Significance

Overview: Our mouse-sized implantable sensor with lamp (MS-ISL) delivers light to any point in the brain of a mouse and provides continuous monitoring of electroencephalogram (EEG), local field potential (LFP), or electromyography (EMG). The MS-ISL will simplify all experiments in the fields of behavior and epilepsy in which optogenetic stimulation must be delivered to the brains of freely-moving animals. Because it provides both an intelligent sensor and an efficient stimulator, the MS-ISL makes possible a new class of experiments in which optogenetic stimulation is delivered immediately in response to events occurring in EEG, LFP, or EMG.

The Research Potential of Optogenetics: Optogenetic stimulation provides a means to control particular populations of neurons in the brain. Transgenic mice, or animals expressing opsins through the injection of adeno-associated virus (AAV), allow us to excite or inhibit cells of a particular type in a particular region of the brain by illuminating that region with light. No other form of stimulation is both as selective and immediate. Optogenetic inhibition of neurons can suppress seizures [21, 22, 23]. When we detect the onset of a seizure in an animal's EEG, we can deliver optogenetic stimulus immediately in an effort to suppress the seizure [24, 27]. We might study social deficits by exciting one population of neurons [2], or disrupt the consolidation of memory by inhibiting another population [28]. When we see a sharp-wave ripple in an animal's EEG, we could deliver optogenetic stimulation immediately, so as to explore the role these waves play in memory consolidation.

The Tools Needed to Exploit This Potential: If we are to take full advantage of the opportunities presented by optogenetic stimulation in the study of behavior and epilepsy, we need a stimulator that can deliver reliable and consistent light pulses to an exact location in the brain. We need a sensor that can monitor at least one biopotential prior to stimulation, and sustain accurate monitoring during stimulation. The stimulator and sensor must be wireless and implantable, or else the subject animals cannot move freely through complex environments, or share the same space with other animals. If we are to respond as rapidly as possible to biopotential events, the sensor must be able to identify these events and initiate the stimulus itself, in a process we call *internal closed-loop control*. The MS-ISL we develop in Phase II will satisfy all the above requirements with one implantable device.

The MS-ISL is the Necessary Tool: The MS-ISL we will develop and bring to market in Phase II will permit optogenetic experiments with internal closed-loop control in freely-moving, cohabiting mice for up to eight weeks. Investigators will be able to configure the device's event-detection algorithm for their own purposes, specify their own stimuli, and be confident in the repeatability of the optical stimulus power.

Innovation

Overview: The innovations of our work promote the energy-efficiency of the sensor, the fidelity of its biopotential recording, and the power of the optical stimulus it delivers. These innovations will allow us to manufacture a mouse-sized implantable sensor with lamp (MS-ISL) that remains operational for eight weeks while implanted, delivers repeatable optical stimuli at any time, and provides biopotential event-detection and response.

The Device Must Be Battery-Powered: When designing an implantable device, there is a great temptation to escape from the energy limitations of a battery by deriving power from an oscillating electromagnetic field [1, 4, 6, 7, 8, 10, 17]. But this is a temptation that we must avoid. Wireless power delivery is unreliable in freely-moving animals. In our collaboration with University of Edinburgh, we found that the Neurolux optogenetic stimulator, which relies upon wireless power delivery, provided anything from zero to full power when we initiated a stimulus, depending upon the location and orientation of the animal, and this uncertainty had to be built into our experimental analysis [27].

When working with our own battery-powered stimulators, we found that the energy of the light pulses had a dramatic effect upon the induced behavior. At low energy, we see no effect, even though the flashes of light are clearly visible through the cement of the head fixture. At medium energy, the animal walks in circles. At high energy, the animal immediately experiences a seizure, with its EEG entrained to a subharmonic of the stimulus frequency, as shown below. These observations show that the optical energy delivered the target region must be known and repeatable.

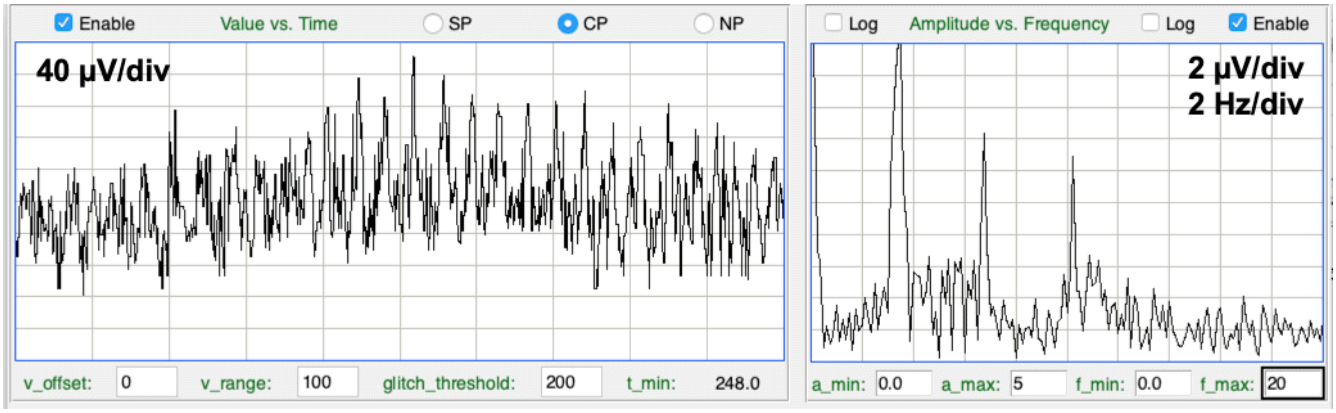

Figure: Eight-Seconds of LFP During Optogenetic Stimulus (Left) and Spectrum (Right). Stimulus is 10-ms Flashes at 10.2 Hz. Spectrum shows fundamental harmonic 3.4 Hz, third harmonic 10.2 Hz. Video recording shows shuddering seizure until a few seconds after stimulus stops.

If we were to power the sensor with an oscillating field, there would be times when the power delivered by the field drops to zero. We would have to equip the sensor with a battery to carry it through such periods, or else the sensor would shut down. If the animal remains in the same position for an hour, the battery would have to supply the sensor for the full hour. We would end up needing a rechargeable battery and sufficient wireless power to run the sensor and charge the battery simultensously.

Even if we could overcome the problems listed above, an oscillating magnetic field sufficient to power an optogenetic stimulator is guaranteed to corrupt any LFP recordings we attempt to make in the subject animal. The oscillating field is rectified by the saline-metal junctions of the LFP recording electrodes, and appears as an artifact on the LFP. Even with constant field power, this artifact fluctuates as the animal moves. Reliable event detection is difficult in the presence of such artifacts. In our collaboration with University of Edinburgh, we had to disable our event detection during wirelessly-powered stimuli in order to avoid radio-frequency artifact [27].

For these reasons, an effective MS-ISL must be battery-powered. Our acceptance of this reality is, we believe, one of our core innovations. In order to maintain isolation between the stimulator and the sensor, the device must, in fact, be powered by two batteries, which is another of our innovations, arrived at in Phase I. At least one of the batteries, the one that powers the lamp, must be capable of delivering 15 mA, which narrows down our choice to miniature lithium-polymer batteries.

Micropower Command Reception. The more energy-efficient the MS-ISL, the longer its operating life. The MS-ISL's command receiver consumes only a few microamps. So far as we can tell, our combination of a crystal radio, split-capacitor tuner, and logic that powers up during command reception, is unique. Having said that, there is nothing new about a crystal radio, nor a circuit that turns on when we need it. But no other manufacturer of implants appears to be doing the same thing. Designing and perfecting miniature microwave circuits is not straightforward. Simulations are of little help. The circuits must be built, tuned manually, and redesigned multiple times before they function as they should.

The World's Most Efficient Fiber-Coupled LEDs. Our fiber-coupled light-emitting diode (FCLED) is

another innovation. Our combination of high-refractive index glass, hand polishing, tapering, and gluing the fiber to a bare LED die took us years to develop. In Phase I we spent months working on our custom tapering machine until we were able to make light guides only 4 mm long out of 270-μm diameter high-index fiber. These light sources are equipped with a steel mounting tube that allows them to be lowered with precision into a skull hole and secured in place during surgery.

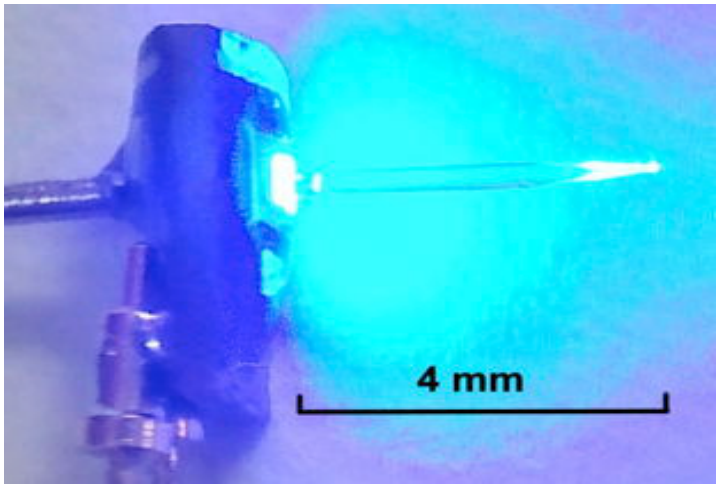

Figure: 4-mm Blue Fiber-Coupled LED. Part number A3036IL-A270-4. The fiber captures 30% of the light emitted by the LED and delivers it to the tapered tip.

These 270-μm fibers deliver 30% of the light emitted by the LED to the taper tip. The LED die we have selected is small, with a bond wire at the edge of the top surface. It is among the most efficient available today. The blue LED converts 30% of electrical energy into light, and the green converts 15%. The Blue FCLED is 10% efficient in transforming electrical power into light radiated at the tip of the fiber. So far as we can tell, our FCLED is ten times more efficient than those made by any other manufacturer. The system described by Anpilov et al. [1], for example, is only 0.05% efficient.

Custom Microprocessor: The MS-ISL event detection requires an efficient, compact microprocessor that can reside in the same field-programmable gate array (FPGA) as the other logic functions required by the circuit. In Phase I we designed a complete open-source, eight-bit, re-configurable (OSR8) microprocessor equipped with multipliers and accumulators optimised for event-detection. The OSR8 is defined in software that we compile and upload into the MS-ISL's programmable logic chip. In the Phase I MS-ISL, the OSR8 consumes 300 pJ per instruction, which compares well to the 150 pJ consumed by the most efficient off-the-shelf microcontrollers, such as the PICXLP devices from Microchip Technology. The result is a circuit that is ten times smaller than any off-the-shelf solution, and at least as efficient.

Event-Detection Algorithms: The algorithms we use to detect features in EEG recordings have been well-proven over the past ten years. They include metrics with names like “coastline”, “spikiness”, “intermittency”, “asymmetry”, and “coherence”. We designed these algorithms to be efficient, so that users of our subcutaneous transmitter (SCT) system could perform event-detection in real time on a dozen EEG signals. In Phase II, we will translate these innovative and efficient metric calculations into the instructions that can be executed on our custom microprocessor.

Isolation of Sensor and Stimulator: One of the difficulties in combining a sensor and a stimulator is the tendency of the stimulation current to corrupt the sensor signal. Even if the stimulation leads are well-insulated with cement where they plug into an FC-LED, they will still be in weak electrical contact with the rest of the body through films of water that penetrate all available cracks and interfaces. We have solved this problem by separating the stimulator and sensor circuits, powering them with separate batteries, and allowing the sensor to control the stimulator through the smallest opto-isolator in the world. Now the stimulator and sensor operate as if they were two separate

implants, except that the sensor can turn on the stimulator at will, so as to permit on-board event detection and response. With two separate batteries, the weak resistive connection between the stimulation electrodes and the sensor electrodes does not affect the sensor measurement, provided there is no breach in the insulation around the stimulator leads.

Battery Recharging Through Stimulator and Sensor Leads. In order to minimize the volume of our MS-ISL, we encapsulate its electronics in the bare minimum of epoxy, and cover it with a thin coat of silicone. It is impossible to remove the batteries for recharging. But the optimum batteries for powering LEDs are rechargeable lithium-polymer cells, on account of their high output voltage, high current capacity, and availability in small packages. It would be a pity to use rechargeable batteries and have no way to recharge them. By adding diodes to the MS-ILS circuit, and designing our own charging circuits, we devised a way to recharge both MS-ISL batteries through the stimulation and sensor leads. Researchers can remove an MS-ISL from one animal, clip its four leads into a recharger circuit, wait twenty-four hours, and implant the device again. There is no need to return the device to the manufacturer for refurbishment.

Approach

Overview: The prototype MS-ISL we designed and built in Phase I has current consumption low enough to support experiments of up to five weeks. Our fiber-coupled light sources are thirty times more efficient at invoking optogenetic response than we anticipated at the beginning of Phase I. On the other hand, the devices failed prematurely when implanted, the implantation of sensor and stimulator was arduous, and the volume of the MS-ISL was too large for adults of the most common species of laboratory mice. We have a promising prototype, but the bulk of our work remains ahead of us. We must refine the prototype to the point where we can manufacture it without error and it will be small enough for a mouse, we must test it and improve its encapsulation until we know it will survive many weeks of implantation, and we must practice and document implantation procedures until we can present them clearly to new users.

Aim 1, On-Board, Closed-Loop Event Detection and Response: In Phase I, we designed a low-power microprocessor equipped with hardware multipliers and accumulators that facilitate the detection of seizures, sharp-wave ripples, muscle activity, heart-rate and respiration. We have well-proven algorithms that perform all these calculations in real-time, but these algorithms exist now in the form of Pascal source code that runs on the external data acquisition computer. In Phase II, we will translate these algorithms into the assembly language of our embedded computer, and so implement closed-loop response in our MS-ISL. We are also working on a Pascal compiler that will automate the translation, but if we are unable to complete the compiler, we will translate the algorithms by hand. We will then run these algorithms on an un-encapsulated MS-ISL connected to a signal generator as a first test of their ability to find pulses and oscillations. At this point, we will be able to estimate the battery current consumed by such event detection. Later, with the help of our collaborating institutes, we will configure MS-ISLs to transmit EEG as well as the results of their own event detection so we can examine their efficacy on real signals while implanted in a moving animal. By refining and simplifying the event detection, we hope to reduce the battery current required by internal closed-loop control to 150 μA. The MS-ISL logic has sufficient memory to store in a buffer several seconds of biopotential signal before an event, to be transmitted once an event is detected, followed by the signal during and a few minutes after the event with little increase in energy consumption. By this means we will be able to study and improve the reliability of event detection.

Aim 2, Identify and Eliminate Sources of Implant Failure: We are not certain of the causes of the premature failures among the twenty stimulators we implanted in our Phase I work. We suspect damage to the circuit when the batteries are being loaded, corrosion around components while implanted, and unwanted activation of the implant by microwave interference when they are not stored in Faraday enclosures.

Damage during manufacture may be occurring when we wash the circuit after loading the battery. We will try spot-welding the battery tabs so as to avoid a water-wash after the batteries are loaded. We will break down the manufacturing process into steps and study each step to see how we can reduce the chance of damaging the circuit. Corrosion is the most complicated problem to resolve, but we have plenty of experience with corrosion and how to slow it down. We will study corrosion resistance with the help of accelerated ageing in hot water, followed by dissection of failed circuits. We will address the problem of implants being activated by interference with MS-ISL configured to flash a warning whenever they are excited by microwave power. It is impossible for us to anticipate all the detailed problems we will have to solve to eliminate the common sources of failure, but our objective is clear: no more than 5% of implanted MS-ISLs will fail prematurely. In order to be confident that our failure rate is less than 5%, we will need to implant the Phase II devices in at least twenty animals. Our plan is to perform sixty implants in six sets of ten animals, resolving reliability issues as we go.

Aim 3, Reduce the Volume of the MS-ISL to 1.3 ml: Our Phase I MS-ISL has volume 1.7 ml, which can be tolerated by a large species of mouse, but is not well-tolerated by the more common laboratory species. Two implants of half this volume are, however, well-tolerated by all common adult mice. In Phase I we divided the MS-ISL into two parts: a stimulator and a sensor, each of volume 0.85 ml. By this means we were able to observe high-fidelity electroencephalogram (EEG) during optogenetic stimulation. But a double-implantation is more difficult than a single-implantation, and by separating the sensor and the stimulator we cannot provide on-board event detection and response. Most of the volume of the MS-ISL implant is its batteries. If we are to reduce the volume of the MS-ISL to 1.3 ml, we must use smaller batteries. In order to use smaller batteries while preserving adequate operating life, we must reduce the current consumption of the device. To reduce the current consumption, we will move to a newer, micro-power logic chip that will reduce the active current consumption of the sensor. By means of modifications to the crystal radio we will reduce its current consumption by a factor of two, extending the sleep life of the sensor. For the stimulator battery, we will exploit our recent discovery that the optical power required to initiate behavioral response with one of our FCLEDs is thirty times less than we believed it to be at the start of Phase I. We will use the smallest LiPo battery we can find. The result will be a single implant of volume 1.3 ml with sensor sleep life 3000 hr, sensor event-detection life of 150 hrs, sensor data transmission life of 75 hrs that can deliver 30 hrs of optogenetic stimulus with the help of an FCLED.

Aim 4, Codify surgical procedures and experimental protocols: No manual for implanting our MS-ISL exists. Researchers eager to work with us are venturing into territory previously unexplored. Only by practicing and repeating the implantation procedure can we provide the clear and detailed protocols necessary for investigators to perform consistent experiments. Academic institutions are willing to try out new procedures on a small scale. Our collaborators at Cornell University will perform the first dozen implantations of our Phase II devices. After that, however, our contract research organization (CRO) partners will perform six sets of ten implantations so that we can observe and resolve problems with infection, implant tolerance, and premature failure of the electronics.

Academic institutions and CROs are set up to perform experiments. We will structure our Phase II testing program as a series of experiments so the program fits more easily into the workflow of our collaborating institutes. We will focus on three use-cases: a closed-loop response demonstration in transgenic mice for epilepsy research, a closed-loop response demonstration in transgenic mice for behavior research, and an open-loop demonstration with AAV-injected mice for epilepsy research. Our demonstrations are experiments that our academic collaborators are confident will work, provided the MS-ISL itself works. In each case, completing the experiment demonstrates the efficacy of the MS-ISL. We suspect that our academic collaborators will find a way to make use of these tests to advance their research program, but it is not our purpose to perform scientific research in our Phase II work. Our purpose is to arrive at a proven and reliable MS-ISL.

First Use-Case: We will induce focal seizures by injecting iron (III) chloride into the cortex of mice [15], an epilepsy model frequently used by our collaborators at Cornell University. We will implant the the MS-ISL subcutaneously. An FCLED will be cemented to the skull so that its tip illuminates the

seizure focus. A depth electrode will record the nearby LFP. It has been previously shown that seizures can be halted by optogenetic activation of inhibitory neurons [21, 22, 23, 24, 25]. We will use a transgenic mouse expressing Ch2 in cortical interneurons: ChR2(H134R)-EGFP mice have a Cre-inducible Ch2 expression and will be crossed with animals expressing tamoxifen-inducible Cre in cortical inhibitory interneurons. The use of these transgenic mice in combination with the MS-ISL will allow for simultaneous monitoring of EEG activity in the brain and optogenetic activation of interneurons during seizure propagation. The MS-ISL will perform on-board event detection and decide randomly whether or not to initiate a stimulus whenever it detects the onset of a seizure. We will compare seizure incidence, duration, and power with and without the stimulus.

Second Use-Case: We demonstrate the disruption of memory consolidation by optogenetic stimulus during the delay period in a behavioral T-maze experiment. We will place the T-maze on one of our 48-cm by 24-cm animal location tracker platforms (ALT) to track the movement of the mouse through the maze and back to the delay area.

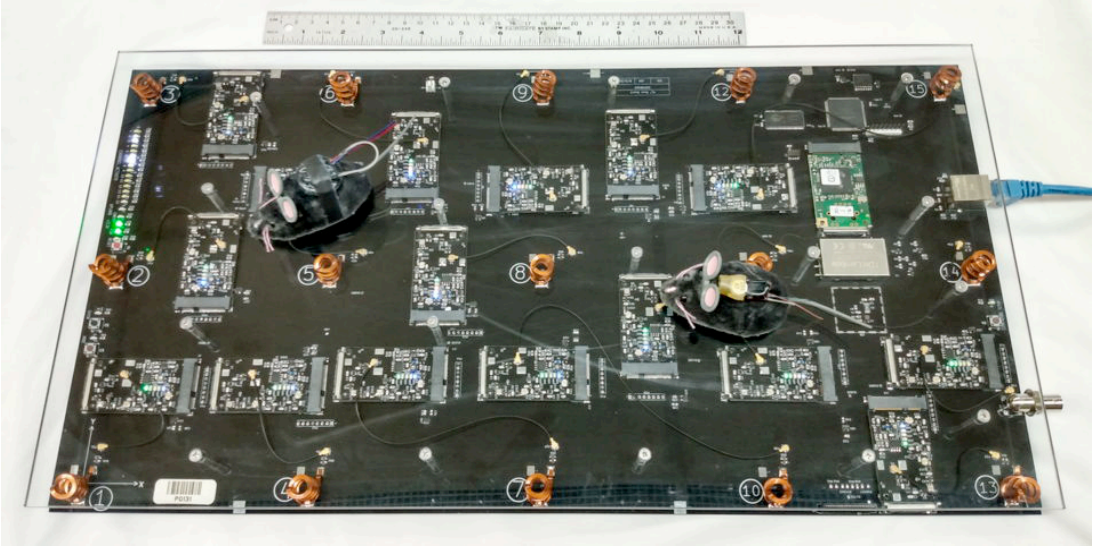

Figure: Animal Location Tracker. This 48 cm by 24 cm array of detector coils is large enough for a small T-maze, and already exists as an OSI product. It measures the location of implanted transmitters with a precision of one or two centimeters, and absolute accuracy five centimeters, which is sufficient to determine which direction a mouse has taken in a T-maze.

There are many studies published on the disruption of memory consolidation by tethered optogenetic stimulus, such as Buzsaki et al., 2021 [28]. We will breed a transgenic mouse suitable for duplicating one such study and do so with our wireless implant, FCLED, and a hippocampal depth electrode.

Third Use Case: Our third use-case makes no use of on-board event detection, but instead uses optogenetic stimulus as a way to provoke a neurological event that we then monitor in detail. Because there is no on-board event detection, there is no need to have the sensor and stimulator combined. We can separate them, and in doing so we extend the operating life of our devices. In Phase I, at the Institute of Neurology, University College London (ION/UCL) we performed this exact experiment as follows. We injected AV-CaMKIIa-hChR2(H134R)-EYFP the motor cortex. We waited six weeks. We performed a second surgery to lower an FCLED into the injection site and inserted depth electrodes at two locations in the cortex. We placed an implantable stimulator-transponder (IST) on one side of the mouse's back and a two-channel 0.0-40 Hz bandwidth subcutaneous transmitter (SCT) on the other side. We observed optogenetic response the next day. With 0.3-ms 10-Hz flashes for 90 s, we saw circling. With 1-ms 10-Hz flashes for 90 s, we saw a seizure. With the lamp turned on continuously for 20 s we sometimes observed cortical spreading depressions (CSDs). To view a CSD, we need two electrodes, so we can see the depression spread from one location to another, and the sensor needs to be able to view direct-current (DC) signals. The electrodes contacts must be made by

crimping, to avoid artifacts generated by solder joints. Our staff and collaborators describe the surgery as arduous, but believe that with practice we can simplify the protocol to the point where a new group can be confident of successful implantation.

Figure: The Implantable Stimulator-Transponder (A3036C). Volume 0.75 ml, mass 1.3 g, battery capacity 10 mAhr. With a blue FCLED, provides 1-ms flashes of 5 mW blue light at 10 Hz for 67 hours. Sleep life is 1400 hrs. Shown with dummy-lamp that prevents short-circuit of stimulus leads and allows testing before implantation. Recharges through lamp leads in the same way as the MS-ISL.

We will perform the above three use-cases at Cornell University. After that, we will work with our contract research organization (CRO) partners to perform each experiment in ten animals. During these thirty implantations performed by professional animal surgeons, we will receive expert advice on how the surgeries can be simplified. Each experiment will last approximately four weeks, during which time we will be able to observe the rate of failure of our implants, as required by Aim 2. After completing the first round of tests, we will give ourselves the opportunity to alter the design of the MS-ISL to improve its reliability and ease of use. We will try the new devices at Cornell University and then have our CRO partners repeat each use-case with the new devices and ten more animals. We will codify, document, and video each of the three surgical procedures. Furthermore, these final thirty implantations will confirm the reliability of our devices