

Specific Aims

Seizures caused by the sudden electrical misfiring of nerves in the brain plague more than 50 million people worldwide. Epileptic seizures are often treated with a mixture of anticonvulsant drugs that have varying levels of success with patients. Implants that send electrical signals through nerves are sometimes used as a drastic measure. If both treatments are unsuccessful, doctors suggest the surgical removal of a bundle of nerves connecting the right and left hemispheres, called the corpus callosum. Patients that have undergone a corpus callosotomy often have speech irregularities, problems with cognition, or movement impediments. We aim to devise an approach to prevent seizures using a long term treatment while not impeding normal brain function. Our novel treatment uses an implantable device that detects irregular electrical signals characteristic of seizures and absorbs these signals to prevent their spread. The device will also keep a record of brain activity allowing for real time monitoring of neural signals by the patient's doctors. With this information, the treatment can be fine tuned to meet the specific needs of each patient.

Aim 1: Detect and respond to irregular brain activity

Hypothesis: Electrical impulses between the left and right hemispheres can be attenuated using passive current-activated microchips that redirect electrical signals.

Method: The neural interface system will be housed in a hermetically sealed titanium enclosure at the base of the skull. The device will interface with the brain through a silicon based multielectrode array (MEA) that is imperceptible to the recipient. Prior studies have shown silicon based brain implants to be capable of both receiving and transmitting neural signals. The proposed device will allow for amplification of neural signals with a 200x gain and transmit the amplified signals through a wireless data link to a receiver up to 1 meter away for signal storage. The proximity between the MEA and receiver reduces power requirements on the MEA, allowing for longer battery life. The recorded signals can be recovered and sent for further analysis.

Aim 2: Integrate synthetic brain tissue scaffold to promote axonal development

Hypothesis: Promotion of axonal regeneration would facilitate interactions between the corpus callosum and our implant.

Method: Prior studies have shown cell-seeded alginate hydrogel scaffolds can promote axon development. Alginate solution will be poured into anodized cylindrical aluminum molds for gelation and dehydrated with acetone to stabilize gel structure. After fabrication, scaffolds will be seeded with neural stem cells. The promotion of axon development in the area surrounding MEAs will increase interactions between the device and neurons. *In vitro* experiments will first be run to assess the extent of neural differentiation and electrical conductivity in the scaffold. This will be followed by *in vivo* implantation of the scaffold and MEA in order to assess the extent of neuron growth surrounding the MEA.

Aim 3: Test device and scaffold in rodent and canine models

Hypothesis: Implantation of the device into typical seizure animal models will provide accurate results for translation into humans.

Method: A rodent model with seizures caused by electroshocks will be used for preliminary in-vivo analysis of the device due to its low cost. The next model will involve a smaller population of rodents exposed to the more expensive kindling technique, one of the most common models used to test the efficacy of anticonvulsant drugs. The final test before clinical trials will involve the implantation of the device into canines with naturally occurring seizures due to accurate translation into humans. The data gathered through the animal trials will provide the constraints of what types of seizures the device can be used for, in addition to information regarding the safety of the device.

Significance

A sudden surge of electrical activity in the brain can cause seizures which affect how a person appears or acts for a short period of time. Epileptic seizures, or recurrent seizures, impact more than 2.5 million Americans and more than 50 million worldwide. Mention of the word seizure often conjures up images of wild convulsions and blackouts. While some who suffer from seizures experience these clearly visible symptoms, other symptoms include a racing heart, blurry vision, and lesser twitching or jerking movements known as tremors. These epileptic fits can severely lower a person's quality of life depending on the frequency with which they occur.

Epileptic seizures are often treated with a mixture of anticonvulsant drugs that have varying levels of success with patients. These drugs can also cause a variety of side effects such as dizziness, rash, and pain. Implants that send electrical signals through nerves are sometimes used in more severe cases as a last resort. If both aforementioned treatments are unsuccessful, another possible alternative involves the surgical removal of a bundle of nerves connecting the right and left hemispheres, called the corpus callosum. Patients that have undergone a corpus callosotomy are met with speech irregularities, problems with cognition, and movement impediments. An ideal alternative for these patients is one that includes long term prevention of seizures and does not impede normal brain function.

Our novel treatment uses an implantable device that detects irregular electrical signals characteristic of seizures and absorbs these signals to prevent their spread. By preventing irregular neural signals from reaching other areas of the brain, seizures can be avoided. Minimal maintenance will be required for the device requiring only one invasive procedure to implant the device. The device will also keep a record of brain activity so doctors can monitor neural signals in real time and use this information to develop improved treatments unique to each patient.

Innovation

1. Current brain implants emit pulses as a response to irregular brain signals that denote the start of a seizure; our device prevents seizures by inhibiting irregular neural signals from reaching other areas of the brain.

The RNS system developed by Neuropace offers a similar treatment for seizures to our proposed device. The RNS system is an implantable neural interface that emits a response to restore normal brain functions when it senses irregular brain activity that signals the beginning of a seizure. This is in contrast to our device which will emit standard pulses to prevent the irregular brain activity that occurs at the start of a seizure from the beginning. By not emitting any pulses, the impact on normal brain function will be minimized. Our proposed approach will have a lower seizure rate than the RNS system as well as a higher quality of life for recipients.

2. Enhancing connection between implant and neurons with scaffolds that promote axonal regeneration

Alginate based scaffolds will be used to enhance axon development near the MEA. This will serve the dual purpose of reducing possible disconnection of the implant from neurons and increase connectivity between the implant and neurons. Prior neural implants do not make use of methods to enhance connectivity between the implant itself and neural tissue. With greater interaction between the circuitry in the device and neurons, a more precise transmission of neural signals will be obtained, leading to the prevention of a wider range of seizures.

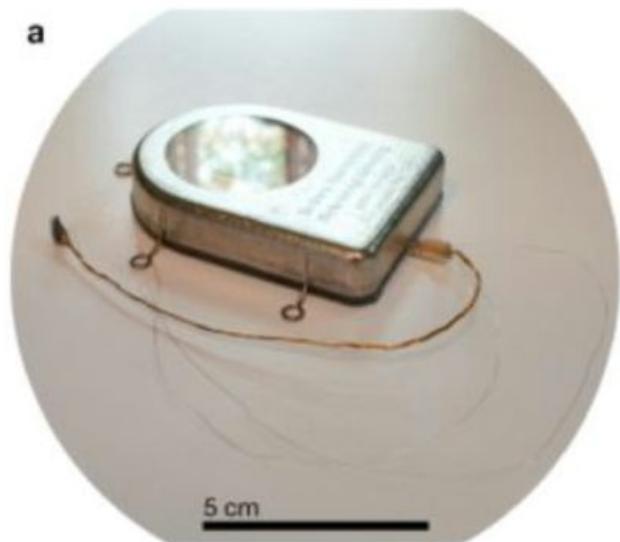


Figure 1 - Image of the transmitter inside the airtight hermetic enclosure [1].

Approach

Aim 1: Irregular brain activity detection and response

Hypothesis: Electrical impulses between the left and right hemispheres can be attenuated using passive current-activated microchips that redirect electrical signals.

Method: The neural interface system will be housed in a hermetically sealed titanium enclosure at the base of the skull. The device will interface with the brain through a silicon based multielectrode array (MEA) that is imperceptible to the recipient. Prior studies have shown silicon based brain implants to be capable of both receiving and transmitting neural signals. The proposed device will allow for amplification of neural signals with a 200x gain and transmit the amplified signals through a wireless data link to a receiver up to 1 meter away for signal storage. The proximity between the MEA and receiver reduces power requirements on the MEA, allowing for longer battery life. The recorded signals can be recovered and sent for further analysis.

1A. Create neural interface system from silicon multielectrode arrays to detect neural signals passing between hemispheres

Researchers have used multielectrode arrays (MEAs) in neural implants to serve as the interface between neurons and electronic circuits. A silicon based multielectrode array has been previously found to be both electrically stable and capable of capturing and emitting neural signals in swine and non-human primate animal models [1]. The power source, signal transmitter, and signal amplifier for our device (see Figure 1) will be enclosed in a 52 mm x 42 mm x 9 mm airtight titanium casing and implanted at the base of the skull. The device weighs a total of 44.9 grams [1]. Titanium has been selected for the enclosure as it is biologically inert and resistant to degradation. MEAs will be connected to the enclosure via electrical wiring cut to ensure tautness around the subject's brain. The MEAs will attach to both hemispheres of the brain near the original corpus callosum attachment points.

1B. Neural signal transmission and storage for further analysis

It is shown that signal transmission data can be successfully transmitted from the neural interface at 24 megabytes per second to a distance up to 1 meter away. An amplification board and a transmission board will be coupled to amplify neural signals with a 200x gain before transmission [2]. This gain allows for easy interpretation and analysis of the neural signals. The device will be wirelessly charged with the use of a medical grade 200 mAh lithium ion battery. It will take around 2 hours to fully charge and takes approximately 7 hours to deplete. Since the device is turned on immediately before the start of a seizure, the battery will remain functional for a longer period of time. The transmission board will have PCM or protection circuit module which protects the battery from over-discharging and over-charging. A portable radio frequency superheterodyne receiver will be used to store up to a week's worth of signals from the wireless subcutaneous implant [3]. The receiver chip will be implanted in a Fitbit or another wearable in order to ensure the device is always in close proximity to the patient. Stored data from the chip can be recovered and analyzed for future seizure prevention research.

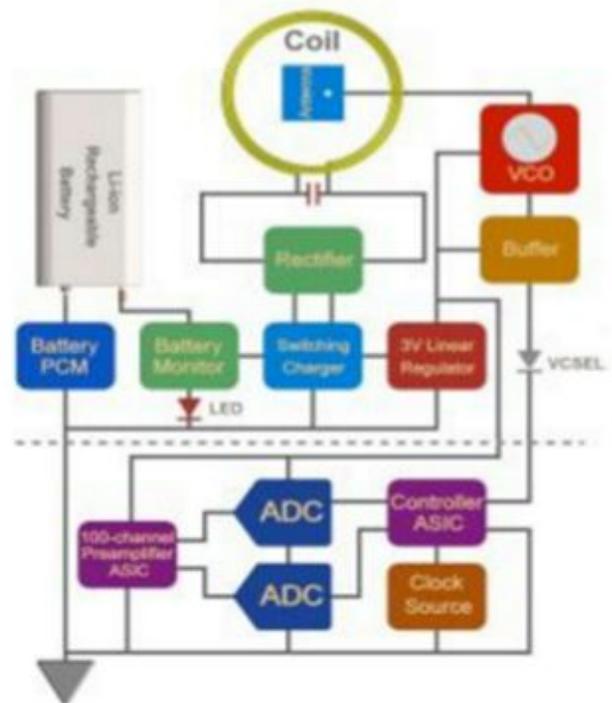


Figure 2 - Block diagram of a transmission board (on top) and an amplification board (on bottom) [1]. The lithium ion battery is part of the transmission board and is wirelessly charged.

1C. Seizure prevention through blocking of irregular neural signals from reaching opposite hemisphere

The MEA will monitor and record electrical activity within the brain. The beginning of most seizures can be characterized by a large spike in brain activity between 3-5 Hz [4]. A seizure can be avoided by inhibiting these irregularities from reaching other sections of the brain. When an irregular pulse begins, the device will activate and prevent the irregular signal from reaching the opposite hemisphere within the brain. The device will be in an off state during normal brain activity. By operating in a passive state, the power requirements of the device can be minimized.

Aim 2: Integrate synthetic brain tissue scaffold that promotes axonal development

Hypothesis: Promotion of axonal regeneration would facilitate interactions between the corpus callosum and our implant.

Method: Prior studies have shown cell-seeded alginate hydrogel scaffolds to promote axon development. Alginate solution will be poured into anodized cylindrical aluminum molds for gelation and dehydrated with acetone to stabilize gel structure. After fabrication, scaffolds will be seeded with neural stem cells. The promotion of axon development in the area surrounding MEAs will increase interactions between the device and neurons. *In vitro* experiments will first be run to assess the extent of neural differentiation and electrical conductivity in the scaffold. This will be followed by *in vivo* implantation of the scaffold and MEA in order to assess the extent of neuron growth surrounding the MEA.

2A. Test potential scaffolds for neuronal differentiation *in vitro*

Biomaterials can influence the chemical and physical environment of neural injuries, and can promote tissue regeneration and reduce secondary damage [6]. Cells are often sensitive to their mechanical and chemical environments, and the differentiation and axonal development of neurons has been shown to be highly dependent on nanoscale surface topography [10]. Previous literature suggests that alginate hydrogels can be used to promote axonal development in induced pluripotent stem cells [7, 8]. A hydrogel scaffold is desirable for neural implant applications because it closely mimics the gelatinous texture of the brain. Alginate is a suitable material for this application because researchers have great control over the porosity and morphology of alginate scaffolds by modulating the characteristics of microspheres during crosslinking [7]. A study by Gunther et al. suggested that smaller pore diameters of 41 μ m were ideal for murine peripheral nervous system axonal regeneration [8]. Alginate hydrogels containing different concentrations of brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) will be tested with neural stem cells for axonal development on a 2D gel. These growth factors are selected because they are commonly known to promote neuronal differentiation and axonal development from stem cells. The porosity of the scaffold will be modulated between 20 and 60 μ m to verify which pore diameter allows for optimal axonal development on our gel.

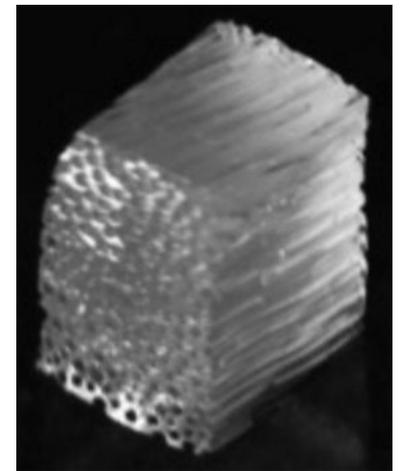


Figure 3 - Alginate scaffold Gunther et al. [8]. Note that the striated topography helps to facilitate axonal development.

Cells will be cultured on each experimental scaffold for a total of 10 days. Axonal development will be measured using luxol blue myelin stain [9] and assessed by length and number of axons developed in a given time period. Neuronal differentiation will be assessed by using qRT-PCR to measuring levels of various neuronal cell genetic markers as described in Yoo et al [10], including Tuj1, VMAT2, TH, DAT, and AADC. Electrophysiology testing will then be conducted. To summarize, after cells are placed in an electrolyte solution, a current-clamp mode is established using a borosilicate coated glass micropipette and suction. The cells are then exposed to step changes in current, and the release of potassium and sodium ions is

recorded, which is correlated to the release of the ions due to the opening of ion channels when the cell reaches its action potential. We would expect to see an action potential of approximately 70 mV in a human neuron.

2B. Test neurons for electrical interface with implant *in vitro*

The second round of electrophysiology testing will be conducted on cells which are grown on a scaffold interfacing with a neuron. The objective of this experiment is to verify that once the cells are grown into our scaffold they are able to contact and conduct electric signals to the implant electrode. To accomplish this, cells will first be cultured in scaffolds connected to the implant. A sharp electrode micropipette will then be inserted into cells on each side of the electrode, and current step changes will be introduced to both sides, and the current across the electrode will be recorded. Small voltage ranges of approximately 1mV will be used. This experiment will also be conducted with a multi-unit electrode patch on each end of the scaffold, allowing us to stimulate and measure the conductivity of the entire network of cells.

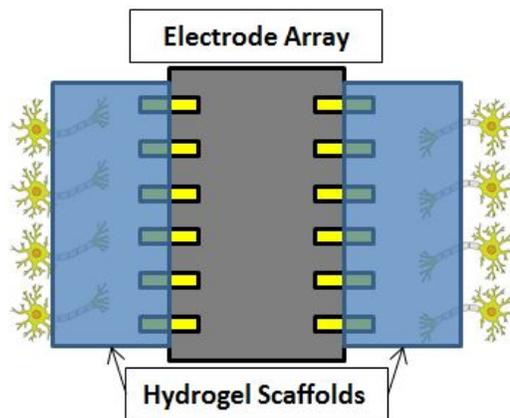


Figure 4 - Depiction of innervated electrode array-scaffold system

2C. Test scaffold for neuronal regeneration *in vivo*

Critical to the function of our device is that neurons are able to successfully integrate into the cell scaffold after implantation. Prior studies have demonstrated in murine models that regeneration of neural tissue (neurons, epithelial cells, and glial cells) can occur in channeled polymeric scaffolds and channeled alginate scaffolds [8, 12]. The corpus callosum of mice will be severed using a scalpel and then replaced with a channeled alginate scaffold which is grown on both sides the electrode microarray. The implants will be harvested from the mice after 1, 5, and 10 week intervals. The scaffolds will be imaged to quantify innervation using Luxol blue myelin staining and conductivity across the array will be tested as described in 2B.

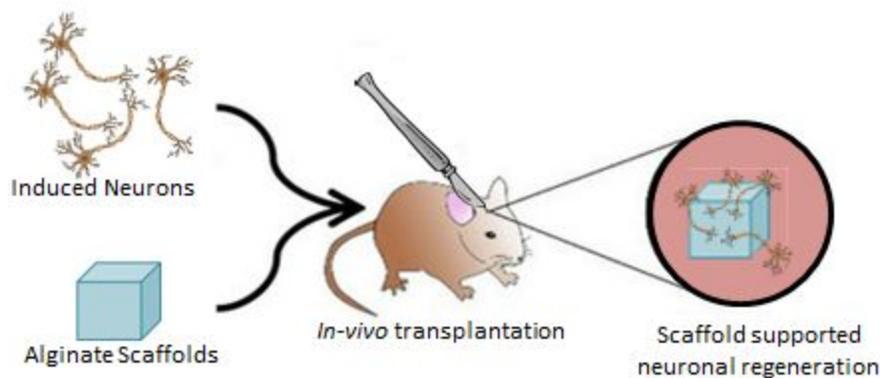


Figure 5 - Testing scaffold for neuronal regeneration *in vivo* (Adapted from Carlson et al [13])

Aim 3: Implantation of the device and scaffold into animal model

Hypothesis: Implantation of the device into typical seizure animal models will provide accurate results for translation into humans.

Method: A rodent model with seizures caused by electroshocks will be used for preliminary in-vivo analysis of the device due to its low cost. The next model will involve a smaller population of rodents exposed to the more expensive kindling technique, one of the most common models used to test the efficacy of anticonvulsant drugs. The final test before clinical trials will involve the implantation of the device into canines with naturally occurring seizures due to accurate translation into humans. The data gathered through the animal trials will provide the constraints of what types of seizures the device can be used for, in addition to information regarding the safety of the device.

3A. Using electroshock rodent model for initial testing

A rodent model with seizures caused by electroshocks (ES) will be used for preliminary in-vivo analysis of the device due to its low cost. In an electroshock model, a single electrical stimulation induces a seizure in a test subject. Seizures are induced via ES in one of two ways: minimal ES or maximal ES [14]. Minimal ES will be used for device testing, as maximal ES has been known to overpower existing models already implemented, successfully, to protect against certain types of seizures in epileptic patients.

3B. Using kindling technique rodent model for secondary testing

After functionality has been demonstrated in the electroshock rodent model, the next model will involve a smaller population of rodents exposed to the more expensive kindling technique. The kindling technique is one of the most common models used to test the efficacy of anticonvulsant drugs. In kindling, rodents are repeatedly exposed to short bursts of electroshocks. Each subsequent time the rodent is stimulated, the effect of the electroshock is increasingly apparent (seen in Figure 6). The duration between shocks and their intensity is varied to test the effectiveness of the treatment in a variety of conditions [14]. This provides data on the efficacy of the device for a variety of seizures and allows discovery of the device's working parameters. The study will last a minimum of 12 weeks with shocks administered once daily. Regular scans of the brain will be analyzed in addition to behavioral observations following each shock. The long-term study also provides data on the long-term stability of the device in-vivo.

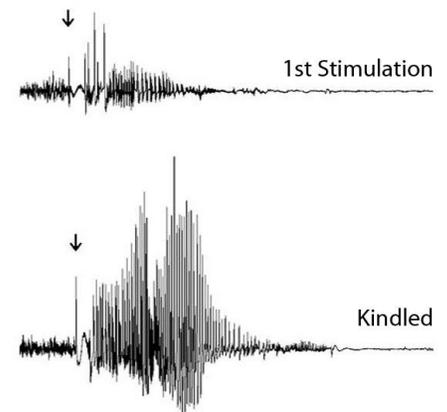


Figure 6: Electrical activity before and after application of kindling technique. Image from: <http://link.springer.com/article/10.1186/1471-2202-14-82>

3C. Using canine seizure model for final preclinical testing

The final test before clinical trials will involve the implantation of the device into canines from with naturally occurring seizures. Canines have been selected as a final test because many breeds suffer from epilepsy, and parallels have been drawn between human and canine epilepsy [15]. Various breeds with natural seizures will provide a deeper insight into the effects of size, genetic, and other variations on our device. Success in these trials indicates device flexibility to accommodate variations between patients. Canines will only be used as a final test subject, rather than throughout, due to the increased cost and ethical problems that go along testing products on canines. However, the benefit of testing on canines will outweigh this cost as long term effects of treatment can be examined [15].

3D. Data analysis for translation into clinical trials

The three animal models are sufficient to enter human clinical trials as the FDA requires testing of treatments on a minimum of 2 proven animal models before human testing. The data gathered through the animal trials will provide the constraints of what types of seizures the device can be used for, in addition to short term and long term safety concerns. Safety in terms of the devices integration into the body is one of the largest issues for

successful implantation as small infections or simple inflammation can be observed in the animal models. The data obtained in the preclinical testing of multiple test subjects will provide insight into the responses of the device under specific neural conditions and symptoms for a given patient.

Potential Pitfalls and Solutions

A major concern with this project is that the device does not necessarily mimic the mechanical properties of brain tissue. The implant consists of a silicon circuit embedded in an alginate hydrogel scaffold. While the alginate scaffold might be expected to have similar mechanical properties to the surrounding gelatinous brain tissues, the silicon circuit could be expected to be much more stiff and dense than the surrounding tissues. Because of a lack of sturdy or inert tissues in the brain to anchor this implant, the weight of the implant becomes an issue. Without proper anchoring, sudden movements of the head may cause displacement of the device and subsequent damage to the surrounding brain tissue. Further exacerbating the issue of weight is the fact that the corpus callosum has a mean area of 655 mm² in men and 657 mm² in women [16]. To overcome this obstacle, the signal transmitter and the amplifier will be placed at the base of the skull.

Another concern is the potential for damage to local neurons and neural support cells caused during the implantation of the device. It is possible to have microhemorrhages occur during surgery which have the potential to cause severe complications in recovery. Additionally, any maintenance of the device involves invasive surgery to cut open the skull and access the device [17]. To mitigate the need for maintenance such as battery replacement, the signal attenuation circuit and transmitter will only be activated when the signal reaches specific frequencies indicative of the start of a seizure. The need for battery replacement is minimized by using a wirelessly rechargeable lithium ion battery.

Immune responses could also produce complications following the implantation of our device. Alginate is shown to be non-immunogenic, and is widely used in the food industry, as well as in a variety of medications. However, if the alginate is not pure, protein contaminants may cause immunogenicity [18]. Nerve growth factor is a native peptide in humans, and is not expected to cause immunogenicity, although contaminants from manufacturing may cause immunogenic reactions. Clinical grade purities of these peptides would be required to mitigate the risk of immune response.

Summary of Goals and Innovation

We propose the development and implantation of a bioactive hydrogel coated microelectrode array into the corpus callosum of canine subjects. A hermetically sealed titanium encapsulated neural interface system will be placed at the base of the skull, and will attenuate the signal through the microelectrode array when currents through reach levels indicative of seizure. This will prevent the spread of the seizure to the opposite hemisphere of the brain, thus allowing the subject to maintain some level of cognitive function and motor control during a seizure. The implant will be coupled with two printed circuit boards: an amplification board to amplify the neuron signals and a transmission board to transmit the signals to a receiver. The receiver will be implanted in a Fitbit like wearable to ensure close proximity and data storage. The device will be wirelessly charged with the help of a medical grade lithium ion battery. Silicon MEAs will be encapsulated within an alginate hydrogel scaffold containing nerve growth factor and hyaluronic acid to promote axonal development and integration of surrounded severed neurons into the implant. Following in-vitro development, sample scaffolds will be placed into murine brains to test for neuronal development. Miniaturized microelectrode arrays will be placed into murine corpus callosum to examine functionality using electroshock testing. After functionality is verified, the kindling method will be applied for 12 weeks to mice to determine the efficacy of the device for a variety of different seizure conditions, over a relative long term, in-vivo. Final in-vivo testing will be conducted in epilepsy prone canine subjects. These subjects are good models for human epilepsy because they have naturally occurring epilepsy, as opposed to that induced by electrical shocks, and they have larger brains which are more comparable to those of humans. Upon success of this research, we will have developed an alternative treatment for split-brain surgery for epilepsy patients.

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