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.

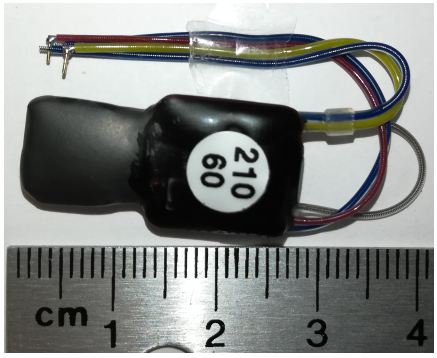

Figure 1a: A visual prototype of the proposed MS-ISL. Total volume is 0.9 mL. The MS-ISL is implanted in the abdomen where it doesn't interfere with animal behavior. Electronics and battery are enclosed in the black epoxy-silicone package. The silver loops are antennas for receiving commands and transmitting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

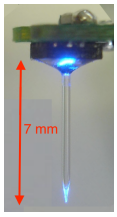

Figure 1b: The Fiber Coupled LED (FCL) is the satellite head fixture that will be driven by the MS-ISL. It is mounted to a hole in the skull with dental cement. Blue light is visible at the LED surface and at the coupled fiber's tapered tip. The FCL pictured is a functional prototype which must be miniaturized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

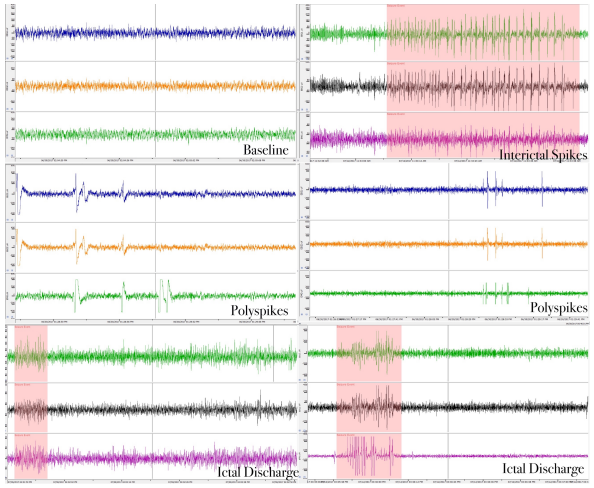

Figure 2. Nanoliters of iron chloride injected into the cortex leads to chronic focal neocortical epilepsy. These are EEG recordings from three implanted electrodes on the mouse's motor cortex. Seizure focus is induced at the placement of the second electrode by nanoinjection of iron chloride. Seizures 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**.

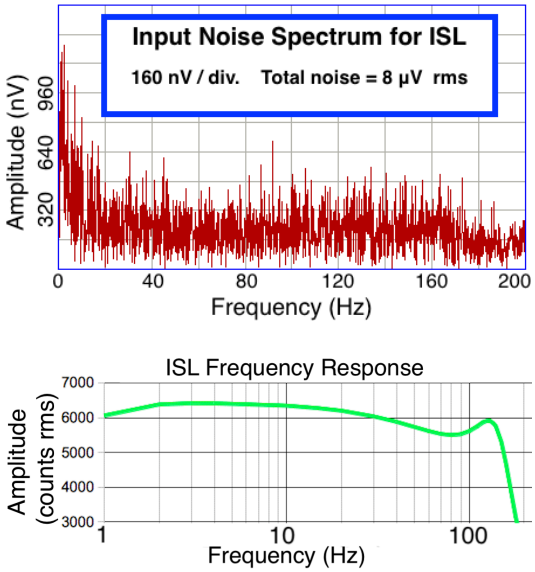

Figure 3 - Biopotential performance of the ISL The proposed instrument will have EEG recording characteristics very similar to the ISL. **Top:** Input noise spectrum for the ISL. **Bottom:** Frequency response of the ISL. A three pole filter is used to limit the contribution of signals above 160 Hz.

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 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.**

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

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.