and applying correcting pulses of closed-loop optogenetic stimulation**. The proposed instrument will be compatible with mouse biology, thus permitting chronic experiments in the enormous pool of transgenic mouse strains available with photosensitive proteins and validated as models of human disease. Aim 1 will develop the necessary hardware by combining core technologies demonstrated in existing products. Aim 2 will enable autonomous EEG event detection in the instrument's micropower logic chip by adapting a computationally efficient algorithm that has been proven capable of classifying EEG events including normal activity, seizures, ictal spikes, inter-ictal spikes, and polyspikes. Aim 3 will test the device's ability to detect seizures, apply correcting optogenetic stimulation, and reduce the duration of focal seizures induced in mice by the nanoinjection of iron chloride. Phase I of this project will make the mouse-compatible instrument available for sale to researchers studying circuit theory of the brain and diseases/disorders characterized by aberrant EEG states such as epilepsy, schizophrenia, Alzheimer's, and obsessive compulsive disorder. Potential follow-on Phase II would develop the technology into a medical instrument that aborts focal seizures in humans who suffer from pharmaceutical-resistant partial epilepsy (approximately 15 million people).

A wireless instrument capable of simultaneous EEG recording and optogenetic stimulation in mice will enable a new class of experiments of epilepsy, schizophrenia, Alzheimer's, obsessive compulsive disorder, and other conditions characterized by aberrant electrophysiology. In addition to aiding basic research and the identification of pharmacological treatments, the instrument will enable a new class of functional neurosurgical intervention. The instrument will demonstrate a new treatment of focal seizures in patients suffering from pharmaceutical-resistant partial epilepsy.

Facilities

Open Source Instruments

Open Source Instruments has its laboratory and manufacturing facility at 5 Pratt Ave, Waltham, MA. Our 2,000 square foot, rented space has an open floor plan. It is well lit with natural and artificial light. There are eight distinct work stations in the space, which are comprised of a work-bench surface, seating, magnifier lights, computers, and specialized equipment. We have three general-purpose electronic assembly stations and an additional three stations dedicated to the manufacture of subcutaneous transmitters. Other stations include an optical fiber stretcher and radio frequency testing space with Faraday enclosure.

The facility is large enough to include space for storing items related to manufacturing such as electronic components, flux, potting epoxy, manufactured parts ready for sale, and Faraday enclosures. There are also shelves for disposable items like mixing tips, paper containers, and wipes. The space is large enough to absorb growth in both projects and people.

The Open Source Instruments Inc. billing and correspondence address is 130 Mt. Auburn Street, Watertown, MA 02472. This is the address under which our paperwork is filed and it is where we receive our mail. Accounting, invoicing, and bill paying happen from this address.

Open Source Instruments is a small company with four regular employees, three of whom are on-site at the manufacturing facility. The relationship between them is collegial. There are several projects on-going within the facility, some of which all participate in and some of which are completed independently. The engineers tend to discuss and trouble shoot each-others' issues. Kevan Hashemi initially recruited Michael Collins, having maintained their relationship while Mr. Collins fulfilled his obligations to the US Services for his education, in order to work closely with him. Mr. Hashemi and Open Source Instruments have supported Mr. Collin's goals and projects and will continue to do so as best we know how, in order to work with a talented, remarkable individual.

Open Source Instruments does not work with any animals in its offices. We have, instead, collaborated with researchers who themselves maintain animal facilities in order to develop all of our implantable devices. We have worked with the Institute for Neurology at University College London, the Department of Physiology at Edinburgh University, Children's Hospital Boston at Harvard University, and Oxford University, for example. For the initial optogenetic device created for a rat, we collaborated with the Institute of Neurology, University College London. For this proposal, we have been fortunate to catch the interest of Dr. Chris Schaffer in the Department of Biomedical Engineering, Cornell University. All animal testing will be conducted under his direction.

Schaffer/Nishimura Lab at Cornell

Cornell University Department of Biomedical Engineering meet all United State regulations for the ethical treatment of laboratory animals, and their facilities are maintained according to the highest standards. Profs. Schaffer and Nishimura have a co-administered, collaborative lab with shared research space, equipment, and other resources.

Laboratory: The shared laboratory, located at Weill Hall at Cornell University, consists of about 3000 sq. ft. of work space, including areas for animal surgery, wet chemistry, cell culture, mechanical and electronic fabrication, and data analysis. The optical imaging experiments are conducted in two dedicated 800 sq. ft. laser labs, which each house a 140 sq. ft. vibration-isolated optical table on which all laser systems and microscopes are constructed. Two ~100 sq. ft. rooms in the animal facilitate are dedicated to behavioral testing and long-term electrophysiological recording for our laboratory.

Clinical: N/A

Animal: Animal husbandry and housing facilities for rodents are located at Weill Hall. The laboratory includes a fully-equipped rodent microsurgery room with gas anesthesia, physiology monitoring, stereotaxic apparatus, stereoscopes, and perfusion setup. Cornell University is fully accredited by AAALAC, and provides 24 hour/7 day veterinary care for all research animals, as well as hands-on training in animal procedures.

Cell Culture: A BSL2 facility for cell culture and viral vector development is located in the lab. The space includes incubators for mammalian cells and bacteria, liquid nitrogen storage, a BSL2 laminar flow hood, and a chemical fume hood.

Computer: Extensive dedicated, networked computer systems are available in the laboratory, including a central 172 TB RAID6 data storage system, machines for data acquisition and instrument control (6 workstations), for data analysis and simulation (12 workstations with parallel computing capability plus six laptop computers), and for preparing papers and presentations for dissemination of our data. Data acquisition, analysis software, and simulation code is largely written in house.

Office: PIs have private offices, while lab members share communal office space and a dedicated conference room. Administrative support is provided by the Department of Biomedical Engineering.

Other Resources: Library resources at Cornell are extensive. Online subscriptions to relevant clinical and research journals are available. Professionally-staffed machine and electronics fabrication shops are available at Cornell, as well as student machine and electronics fabrication shops (where students can work after completing required training). A professionally-staffed histological service is available through the Cornell Veterinary College. This group is not only able to help with slicing tissue and standard histology staining, but also with antibody staining.

Research Environment:
These experiments take advantage of unique resources and capabilities available in Prof. Nishimura and Schaffer's laboratories at Cornell University. To clarify for reviewers how our dual-PI lab operates, we briefly review the history of Profs. Schaffer and Nishimura's long collaboration. Prof. Nishimura was previously a postdoc and then research associate in Prof. Schaffer's lab at Cornell, before that both were members of David Kleinfeld's lab at UCSD (Nishimura for PhD, Schaffer for post-doc), and before that both worked with Eric Mazur at Harvard (Nishimura as undergraduate researcher, Schaffer for PhD). Now both Nishimura and Schaffer are Associate Professors at Cornell and they run a joint lab together, where resources are shared and about two-thirds of the lab's projects are collaborative, while others are independent, with some led by Schaffer and some by Nishimura. During more than 20 years of working in the same labs, Profs. Schaffer and Nishimura have developed a unique and effective working relationship. It was to maintain this highly effective collaborative approach as well as to maximize utilization of expensive experimental resources that Profs. Schaffer and Nishimura chose to organize as a dual-PI lab at Cornell. Profs. Schaffer and Nishimura have also been partners in life for over 20 years.

Few labs have both the resources and expertise in optical design and nonlinear optics as well as in vivo animal work and biology to conduct the proposed studies. The lab has a long history of studying cellular interactions in the normal and disease state central nervous system of rodents using nonlinear optical tools as well as developing novel applications for laser technologies. In addition, Cornell University is a renowned center of expertise for in vivo microscopy and the use of nonlinear optical techniques for biology research. The Schaffer/Nishimura lab both contributes to this technology development and benefits from it, for example in the use of longer wavelength light for very deep in vivo two- and three-photon imaging (in collaboration with Prof. Xu (Applied Physics)). Our lab also collaborates extensively with neurobiologists at Cornell (e.g. several papers with Prof Fetcho (Neurobiology)) and with researchers at Weill Cornell Medical (e.g. several papers with Prof. Iadecola (Neurology) and Prof. Schwartz (Neurosurgery)).

Equipment

Open Source Instruments

In its laboratory, Open Source Instruments has all equipment necessary for electronic design and assembly. Items include:

Six soldering irons at the electronic assembly stations

Three complete telemetry set-ups for testing and programming transmitters, which have LWDAQ Drivers and Octal Data Receivers, antennas, and spectrometers

Optical fiber stretcher to heat and divide optical fibers to create tapers

Two oscilloscopes

One Vector Voltmeter

Photometers

Lab oven for elevating temperature during accelerated testing

Inspection optics

Anti-static mats

Cleaning station with hot water and specialized brushes for electronics

Compressed nitrogen

Compressed air

Vacuum chamber for device encapsulation

Motorized rotators for encapsulation curing

A custom silicone curing enclosure

Custom-made Faraday enclosures

Cornell University

The Schaffer lab comes with the support of the Biomedical Engineering College at Cornell University.

Major Equipment: Schaffer/Nishimura Lab

Surgical facility (located in Nishimura and Schaffer laboratories):
Three surgical stereotaxic setups, three high-quality surgical stereoscopes, gas or injectable anesthesia, animal ventilator, blood pressure monitor, two pulse oximeters, exhaled carbon dioxide monitor, five closed-loop core temperature thermometer and heating blankets, animal perfusion setup, micropipette puller. Full histology capabilities, including cryostat, setup for stains as well as immunohistology, and Zeiss widefield fluorescence microscope.

Optical imaging facility (located in Nishimura and Schaffer laboratory):
High-power commercial femtosecond laser oscillators:
Manually-tuned, high-power Ti:Sapphire laser: 720-980-nm wavelength, 80-fs pulse duration, 4-W average power at 800 nm, 76-MHz repetition rate
Two automated-tuning Ti:Sapphire laser: 700-1030-nm wavelength, 75-fs pulse duration, 3-W average power at 800 nm, 76-MHz repetition rate
Yb:fiber laser: 1030-nm wavelength, 300-fs pulse duration, 4-W average power, 6-MHz repetition rate

Yb-fiber laser driven optical parametric amplifier: 1330-nm wavelength, 75-fs pulse duration, 250-mW average power, 1 MHz repetition rate
Er:fiber laser: 1550-nm wavelength, 500-fs pulse duration, 2-W average power, 0.25 – 5 MHz repetition rate; this light is Raman shifted in a photonic crystal rod to 1700-nm wavelength, with ~50-mW average power

Commercial femtosecond laser amplifier:
Ti:Sapphire amplifier: 800-nm wavelength, 50-fs pulse duration, 1-mJ pulse energy, 1-kHz repetition rate, with separate femtosecond seed oscillator

Nonlinear microscopes:
Four home-built two-photon excited fluorescence microscopes designed specifically for in vivo animal imaging and targeted optical manipulation. Optics are designed for low-loss delivery of femtosecond pulses from any of the imaging lasers described above, compensation of dispersion to achieve shortest pulse duration at the focus, and high efficiency collection and detection of emitted fluorescence or harmonic radiation on four separate detection channels. Additional laser beams are integrated into the microscopes for sample manipulation through photochemistry and optical ablation with the amplified femtosecond laser. High-resolution three-dimensional positioning of sample is achieved through computer-controlled translation stages. The microscope uses custom data acquisition software that allows fast frame and volume imaging and rotatable line-scans. One microscope is a custom-designed hyperspectral multiphoton microscope that provides 48 channels of excitation/emission information at each voxel in a three-dimensional image. One four-channel system is additionally capable of fast scanning with resonant scanners enabling approximately 8x faster image acquisition. The 1,300 and 1,700 nm imaging sources are used to drive three-photon excited fluorescence, which enables imaging deeper into scattering samples than conventional two-photon excitation.

Conventional, fluorescence microscope:
Zeiss Examiner.D1 with bright-field and epifluorescence imaging, cooled CCD camera.

Histology:
Cryotome for sectioning tissue and facilities for immunohistology and staining.
Cell culture:

BSL II cell culture hood, mammalian and bacterial incubators, desktop centrifuges, ultracentrifuge, qPCR machine, inverted microscope.

Animal behavior:
Automated animal tracking camera system and software as well as appropriate mouse “mazes” to enable a broad range of behavioral testing, including locomotor function, spatial and working memory, sensory sensitivity.

Electrophysiology:
Dual setups for recording three-channel field potential with concurrent video monitoring of awake animals.

Subcutaneous Transmitter Equipment Set-Up provided by Open Source Instruments:
Octal Data Receiver
Eight Loop Antenna
LWDAQ Driver
Faraday Enclosure
Subcutaneous Transmitters for testing the system

SBIR · STTR
America's Seed Fund™
POWERED BY SBA

SBIR.gov SBC Registration

SBC Control ID:	SBC_000814955		
Company Name:	OPEN SOURCE INSTRUMENTS INC.		
Address:	130 MOUNT AUBURN ST		
City:	WATERTOWN		
State:	MA	**Zip:**	02472-3932
EIN (TIN):	141911312	**DUNS:**	046838184
Company URL:	opensourceinstruments.com		
Number of Employees:			6
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?			No
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?			0.00%

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Kevan Middle Name Last Name*: Hashemi Suffix:

Position/Title*: President and Founder
Organization Name*: Open Source Instruments, Inc.
Department:
Division:
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: WATERTOWN
County: Massachusetts
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 024723932

Phone Number*: 6173353472 Fax Number:

E-Mail*: hashemi@opensourceinstruments.com

Credential, e.g., agency login: KEVANSHASHEMI

Project Role*: PD/PI Other Project Role Category:

Degree Type: Master of Science Degree Year: 1992

Attach Biographical Sketch*: File Name: Biosketch-Hashemi-20190405-1438.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12832361 Funding Opportunity Number: PA-18-871 . Received Date: 2019-04-05T17:35:27.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Michael Middle Name London Last Name*: Collins Suffix:

Position/Title*: Engineer
Organization Name*: Open Source Instruments, Inc.
Department:
Division:
Street1*: 130 MOUNT AUBURN STREET
Street2:
City*: WATERTOWN
County: Massachusetts
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 024723932

Phone Number*: 978-335-3137 Fax Number:

E-Mail*: collins@opensourceinstruments.co

Credential, e.g., agency login: MICHAELCOLLINS

Project Role*: Co-Investigator Other Project Role Category:

Degree Type: Master of Science Degree Year: 2014

Attach Biographical Sketch*: File Name: Biosketch-Collins-20190405-1438.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: James Middle Name Last Name*: Bensinger Suffix:

Position/Title*: Professor of Physics
Organization Name*: Brandeis University
Department:
Division:
Street1*: 415 South Street
Street2:
City*: Waltham
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 024532700

Phone Number*: 6176104316 Fax Number:

E-Mail*: bensinger@opensourceinstruments.com

Credential, e.g., agency login: JAMESBENSINGER

Project Role*: Other Professional Other Project Role Category: Project Manager and Physicist

Degree Type: PhD Degree Year: 1970

Attach Biographical Sketch*: File Name: biosketch-Bensinger_Final.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Chris Middle Name Last Name*: Schaffer Suffix:

Position/Title*: Associate Professor; Associate Dean of the Fa
Organization Name*: Cornell University
Department: Biomedical Engineering
Division:
Street1*: B54 Weill Hall
Street2: 526 Campus Rd.
City*: Ithaca
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 148537202

Phone Number*: (607)342-7737 Fax Number:

E-Mail*: cs385@cornell.edu

Credential, e.g., agency login: CBSCHAFFER

Project Role*: Other Professional Other Project Role Category: Biomedical Engineer

Degree Type: Doctor of Philosophy in Physics Degree Year: 2001

Attach Biographical Sketch*: File Name: Schaffer_NIH_Biosketch.pdf

Attach Current & Pending Support: File Name: Schaffer_Support.pdf

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Theodore Middle Name Last Name*: Schwatrz Suffix:

Position/Title*: Professor Neurological Surgery, Otolaryngoloy
Organization Name*: Weill Cornell Medical College
Department: Neurosurgery
Division:
Street1*: 407 East 61st Street
Street2:
City*: New York
County:
State*: NY: New York
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 100651234

Phone Number*: (212) 821-0850 Fax Number:

E-Mail*: schwarh@med.cornell.edu

Credential, e.g., agency login: THSCHWARTZ

Project Role*: Consultant Other Project Role Category:

Degree Type: Doctor of Medicine, post docs in Neurobiology and Epilepsy Surgery Degree Year: 1993

Attach Biographical Sketch*: File Name: Biosketch_Schwartz.pdf

Attach Current & Pending Support: File Name: Support_Schwartz.pdf

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kevan Hashemi

eRA COMMONS USER NAME (credential, e.g., agency login): KEVANSHASHEMI

POSITION TITLE: Electrical Engineer, Adjunct Faculty, Brandeis University

President and Founder, Open Source Instruments

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cambridge University, Cambridge, UK	B.S.	05/01/87	General Engineering
Cornell University, Ithaca, NY	M.S.	05/01/92	Electrical Engineering

A. Personal Statement

In 2004, I founded Open Source Instruments Inc. to build electronic devices for researchers. Shortly after founding, I was asked by Matthew Walker, director of Clinical and Experimental Epilepsy at the Institute of Neurology, University College London (ION), to develop a wireless EEG transmitter for research animals. By 2008 we had a working product which permitted ION to publish its first paper using our device (2011) and since then there have been 12 study results published in reputable journals all using data gathered from our system. Through individual, electronic designs and customized software analysis, I create products that are adapted to the specific needs of the researchers' project.

As founder and Chief Executive of Open Source Instruments, I bring to this proposed project extensive experience in product development and a proven team of technical staff, most importantly, those with expertise in radio-frequency and optics. I have been working for Professor Bensinger at Brandeis University for twenty-five years, designing and manufacturing opto-electronics for High Energy Physics experiments. Michael Collins worked for Prof. Bensinger and I at Brandeis in 2010. Now, he works at Open Source Instruments. Open Source Instruments, is supported by board members and staff with expertise in mechanical engineering, electronics, and ASIC design.

All of Open Source Instruments' telemetry devices are designed to specifications that evolve from open discussion with the customers. This open discussion is possible only because we are open source. **Our designs and software are free for all to look at, just as the experimental methods of our customers are free for all to read. This open relationship allows us to design instruments**

that are of real, lasting value to scientists. Open Source Instrument's intellectual property is covered by the GNU General Public License. The telemetry system we provided to ION has allowed their experimental epilepsy group to accelerate and significantly expand its research over the last ten years.

Currently, our products are being used for research in about 15 laboratories around the world. The growing publication record speaks for the reliability and fidelity of the EEG recordings which researchers obtain with our equipment.

The wireless optogenetics market, which our customers have asked us to become part of, has seen several ingenious but impractical devices. Our proposed device is different. It will work, and it will bring real value to researchers. This project is the next best step for me and Open Source Instruments in our journey of building effective research tools for scientists.

B. Positions and Honors

2004-present	President, Open Source Instruments, Watertown and Waltham, MA
1995-present	Electrical Engineer and Adjunct Faculty, Martin A. Fisher School of Physics, Brandeis University, Waltham, MA
1992-1994	Electrical Engineer, Superconducting Super Collider, Harvard University, Cambridge, MA
1987-1989	Electrical Engineer, Meta Machines, Ltd, Abingdon, Oxfordshire, UK

C. Contributions to Science

1. In the 1990's, a dozen research groups around the world were working on their own laser rangefinder designs. I was an important member of one such group at the Harvard High Energy Physics Laboratory. Our purpose was to figure out how to build a rangefinder accurate to a tenth of a millimeter that could be manufactured for a few thousand dollars and installed by the hundreds in the Superconducting Super-Collider (SSC) in Texas. We built a successful prototype, which we describe in our paper "*Sources of Error in a Laser Rangefinder*" (Hashemi et al. 1994 Review of Scientific Instruments. 65. 3165 - 3171. 10.1063/1.1144545). Our work formed part of the successful global effort that produced the economical and effective laser rangefinders available today.

2. From 1995 to the present day I have been the principle electrical engineer at Brandeis designing opto-electronic alignment systems for High Energy Physics particle detectors. In particular, we designed and build the alignment system of the ATLAS end-cap muon spectrometer. There were several opto-electronic devices competing for installation in this monitoring system at the time we joined the collaboration in 1995. Our design was the Brandeis CCD Angle Monitor (BCAM), a radiation-resistant, solid-state camera with a stable chassis that we could calibrate to provide accuracy better than fifty microradians and precision better than five microradians. The BCAM won through as the best device for the job, and we installed thousands of them in the ATLAS detector. We report on the performance of the BCAM in the ATLAS alignment system in "*The Optical Alignment System of the ATLAS Muon Spectrometer Endcaps*" (Amelung et al. 2008 ATLAS Muon Note ATL-MUON-PUB-2008-003), a technical paper published by the ATLAS collaboration. I was the principle inventor of the BCAM, and I designed all the electronics used in the device. I also wrote the core data acquisition software, image analysis software, and software that takes calibration measurements and calculates the parameters that define each BCAM camera.

As members of the ATLAS Collaboration, we are co-authors on the landmark physics paper "*Observation of a new particle in the search for the Standard Model Higgs boson with the ATLAS detector at the LHC*" (Aad et al. 2012, Physics Letters B, Volume 716, Issue 1, Pages 1-29), along

with several thousand other people. In the past ten years six new versions of the BCAM have been designed and built, and these have been installed in half a dozen new detectors in Europe, the United States, South America, and India.

3. I began my collaboration with Dr. Matthew Walker of the Institute of Neurology (ION) at University College London in 2005. Our purpose was to design and build a battery-powered device that could be implanted subcutaneously in a rat and record high-fidelity EEG for two months. We produced such a device in 2010 ("*A Novel Telemetry System for Recording EEG in Small Animals*" Chang et al. 2011, Journal of Neuroscience Methods, 201(1): 106-115). These rat-sized implants made possible an entire series of experiments at ION.

With the OSI telemetry system in place, researchers at ION could acquire tens of thousands of hours of high-fidelity EEG from freely-moving animals. For efficient use of time, they had to figure out how to search through the recordings automatically to count seizures, spikes, and other unusual events. I developed the Event Classifier software for this purpose. ION describes their use of the Event Classifier in the Methods section of "*Optogenetic and Potassium Channel Gene Therapy in a Rodent Model of Focal Neocortical Epilepsy*" (Wykes et al. 2012, Science Translational Medicine, DOI: 10.1126/scitranslmed.3004190), a paper in which I am a co-author for my contribution to their analysis. I am likewise a co-author on these other published neuroscience papers for similar contributions to EEG analysis: Brown et al. 2018, eNeuro, ENEURO.0426-17; Wright et al. 2015, BRAIN Journal of Neurology, Oxford University Press, 138(9); Snowball et al. 2019, Journal of Neuroscience, doi: 10.1523/JNEUROSCI.1143-178.

4. My collaboration with Dr. Louise Upton of the Department of Physiology, Oxford University, in 2012 developed a subcutaneous, wireless transmitter small enough to be implanted in mice. In 2015, Dr. Upton and her group completed their first study with the mouse-sized implants, as reported in "*Epileptogenic effects of NMDAR antibodies in a passive transfer mouse model*" (Wright et al. 2015, BRAIN Journal of Neurology, Oxford University Press, 138(9)).

D. Additional Information: Research Support and/or Scholastic Performance

Completed list of published work for Kevan Hashemi can be found here: https://orcid.org/0000-0002-6007-893X/print

List of publications based on strength of data gathered with Open Source Instruments transmitters can be found here http://www.opensourceinstruments.com/SCT/#Published%20Papers

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Michael Collins

eRA COMMONS USER NAME (credential, e.g., agency login): MICHAELCOLLINS

POSITION TITLE: Electrical Engineer

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Brandeis University	B.A.	06/2012	Economics
Northeastern University, Boston, MA	M.S.	08/2014	Electrical and Computer Engineering

A. Personal Statement

I have worked on projects for OSI since 2012 and have been at OSI full-time since January 2017. I have experience with optical fibers, miniaturized optoelectronics, circuit design, circuit layout, firmware development, electronics testing, and cost estimation. These are the skills necessary to build a commercially viable optogenetic stimulator that will perform to the specifications defined in this proposal. In several years of discussions with our customers, it has become obvious to me that the market has failed to meet scientists' demands for a wireless optical stimulator. I have spoken with scientists who have attempted to use the optical stimulator systems advertised by other companies, and they have found those systems to be fatally flawed and impractical for real experimental work. I have worked with Mr. Hashemi, Dr. Schaffer, and other neuroscientists to ensure that our proposed product is not only a technological leap, but that it is also practical, profitable, and commercially viable.

While working for NATO's Centre for Maritime Research and Experimentation, I was promoted to “Junior Scientist” as a direct result of my research reports on the effect of underwater electronics on maritime security and my successful interactions with our stakeholders. For context, the official job description of “Junior Scientist” lists “PhD” as a requirement.

I founded my own company in 2017 to design, manufacture, and sell hardware for managing automotive inventory for a niche industrial market. This company sold products to a NASDAQ-listed corporation where they are undergoing evaluation.

B. Positions and Honors

2009 - 2012 Research Assistant; High Energy Physics Electronics, Brandeis; Waltham, MA
2013 (Summer) Research Intern; Los Alamos National Laboratory; Los Alamos, NM
2012 - 2014 Scientist; Open Source Instruments; Waltham, MA

2012 - 2014 Research Assistant; Northeastern University; Boston, MA
2014 - 2016 Junior Scientist; Centre for Maritime Research and Experimentation; La Spezia, Italy
2017 – Present Scientist; Open Source Instruments; Waltham, MA

C. Contributions to Science

I began work on OSI's optogenetic hardware in 2012. I was responsible for developing OSI's method of coupling LED light into optical fibers for optogenetic stimulation.The solution uses custom LED dies, custom glass material, and custom tapering of the glass fiber tip. This solution is a breakthrough in optogenetic instrumentation for several reasons. 1. It is an order of magnitude more energy efficient than conventional methods of illuminating deep neural tissue. The energy savings permit OSI to target deep brain structures with optical stimulation using a completely wireless, implantable device. Without this innovation, deep brain stimulation is only practical with tethered equipment. 2. The solution I developed is guaranteed to deliver consistent power during every stimulation, compared to commercially available devices whose power output varies randomly depending on the orientation of the animal within the enclosure. 3. The method is self-contained in the instrument and is not dependent upon wireless external power. This allows us to record EEG simultaneous to providing optical stimulus. 4. The stimulus is independently addressable, allowing it to be used with cohabiting animals. The final optogenetic head fixture that I developed is now commercially available to scientists and undergoing trials in rats at University College London.

1. Collins, Michael and Kevin D. LePage. “Civilian Monitoring Network Risks and Recommendations.” Full Report. La Spezia: STO-CMRE, 2017.
2. Collins, Michael. “Civilian Monitoring Network Workshop Outcomes.” Full Report. La Spezia: STO-CMRE, 2017.
3. Collins, Michael. “Taxonomy of civilian monitoring networks.” Memorandum Report. CMRE-MR-2015-019. La Spezia: STO-CMRE, 2016.
4. Collins, Michael. “Civilian Monitoring Network (CMN) high level report.” Memorandum Report.CMRE-MR-2015-018. La Spezia: STO-CMRE, 2016.
5. Collins, Michael L. “Detecting body cavity bombs with nuclear quadrupole resonance.” MS Thesis. Boston: Northeastern University, 2014.

D. Additional Information: Research Support and/or Scholastic Performance

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: James Bensinger

eRA COMMONS USER NAME (credential, e.g., agency login): JAMESBENSINGER

POSITION TITLE: Professor of Physics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Bucknell University	BSc	05/01/63	Physics
University of Wisconsin	PhD	06/01/70	Physics

A. Personal Statement

I am pleased to be part of this application for an PA-18-871 award. The technical people in this group are the same people that designed our profitable line of implantable telemetry devices for Open Source Instruments. Prior to that, the team worked together at Brandeis University to design, manufacture, and install the opto-electronic system that monitors the deformations of the ATLAS particle detector at CERN.

I am a minority owner of Open Source Instruments Inc., an investor in the company, and its acting treasurer. The company started with a project which grew out of research at Brandeis University, where I work with Kevan Hashemi, Open Source Instruments Founder and President. I met Kevan when we were both working on an experiment at CERN in 1993 while Kevan was at Harvard. When the funding on the Harvard project ended, I took the opportunity to hire Kevan at Brandeis, where he has been since. We have worked together for 23 years.

I bring to this project my knowledge of physics and in particular that of the propagation of light through optical fibers. In any development like this, there are always surprising physical phenomena that arise as obstacles to the development. My contribution to the development efforts at Open Source Instruments is often that of studying and understanding such surprises, a function for which my background in physics is of great value.

I will be working part-time at Brandeis University starting next year, allowing me to spend more time working on research at Open Source Instruments. I look forward to working on independent projects with a talented group of scientists. I very much hope to be able to work on efficient light injection and fiber-optic delivery for a mouse-sized ISL.

"Reference Bars for the Alignment of the ATLAS Muon Spectrometer" C. Amelung, J.R. Bensinger, et al., Nuclear Instruments and Methods A555, 36-47 (2005).

"The Optical Alignment System of the ATLAS Muon Spectrometer Endcaps," C. Amelung, et al., JINST 3:P11005, 2008.

"The ATLAS Experiment at the CERN Large Hadron Collider," The ATLAS Collaboration, JINST 3:S08003, 2008.

"System test of the ATLAS muon spectrometer in the H8 beam at the CERN SPS," The ATLAS Muon Collaboration, Nucl. Instrum. Meth. A593:232-254,2008. May 2008.

"Study of the ATLAS MDT spectrometer using high energy CERN combined test beam data," C. Adorisio, et al., Nucl. Instrum. Meth. A598:400-415, 2009.

B. Positions and Honors

1970 – 73: Instructor/Assistant Professor, University of Pennsylvania
1973 – 74: Research Associate, Brandeis University
1974 – 80: Assistant Professor, Brandeis University
1980 – 89: Associate Professor, Brandeis University
1989 – Present: Professor, Brandeis University
1996 – 2000: Chair, Brandeis University Physics Department

C. Contributions to Science

1. "Measurement of transverse energy--energy correlations in multi-jet events in pp collisions at $\sqrt{s}$ = 7 TeV using the ATLAS detector and determination of the strong coupling constant $\alpha_s(m_Z)$" ATLAS Collaboration, Physics Letters B 750 (2015) 427-447.
2. "Measurement of the branching ratio $\Gamma(\Lambda^0_b \to \psi(2S)\Lambda^0)/\Gamma(\Lambda^0_b \to J/\psi\Lambda^0)$ with the ATLAS detector" ATLAS Collaboration, Physics Letters B 751 (2015) 63-80.
3. "Summary of the searches for squarks and gluinos using $\sqrt{s}$ = 8 TeV pp collisions with the ATLAS experiment at the LHC" ATLAS Collaboration, JHEP 10 (2015) 054.
4. "Search for photonic signatures of gauge-mediated supersymmetry in 8 TeV pp collisions with the ATLAS detector" ATLAS Collaboration, Phys. Rev. D 92 (2015) 072001.
5. "Determination of the top-quark pole mass using tt-bar + 1-jet events collected with the ATLAS experiment in 7 TeV pp collisions" ATLAS Collaboration, JHEP 10 (2015) 121,

Collaborators & Other Affiliations (2000-present)

ATLAS Collaboration

D. Additional Information: Research Support and/or Scholastic Performance

Graduate and Postdoctoral Advisors

Alvin Erwin, University of Wisconsin.
Walter Selove, University of Pennsylvania

Thesis and Postdoc Sponsor

Postdoctoral (3 total):

Saminder Dhaliwal 2014-Present

Dmitri Kotchetkov	2005-2007
David Dagenhart	2001-2009

PhD students (9 total):

Keith Zengel	2015
Laurel Coffey	2014
Serdar Gozpinar	2012
Dan Pomeroy	2012
Scott Aefsky	2011
David Clark	2010
Natasa Kravchenko	2008
Hongquan Niu	2003

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

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NAME: Schaffer, Chris B.

eRA COMMONS USER NAME (credential, e.g., agency login): CBSCHAFFER

POSITION TITLE: Associate Professor; Associate Dean of the Faculty

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Florida, Gainesville, FL	BS	12/1995	Physics
Harvard University, Cambridge, MA	PHD	06/2001	Physics
Univ. of California, San Diego, La Jolla, CA	Postdoctoral Fellow	06/2002	Biophysics and Neuroscience

A. Personal Statement

My lab develops and uses advanced optical techniques to observe and manipulate in vivo biological systems, with the goal of constructing a microscopic-scale understanding of normal and disease-state physiological processes in the central nervous system. The scientific questions we address center principally on elucidating the cellular-scale interactions that lead to brain cell dysfunction in neurological diseases. We develop novel optical methods that enable us to attack these problems in ways not previously possible, and because many of our research questions involve interactions among different components of an organism (e.g. effect of altered blood flow on brain cell health) we focus almost exclusively on in vivo approaches. We study animal models of a variety of neurological diseases, including microvascular stroke, Alzheimer's disease, spinal cord injury, and epilepsy. In exciting new work, we are beginning to elucidate the pathways by which cortical microvascular dysfunction interacts with and exacerbates Alzheimer's disease. We have also recently developed capabilities for long-term in vivo imaging in the mouse spinal cord, opening the door to optical imaging studies of normal and diseased cell dynamics in the spinal cord. I am also active in developing novel educational strategies to teach science as a dynamic process for discovery. These approaches are used in outreach settings in middle and high-school science classes as well as in my undergraduate and graduate level courses. I also have a strong interest in science policy and recently spent a one-year sabbatical in Washington, DC, working as a science policy fellow for Senator Edward Markey in the United States Congress. I continue to be active in science policy, including through a course I teach on this topic. In recent work, we demonstrated a laser-based surgical therapy that blocks the propagation of focally initiated seizures in rodents [1]. In ongoing work to evaluate the long-term efficacy of that approach, we have established a robust chronic rodent model of focal epilepsy in our lab and it is this model that we would use for the proposed testing of the implanted wireless EEG/optogenetic feedback device from Open Source Instruments

[1] Nagappan S, Liu L, Fetcho R, Nguyen J, Nishimura N, Radwanski R, Lieberman S, Baird-Daniel E, Ma H, Zhao M, Schaffer CB, Schwartz TH. In vivo femtosecond laser sub-surface cortical microtransections attenuate acute rat focal seizures. Cerebral Cortex doi: 10.1093/cercor/bhy210 (2018).

B. Positions and Honors

Positions and Employment

2002 - 2005	Assistant Project Scientist and La Jolla Interfaces in Science Fellow, Dept. of Physics, Univ. of California at San Diego, Advisors: David Kleinfeld and Patrick Lyden
2006 - 2011	Assistant Professor, Department of Biomedical Engineering, Cornell University
2011 -	Associate Professor, Meinig School of Biomedical Engineering, Cornell University
2012 - 2013	OSA/SPIE Arthur H. Guenther Congressional Science Policy Fellow, Office of Senator Edward J. Markey, United States Congress
2013 - 2016	Director of Graduate Studies, Graduate Field of Biomedical Engineering, Cornell University
2014 -	Associate Professor, Dept. of Neuroscience, Brain and Mind Research Institute, Weill Cornell Medical College

2016 - Associate Dean of the Faculty, Cornell University

Other Experience and Professional Memberships

2002 - 2006 Vice-Chair (02-04) and Chair (04-06), Ultrafast Optical Phenomena Technical Group, Optical Society of America Science and Engineering Council and Annual Meeting committee
2002 - 2007 Chair, Commercial and Biomedical Applications of Ultrafast Lasers SPIE's Photonics West
2005 - 2007 Chair, Optical Microscopy and OCT Technical Group, Optical Society of America Science and Engineering Council and Annual Meeting committee
2006 - 2010 Vice-Chair (06-07) and Chair (07-10), Biomedical Optics, OSA Board of Meetings
2009 - present Reviewer, National Science Foundation, MRI-R2 (2009, 2010) and BISH (2009) panels; ad-hoc reviewer
2009 - 2014 Reviewer, Alzheimer's Association
2010 - 2010 Reviewer, Congressionally Directed Medical Program: Epilepsy panel (2010)
2011 - 2012 Working Group Member, NIH/NINDS 2012 Stroke Progress Review Group
2012 - 2013 Program Committee Member, Brain 2013, XXVIth International Symposium on Cerebral Blood Flow, Metabolism, and Function
2014 - present Associate Editor, Neurophotonics
2016 - present Associate Editor, Optica

Honors

1995 Outstanding Four Year Scholar, University of Florida, awarded to two graduates out of a class of 4000 for academic excellence in undergraduate studies
1996 National Defense Science and Engineering Graduate Fellowship, US Department of Defense
1996 Apker Award, American Physical Society, awarded to one undergraduate from a Ph.D.-granting institution nationally for achievements in research and academics
2001 New Focus Student Award, Optical Society of America, recognizes Ph.D. students for research excellence, presentation skills, and leadership in the optics community
2002 La Jolla Interfaces in Science Post-Doctoral Fellowship, Burroughs Wellcome fund
2008 Dorothy G. Swanson Excellence in Teaching Award, College of Engineering at Cornell University, highest award for teaching excellence in the College
2009 Biomedical Engineering Teaching Award, American Society for Engineering Education, national award for contributions to biomedical engineering education by a junior faculty member
2010 Zellman Warhaft Commitment to Diversity Faculty Award, College of Engineering at Cornell, for outstanding involvement in and support of diversity activities in the College
2012 Arthur H. Guenther Congressional Science Policy Fellowship, OSA and SPIE, supports one year of service as a science policy advisor in the United States Congress through a program administered by AAAS
2014 James M. and Marsha D. McCormick Award for Outstanding Advising of First-Year Engineering Students, College of Engineering at Cornell, highest award for advising in the college
2016 Mr. and Mrs. Richard F. Tucker Excellence in Teaching Award from the College of Engineering at Cornell University, highest award for teaching excellence in the College

C. Contribution to Science

1. Blood flow to the brain is reduced by about one third in patients with Alzheimer's disease. This decreased brain blood flow contributes to the memory and cognitive problems seen in Alzheimer's and may accelerate progression of the disease. The mechanism causing this poor brain blood flow, however, has remained undiscovered. Using high-resolution in vivo imaging of blood flow in mouse models of Alzheimer's disease, we have identified the plugging of capillary segments by firmly adhered white blood cells as a mechanism that contributes to this blood flow decrease. In Alzheimer's mice, nearly 2% of capillaries have stalled blood flow due to an adhered leukocyte, while wild type mice have stalls in less than 0.5% of capillaries. Because one stalled capillary decreases blood flow in many downstream branches, this leads to substantial blood flow decreases. When we blocked leukocyte adhesion, cortical blood flow increased by ~30%. This increase in brain blood flow was accompanied by an immediate

improvement in cognitive performance of mice on spatial and working memory tasks. These data suggest that white blood cells sticking in capillaries may be responsible for the reduced blood flow to the brain seen in Alzheimer's patients and that treating this could both improve cognitive function and slow disease progression. We have further elucidated the role of stalling capillaries in the normal brain and in mouse models of polycythemia vera.

a. Cruz Hernández JC, Bracko O, Kersbergen CJ, Muse V, Haft-Javaherian M, Berg M, Park L, Vinarcsik LK, Ivasyk I, Rivera DA, Kang Y, Cortes-Canteli M, Peyrounette M, Doyeux V, Smith A, Zhou J, Otte G, Beverly JD, Davenport E, Davit Y, Lin CP, Strickland S, Iadecola C, Lorthois S, Nishimura N, Schaffer CB. Neutrophil adhesion in brain capillaries reduces cortical blood flow and impairs memory function in Alzheimer's disease mouse models. Nat Neurosci. 2019 Feb 11;. doi: 10.1038/s41593-018-0329-4. [Epub ahead of print] PubMed PMID: 30742116.

b. Erdener ŞE, Tang J, Sajjadi A, Kılıç K, Kura S, Schaffer CB, Boas DA. Spatio-temporal dynamics of cerebral capillary segments with stalling red blood cells. J Cereb Blood Flow Metab. 2017 Jan 1;:271678X17743877. doi: 10.1177/0271678X17743877. [Epub ahead of print] PubMed PMID: 29168661.

c. Santisakultarm TP, Paduano CQ, Stokol T, Southard TL, Nishimura N, Skoda RC, Olbricht WL, Schafer AI, Silver RT, Schaffer CB. Stalled cerebral capillary blood flow in mouse models of essential thrombocythemia and polycythemia vera revealed by in vivo two-photon imaging. J Thromb Haemost. 2014 Dec;12(12):2120-30. doi: 10.1111/jth.12738. Epub 2014 Oct 29. PubMed PMID: 25263265.

2. Even subtle alterations in cerebral blood flow can impact the health and function of brain cells and are linked to cognitive decline and dementia. My lab has made significant strides toward understanding how blood flow is altered in the brain by microvascular occlusions. As a post-doc, I helped develop optical tools to occlude targeted brain microvessels, providing a much-needed animal model for small strokes. I contributed to studies that used this model to quantify the blood flow changes that result from the occlusion of brain arterioles and capillaries. In my lab at Cornell, we have completed this story by quantifying the flow changes that result from venule occlusions and by examining the role of active vascular regulation in blood flow rerouting after a vessel occlusion. This work was essential to understanding the immediate impact of microvascular occlusion on the brain (altered blood flow) and set the stage for studies of the effect of these lesions on the health and function brain cells, now being conducted in my and other labs using the approaches we developed.

a. Schaffer CB, Friedman B, Nishimura N, Schroeder LF, Tsai PS, et al. Two-photon imaging of cortical surface microvessels reveals a robust redistribution in blood flow after vascular occlusion. PLoS Biol. 2006 Feb;4(2):e22. PubMed PMID: 16379497; PubMed Central PMCID: PMC1324794.

b. Nishimura N, Schaffer CB, Friedman B, Lyden PD, Kleinfeld D. Penetrating arterioles are a bottleneck in the perfusion of neocortex. Proc Natl Acad Sci U S A. 2007 Jan 2;104(1):365-70. PubMed PMID: 17190804; PubMed Central PMCID: PMC1765467.

c. Nishimura N, Rosidi NL, Iadecola C, Schaffer CB. Limitations of collateral flow after occlusion of a single cortical penetrating arteriole. J Cereb Blood Flow Metab. 2010 Dec;30(12):1914-27. PubMed PMID: 20842163; PubMed Central PMCID: PMC3002886.

d. Nguyen J, Nishimura N, Fetcho RN, Iadecola C, Schaffer CB. Occlusion of cortical ascending venules causes blood flow decreases, reversals in flow direction, and vessel dilation in upstream capillaries. J Cereb Blood Flow Metab. 2011 Nov;31(11):2243-54. PubMed PMID: 21712834; PubMed Central PMCID: PMC3210348.

3. Recent clinical evidence suggests that small hemorrhages from the rupture of microvessels in the brain are linked to increased risk of neurodegenerative diseases, as well as to more precipitous cognitive decline with age. However, it remains unclear how severely and by what mechanism a microhemorrhage causes death or dysfunction in brain cells. This is due, in part, to a lack of good animal models of microhemorrhage. We used in vivo imaging coupled with our unique animal model of microvascular hemorrhage to study how such lesions affect the function and structure of neurons and other brain cells. We found, surprisingly, that microhemorrhages do not lead to structural degeneration or long-term functional impairment in neurons, but rather cause a rapid inflammatory response in microglia that is sustained in the vicinity of the microhemorrhage over weeks after the lesion. This data suggests that chronic, local inflammation may underlie the brain dysfunction that results from microhemorrhages rather

than direct neuronal damage, a hypothesis we are currently pursuing. We have also used this hemorrhage model in collaborative studies of the impact of anticoagulants and thrombolytics on brain hemorrhage size.

a. Rosidi NL, Zhou J, Pattanaik S, Wang P, Jin W, et al. Cortical microhemorrhages cause local inflammation but do not trigger widespread dendrite degeneration. PLoS One. 2011;6(10):e26612. PubMed PMID: 22028924; PubMed Central PMCID: PMC3197572.

b. Cianchetti FA, Kim DH, Dimiduk S, Nishimura N, Schaffer CB. Stimulus-evoked calcium transients in somatosensory cortex are temporarily inhibited by a nearby microhemorrhage. PLoS One. 2013;8(5):e65663. PubMed PMID: 23724147; PubMed Central PMCID: PMC3665593.

c. Lauer A, Pfeilschifter W, Schaffer CB, Lo EH, Foerch C. Intracerebral haemorrhage associated with antithrombotic treatment: translational insights from experimental studies. Lancet Neurol. 2013 Apr;12(4):394-405. PubMed PMID: 23518332; PubMed Central PMCID: PMC3702044.

d. Nishimura N, Schaffer CB. Big effects from tiny vessels: imaging the impact of microvascular clots and hemorrhages on the brain. Stroke. 2013 Jun;44(6 Suppl 1):S90-2. PubMed PMID: 23709743; PubMed Central PMCID: PMC3862170.

4. Long-term in vivo imaging in the cortex of mice has become a powerful tool for dissecting the cellular interactions that underlie normal and disease state physiological processes. We have recently worked to develop animal surgical preparations and imaging strategies that would allow such in vivo imaging approaches to be used in the spinal cord of mice. The surgical preparation we developed was the first to enable long-term optical access to the murine spinal cord, and we used this preparation to examine the heterogeneity of axon dieback and the inflammatory response after a mild spinal cord injury. We have further developed label-free optical methods for visualizing myelin in the spinal cord, and recently used our animal preparation to examine the impact of vascular occlusions on spinal cord blood flow. Recently, we contributed to studies showing that using higher order nonlinear processes for imaging allows deeper penetration into scattering tissue, such as the white matter tracks on the dorsal spinal cord. Taken together, we are moving toward the capability to directly image structure and function (e.g. neural activity) of all cellular constituents through most of the spinal cord over time in wildtype or disease-model mice.

a. Farrar MJ, Wise FW, Fetcho JR, Schaffer CB. In vivo imaging of myelin in the vertebrate central nervous system using third harmonic generation microscopy. Biophys J. 2011 Mar 2;100(5):1362-71. PubMed PMID: 21354410; PubMed Central PMCID: PMC3043202.

b. Farrar MJ, Bernstein IM, Schlafer DH, Cleland TA, Fetcho JR, et al. Chronic in vivo imaging in the mouse spinal cord using an implanted chamber. Nat Methods. 2012 Jan 22;9(3):297-302. PubMed PMID: 22266542; PubMed Central PMCID: PMC3429123.

c. Horton NG, Wang K, Kobat D, Clark CG, Wise FW, et al. In vivo three-photon microscopy of subcortical structures within an intact mouse brain. Nat Photonics. 2013 Mar 1;7(3)PubMed PMID: 24353743; PubMed Central PMCID: PMC3864872.

d. Farrar MJ, Rubin JD, Diago DM, Schaffer CB. Characterization of blood flow in the mouse dorsal spinal venous system before and after dorsal spinal vein occlusion. J Cereb Blood Flow Metab. 2015 Jan 7;PubMed PMID: 25564237.

5. When tightly-focused into biological samples or tissue, femtosecond duration laser pulses can produce micrometer-scale disruption, while causing minimal collateral damage to structures around the targeted region. Essentially, the laser acts as a light scalpel that can cut with sub-cellular precision deep inside a sample without affecting the overlying tissue. As a graduate student, I studied the laser-material interactions that enable this sub-surface disruption. As a post-doc, I worked on the use of this capability to trigger the clotting or hemorrhage of small blood vessels in rodent cortex, producing an excellent animal model of microvascular stroke (which my and other labs continue to use today). In my lab at Cornell, we have further studied the use of this laser scalpel to create a transient pore in a cell membrane to introduce foreign DNA into a targeted cell. We have also pioneered the use of this laser scalpel to produce sub-surface cuts in the cortex, which we are now using to explore laser-based surgical strategies to inhibit the propagation of focal epileptic seizures.

a. Schaffer CB, Brodeur A, García JF, Mazur E. Micromachining bulk glass by use of femtosecond laser pulses with nanojoule energy. Opt Lett. 2001 Jan 15;26(2):93-5. PubMed PMID: 18033517.

b. Nishimura N, Schaffer CB, Friedman B, Tsai PS, Lyden PD, et al. Targeted insult to subsurface cortical blood vessels using ultrashort laser pulses: three models of stroke. Nat Methods. 2006 Feb;3(2):99-108. PubMed PMID: 16432519.

c. Nguyen J, Ferdman J, Zhao M, Huland D, Saqqa S, et al. Sub-surface, micrometer-scale incisions produced in rodent cortex using tightly-focused femtosecond laser pulses. Lasers Surg Med. 2011 Jul;43(5):382-91. PubMed PMID: 21674543.

d. Davis AA, Farrar MJ, Nishimura N, Jin MM, Schaffer CB. Optoporation and genetic manipulation of cells using femtosecond laser pulses. Biophys J. 2013 Aug 20;105(4):862-71. PubMed PMID: 23972838; PubMed Central PMCID: PMC3752125.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/myncbi/chris.schaffer.1/bibliography/40950403/public/?sort=date&direction=ascending

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Schwartz, Theodore H.

eRA COMMONS USER NAME THSCHWARTZ

POSITION TITLE: Professor, Neurological Surgery, Otolaryngology, Neuroscience

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Harvard College, Cambridge, MA	B. A.	1983-1987	Philosophy
Harvard Medical School, Boston, MA	M. D.	1988-1993	Medicine
Columbia University, New York, NY	Post-doc	1996-1997	Neurobiology
Max-Planck Institute for Neurobiology, Munich, DE	Post-doc	1999-2000	Neurobiology
Yale University, New Haven, CT	Post-doc	1999-2000	Epilepsy Surgery

A. Personal Statement

I have been the Director of the Epilepsy Research Laboratory at Weill Cornell Medical College for the last 15 years. My lab uses optical imaging techniques, including calcium, voltage and intrinsic, to map the initiation and spread of epilepsy and understand its neurovascular coupling mechanisms in both rats and humans. As a practicing neurosurgeon and Director of Epilepsy Surgery, I also have access to human cortex for investigation. The laboratory has had NIH funding as well as funding from several private foundations. We also collaborate with several other basic science laboratories that specialize in two photon imaging (Drs. Shaffer, Aksay and Yuste), zebrafish physiology (Dr. Aksay), organic thin film imaging (Kymissis) and multicontact electrode arrays (Dr. Shevone). I am fully committed to consulting with Dr. Chris Schaffer in his SBIR proposal develop a mouse-implantable field potential recording system that can be programmed to recognize features in the recordings and trigger an LED to turn on to drive optogenetic channels in the treatment of epilepsy.

B. Positions and Honors

Positions

1991-92 Research Fellow, Dept. of Neurosurgery, University of Washington, Seattle, WA
94. Intern, Dept. of Surgery, Columbia Presbyterian Med. Center, New York, NY
1994-98 Resident, Dept. of Neurosurgery, The Neurological Institute of New York, NY
1998-99 Chief Resident, Dept. of Neurosurgery, The Neurological Institute of New York, NY
2000-01 Assist. Professor, Dept. of Neurological Surgery, UMDNJ-NJ Med. School, Newark, NJ
2000-01 Adjunct Assist. Professor, Dept. of Neuroscience, UMDNJ-NJ Med. School, Newark, NJ
2000-01 Director of Epilepsy Surgery, UMDNJ-NJ Med. School, Newark, NJ
2000-01 Assoc. Research Scientist, Dept. of Biology, Columbia University, New York, NY
2001-05 Assist. Professor, Dept. of Neurosurgery, Weill Cornell Med. College, New York, NY
2001- Director of Epilepsy Surgery, Weill Cornell Med. College, New York, NY
2004-05 Assist. Professor, Dept. of Neurology & Neurosci., Weill Cornell Med. College, New York, NY
2005-09 Assoc. Professor, Dept. of Neurosurgery, Weill Cornell Med. College, New York, NY
2005- Assoc. Professor, Dept. of Neurology & Neurosci., Weill Cornell Med. College, New York, NY
2008- Assoc. Professor, Dept. of Otorhinolaryngology, Weill Cornell Med. College, New York, NY
2009- Professor, Dept. of Neurosurgery, Weill Cornell Med. College, New York, NY

2014- David and Ursel Barnes Endowed Professor of Minimally Invasive Neurosurgery

Honors/Awards

John Harvard Scholarship, Harvard College (1985-87), Hoopes Prize, Harvard College (1987), Magna Cum Laude, Harvard College (1987), Graduate Research Grant, UW (1991-92), Student Research Fellowship, Harvard Medical School (1991-92), Magna Cum Laude, Harvard Medical School (1993), Junior Investigator Award, AES (1997), German Academic Exchange (DAAD) Award (1999), William P. Van Wagenen Fellowship, AANS (1999), Alexander Von Humboldt Research Fellowship (1999), Research/Clinical Training Fellowship, EFA (1999), Editorial Board, Epilepsy Currents (2004-2009), NCRR GCRC Site visit, Albert Einstein College of Med. (2005), Scientific Advisory Board CURE (2005), NINDS Study Section NSD C (2005-06), NINDS Research Supplement for Minorities (2005-2006), Mentor-Post-Doctoral Research Fellowship, EFA (2006-2007), MHHC Foundation Health Leadership Award (2006), NINDS NSD-C Permanent member (2006-10), NIBIB Study Section 06-003 (2007), NINDS ZNS1 SRB-B (2008), George Ehni Lectureship, Baylor (2008), New York Best Doctors (2008-present), NINDS ZNS1 SRB-S (2008), NINDS ZNS1 SRB-B 12 (2009), America's Top Surgeons (2009-present), NINDS ZNS1 SRB-B 17 (2009), NINDS study section ZRG1 ETTN-B (2010), Editorial boards: World Neurosurgery (2010-present), Journal of Neurosurgery (2013-present), Acta Neurochirurgica (2016-present) Gentle Giant Award, PNA (2013), Endowed Professorship (2014), K12 Study Section (2016), Chair, Editorial Board, Journal of Neurosurgery (2019)

C. Contributions to Science

1. Optical and electrical mapping of epilepsy and surround inhibition in animals and humans using intrinsic signals. The gold standard in seizure mapping for years has always been electrode recordings either field potentials or unit activity. We were one of the first lab to demonstrate the inter-ictal and well as ictal events could be imaged based on changes in reflectance of light. Moreover, we were the first to demonstrate that surround inhibition could be imaged optically. As a practicing neurosurgeon, these findings were then reproduced in the human brain during neurosurgical operations indicating the feasibility of using optical measurements to map the onset and propagation of seizures in the human. 1) Schwartz TH, Bonhoeffer T. (2001) *In vivo* optical mapping of neocortical epilepsy and inhibitory surround in ferret cerebral cortex**. Nat Medicine** 7;9:1063-1067. PMID 11533712 2) Zhao M, Suh M, Ma H, Perry C, Geneslaw A, Schwartz TH. (2007) Focal increases in perfusion and decreases in hemoglobin oxygenation precede seizure onset in spontaneous human epilepsy. **Epilepsia**; 48;11:2059-2067. PMID 17666071 3) Ma H, Suh M, Zhao M, Perry C, Geneslaw A, Schwartz TH. (2009) The importance of latency in the focality of perfusion and oxygenation changes associated with triggered afterdischarges in human cortex. **J Cereb Blood Flow Metab** 29;5:1003-1. PMID 19293822 4) Zhao M, Nguyen J, Ma, H, Mozomi N, Schaffer CB, Schwartz TH. (2011) Pre-ictal and ictal neurovascular and metabolic coupling surrounding an ictal focus. **J Neurosci** 31;37:13292-300. PMID 21917812. 5) Liou J-Y, Ma H, Wenzel M, Zhao M, Baird-Daniel E, Smith EH, Daniel A, Emerson R,Yuste R, **Schwartz TH**, Schevon C**.** (2018) The role of inhibitory control in modulating long-range spread of focal ictal activity 141;7:2083-2097 PMID. 29754397
2. Ictal and inter-ictal hemodynamic coupling. Whether the influx of oxygenated blood is sufficient to meet the metabolic demands of neurons participating in a seizure has been debated for several decades. Our lab showed definitively in both animal models as well as the human that ictal onset is accompanied by focal decreases in hemoglobin oxygenation. These findings were confirmed measuring cortical oxygenation and also demonstrated in interictal as well as ictal events. We labeled this phenomenon the "epileptic dip", reminiscent of the initial dip measured at the onset of normal sensory processing. 1) Suh M, Bahar S, Mehta A, Schwartz TH. (2005) Temporal dependence in uncoupling of blood volume and oxygenation during interictal epileptiform events in rat neocortex. **J Neurosci** 25:1;68-77. PMID 15634768 2) Zhao M, Suh M, Ma H, Perry C, Geneslaw A, Schwartz TH.(2007) Focal increases in perfusion and decreases in hemoglobin oxygenation precede seizure onset in spontaneous human epilepsy. **Epilepsia**;48;11:2059-2067. PMID 17666071 3) Zhao M, Ma H, Suh M, Schwartz TH. (2009) Spatio-temporal dynamics of perfusion and oximetry signals during ictal discharges in the rat neocortex. **J Neurosci** 9;2814-2823. PMID 19261877 3 4) Geneslaw A, Zhao M, Ma H, Schwartz TH. (2011) Tissue hypoxia correlates with intensity of interictal spikes. **J Cereb Blood Flow Metab** 31(6): 1394-402. PMID 21343943 5) Harris S, Boorman LW, Kennerley AJ, Sharp PS, Martin C, Redgrave P, **Schwartz TH,** Berwick J. (2018) Seizure epicenter depth and translaminar field potential synchrony underlie complex variation in tissue oxygenation during ictal initiation. NeuroImage 1;171:165-175.PMID 29294386.
3. Subthreshold multifocal wave propagation during seizure evolution. Using optical measurements of subthreshold axonal and dendritic activity including voltage-sensitive dyes and calcium imaging, our laboratory showed that seizures do not evolve on a subthreshold level by a Jacksonian march. Rather,

widely propagating waves of subthreshold activity with multidirectional directionality occur throughout the evolving seizure from its very beginning. The correlation between brain perfusion and these widely propagating waves is highly uncoupled in both space and time. 1) Schwartz TH, Rabinowitz D, Unni V, Kumar VS, Smetters D, Yuste R. (1998) Networks of coactive neurons in developing layer I. **Neuron**; 20:541-552. PMID 9539127 2) Ma H, Suh M, Zhao M, Perry C, Schwartz TH. (2009) Hemodynamics surrogates for neuronal activity maps during interictal epileptiform events in rat neocortex. **J Neurophysiol** 101:2550-2562.PMID 19244357 3) Ma H, Schwartz TH. (2013) Dynamic neurovascular coupling and uncoupling during ictal onset, propagation and termination revealed by simultaneous in vivo optical imaging of neural activity and local blood volume. **Cereb Cortex** 23;4:889-95 PMID 22499798 4) Ma H, Harris S, Rahmani R, Lacefield C, Zhao M, Daniel AS, Shou Z, Bruno R, Berwick J, Schwartz TH. (2014) Wide-field in vivo neocortical calcium dye imaging using a novel convection-enhanced loading technique combined with simultaneous multi-wavelength imaging of voltage sensitive dyes and hemodynamic signals. **Neurophotonics** 1;1:015003 PMID: 25525611 5) Baird-Daniel E, Daniel AGS, Wenzel M, Li D, Liou J-Y, Laffont P, Zhao M, Yuste R, Ma H, **Schwartz TH** (2017) Glial waves triggered by seizure activity are not essential for initiating ictal onset or neurovascular coupling. **Cerebral Cortex** 27;6:3318-3330. PMID 29369176

4. Pre-ictal hemodynamic changes. The ability to predict seizure onset has been a critical aspect of seizure research and particularly important for triggering therapy. Our lab was the first two show several different mechanisms whereby increases in focal blood perfusion and decreases in hemoglobin precede seizure onset by as long as 20 seconds in the human brain. Additionally, arteriolar vasoconstriction in surrounding cortex occurs roughly 5 seconds before seizure onset in animals indicating that distant cortical areas are involved in pre-ictal brain preparation either by shunting blood to imminently active areas or away from areas where inhibition occurs. 1) Zhao M, Suh M, Ma H, Perry C, Geneslaw A, Schwartz TH. (2007) Focal increases in perfusion and decreases in hemoglobin oxygenation precede seizure onset in spontaneous human epilepsy. **Epilepsia**;48;11:2059-2067. PMID 1766607 2) Schwartz TH**,** Hong SB, Bagshaw AP, Chauvel P, Benar C.G. (2011) Preictal changes in cerebral haemodynamics: review of findings and insights from intracerebral EEG. **Epilepsy Res** 97(3): 252-66 PMID 21855297 3) Zhao M, Nguyen J, Ma, H, Mozomi N, Schaffer CB, Schwartz TH. (2011) Pre-ictal and ictal neurovascular and metabolic coupling surrounding an ictal focus. **J Neurosci** 31;37:13292-300. PMID 21917812.
5. Effects of seizures on cortical processing. The impact of an epileptic focus on normal cortical architecture is often profound both during and in between seizures. We have shown that seizures can not only be triggered by sensory stimulation but alter the receptive fields of the involved neurons. Moreover, these changes occur not only ipsilateral to the seizure focus but contralateral as well. 1) Schwartz TH (2003) Optical imaging of epileptiform events in visual cortex in response to patterned photic stimulation. **Cerebral Cortex**; 13;12:1287-1928 PMID: 14615295. 2) Schwartz TH**,** Chen, L-M, Friedman RM, Spencer DD, Roe AW. (2004) Intraoperative optical imaging of face topography in human somatosensory cortex. **Neuroreport**;15;1527-1532. 3) Harris S, Bruyns-Haylett M, Kennerley A, Boorman L, Overton P, Ma H, Zhao M, Schwartz TH, Berwick J (2013). The effects of focal epileptic activity on regional sensory-evoked neurovascular coupling and post-ictal modulation of bilateral sensory processing. **J Cereb Blood Flow Metab** 33;10:1595-604 PMID 23860375 4) Harris S, Boorman L, Bruyns-Haylett M, Kennerley A, Overton PG, Ma H, Zhao M, Schwartz TH**,** Berwick J. (2014) Contralateral dissociation between neural activity and cerebral blood volume during recurrent focal neocortical seizures. **Epilepsia** 55;9:1423-30 PMID: 25053117. 5) Harris SS, Boorman LW, Das D, Kennerley AJ, Sharp PS, Martin C, Redgrave P, **Schwartz TH**, Berwick J. (2019) Physiological and pathological brain activation in the anesthetized rat produces hemodynamic-dependent cortical temperature increases that can confound BOLD fMRI signal. **Frontiers in Neuroscience** 12;550: PMID: 30154690

MyBibliography.
http://www.ncbi.nlm.nih.gov/sites/myncbi/1paGsdBadpOQO/bibliograpahy/47946334/public/?sort=date&direction=ascending

Chris B. Schaffer

Current Support

D. Research Support

Ongoing Research Support

R01 AG049952-01 Schaffer (PI) 05/15/2015 – 04/30/2020
National Institute on Aging (NIA)
Stalled capillary flow: A novel mechanism for hypoperfusion in Alzheimer's disease
This proposal tests the idea that reactive oxygen species produced in the Alzheimer brain leads to upregulation of cell adhesion markers in the endothelium, causing leukocyte adhesion in a subset of brain capillaries, thereby causing a global decrease in blood flow.
Role: PI

R21 NS096669 Schaffer (PI) 10/01/2016 – 09/30/2018
National Institutes of Health
Three Photon Imaging Of Neural Activity In The Spinal Cord Of Awake Mice
Neurons in the spinal cord form circuits that control rhythmic motions such as walking and running. Despite the importance of understanding how these circuits work and how they fail after spinal cord injury or in diseases such as amyotrophic lateral sclerosis, there is no existing approach to directly measure the patterns of activity across a large ensemble of spinal cord neurons in awake, moving animals.
Role: PI

DOH01-C32094GG Schaffer (PI) 01/01/2017 – 12/31/2018
New York State Department of Health

Imaging neural activity in the spinal cord of awake mice after spinal cord injury

This project will establish the capability to image patterns of neural activity in the spinal cord of awake, behaving mice with and without spinal cord injury and demonstrate the utility of this approach in a study of the motion-correlated activity patterns of one class of spinal cord neurons.

Role: PI

A2017488S Schaffer (PI) 07/01/2017 – 06/30/2019
Brightfocus Foundation
Blocking neutrophil adhesion to improve brain blood flow in Alzheimer's disease
We propose to screen drugs that interfere with leukoctye adhesion and that are proven to be safe in humans to find compounds that block the capillary stalling phenomena we found to be responsible for reduced brain blood flow and some cognitive dysfunction in AD mouse models.
Role: PI

1707312 Xu (PI) 09/01/2017 – 08/31/2022
National Science Foundation
NeuroNex Technology Hub: Optical technologies for large scale, noninvasive recording of neural activity
This center grant focuses on developing advanced optical imaging techniques, including three-photon excited fluorescence microscopy, to image patterns of neural activity with single-cell resolution and across large areas of the central nervous system in multiple animal models during complex behavioral tasks.
Role: Co-PI

R01 EB002019 Wise (PI) 09/01/2016 – 08/31/2020
National Institutes of Health

Laser and microscope development for multicolor nonlinear imaging deep in tissue

The major goals are the development of practical sources of high-energy ultrashort light pulses, along with novel microscope designs that will dramatically increase penetration depth and information density, which will enable novel studies of health and disease in animal models.

Role: Co-I

Theodore Schwartz
Current Support

Research support

Research Support in last 3 years
Ongoing
None

Completed (in last 3 years)

2015-16 **Daedalus Fund** (PI) (0% salary)
Femtosecond laser-produced subsurface cuts to halt focal epileptic seizures Use of a 2-photon laser to transect the cortex in rats to stop seizure initiation and propagation

2015-16 **Epilepsy Research UK** (Co-I) (0% salary)
How activation of sensory regions can promote propagation of adjacent focal neocortical seizures

2013-16 **NSF CBET**-1264928 Co-I (2%)
Collaborative research in biophotonics: Implantable sensor Develop an implantable sensory for optical mapping of cerebral hemodynamics

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 0468381840000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: OPEN SOURCE INSTRUMENTS, INC.

Start Date*: 09-09-2019 **End Date*:** 08-30-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Kevan		Hashemi		PD/PI		12.0			70,000.00	28,000.00	98,000.00
2 .		Michael		London		Engineer		4.0			16,000.00	1,224.00	17,224.00
3 .	Dr.	James		Bensinger		Physicist and Project Manager		1.0			4,000.00	306.00	4,306.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **119,530.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	3.0			6,000.00	459.00	6,459.00
1	**Total Number Other Personnel**					**Total Other Personnel**	**6,459.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**125,989.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-871 . Received Date: 2019-04-05T17:35:27.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 0468381840000

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: OPEN SOURCE INSTRUMENTS, INC.

Start Date*: 09-09-2019 **End Date*:** 08-30-2020 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	980.00
2. Foreign Travel Costs	
Total Travel Cost	**980.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 0468381840000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: OPEN SOURCE INSTRUMENTS, INC.

Start Date*: 09-09-2019 **End Date*:** 08-30-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,500.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	61,212.00
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**100,712.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**227,681.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Rent			5,400.00
2 . General Supplies			180.00
3 . Accountant			4,000.00
4 . Workers Compensation Insurance			500.00
		Total Indirect Costs	**10,080.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**237,761.00**

J. Fee	Funds Requested ($)*
	8,000.00

K. Total Costs and Fee	Funds Requested ($)*
	245,761.00

L. Budget Justification*	File Name: Budget_Justification_v1.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification

A. Senior/Key Personnel
Salaries and wages are based on standard rates/salaries which are comparable to others doing similar effort.

Fringe benefit rate of 40% is requested for employees whose primary role will be with Open Source Instruments. For other contributing personnel we request a fringe benefit rate of 7.65% for FICA.

B. Other Personnel
Salaries and wages are based on standard rates/salaries which are comparable to others doing similar effort.

Funds are requested to provide salary support for the technician and treasurer for 6 months time, or half their duties during the period of the grant.

A fringe benefit rate of 7.65% is requested to cover FICA.

D. Travel
The budget accounts for two trips to Ithaca, New York for the PI to meet with Animal Trial Director. The trip is 640 miles round trip at $0.54 per mile = $364. Added to the cost of driving is a per diem of $126 per day for two trips. $490 x 2 = $980

F. Other Direct Costs
Materials and Supplies

Assembled circuit boards $4,000 x 3 runs = $16,000
Springs for flexible leads $2,000 x 2 batches = $4,000
Optical fiber $2,000 x 2 orders = $4,000
LED die chip minimum orders $1,000 x 3 = $3,000
Eutectic mounting of LED die chips $3,000 x 3 = $9,000
Encapsulation tooling $3,500

H. Indirect Costs
Rent of office space is $1,800 per month. This project would be a third of the effort of the organization so rent for this project would be $600 per month for the 9 months of the project.

General Supplies include: paper, ink cartridges, syringes for manufacture, epoxy for coating, silicone, solder iron tips, and other items used daily for design and manufacture in our facility.

Accountant and annual tax audit costs $12,000. A third of the audit will be accounting for funds from this grant, if awarded.

Operational office fees such as Nitrogen tanks and Internet at $60 per month. This project would be a third of the effort of the organization so $20 per month for 9 months of the project.

Insurance annually is $1,500 for Open Source Instruments. This project would be a third of the organization's effort so the charge to this project would be $500.

J. Fee
A fee to Open Source Instruments of not more than 7% of combined direct and in-direct costs is requested. $8,000

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		119,530.00
Section B, Other Personnel		6,459.00
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		125,989.00
Section C, Equipment		0.00
Section D, Travel		980.00
1. Domestic	980.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		100,712.00
1. Materials and Supplies	39,500.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	61,212.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		227,681.00
Section H, Indirect Costs		10,080.00
Section I, Total Direct and Indirect Costs (G + H)		237,761.00
Section J, Fee		8,000.00
Section K, Total Costs and Fee (I + J)		245,761.00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 8726124450000

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Cornell University

Start Date*: 09-01-2019 **End Date*:** 08-30-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Theodore		Schwatz		Consultant Neuroscientist			0.09		0.00	0.00	0.00
2 .	Dr.	Chris		Schaffer		Biomedical Engineer	142,200.00		0.09		1,422.00	508.00	1,930.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **1,930.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6.0			18,147.00	0.00	18,147.00
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	**18,147.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**20,077.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-871 . Received Date: 2019-04-05T17:35:27.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 8726124450000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Cornell University

Start Date*: 09-01-2019 **End Date*:** 08-30-2020 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12832361 Funding Opportunity Number: PA-18-871 . Received Date: 2019-04-05T17:35:27.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 8726124450000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Cornell University

Start Date*: 09-01-2019 **End Date*:** 08-30-2020 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	7,368.00
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Animal Costs	5,700.00
9 . GRA Tuition	5,297.00
10 . Mandatory Health Fee	1,558.00
Total Other Direct Costs	**19,923.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**40,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Facilities & Administration Costs - Endowed Research	64.0	33,145.00	21,212.00
		Total Indirect Costs	**21,212.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**61,212.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	61,212.00

L. Budget Justification*	File Name: 89743_Schaffer-Resub_BudgetJustification_v1.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification – Cornell University

A. Senior/Key Person

Subcontract Principal Investigator, Prof. Chris Schaffer, Ph.D. (9-month appt.): This proposal requests salary support for 0.09 month academic year effort each budget year for Dr. Chris Schaffer. Dr. Schaffer will oversee the subcontract work. He will also guide the graduate research assistant in the planning and execution of experiments, in the analysis of data, and in the preparation of manuscripts and conference presentations for dissemination of the work.

B. Other Personnel

Graduate Research Assistant, To Be Named (12-month appt.): This proposal requests salary support for 6 calendar months of effort for a basic GRA appointment for 1 graduate students in each budget year. The salary support includes stipend each period. Stipends are budgeted with a 3% increase at the beginning of each academic year (August 16th). The GRA will complete the research as outlined in the Cornell statement of work under the supervision of Dr. Schaffer. GRA total support is calculated at the NIH cap.

GRA Stipend Support	Period 1	Total
GRA AY Stipend	$ 13,610	$ 13,610
GRA Summer Stipend	$ 4,537	$ 4,537
Total GRA Stipend	**$ 18,147**	**$ 18,147**

All Cornell University non-student salaries are budgeted with an increase in July of each budget period in accordance with Cornell University policy with the exception of personnel with salaries above the current NIH salary cap. Salaries are based on current FY 18/19.

Consistent with federal cost principles, Cornell University estimates personnel time on a percentage of total effort. Cornell University does not track work hours for FLSA (Fair Labor Standard Act) exempt staff, and is unable to provide billing or time records based on hours. In accordance with OMB 2 CFR Part 200 §430(i), Cornell allocates a level of effort utilizing a Plan Confirmation System. The percentage of effort has been converted into months of effort in this budget proposal.

Employee Benefits

Employee Benefits have been proposed at a rate of 34.9% for all non-student compensation through June 30, 2019, 35.3% from July 1, 2019 through June 30, 2020 and 35.7% effective July 1, 2020 for Cornell's endowed colleges. These rates are approved by the Department of Health and Human Services. See *https://www.dfa.cornell.edu/capitalassets/cost/employee* for more information about Employee benefit rates.

Materials and Supplies

Funds are requested in each budget year for purchase of materials and supplies necessary to complete the proposed project. These include, but are not limited to, disposable surgical supplies and drugs for inducing seizures and implanting the EEG/optogenetic devices in mice as well as miscellaneous laboratory needs including personal protective equipment and general use reagents and labware.

Other Direct Costs

Graduate Research Assistant, To Be Named (12-month appt.): This proposal requests support for 6 calendar months of effort for a basic GRA appointment for 1 graduate students in each budget year. The support includes tuition and mandatory health insurance fees each period. The support includes tuition and mandatory health insurance fees each period. Health insurance fees are budgeted with a 10% increase effective August 1st of each year, and tuition is budgeted with no projected increases. The GRA will complete the research as outlined in the Cornell statement of work under the supervision of Dr. Schaffer. GRA total support is calculated at the NIH cap.

GRA Other Direct Costs	Period 1	Total
GRA AY Tuition	$ 5,297	$ 5,297
GRA Health Insurance Fee	$ 1,558	$ 1,558
Total GRA ODC's	**$ 6,855**	**$ 6,855**

Animal Costs: Funds are requested for animal costs necessary to complete the proposed research. These include, but are not limited to, the purchase, breeding, genotyping, housing, and veterinary care of the mice needed for the proposed research. Housing costs are $0.72/cage/day. Cornell staff bill hourly for husbandry services. Genotyping is conducted by an external contractor.

Facilities and Administrative Costs (F&A)
F&A costs have been proposed at a rate of 64% for Endowed Research effective July 1, 2018. The rate is approved by the Department of Health and Human Services. See *http://www.dfa.cornell.edu/sites/default/files/dhhsrateagreement.pdf.*
Modified Total Direct Cost exclusions include Capital Equipment, GRA Tuition and Health Fees, and Subcontract costs in excess of $25,000 per subcontract.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		1,930.00
Section B, Other Personnel		18,147.00
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		20,077.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		19,923.00
1. Materials and Supplies	7,368.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	5,700.00	
9. Other 2	5,297.00	
10. Other 3	1,558.00	
Section G, Direct Costs (A thru F)		40,000.00
Section H, Indirect Costs		21,212.00
Section I, Total Direct and Indirect Costs (G + H)		61,212.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		61,212.00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	206,469	0	0	0	0	**206,469**

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

Agency to which you are applying (select only one)*

❍ DOE ❍ HHS ❍ USDA ● Other: BRAIN NIH

SBC Control ID:* 000814955

Program Type (select only one)*

● SBIR ❍ STTR

❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

Application Type (select only one)*

● Phase I ❍ Phase II ❍ Fast-Track ❍ Direct Phase II ❍ Phase IIA ❍ Phase IIB

❍ Commercialization Readiness Program (See agency-specific instructions to determine application type participation.)

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?*	● Yes	❍ No
1b. Anticipated Number of personnel to be employed at your organization at the time of award.*	4	
1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?*	❍ Yes	● No
1d. Is your small business a Faculty or Student-Owned entity?*	❍ Yes	● No

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ❍ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and email address of the official signing for the applicant organization to state-level economic development organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ❍ No

7.Commercialization Plan: The following applications require a Commercialization Plan: Phase I (DOE only), Phase II (all agencies), Phase I/II Fast-Track (all agencies). Include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ❍ Yes ● No

Attach File:*

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ● Yes ❍ No

STTR-Specific Questions:

Questions 10 - 12 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 - 12 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ❍ Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ❍ Yes ❍ No

12. Provide DUNS Number of non-profit research partner for STTR.*

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

Tracking Number: GRANT12832361

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3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

☑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name: Michael

Middle Name: London

*Last Name: Collins

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12832361 Funding Opportunity Number: PA-18-871 . Received Date: 2019-04-05T17:35:27.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	Introduction-20190405-1611.pdf
Research Plan Section	
2. Specific Aims	Specific_Aims-20180905-0725.pdf
3. Research Strategy*	Research_Strategy_20190405-1556.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	Vertebrate_Animals_cs2.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	Consortium_Arrangement_OSI_Cornell.pdf
9. Letters of Support	Support_All_040519.pdf
10. Resource Sharing Plan(s)	
11. Authentication of Key Biological and/or Chemical Resources	
Appendix	
12. Appendix	

Tracking Number: GRANT12832361
Funding Opportunity Number: PA-18-871. Received Date: 2019-04-05T17:35:27.000-04:00

Introduction This resubmission of a Phase I application proposes a neurosurgical device capable of real-time electrophysiological event detection and closed-loop optogenetic response. As part of the Phase I award, OSI will test the device's ability to terminate epileptic seizures in transgenic mice. Reviewers of the original submission stated that the proposal **"has solid scientific premise"** and noted that enabling a new class of neuroscience experiments will give OSI **"entry to a potentially large market."** We welcome this opportunity to answer reviewer's questions. Since the device is **"clearly informed by the needs of researchers in the field of epilepsy",** reviewers wonder **"whether such a product would find more general application"**. Yes, OSI will pursue the larger market opportunity beyond epilepsy. The Research Strategy (RS) expands upon how functional optogenetic intervention may be used to treat disorder states such as Alzheimer's, OCD, schizophrenia, and chronic pain. As an example of wider market demand, this resubmission includes a letter from Dr. Maria Fitzgerald, a global leader in the biology of pain. She describes how the proposed device may be used to treat the chronic or intractable pain associated with spinal cord injuries and/or neuropathy and highlights the relevance to the nations' opioid crisis. This resubmission also includes a letter of support from Dr. Bauer describing how increasing regulatory limits on tethered neuroscience instruments is creating a market opportunity for the proposed device. To support efforts outside of the field of epilepsy, OSI agrees that **"other embedded software (e.g., to detect different sleep states) might offer a wider potential market"**. Offering customized software is an integral part of OSI's business model. OSI's EEG classification software can be used to detect customer-specific EEG states and LFP patterns other than epileptic seizures. As an example, Alzheimer's researchers used OSI's software to identify interictal spikes in the J20 mouse model of Alzheimer's disease. Processing 3500 hours of data from J20 and wild-type mice, the software had a 0.21% false positive rate (1.9% false positive risk)[6,64]. The proposed device is a base product upon which OSI will offer embedded software that is inexpensively tailored to the needs of labs studying LFP patterns associated with Alzheimer's, sleep, etc. **"Lack of details on the computationally efficient EEG event classification algorithm and its performance metrics such as specificity, sensitivity and false alarm rate has diminished the enthusiasm for the project."** The efficacy of OSI's event classifier is documented in numerous published studies, each of which relied upon fully-automated event-counting applied to tens of thousands of hours of EEG[6, 10, 11, 24, 26, 30, 50, 51, 59, 60]. Tables of specificity, sensitivity, and false alarm are available in the OSI documentation [63]. **"Early seizure onset detection is a challenging task. It is not clear if the investigators already have the algorithm."** In recent work on early detection of kainic acid seizures in mice[65], OSI classified 190,000 one-second EEG intervals as ictal or non-ictal. The classifier's detection threshold was chosen to ensure that no seizures were missed (sensitivity > 0.99). 2.96% of the 190,000 intervals were classified as ictal; the false positive rate = 0.0086; and the false positive risk = 0.29. Therefore, OSI's software can detect a seizure within 1 second of onset with sensitivity > 0.99 and specificity = 0.9914. Although most applications would employ a longer detection window than a single second to increase the specificity, the one second window discussed here is appropriate for this proposal's *in vivo* test. **"The PI does not appear to have significant prior education or experience in EEG analysis."** The budget has been adjusted to allow Kevan Hashemi to serve as the resubmission's PI. Mr. Hashemi was previously identified as **"Co-PI…non-PhD, has only one publication"**. The NIH has no requirement that the PI hold a PhD. The resubmission updates Mr. Hashemi's biosketch to include his 24 published works, including 4 journal articles on automated EEG analysis. **"No biosketch was provided for Professor Nishimura and Professor Schaffer does not seem to have much experience with epilepsy research."** The Facilities section elaborates upon the close collaboration between Professor Nishimura, Professor Schaffer, and Professor Schwartz. Dr. Schwartz is the director of Cornell's Epilepsy Research Laboratory at the Weill Cornell Brain and Spine Center. This resubmission formally includes Dr. Schwartz as a consultant and provides his biosketch. We apologize for inadvertently truncating the author list on the recent epilepsy-related paper in Prof. Schaffer's Biosketch. In fact, he is a co-corresponding author (with Prof. Schwartz) on one epilepsy-related paper that appeared in *Cerebral Cortex*, with the majority of the experimental work in that paper occurring in his lab in Ithaca. He has also published two additional papers with Prof. Schwartz, one related to epilepsy that appeared in the *Journal of Neuroscience.* **"Exactly what is expected from the animal experiments could have been described better."** This section has been rewritten for clarity. The experiment will use an iron chloride epilepsy model to induce seizures in mice. This model induces 25 seizures per hour, permitting each instrument to observe 9,000 seizures over a period of 14 weeks while recording for 4 hours per day. All EEG data will be recorded. Embedded EEG classification onboard the instrument will determine the onset of a seizure within one second with expected sensitivity > 0.99 and specificity = 0.9914. The instrument will apply optogenetic correction to a randomly selected portion of the seizures. With the large volume of data collected, the researchers expect to observe a statistical decrease in the duration or intensity of seizures when the optogenetic correction is applied. **"The number of mice and their genders needed for a reliable result was not detailed."** The RS and Vertebrate Animals sections now include these details, including explicit consideration of sex as a biological variable. **"The new size and design aspects of the device has not been discussed"**. The instrument is implanted in the abdomen and will have a total volume of 0.9mL and max dimensions of 26 x 13 x 3.5 mm, a form factor successful for implantations in mice for experiments lasting several months. Subcutaneous leads with a diameter of 0.5 mm connect the instrument to the head fixture containing the LED-coupled fiber. The head fixture has dimensions 3 x 3 x 2 mm and is mounted to the skull with dental cement. A 3 mm long optical fiber taper with a 220 m diameter base protrudes from the head fixture into the brain. The RS now includes a visual prototype of the proposed device.

Specific Aims: Optogenetics is a research field that utilizes light to activate transgenically or virally transduced photosensitive protein channels to excite or inhibit neurons[13,19,20,52,57]. Researchers can not only target specific regions of the brain through focused illumination but they can also genetically target specific groups of neurons within that region by utilizing promotor driven cell-specific expression of photosensitive channels. The high specificity offered by optogenetics creates many *in vivo* research opportunities to study psychiatric and neurological conditions. Conditions including epilepsy, Alzheimer's, OCD, and schizophrenia have characteristic abnormal EEG patterns[5,6,16,29,45,57]. To study these conditions and find treatments, researchers utilize **mouse models to genetically and spatially target neuron groups for activation and inhibition while monitoring electrophysiology in one or more brain regions**. At the moment, these experiments are very difficult to perform due to the lack of a device that can perform wireless EEG monitoring and optical stimulation in mice. While tethered equipment has been sufficient to show the promise of optogenetics, it has severe practical shortcomings including short testing times and prohibiting cohabition which limit experiment design[32]. Therefore, **neuroscientists in several research areas have asked us for a wireless instrument capable of EEG recording and optical stimulation in mice.** We propose an instrument that not only delivers this capability, but that also paves the way for **optogenetics as a functional neurosurgical intervention**. The instrument will be able to detect aberrant EEG patterns and then deliver optogenetic stimulation per closed-loop feedback. This will allow our customers to deliver functional intervention in neural circuits exhibiting a disease state while having little or no impact on healthy brain function. We will test the intervention hypothesis on a mouse model of focal epilepsy. This will advance our long-term goal of creating **a fully implantable medical device that detects the onset of seizures and aborts them with optical stimulation** akin to how implantable defibrillators detect and abort arrhythmias.

Aim 1: Build a fully implantable EEG monitor with optical stimulator that is suitable for use with transgenic mice. It will be called the Mouse-Sized Implantable Stimulator with Lamp (MS-ISL). OSI has already developed each of the core building blocks necessary for the instrument. Our main product is a fully implantable instrument that transmits high-fidelity EEG data in mice or rats for over four months at a time[26]. We've also developed and tested an optogenetic stimulator with EEG recording that was proven capable of inducing optogenetic effects in rats. This 4.2 mL product was built as a proof-of-concept; It has very limited research utility on its own due to the difficulty of designing experiments for rats compared to mice. To access the enormous pool of transgenic mouse strains that are validated as disease models and that are sensitive to optogenetic stimulation, our customers request that we provide an optogenetic stimulation device compatible with mice. We will design an instrument that has a volume of 1.5 mL that is capable of recording high-fidelity EEG and applying optogenetic stimulation upon receipt of wireless, individually addressed commands. This will give researchers access to the diverse pool of transgenic mice.

Aim 2: Embed OSI's proven PC-based EEG event detection software in a 2.5 x 2.5 mm logic chip, enabling completely autonomous, closed-loop response. The MS-ISL will wirelessly transmit EEG data to our existing PC-based data acquisition system in real-time. The PC-based software detects events and commands optical stimulation[60,61]. To move toward a self-contained medical device, we will embed our computationally efficient detection algorithm directly in the MS-ISL itself, permitting it to be used without the PC. We will take 200 hours of archival EEG data recorded by our customers and play it into the logic chip. We will show that it can calculate each classification metric and autonomously classify events including seizures, ictal pulses, and post-ictal depression in real-time. It can respond with stimulation or simply report the events.

Aim 3: Demonstrate the MS-ISL's ability to detect seizures and interfere with them by applying closed-loop optogenetic stimulation. Our goal is to test the utility of the MS-ISL for monitoring brain activity, identifying aberrant activity patterns, and modulating the firing of genetically-targeted neurons through optogenetic stimulation. We will demonstrate this in a mouse model of focal epilepsy, which provides an ideal testbed because the appropriate site for electrophysiological recording and optogenetic activation is clear. The subcutaneously-implanted, wireless MS-ISL will monitor cortical EEG at an epileptic focus, identify seizure initiation, and turn on the fiber-coupled LED to optogenetically stimulate neurons to interrupt the seizure. We will test this scheme both by exciting inhibitory interneurons using Channelrhodopsin-2 (Ch2) in one group of transgenic animals and by inhibiting excitatory pyramidal neurons using Halorhodopsin (HR) in another. We will induce chronic focal neocortical epilepsy by intracranial nanoinjection of iron chloride, which simulates the conditions of traumatic focal epilepsy induction and leads to the development of seizures over time. The MS-ISL will be implanted and left off for about two weeks until the epilepsy develops so that the mice are having about 25 seizures an hour. We will then begin recording sessions with half the time allocated to just recording brain activity and the other half with the optogenetic feedback turned on. We will demonstrate the effect of the MS-ISL's optogenetic feedback in a 14-week, chronic experiment. Our primary assay will be a statistical reduction in the duration of seizures when closed-loop optogenetic stimulation is applied.

1. Significance

We propose to build a fully implantable EEG monitor combined with an optogenetic stimulator. The instrument will both: **1.** Fulfill an immediate commercial need that advances basic neuroscience and disease research; and **2.** Advance our long-term objective of creating a medical device that uses closed-loop optogenetic response as functional intervention to treat disease states such as focal seizures.

1.1 An instrument that advances our understanding of the brain and human disease

In the last decade, optogenetics has grown from being the subject of just a dozen papers in 2009 to over 950 in 2017[38]. It evolved from being an experimental technique for activating neurons *in vitro* to being a means of studying neuropsychiatric diseases *in vivo* with mouse models of conditions including schizophrenia, epilepsy, and Parkinson's[13,19,20,52,57]. The promise of the technology comes from its ability to target highly specific neuron groups both spatially, temporally, and genetically. For example, researchers can design an experiment in which a mouse model expresses Halorhodopsin (HR) in cortical pyramidal neurons, and they can subsequently activate those neurons with pulses of light [21,43]. Many neuroscientists want to apply optogenetics to their own research topics. However, they are often unable to proceed due to a lack of suitable equipment. Instrumentation companies have failed to keep pace with the progress and imagination of neuroscientists. This has created a market opportunity.

Commercially available hardware typically requires that animals are tethered by their heads to benchtop equipment. This places severe constraints on experiment design, and adds a cost to obtaining usable data that is often prohibitive. For example, *i)* regulations limit the amount of time that a tether can be used each day; *ii)* tethers require frequent human intervention throughout the course of the experiment; *iii)* tethers are incompatible with experiment methodologies such as operant chambers and mazes; *iv)* animals cannot cohabit; *v)* animals cannot engage in natural behavior during recording (socialization, moving into enclosed spaces, using exercise wheels, etc); *vi)* externally protruding hardware is prone to animal self-mutilation[32]; and *vii)* the physical tether often introduces movement artifacts in data that increase the cost of data analysis. To make optogenetics more accessible, several groups have developed wireless optogenetic stimulators. However, they are all **fundamentally incompatible with electrophysiological recording**, limiting their use to behavioral experiments.

Consider the class of conditions in which abnormal, characteristic EEG patterns are present. These include epilepsy, Alzheimer's, OCD, and schizophrenia[5,6,16,29,45,57]. To study these conditions and their cures, researchers would like to **simultaneously monitor EEG and apply optogenetic stimulation**. For these experiments to be practical, researchers need a wireless, fully implantable instrument capable of both EEG recording and optical stimulation. Open Source Instruments Inc (OSI) is capable of designing this instrument and subsequently making it available for sale alongside its existing EEG telemetry products. We will call the instrument the Mouse-Sized Implantable Stimulator with Lamp (MS-ISL).

> "I confirm that my laboratory would be keen to purchase the proposed Mouse-Sized Implantable Sensor with Lamp (MS-ISL) for implantation in mice. … The proposed development work represents important steps both towards clinical translation and towards testing circuit theories of brain function. I foresee many additional applications by our group and others." - *Dr. Dimitri Kullmann, Professor of Neurology, University College London (U.K.)*
>
> "Therefore, I confirm that my lab would use the proposed mouse-sized Implantable Stimulator and Monitors with fiber-coupled LEDs. The development of the new technologies described by Kevan Hashemi in this proposal is, in my opinion, a fundamental step towards making discoveries that contribute to ameliorate the burden of mental health disease through the world." - *Dr. Analisa Scimemi, Assistant Professor, University at Albany (New York)*

The proposed device will allow researchers to test for abnormal brain activity and modify it at the same time, leading to a better understanding of diseases like Parkinson's disease, dementia, Alzheimer's disease, depression, epilepsy and more. As an example, consider the recent advances in Parkinson's disease showing that there is extra activity within the beta band of electrorheological records which can be picked up in both the motor cortex and the subthalamic nucleus of the basal ganglia[66]. The proposed device could be used to inhibit subthalamic neurons by optogenetic suppression to block the rhythmic synchronous activity associated with the beta burst. Simultaneously, the device would record in the motor cortex to determine if the optogenetic stimulus is able to relieve the synchronous beta burst that is correlated with Parkinson's disease and motor abnormalities. The real time feedback of the proposed device could allow researchers to make corrections of abnormal activity such as increased activity in the bata band and determine the effect on disease states like Parkinson's. The proposed device could be used to study sleep disorders and memory as well. Recent studies show that sharp waves or ripples in the hippocampus during sleep are correlated with consolidation of spatial memory[67,68,69]. The proposed device would help researchers determine how hippocampus activity and memory consolidation are linked. For example, researchers could perform EEG recording in regions where memory consolidation takes place while optogenetically driving or inhibiting CA1 hippocampus neurons. Another use of the MS-ISL will be developing optogenetic pain treatments that may alleviate the opioid crisis.

1.2 A medical device that uses optogenetics as functional neurosurgical intervention

The MS-ISL will not only create the basic research opportunities described above, but will be immediately used as a means of functional intervention to treat disease states in the brain. Our long term objective is to provide medical devices that monitor EEG activity and automatically respond to aberrant events by applying

correcting pulses of optogenetic stimulation. The specificity of stimulation means that disease states can be treated with minimal impact on healthy brain function[3,4,44,51]. While many disorders may be treatable with this approach, we will start with focal seizures for the reasons described below.

Several groups, including one of our customers, have shown that seizures can be halted or reduced by the optogenetic activation of inhibitory neurons with the use of Chennelrhodopsin-2 (Ch2)[12,28,43,55,60]. It has also been shown that expressing Halorhodopsin (HR) in cortical pyramidal neurons can reduce seizure propagation[28,31]. We will test the ability of the MS-ISL to interfere with seizures via both the Ch2 and HR approaches at the Schaffer Lab at Cornell. Focal seizures initiate in one location and propagate out to other regions in the brain. This means that it is obvious which region of the brain we must target for both EEG monitoring and optical stimulation. Furthermore, we have analyzed the EEG signals of focal seizures and have already demonstrated the ability of our PC-based software to automatically identify them in real time[24,60].

We will work with focal epilepsy models not just because it is an ideal testbed for the technology, but because it could have a direct impact on human health. Localization-related epilepsy is the most common seizure disorder, affecting approximately one half of the 50 - 60 million people who suffer from epilepsy[7,17,48]. Approximately 45% of these patients experience medically untreatable focal seizures that are physically disabling[15]. In the future, we could evolve the MS-ISL into a a **fully implantable medical device for humans that detects the onset of seizures and aborts them with optical stimulation** akin to how implantable defibrillators detect and treat arrhythmias.

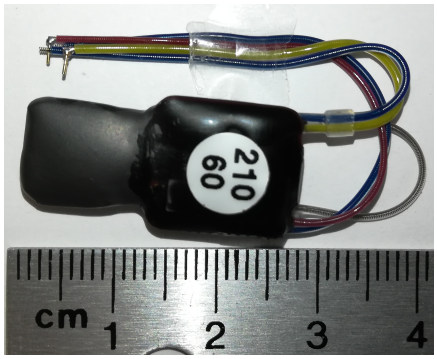

Figure 1a: A visual prototype of the proposed MS-ISL. Total volume is 0.9 mL. The MS-ISL is implanted in the abdomen where it doesn't interfere with animal behavior. Electronics and battery are enclosed in the black epoxy-silicone package. The silver loops are antennas for receiving commands and transmitting live data. The colored leads record EEG signals and drive the LED.

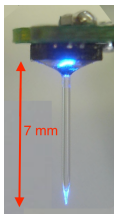

Figure 1b: The Fiber Coupled LED (FCL) is the satellite head fixture that will be driven by the MS-ISL. It is mounted to a hole in the skull with dental cement. Blue light is visible at the LED surface and at the coupled fiber's tapered tip. The FCL pictured is a functional prototype which must be miniaturized to 3 x 3 mm for use with the MS-ISL.

2. Innovation

2.1 Biometric Instruments at Open Source Instruments, Inc.

Open Source Instruments Inc. (OSI) was founded in 2004 to design equipment for scientific research[35]. OSI appeals to scientific customers by providing complete characterization of all of its instruments and software. Soon after its founding, OSI entered a collaboration with Dr. Matthew Walker, Institute of Neurology (ION), University College London (UCL), to develop an implantable, wireless EEG monitor for epilepsy studies in rats. After five years of collaboration, OSI demonstrated an effective and reliable wireless EEG monitor[9,41]. OSI's fully implantable monitor produces recordings free of noise and artifact, which allows us to automatically detect EEG events such as seizures, ictal spikes, inter-ictal spikes, etc in our PC software. OSI telemetry products and software are now used in both mice and rats [6,9,10,11,18,24,26,30,50,51,59,60]. Our telemetry products have been profitable since 2009.

OSI's experience with EEG monitors and automatic seizure detection gives it two of the three core technologies necessary to build an optgoenetic stimulator with closed-loop response. The final ingredient is the ability to inject light into neural tissue. To develop a proof-of-concept, OSI added an optogenetic stimulus capability for its rat-sized EEG monitor. We developed this device in collaboration with Dr. Dimitri Kullmann (ION, UCL) and called it the Implantable Sensor with Lamp (ISL) [42] [Figure 1]. **The ISL is a proof of concept. It is a wireless, subcutaneous device implanted in a rat's abdomen. It contains a battery, antennas, and the electronics required for command reception, EEG recording, live EEG data transmission, and driving an LED for optogenetic stimulation.** The device does not use an external tether during any stage of deployment and there are no external components that can be scratched or chewed.

Subcutaneous leads connect the ISL to a satellite head fixture that we call the Fiber-Coupled LED (FCL). The FCL houses an LED coupled to an optical fiber that is tapered to a sharp point that minimizes the formation of scar tissue. The ISL has been implanted in rats and is proven to provoke an optogenetic response (behavioral changes and induction of seizure)[34]. Preliminary results indicate its ability to abort seizures [61]. While the ISL demonstrated the principle of an optogenetic medical device, its research utility is actually severely limited by the lack of transgenic rats that express opsins or which are validated as disease models. The proof-of-concept ISL cannot classify EEG events on its own, but relies upon processing in PC software. It is also a disposable device, usable for only a single experiment. The proposed MS-ISL will be re-chargeable and will be capable of autonomous, closed-loop EEG event classification.

2.2 The Need for a Mouse-sized Device

When rats are used in optogenetic experiments, a viral vector must be used to express the photosensitive protein that controls ion channels. This method is unreliable, imprecise, and requires expertise not available to many laboratories. By contrast, a wide variety of transgenic mouse strains are readily available which express opsins in specific neuron subsets. Furthermore, many mouse strains are available as validated models of

"[The proposed project] would allow us to make use of the wide range of genetically modified mouse strains that allow optogenetic actuators (opsins) to be expressed selectively in different populations of neurons (either excitatory or inhibitory). There are also many strains that have been validated as models of epilepsy, schizophrenia and other neurological disorders, further underlining the need to perform these experiments in mice." - *Dr. Dimitri Kullmann, Professor of Neurology, University College London*

"Most optogenetics projects are conducted in mice (rather than rats) given the exclusive availability of a large variety of transgenic mice allowing the targeting of many specific cell types. So, an optogenetic stimulator suitable for implantation in mice is of great value to the wider research community!" - *Dr. Dennis Kätzel, University of Ulm*

human disease. **Mouse models offer far more opportunities for optogenetic experimentation than rats**. To be commercially viable and have a significant impact on neuroscience, we must take the technologies we demonstrated in the ISL, and implement them in a device that is compatible with mice. This is not an iteration, but **a redesign that will result in a commercial product that has 0.9 mL volume compared to the proof-of-concept ISL's 4.2 mL volume**, thus enabling a whole new class of experiments.

2.3 Closed-loop Autonomous Response

A major barrier to functional intervention devices is the ability to reliably detect subtle EEG abnormalities with a tiny, low-power device that may have to run for a decade on a single battery. The MS-ISL will the **the first implantable device in the world that is capable of autonomously classifying EEG events** to determine when intervention is necessary. We will use the computationally-efficient event classification algorithm that OSI developed and proved successful in its PC-based software, ECP19 [24,60]. We will implement the algorithm in a 2.5 x 2.5 mm logic chip optimized for EEG event classification. It will detect events such as seizures, ictal pulses, inter-ictal spikes, polyspikes, and post-ictal depression. The MS-ISL can be used *either* with the the PC-based ECP19 forming a part of the closed-loop *or* completely autonomously, with processing done onboard the MS-ISL.

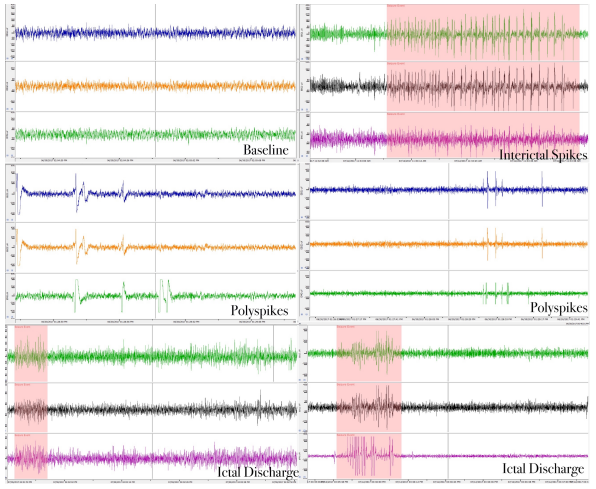

Figure 2. Nanoliters of iron chloride injected into the cortex leads to chronic focal neocortical epilepsy. These are EEG recordings from three implanted electrodes on the mouse's motor cortex. Seizure focus is induced at the placement of the second electrode by nanoinjection of iron chloride. Seizures then propagate to electrodes three (~1 mm away) and then finally electrode one (~2 mm away). Box shows multiple recordings of characteristic epileptiforms from local field potentials including polyspikes, ictal discharge, and interictal spikes.

2.4 A Focal Seizure Model to Test the MS-ISL

To demonstrate the MS-ISL's utility as an intervention device, we will use a recently developed model of traumatic injury and cerebral microhemorrhage using a nanojnjection of iron chloride. Cerebral microhemorrhages are common in both traumatic brain injuries and aging brains, especially ones with degenerative diseases. These small bleeds can lead to increased incidences of inflammation as well as an increase in the loss of the contents of plasma into the brain tissue. The increase in certain compounds like hemoglobin and iron within the brain tissue creates an accumulation of oxygen and reactive oxygen species (ROS). The increase in iron attracts more oxygen and ROS to the micohemorrhage and as well as the increase in extracellular plasma brings more glutamate to the site causing excitotoxicity. The increase in ROS can cause neural rewiring that induces a focus for seizure activity[49,56]. In order to simulate the environmental factors that occur during traumatic focal epilepsy induction, an iron injection is used to simulate the damaging effects of a microhemorrhage that lead to the creation of an epileptic focus[23,58]. This model has shown 70% viability to mice and shows that about 25 seizures an hour with a length of about 4 seconds per event[23]. This model is an ideal testbed for the MS-ISL, as it is clear that we would want to monitor EEG and modulate activity at the epileptic focus, and the high rate of events will provide ample data.

3. Approach

3.1 OSI's approach compared to existing technology

Several companies provide optogenetics technology which at first appears suitable for transgenic mouse experiments. To overcome the shortfalls of tethered equipment, groups have developed wireless instruments that can deliver optogenetic stimulation to mice [21,25,32,53]. The existing products are compared to our proposed instrument in Table 1. None of them are adequate for experiments requiring biopotential monitoring, as described below. In his letter of support, Dr. Alfredo Gonzalez recounts his attempts to use commercially available optogenetics instruments in his work with mice. He has found that no product currently on the market can meet the modest demands of his research.

None of the competing devices are compatible with electrophysiology recording. Our proposed instrument will be capable of EEG recording with performance very similar to that of our commercially available monitors (Figure 3). **Key specifications:** *i)* records at 512 samples per second with performance optimized for signals between 0 and 160 Hz; *ii)* total noise is 8 µV root mean square (rms); *iii)* 20 mV dynamic range; *iv)* no

artifacts due to instrument movement. For reference, baseline EEG levels are around 50μV rms and may exceed 1000μV rms during seizures (SNR ranges between 5 and 100). The digitized signal is transmitted using ultra low power 915 MHz telemetry which has no observable effect on the EEG measurement.

Some wireless optogenetic equipment relies on wireless power transfer to run the instrument because their electronics are not efficient enough to be powered by a battery[25,27,32]. The contact between brain tissue and an EEG electrode demodulates the wireless power oscillations and produces low-frequency artifacts in the EEG recording. **Wireless power systems are certain to irrevocably corrupt EEG signals in all cases.** In his letter of support, Dr. Gonzalez discusses how the Neurolux system that he purchased suffers from this issue and corrupts EEG recordings.

We believe that the only way to make a practical implantable optogenetic stimulator with monitoring capability is to provide power with a battery, as proven effective by our available products. Unlike other devices which rely on battery power, our circuits are ultra-efficient and capable of running an entire experiment without being recharged. **Our proposed device will be capable of 160 days of standby time, or over one week of continuous data recording and periodic optical stimulation [Table 2].** This compares to a standby time of less than 1 day for competitors' devices [Table 1]. The device will be surgically implanted in standby mode. Once the model has fully recovered and researchers are ready to begin collecting data, they send a command to switch the instrument on; it is only then that the device begins consuming significant power. Experiments which do not require continuous EEG monitoring can set the device to standby mode when not in use. For example, **if an experiment requires 4 hours of EEG monitoring per day, the device will run for 32 days**.

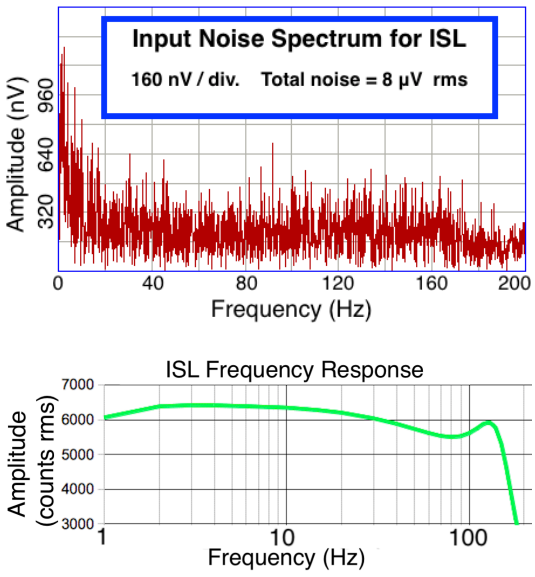

Figure 3 - Biopotential performance of the ISL The proposed instrument will have EEG recording characteristics very similar to the ISL. **Top:** Input noise spectrum for the ISL. **Bottom:** Frequency response of the ISL. A three pole filter is used to limit the contribution of signals above 160 Hz.

Another major advantage of our proposed device compared to others is the physical packaging. All of the mouse-sized optogenetic stimulators for sale today are mounted external to the skull of the animal, presenting several issues: *i)* external systems weigh approximately the same as the mouse's head, making them

Table 1: Survey of Current Wireless Optogenetic Devices

	OSI MS-ISL	Kendall [25]	Teleopto [53]	Riken [21]	Poon Lab [32]	NeuroLux [33]
EEG monitoring	Yes	No	No	No	No	No
Location	subcutaneous	head mounted	head mounted	head mounted	subcutaneous	subcutaneous
Volume	0.9mL	~3mL	1.6mL	~2mL	0.1 mL	0.1 mL
Power Source	Battery	RF	Battery	Battery	RF	RF
Standby Time	3800 hours	NA	17 hours	20 hours	Indefinite	Indefinite
Programmable	Yes	Yes	No	No	Yes	Yes
Individual Control	Yes	Yes	Yes	Yes	No	No
For Sale	Planned	No Longer	Yes	No	No	Yes
Can target deep brain	Yes	?	Yes	Yes	Yes	No
Consistent stimulus	Yes	?	Yes	Yes	No	No

unwearable for continuous use, *ii)* the large, externally protruding device inhibits normal behavior and social interactions with other mice, and *iii)* mice will injure themselves and each other by scratching and chewing on external devices[32]. Our instrument design avoids these shortcomings by using a fully implantable, two-part design [Figure 1]. **The benefits include animal cohabitation and improved welfare; the ability to record data 24 hours per day; the elimination of external components prone to scratching or chewing; and improved reliability.**

Our proposed instrument will be much more practical than any other on the market. **Once implanted, the device will require absolutely no physical interaction by the researcher.** It is completely controlled by wireless commands, and will easily endure over 100 days of implantation per charge.

3.2 Aim 1: Build a fully implantable EEG monitor with optical stimulator

We will use the existing ISL circuit as a starting point for the MS-ISL design. We will substitute all of the components in the current design with similar components in smaller packages. For example, the logic chip is 16 mm on each side, but can be substituted with a chip that is functionally identical, but only 2.5 mm on each side. We will further reduce the volume by designing a single dual purpose antenna that replaces the two antennas in our proof-of-concept, ISL. The total volume of the circuit board and battery will be 0.9 mL once assembled and coated.

Our proof-of-concept ISL is a disposable device designed for a single experiment. For the commercial MS-ISL proposed here, we will design recharging circuitry that allows the instrument to be reused. Researchers can return the MS-ISL after explantation for refurbishment and re-certification. **SBIR Phase I development will allow us to profitably sell the MS-ISL for $700 new and $350 refurbished** (including a new optical head fixture). The **fixed cost of all bench top equipment required to use the MS-ISL is $12,500 and supports up to 30 cohabiting mice**. The bench top equipment is already available for sale per our EEG telemetry product line.

Table 2: Calculated runtime for the MS-ISL per charge

Operation Mode	Current draw	Runtime
Standby Mode	5 µA	> 158 days
Optical stimulation for 30 minutes per day	28 µA	30 days
Epilepsy Experiment	25 µA	32 days

Runtime for the proposed device is estimated based on current consumption measurements taken in our lab.
Standby mode: the device is inactive but able to receive commands and start recording or optical stimulation
Optical Stimulation 30 minutes per day: the lamp is switched on for 2 ms pulses at 10 Hz repetition with 9 mW optical power at the fiber tip. This intensity and duty cycle has been shown to induce behavioral changes [61].
Epilepsy Experiment: Uses 4 hours of EEG recording per day and optogenetic stimulation in response to each of 25 seizures per hour (10 s stimulation per seizure)

One of the largest barriers to creating a mouse-sized optogenetic instrument is the challenge of delivering sufficient optical power to activate opsins without rapidly depleting the battery. Gathering light from an LED surface and injecting it into an optical fiber is inefficient when using commercially available LEDs and fibers. We have tested solutions to this problem by *1)* having custom optical fiber manufactured with a refractive index of 1.63; *2)* mounting the fiber to a custom wire-bonded bare LED die, and *3)* building a machine that tapers the optical fiber tip to maximize radiant flux and minimize the formation of opaque scar tissue. To fit the optical component into a mouse-sized device, we will use the Cree TR2227 LED die. Since this chip is just 240 x 320 µm across, we will need special procedures to handle it. We have consulted with a reliable supplier who is confident in their ability to mount TR2227 dies for us using their equipment. The TR2227 will be mounted on our substrate with a eutectic gold bond. We will re-tool our machine to craft 220 µm diameter fiber tapers. Optical fiber length may be specified by the customer. Customers who only need to illuminate the surface of the brain rather than targeting a deep region will be able to order the instrument without a coupled fiber and simply rely on the LED illuminating brain tissue through a section of thinned skull.

The MS-ISL will produce at least 4 mW of 460 nm light from its fiber tip at its default operating current of 40 mA. Even 2 mW is sufficient to activate channelrhodopsin-2 (Ch2) and halorhodopsin (HR) molecules in mammalian neurons [4]. These custom procedures will allow us to **deliver far more optical power into deep neural tissue than any other company for the same amount of electrical energy expended**. Customers can modulate the optical power by selecting different LED duty cycles. Customers can order either a blue version (~460 nm) for use with Ch2 or green version (~530 nm) for use with HR.

3.3 Aim 2: Embed OSI's proven PC-based EEG event detection software in a 2.5 x 2.5 mm logic chip, enabling completely autonomous, closed-loop response.

OSI provides Event Classification Processor software (ECP19) to automatically classify EEG events. ECP19 calculates 7 metrics on EEG data including power, coastline, intermittency, coherence, asymmetry, and spikiness. Using recordings that our customers have made with our instruments, we have developed an Event Library that includes baseline, ictal spikes, inter-ictal spikes, polyspikes, and various kinds of seizures. ECP19 automatically classifies data as belonging to the appropriate event. In addition to using the events defined by OSI, the software allows our customers to develop their own event definitions. ECP methodology was published in [60] and it has been used to replace or augment human classification in several published papers since then. ECP19 runs on a PC (Linux/Mac/Microsoft). It can be used in real-time or after an experiment to classify thousands of hours of data.

ECP19 can be used with the MS-ISL to provide closed-loop feedback. ECP19 will process MS-ISL EEG data in real time, and ECP19 will wirelessly command optogenetic stimulation in response to appropriate events. This has already been tested using the proof-of-concept device [61].

In order to provide functional intervention, a medical device will have to perform event classification autonomously. The challenge is that the processing could run down the device's battery quickly and make the device impractical. This is especially true as the device must be capable of distinguishing between subtle differences in EEG events. Fortunately, the algorithm underlying our ECP19 software is fundamentally computationally efficient. We will use the MS-ISL to prove that autonomous processing is practical.

We will build a microprocessor in the same programmable logic chip that handles EEG recording, telemetry, and command reception (LCMXO2-1200ZE). This chip is highly energy efficient. The microprocessor will be programmed in a custom version of Z80 assembler. It will be optimized for the fundamental mathematical operations that underly our calculation of EEG metrics. We can then program the MS-ISL to trigger on any particular event type that can be classified according to the underlying metrics. We will test the device in our lab by feeding it at least 200 hours of data recorded from at least four independent customers. We will compare its ability to calculate EEG metrics to the capability of the PC-based ECP19.

For the *in vivo* test in Aim 3, the PC-version of ECP19 will command optical stimulation. Independent of the closed-loop desktop processing, the MS-ISL will use telemetry to report when it independently detects seizures. We will compare the embedded MS-ISL software performance to the proven PC version.

3.4 Aim 3: Test the MS-ISL *in vivo.* We will interfere with focal seizures using the implantable EEG recording and optogenetic stimulation device.

Seizures are an abnormal increase in excitatory activity within the brain. Focal seizures initiate in one location and propagate out to other regions in the brain. These seizures can't be medically managed in 45% of human patients[7]. It has been previously shown that seizures have the ability to be halted or reduced by optogenetic activation of inhibitory neurons with the use of Channelrhodopsin-2[12,28,43,55,60]. It has also been shown that expressing Halorhodopsin (HR) in cortical pyramidal neurons can also reduce seizure propagation[28,31]. Therefore, we propose to utilize two different types of transgenic mice: one expressing Ch2 in cortical interneurons and one expressing HR in pyramidal cortical neurons. ChR2(H134R)-EGFP transgenic mice from Jackson Labs have a Cre-inducible Channelrhodopsin-2 (Ch2) knock-in and will be crossed with animals expressing a transgene for the tamoxifen-inducible Cre in cortical interneurons. This creates mice that have Ch2 expressed in cortical interneurons, so that when blue light (~460 nm) illuminates the tissue it will cause a conformational change in the channel allowing cations in to depolarize the membrane[2,19,54,62]. This activation of inhibitory neurons can directly inhibit cortical pyramidal neurons during seizures to reduce the excessive synchronous activation of the brain. Another subset of transgenic mice, Thy1-eNpHR2.0-EYFP, will be used in a similar fashion to express HR in cortical pyramidal neurons. These neurons when illuminated with yellow light (~600 nm) will induce a conformational change in a chloride channel causing the neurons to hyperpolarize and can reduce seizure activity[20]. The use of these transgenic mice in combination with the wireless ISL will allow for simultaneous monitoring of EEG activity in the brain and optogenetic activation of interneurons or inhibition of cortical pyramidal neurons through the FCL during seizure propagation. We will evaluate whether closed-loop stimulation shortens seizure length.

Methods: We have constructed a paradigm to investigate the ability of the ISL to monitor brain activity over time while optogentically minimizing seizure propagation. First, we will create a burr hole in the skull of our transgenic mice to induce chronic focal neocortical epilepsy and introduce the ISL. We will utilize a recently-published model of focal epilepsy in rodents that relies on the microinjection of an iron chloride solution into the cortex [23]. Briefly, we will inject 350 nL of 100 mM $FeCl_3$ at the center of the burr hole at a depth of ~500 µm beneath the cortical surface. In preliminary data, we have found that all iron-injected animals showed epileptic activity within a week or two, including polyspikes, interictal spikes, and full ictal discharges (Figure 2). We observed ~25 seizures per hour with this model. Immediately after iron chloride injection the FCL and EEG recording electrode will be inserted into the cortex at the injection site, and the ISL will be subcutaneously implanted. Previous recordings from the Schaffer lab will be used to determine parameters for seizure detection by the ISL. During testing, the device will record activity only for half of the session, then during the other half of the session the device will record and optogenetically stimulate whenever the ILS recognizes seizure-like activity. The light will flash for 10 s at 10 Hz to elicit optogenetic stimulation. Each group will have the device turned off for the first two weeks to allow time for the epilepsy model to develop. In this proposed study, we will break animals up into two groups based on the transgenic model. One group will include mice with Ch2 gene expressed in cortical interneurons and the other will express the HR gene in cortical pyramidal neurons. Each group will utilize the surgery described above. Both groups will have weekly four-hour recording session for 100 days with ten animals per group. We will have five female and five male animals per group to distinguish if there are any differences between sexes, but we do not expect any. These recordings sessions will determine the ability of the device to monitor brain activity, detect seizures, and optogenetically reduce seizure propagation over long periods of time. We will video record sessions to examine behavioral changes during seizures and optogenetic manipulation of brain activity. We will compare the frequency, amplitude, and duration of seizures, as well as behavioral correlates, with the optogenetic feedback on vs. off in both the Ch2 transgenic mice and HR transgenic mice.

Expected Outcomes: Chronic recording of animals with iron chloride induced focal neocortical epilepsy should correlate with previous data from the Schaffer lab and show frequent focal seizures. The MS-ISL will collects 56 hours of data over a 14 week period, thus capturing approximately 1,400 potential seizures. Optogenetic stimulation in half of these potential seizures should lead to a reduction in the frequency, amplitude, and duration of the seizures. Our primary assay will be the observation of a statistical shortening of seizure length in response to closed-loop optogenetic stimulation. Overall, the wireless system should show its ability to monitor brain activity while simultaneously detecting seizures and optogenetically minimize their activity.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	❍ Yes ● No

Other Requested information

USE OF VERTEBRAL ANIMAL SUBJECTS

Our studies of seizure propagation necessitate the use of laboratory animals. We take every effort to ensure safe and humane handling and treatment of our animals and extract the maximum amount of useful data from each animal we use.

Animals will be obtained commercially and housed at an animal care facility that is located at Weill Hall, down the hall from the Prof. Schaffer's laboratory. Animals used for chronic EEG recordings will be housed in the same animal facility.

The care and experimental manipulation of our animals has been reviewed and approved by the Institutional Animal Care and Use Committee at Cornell University. We now address the five points mentioned in the Instructions.

1. Proposed use of animals. Our subjects are 15-40 g, heterozygous ChR2(H134R)-EGFP transgenic mice on a C57BL/6 background mice (20 per year, half male/half female), and heterozygous Thy1-eNpHR2.0-EYFP transgenic mice on a C57BL/6 background mice (20 per year, half male/half female). We will conduct experiments in both male and female mice and compare results to explicitly assess if there are any sex-based differences. Since our experiments involve propagation of complicated neural activity patterns, such as seizures, cell culture cannot be used.

Anesthesia

During the surgery all subjects will be maintained at sufficiently deep anesthetic levels to eliminate any response to a firm footpad pinch. We will use inhaled isofluorane for surgery.

Surgery

Chronic epilepsy induction with mouse sized implantable sensor with lamp

Glycopyrolate is delivered by subcutaneous injection (500 µg per kg mouse) at the start of surgery. We further supplement with 5 % (w/v) glucose in physiological saline (5 ml per kg mouse) every hour. Body temperature is measured and is maintained at 37.5° C with a heating blanket. Blood oxygen levels and heart rate are monitored with a pulse oximeter. Eyes are protected with a covering of veterinary eye ointment. In acute animals, blood pressure is monitored through a catheter in the femoral artery. All surgical areas are shaved and cleansed with Povidone-iodine followed by swabbing with 70 % (v/v) alcohol. Bupivacaine is injected into the skin at each incision. A burr hole is drilled into the skull on the right side near lamda where chronic forcal epilepsy induction will occur through nanoinjection followed by introduction of and electrode and the fiber coupled LED. A glass pipet attached to a nanoinject will be inserted 500 µm into the motor cortex near the burr hole. Once the glass pipet has been at the correct depth in the cortex for 5 minutes an injection of 350 nL of either saline or iron chloride will be made into the mouse's brain. The glass pipet must wait in the brain for a minimum of half an hour to avoid backflow. Once the glass pipet is removed the fiber coupled LED and electrode will be lowered to the new epileptic focus. Then the subcutaneous implant will be placed in the abdomen and sutured closed with wires and fibers that run subcutaneously to the head where the fiber coupled LED will be mounted and cemented down with C&B Metabond.

Chronic post-operative care

Chronic animals will be housed one to a cage after chamber attachment. The animals are judged to be in distress if there is a loss of grooming or appetitive or if the animals exhibit a reluctance to rise and move. Analgesics will be administered post-operatively if the animal is in pain as diagnosed by cowering in a corner of the cage, sustained immobility, or not eating or drinking. We use Ketoprofen at a dosage of 0.1 - 0.5 mg per 100 g animal weight, administered sub-cutaneously, once following surgery and again 24 and 48 hours later. Post-operative maintenance will be carried out continuously until the animal is awake and can regulate its body temperature. Typically, this occurs within 1 hour after surgery. At this time the animal is transferred to the animal housing facility.

Electrophysiology/Optogenetic Stimulation

In chronic animals, there will be an implanted electrode and monitored for 4 hours every week for 100 days. During half of the recording time each week the full device will be turned on which will detect seizure initiation and activate the fiber coupled LED to flash for 10 s at 10 Hz to elicit optogenetic stimulation.

Epilepsy models

In chronic animals, a microinjection of 350 nL of 100 mM $FeCl_3$ will be made at a depth of ~500 µm beneath the cortical surface. This model leads to interictal spikes and ictal events after about 1-2 weeks.

2. Justification of use of animals. Our studies will require the use of approximately 20 transgenic mice per year, based on an average usage of 3 per week. Because this work involves the evaluation of layer-specific seizure propagation as well as neural activity in response to peripheral stimuli, it is not possible to use cell culture or other non-*in vivo* methods to obtain the results. We have chosen mice as our model system because of the

large body of knowledge on seizure propagation in mice as well as reliable epilepsy animal models in mice. The use of mice also builds on the experience of the co-P.I.s and other laboratory personnel with these animals.

We emphasize that every effort will be made to get as much useful data as possible out of each animal, as a means to minimize the total number of animals required. In all cases, enough experiments will be performed to ensure statistically-significant results are obtained.

Cornell University is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC effective date: June, 2015), and has a current PHS Animal Welfare Assurance (ID: A3347-01). Animals used in the proposed research will be purchased commercially and housed at an on-site animal housing facility at Weill Hall. The animal housing facilities are located approximately 100 m from the P.I.'s laboratory, in the same building. The animal facility provides 24 hour/ 7 day animal care, including weekends and holidays, by a highly-trained staff of veterinarians and animal health technicians.

Cornell-wide support services include veterinary care and consultation as well as training classes and seminars, including hands-on training labs. Access to surgical suites and other appropriate core facilities is also provided.

3. Minimization of pain, stress, and discomfort for laboratory animals. During surgery animals will be anesthetized (see Section 1, above) to provide a calm and pain-free state. The animal is not mounted into the stereotaxic holder for surgery until after Level 3 anesthesia is reached, as assessed by the loss of eye blink and pedal reflexes. This plane of anesthesia is maintained throughout the surgery and imaging session by vigilant physiological monitoring and frequent anesthesia supplements. The animals are injected sub-cutaneously with glycopyrolate (500 µg per kg animal weight) to prevent secretions that may block the trachea and throat. Fluids and sugar are provided by sub-cutaneous injection of 5% glucose in saline (5 ml per kg animal weight each hour). Ophthalmic ointment is applied to keep the eyes from dehydrating. A heating blanket that is feedback controlled by readings from a rectal thermometer is used to maintain the animal's core temperature.

All animals in the study will be weighed once per week and checked for signs of distress. Loss of more than 20% of body weight indicates that the animal will be evaluated by the veterinary staff.

After the surgery session is completed the animals will be taken off the isofluorane anesthesia. The animals will be continuously monitored until they are awake and behaving normally, usually about 2 hours, then returned, housed one to a cage, to the animal facility. The surgical wounds will be inspected for signs of infection. Analgesics will be administered post-operatively for three days and later if the animal shows signs of pain (cowering in a corner of the cage, immobility, lack of appetite or thirst). We will use the NSAID ketoprofen for this pain management.

4. Method of euthanasia for laboratory animals. The animals will be euthanized by an inter-peritoneal injection of a lethal overdose of pentobarbital (250 mg per kg rodent weight). This method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. After loss of eyeblink and pedal reflexes, a needle is inserted into the heart to take blood. Then, the chest cavity of the animal is opened to permit transcardial perfusion of 100 ml of saline, followed by 100 ml of 4% paraformaldehyde in saline for fixation. After adding fiducial marks that identify the location of imaged region, the brain of the animal is removed, cryoprotected in 25%, then 50% sucrose, and finally cut on a cryostat. Brain slices near the imaging location are identified and analyzed histologically. The carcass of the animal is stored at –4 degrees Celsius, and later properly disposed of.

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Consortium and Contractural Arrangement

This proposal is a collaborative effort between Open Source Instruments, Inc. and Cornell University's Schaffer Laboratory in the Meining School of Biomedical Engineering. Open Source Instruments will serve as the prime institution for the project, and Cornell University will serve as the sub-recipient.

Open Source Instruments will design and build an optogenetic brain implant with EEG monitoring and response for mice. This will be a research tool to be placed subcutaneously in animals and will be designed in order to support the search for effective therapeutic interventions for Epilepsy.

Prof. Schaffer's laboratory at Cornell will take the wireless EEG/optogenetic devices produced by Open Source Instruments and test how well they interrupt epileptic seizures in rodents. Prof. Schaffer will also assist with the design of the experiments and interpretation of results, as well as dissemination of findings to interested communities.

Substantial coordination of efforts will be accomplished via internet communications. The travel budget additionally includes support for two, face-to-face meetings of the key investigators during the duration of this phase of the project, one year.

Weill Cornell Medicine NewYork-Presbyterian

Theodore H. Schwartz, M.D., F.A.C.S.
David and Ursel Barnes Professor of Minimally Invasive Neurosurgery
Director, Anterior Skull Base and Pituitary Surgery
Director, Epilepsy Research Laboratory
Professor of Neurological Surgery, Otolaryngology and Neuroscience

Kevan Hashemi
President
Open Source Instruments
hashemi@opensourceinstruments.com

Dear Kevan,

I am writing to convey my willingness to serve as a consultant for your SBIR proposal with Chris Schaffer from Cornell University entitled, "An optogenetic brain implant with EEG monitoring and response for mice." As you know, I am a faculty member in the Neurological Surgery department at Weill Cornell Medical, where I see patients and run a research group focused on understanding and treating epilepsy. I have worked on several projects with Prof. Schaffer over the past ten years that have focused on using advance optical techniques to study seizure propagation and explore novel neurosurgical approaches to treating epilepsy in rodent models. For example, we have a paper that just appeared in Cerebral Cortex describing the efficacy of a laser-based surgical therapy to block the propagation of focally-initiated cortical seizures.

The device your company proposes to build in this proposal would be of great utility for the necessary animal studies that could guide future functional neurosurgical interventions in humans. Following neural activity patterns in one part of the brain, detecting aberrant activity patterns, and then delivering optogenetic stimulation (either excitatory or inhibitory) to modulate the activity patterns of a genetically-defined population of neurons in the same or another area of the brain is an emerging approach that has the potential to treat a wide variety of neurological and psychiatric diseases. To develop and test such therapies, we need the ability to do experiments like this in murine animal models. Your device exactly fills this need.

The decision to test the device in a rodent model of focal epilepsy makes sense to me, as it is clear in this model exactly where brain activity should be monitored and what cell types (and where) we should optogenetically modulate activity. My and Chris's lab have extensive experience with this model (in fact a former post-doc from my lab devised it) and I am happy to provide any technical assistance or advice needed to help you complete the proposed work.

I look froward to working with you on this exciting project.

Sincerely,

Theodore H. Schwartz

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Professor Dimitri M Kullmann, FMedSci FRS
UCL INSTITUTE OF NEUROLOGY
Department of Clinical and Experimental Epilepsy

10/08/2018

Re: SBIR grant proposal – Mouse-Sized Implantable Sensor with Lamp (MS-ISL)

This letter is to confirm my enthusiastic support for the proposal by Open Source Instruments, Inc, to develop and miniaturize a self-contained device to analyse electrocorticography signals and deliver light to the brain. Working with wireless transmitters developed by Open Source Instruments, my laboratory was the first to report that seizures in experimental animals could be suppressed optogenetically (Wykes et al., Sci Transl Med 2012 Nov 21;4(161):161ra1520). Since then we have made progress in automatic detection of seizures, new seizure models, improved targeting of neuron subtypes, and new gene therapy strategies that are amenable to clinical translation (Kätzel et al., Nat Commun. 2014 May 27;5:3847; Lieb et al., Nat Med. 2018 Jul 9. doi: 10.1038/s41591-018-0103-x). All of this work has relied heavily on devices purchased from Open Source Instruments. Our long-term goal is to use an entirely self-contained closed-loop device akin to automated implantable defibrillators used to detect and terminate cardiac arrhythmias. Similar technology could be applied to a variety of neurological disorders where abnormal brain rhythms are thought to play a role, including Parkinson's disease and schizophrenia. Our work to date has relied on rat models of epilepsy, with external computation of EEG patterns and fiber-optic light delivery. For us to make progress we would need a miniaturized device that could be implanted in a mouse, with built-in circuitry to control a light-emitting diode mounted on the skull. This would allow us to make use of the wide range of genetically modified mouse strains that allow optogenetic actuators (opsins) to be expressed selectively in different populations of neurons (either excitatory or inhibitory). There are also many mouse strains that have been validated as models of epilepsy, schizophrenia and other neurological disorders, further underlining the need to perform these experiments in mice.

I confirm that my laboratory would be keen to purchase the proposed Mouse-Sized Implantable Sensor with Lamp (MS-ISL) for implantation in mice. In the first instance, we would perform proof-of-principle studies that do not require the animals to be epileptic, to confirm that we are able to manipulate an observable behaviour (for instance turning when activating principal cells in the supplementary motor cortex). Furthermore, because of the inevitably limited battery life of implantable systems, we are keen for Open Source Instruments to enable the devices to be recharged.

The proposed development work represents important steps both towards clinical translation and towards testing circuit theories of brain function. I foresee many additional applications by our group and others.

In summary, I fully support the proposal by Open Source Instruments, and look forward to using the devices in my laboratory.

Yours sincerely,
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Yours sincerely

Dimitri M Kullmann
Professor of Neurology

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LONDON'S GLOBAL UNIVERSITY

Maria Fitzgerald FMedSci FRS
Professor of Developmental Neurobiology
UCL Neuroscience, Physiology & Pharmacology

26th March 2019

Brain Initiative,
National Institute of Neurological Disorders and Stroke

Dear Sir/Madam

Re: Open Source Instruments, Inc. SBIR Application

I am writing to express my support for this application and to emphasise the importance of the proposed project to develop a wireless instrument capable of simultaneous EEG recording and optogenetic stimulation in mice.

I am in an ideal position to do this as I have had direct experience of working with Open Source Instruments in my own research. My scientific career has been dedicated to understanding the biology of pain and studying how pain experience in childhood can influence susceptibility for chronic pain in later life. We have successfully used Open Source fully implantable, wireless EEG monitors in young and adolescent rats and mice to study how pain is processed in young rodents*. This was the first time that it was possible for animals to move freely (untethered) in a natural environment while measuring, in real time, their brain activity in response to noxious (painful) stimulation. The proposal to include optogenetic stimulation in such a system would be of enormous benefit, as it would allow concurrent modulation of specific groups of neurons in the brain that might modify pain experience.

The USA is currently undergoing a crisis in the use and misuse of opioids in chronic pain. To address this, we need a much better understanding of how and why some individuals are susceptible to chronic pain and to develop new treatments that avoid addictive drugs. The Hashemi proposal offers a practical and much needed resource for studying pain processing in the brain in mouse models of neuropathic pain, such as that following spinal injury and inflammatory pain, such as osteoarthritis and will bring substantial advances to this important field.

Best wishes,

Maria Fitzgerald FMedSci FRS

*Chang P, Fabrizi L, Olhede S, Fitzgerald M. The Development of Nociceptive Network Activity in the Somatosensory Cortex of Freely Moving Rat Pups. Cereb Cortex. 2016 ;26(12):4513-4523. doi: 10.1093

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April 2nd, 2019

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I am writing to establish my full and enthusiastic support for Open Source Instruments' (OSI) re-submission to the BRAIN Initiative to develop optogenetics for their wireless mouse electrophysiology systems. I am a principal investigator at the University of Edinburgh whose work is focused on testing optogenetic strategies to block seizures in rodent models of temporal lobe epilepsy (TLE). I am funded by Epilepsy Research UK, a charity in Great Britain dedicated to research in the disease.

I have personally attempted wireless optogenetics in conjunction with electrophysiology from mice over the past year and I attest that experiments would be greatly helped by the developments proposed by OSI. OSI electrophysiology devices themselves are very effective at recording from rodents continuously for 24 hours per day over weeks. This is wonderful for animal husbandry as long-term wireless recordings have less of an impact on the well-being of the animals than tethered protocols. Chronic wireless recordings are incredibly useful, as the field of epilepsy is shifting to models that better mimic the symptoms of the disease to improve our understanding of the mechanism leading to seizures and, to therefore have a greater chance of discovering novel translational approaches. One important characteristic of epilepsy is that seizures occur sporadically and are difficult to predict. Consequently, the use of chronic models of TLE, in which seizures occur spontaneously and often only once or twice per day, are becoming more prevalent in high impact publications. However, in order to detect these seizures and have sufficient samples for accurate statistics, it is necessary to record continuously over days or weeks.

Optogenetics allows us to modulate the activity of specific neuronal populations in experimental animals with a high degree of temporal precision. This is important as we can test how groups of cells may contribute to the epileptic circuit and determine whether these manipulations may have therapeutic value. These findings may eventually be translated to the clinic through deep brain stimulation of specific brain areas, genetic therapy or through implementation of optogenetics itself given significant future advances in these technologies.

First generation wireless recordings and optogenetics systems have begun to be sold by multiple companies. However, very few of these technologies have the publication record that Open Source Instrument devices do. Furthermore, the majority of wireless technologies are designed for high sampling rate paradigms in order to record action potentials from individual cells. Consequently, the battery life of these systems is limited to a few hours. No clear solution has been designed yet for long-duration recordings in combination with optogenetics.

In order to attempt to complete the goals of our project I have utilized a wireless optogenetic stimulator from a company called Neurolux in conjunction with OSI's electrophysiology telemetry system. I detected the onset of seizures in real-time using OSI's event classifier. When a seizure began, the Neurolux wireless device was activated through a TTL pulse from the logic output on the front of OSI's radio receiver. The Neurolux system is powered by radio-frequency induction, which unfortunately, depending on the animal's position in the cage, generates electrophysiological signal loss. Worse signal loss is seen when recording with traditional tethered systems in conjunction with wireless optogenetics. Consequently, reliable signal classification is sub-optimal upon light stimulation.

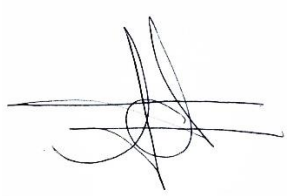
Another feature of radio-frequency power induction, such as that used by Neurolux, is that the optical power delivered by the light varies with the location and head-orientation of the animal. Therefore, it is unclear whether at certain locations, the power is sufficient to activate opsins. The Mouse-Sized Implantable Sensor with Lamp (MS-ISL), on the other hand, delivers a fixed power to its fibre tip, and transmits an acknowledgment to confirm activation of a stimulus. Another disadvantage of the Neurolux system is its inability to distinguish between host animals. It is necessary to install a separate stimulation system for each of the animals in our study, which is not cost-effective, and each animal must be caged separately. It also means that recording from a large group of animals over to achieve statistical power takes months.

The proposed MS-ISL from OSI provides its own battery power for illumination, so there will be no induction of circulating currents in the brain by radio-frequency waves. This would improve event classification and a single MS-ISL command transmitter would allow us to work with potentially dozens of co-housed animals in a compact Faraday enclosure, saving us both money and space.

In conclusion, the field of epilepsy research, like other neuroscience sub-disciplines, is undergoing a revival thanks to the advent of optogenetics. The technology, in combination with genetic advances in mice has the potential to make important breakthroughs in deciphering the circuits underlying epilepsy and developing novel translational strategies for the disease. Furthermore, the specific technological advance proposed by OSI, wireless optogenetic devices optimized for compatibility with long-term electrophysiological recordings, is a critical element in order to advance this field of research.

Please do not hesitate to contact me if you have further questions.

Yours sincerely,



Alfredo Gonzalez-Sulser, PhD
Epilepsy Research UK Fellow

Epilepsiezentrum Frankfurt Rhein-Main
Leiter: Prof. Dr. Felix Rosenow

Universitätsklinikum · ZNN-Epilepsiezentrum· Schleusenweg 2-16 · 60528 Frankfurt a. M.

Brain Initiative
National Institute of Neurological Disorders and Stroke

Bearbeiter/in: Dr. S. Bauer
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Fax: +49 69-63 01-84466
Datum: 26 March 2019

Regarding: Open Source Instruments, Inc. SBIR Application.

Dear Sir, dear Madam,

as head of the „Translational and Experimental Epileptology" laboratory (formerly Philipps University Marburg, Germany; currently Goethe-University, Frankfurt, Germany), I have been using the EEG telemetry system developed and provided by Open Source Instruments (including wireless EEG transmitters A3028R and A3028B, LWDAQ device, Octal data receiver, recording and Neuroarchiver software) for more than 5 years. During this time, well over 150 EEG transmitters were implanted into both mice and rats by my lab members and me. The devices proved to be highly reliable in continuous long-term recording of low-noise high-quality EEG for more than 3 months per rat or 2 weeks per mouse. I was always highly satisfied with both OSI's product quality and service, and I will continue using OSI's EEG transmitters in future projects.
Depending on site and parameters (e. g. frequency, voltage), electrical deep brain stimulation (DBS) can modify brain functions in very different ways. Stimulation of the perforant pathway, for example, can induce seizures, status epilepticus, epilepsy or epileptic tolerance. Our latest experiments included open-loop long-term electrical DBS of various targets, such as fimbria/fornix, dentate gyrus, perforant pathway and ventral hippocampal commissure (VHC). We could demonstrate a pronounced anti-epileptogenic effect of low-frequency (1 Hz) low-amplitude (1 V) VHC stimulation in a rat model of temporal lobe epilepsy with hippocampal sclerosis (manuscript submitted to "Brain Stimulation"). However, for administration of this electrical stimulation, it is still necessary to tether the animals via a cable and a swivel commutator to a stimulation device, which considerably impairs the animal's mobility. The impact of experimental procedures on animal wellbeing is rated with ever-increasing strictness by the regulatory authorities. In particular, immobilization of animals during DBS procedures was subject of intense discussions with authorities during our latest applications. Therefore, I was very excited to learn that OSI plans to develop an implantable device which will be able to both record EEG in the accustomed high quality and deliver electrical pulses to a connected stimulation electrode. The product is of high interest for future DBS projects in our lab. Hence, I strongly support the idea to develop such a device.

Frankfurt, 26 March 2019

Dr. Sebastian Bauer
Senior physician, Head of Translational and Experimental Epileptology Group
Epilepsy Center Frankfurt Rhine-Main, Dept. of Neurology, University Hospital Frankfurt, Goethe-University Frankfurt, Germany

UCL INSTITUTE OF NEUROLOGY
QUEEN SQUARE

THE NATIONAL HOSPITAL FOR NEUROLOGY AND
NEUROSURGERY
QUEEN SQUARE
LONDON WC1N 3BG

To whom it may concern,

I am a Senior Research Fellow at the UCL Institute of Neurology, in the Department of Clinical and Experimental Epilepsy.

I have been using telemetry devices manufactured by Open Source Instruments for the last 8 years and found them invaluable for my epilepsy research. These devices have allowed me to record EEG/ECoG continuously for months in freely moving rodents. I have found these devices reliable and robust.

Recently I have successfully implanted and tested ISL devices for *in vivo* optogenetic control of neurons in rats. I have successfully demonstrated that they can be used to drive population firing of neurons in the cortex to induce physiological behaviours (circling behaviour induced by 10Hz stimulation of opsin expressing neurons in the motor cortex, M2 area). I am currently using these devices in combination with an Open Source Instruments online seizure detection system to provide close-loop stimulation to suppress seizures in several rat models of neocortical epilepsy.

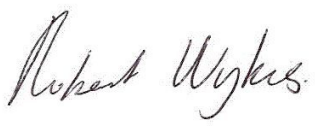
Future research aims will be to use miniaturised versions to allow similar experiments in mice.
There are 2 main reasons I would prefer to do some experiments in mice instead of rats and thus need the ISL miniaturised. Both are for transgenic reasons.

1. In rats we must use viral vectors to express an opsin and have limited ability to express our opsin in defined subsets or sub-populations of neurons. In mice we can use transgenic animals to express an opsin wherever we want it. There is much greater specificity and expression without the need to inject a virus.

2. I wish to conduct our experiments in animals that model human diseases. In our facility we have transgenic mice that model diseases such as Alzheimer's and Down syndrome both of which have abnormal EEG and hence the ability to manipulate neuronal firing *in vivo* using optogenetics is a research aim of ours. We also have other transgenic mice that harbour mutations in genes known in humans to cause epilepsy. These experiments can only be done in mice and not rats - hence the reason why we need a miniaturised ISL to meet our research aims.

I will use miniaturised versions of Open Source Instruments ISL devices when they become available to advance my telemetry research.

Yours sincerely,



Dr. Robert Wykes
UCL Institute of Neurology
R.Wykes@ucl.ac.uk
August 5th 2018

UNIVERSITY AT ALBANY
State University of New York

Annalisa Scimemi, PhD
Assistant Professor
Department of Biology

To: Brain Initiative, National Institute of Neurological Disorders and Stroke
Re: Open Source Instruments, Inc. SBIR Application

March 27, 2019

Dear Sir/Madam,

I am writing to express my enthusiastic support for Kevan Hashemi's application for a SBIR grant to support the commercialization of the implantable fiber-coupled LED for rodent optogenetics.

I am an Assistant Professor at SUNY Albany, with extensive experience in synaptic physiology, imaging and modeling. Recently, my lab has been in contact with Kevan Hashemi to purchase wireless transmitters to perform chronic EEG recordings from mouse pups. This is a challenging task not only because of the small size of the pups, but also because many of the recording tools available from other competitors are either wired or lack the ability to perform continuous recordings over the course of a week.

My lab is currently involved in a number of projects to determine how glutamate transporters shape synaptic, cell and circuit function in two regions of the brain, the hippocampus and the striatum, which play a fundamental role in the control of spatial navigation (the hippocampus), movement execution and reward (the striatum). Although performing EEG recordings allows monitoring the spontaneous electrical activity in the brain of living animals, we would like to expand our analysis to evoked responses triggered by activating implantable LEDs in the brain of genetically engineered mouse lines expressing the light-gated channel ChR2 under different cell-specific promoters. Through these experiments, we would be able to make direct inferences between the role of distinct classes of glutamate transporters and the execution of specific behaviors.

Therefore, I confirm that my lab would use the proposed mouse-sized Implantable Stimulator and Monitors with fiber-coupled LEDs. I would also like to mention that the development of the new technologies described by Kevan Hashemi in this proposal is, in my opinion, a fundamental step towards making discoveries that contribute to ameliorate the burden of mental health disease throughout the world. There is essentially no limit to use this type of tool in any animal model of brain disease. I fully support the proposal that Kevan Hashemi is submitting to your attention and I look forward to testing his new equipment in my lab. Should you require further information, please do not hesitate to contact me.

Sincerely,

Annalisa Scimemi

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