Design of Brain Implants for Long-Term Biocompatibility and Functionality

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Abstract

Implanted brain electrodes have significant potential to alleviate clinical symptoms associated with neurological disorders and traumatic injury as well as provide mobility solutions for those who suffer from paralysis. These electrodes are chronically implanted into the patient's cortical tissue into order to record neuronal signaling and stimulate certain sections of the brain. However, these implants are susceptible to chronic inflammation, which severely degrades their recording and stimulative capabilities, reducing the efficacy of the treatments after a certain period of time. We aim to reduce the chronic inflammation utilizing three modifications to the design of these brain implants: (1) Construction of a softer polymer-coated electrode with PEDOT doped with PSS to alleviate the mechanical trauma on the surrounding tissue; (2) Incorporation of a drug-eluting polymer with interleukin-1 antagonist to suppress the immune response; (3) Protein-coat the implant with RGD to enhance cell-implant connectivity, maximizing the signal transfer.

To quantify the degree of inflammation change due to these design modifications, *in vitro* experiments will first be conducted. These will involve direct contact of microglia cells with the implant, in which release of nitric oxide will indicate an undesirable inflammatory response of activated microglia. The surface of the implant will be visualized with scanning electron microscopy to investigate cell integration with the implant. *In Vivo* experiments in rodent models will be used to understand systemic and local inflammatory responses to the implant at regular time intervals. The tissue response be quantified via various staining methods to characterize the concentration of cytokines and degree of glial scarring, two strong indicators of a chronic inflammatory response. We predict that the design modifications to the brain implant should reduce cytokine concentration and glial scarring, thereby demonstrating proof of concept for reducing chronic inflammation.

Objective, Hypothesis and Aims

Neurological disorders and trauma are a severe burden on the patients' quality of life as well as the healthcare system. Brain implants and their use in neuroprosthetics promise to alleviate many of the symptoms of neurological disorders, but their long-term effectiveness is limited by chronic inflammation. We aim to design a brain implant, which reduces chronic inflammation and increases the functional longevity in order to maximize therapeutic treatment. **We hypothesize that a BCI polymer coating consisting of poly-3,4-ethylene dioxythiophene (PEDOT), polystyrene sulfonate (PSS), and controlled release of interleukin(IL)-1 antagonist will significantly reduce the body's chronic inflammatory response and increase longevity and functionality of a BCI implant.**

Specific Aim 1: Construct a polymer-coated electrode with optimum electrical properties. We will coat the surface of gold electrodes with PEDOT and dope this layer with PSS to enhance the electrical conductivity of the BCI implant. In order to mimic stresses typical of surgical implantation, the electrode will be mechanically tested to determine the yield strength. Next, we will characterize the electrical activity of *in vitro* neurons, glial cells, and astrocytes in response to stimulation from the different electrodes. Long-term testing (up to 1 year) will be undertaken to examine the electrical and mechanical stability over time and to verify that a polymer-coated BCI implant is more effective than traditional non-coated BCI electrodes.

Specific Aim 2: Characterize the diffusion and time-dependent concentration of slow-release IL-1 antagonist from polymer coating. We will place the device in PBS or microglial cell culture medium, which will be changed every 24 hr. The amount of IL-1 antagonist released every 24 hour will be determined by the difference between UV absorptions at 215 and 225 nm with a microplate reader. We will also examine the morphology of the electrode surface post-release using SEM and profilometry.

Specific Aim 3: Conduct *in vivo* experiments to quantify inflammation. Rodent models will be used to assess inflammation and glial scarring in response to the brain implant modified by Aims 1 and 2. Chronic inflammation will be quantified by astrocyte reactivity and cytokine concentrations. Neuronal slices will be obtained after removal of implant to stain for astrocytes and cytokines. A statistically significant reduction in long-term inflammation of our electrode compared to the standard non-coated electrodes will establish the success of Specific Aim 3 (and by inference Aims 1 and 2). This would correlate to longer-term functionality of the implant, which would be further validated in animal models or humans.

If these aims are successful, further validation in a larger-scale study with rodent models will be conducted followed by a pilot study in non-human primate models to assess long-term functionality of the brain-computer interface in its intended task.

Background and Significance

Current Statistics of Neurological Disorders

Neurological disorders, traumatic brain injury (TBI), and spinal cord injury can affect motor coordination, sensory perception (i.e. auditory and visual), and cognitive functioning. These disorders affect approximately 55 million[1,2] people in the United States, exceeding $500 billion in healthcare costs. The high cost is not due only to the treatments but also to the cost of the caregivers and lost productivity of patients[3].

Brain-computer interfaces (BCI) in the form of brain implants, as shown in Figure 1, attempt to offer a treatment solution by generating new signal pathways to artificially restore cognitive, sensory, and motor functioning.

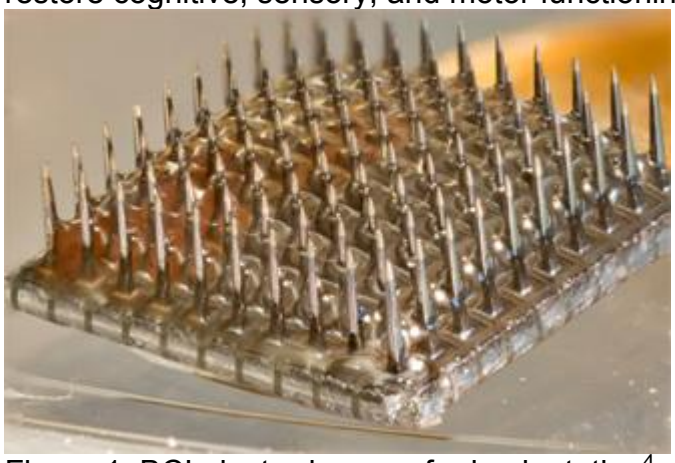


Figure 1. BCI electrode array for implantation[4]

Applications of BCI

One of the current uses of BCIs is in deep-brain stimulation (DBS) in application for Parkinson's disease. Electrical stimulation of the motor cortex can alleviate many of the motion-oriented symptoms such as tremors, freezing of gait, and dyskinesias.[19] Although the exact mechanism is still unknown, one prevailing theory is that the stimulation inhibits abnormal neuronal activity by disrupting signal transmission of targeted areas, such as the subthalamic nucleus[5]. BCI's also hold promise in application toward victims of paralysis. Whether the cause is stroke, injury, or neurodegenerative disorders like Multiple Sclerosis, an implanted BCI translates neurological signals from the brain to the motor neurons in the muscles, allowing patients to bypass damaged portions of the nervous system and still maintain some degree of mobility[6].

However, implantations made from foreign materials generally cause issues with biocompatibility. Chronic inflammation can cause degradation in signal quality over time. Recording from such microelectrodes for BCI systems results in <40% of the electrodes remaining functional for prolonged periods of time[7]. One of the reasons for this poor performance is the degree of inflammation and trauma within the tissue when the implant is surgically inserted[8]. Understanding the biological mechanisms within the brain

and how to limit immune responses can potentially prolong the life of implanted electrodes, leading to improved BCIs in clinical application.

Physiology of Brain and Foreign Body Response

A permanent implant, such as a BCI microchip, typically induces an immune response, making chronic inflammation a key concern. The first encounter of cortical tissue with a needle-like electrode is violent, severing capillaries, the extracellular matrix, glial and neuronal cell processes. The mechanical trauma initiates the CNS wound healing response, and disruption of blood vessels releases erythrocytes, activating platelets and clotting factors. In terms of long-term inflammation, we see a chronic foreign body reaction once the acute inflammatory response declines. This reaction is characterized by reactive astrocytes, and activated microglia, which forms a glial scar. Tissue scarring is associated with loss of signal acquisition[9]. The main protein involved in inflammatory response is interleukin-1 (IL-1), which is a cytokine that participates in regulating immune responses[11]. This biocompatibility issue over time starting from IL-1 to glial scarring is what prevents long-term functionality of microelectrodes. Figure 2 shows the long-term effect of the electrode implant on the surrounding cellular network and how the reduced density of the cellular network lowers signal quality.

Additionally, this inflammation causes significant variability from subject to subject on long-term stability of the signal despite improvements in instrumentation design of the BCI's.[8] During a tissue response in the brain, microglia (macrophage analogs of the brain) secrete proteolytic enzymes at the site of inflammation[8]. Since microglia have multiple pathways of secretion and is responsible for both neuronal growth and cell death, it is difficult to ascertain their specific response to the implant. It is possible that since implants do not degrade as most foreign biological matter, the microglia continually try to phagocytose the device, resulting in a continuous release of toxic substances.[8]

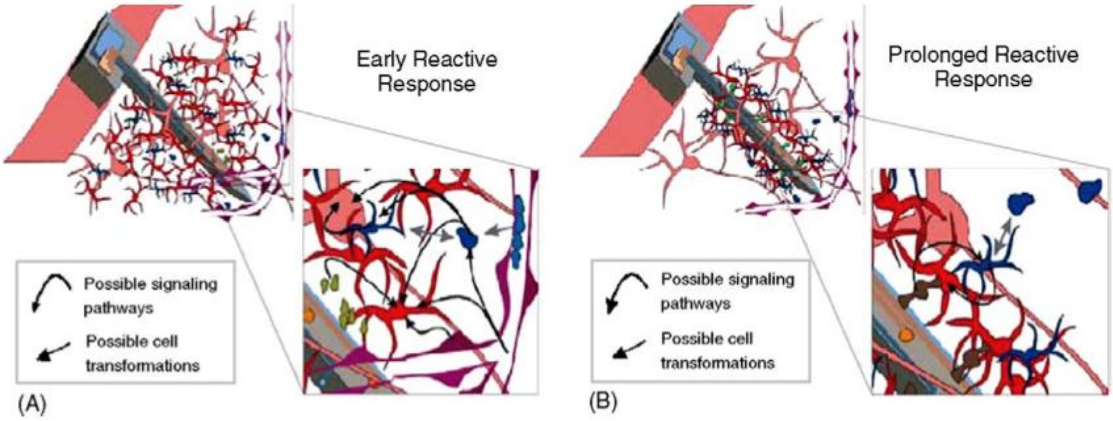


Figure 2. Initial cellular response on electrode array can be seen in 'A'. A fewer concentration of cells around the array in 'B' can be seen in the prolonged response.[7]

These consequences also arise due to metal corrosion and degradation of the plastic insulation layer, which are prominent issues apparent within 8 hours after an electrode is immersed in an electrolytic solution. The unavoidable corrosion that occurs is

particularly undesirable because metal ions in the brain can elicit further immune response, among other deleterious effects associated with corrosion of the electrode material[10].

The severing of blood vessels during device desertion is another event that leads to inflammation. Implanted neural prosthetic devices will need to address many concerns in order to be considered a viable biomaterial for long-term clinical applications. Such concerns include specific immune response of the patient to the implant material, and the frequency of implant replacement. A key step to stem the tissue response of the CNS is implanted “needle” electrodes, which minimizes the trauma[8].

Current Strategies for Dealing with Biocompatibility for BCIs

It is also critical to understand how the material choice affects the signal transmission between the brain and the prosthesis. Due to the neuronal plasticity and consequently the continually adapting signal transduction, a biomaterial that can integrate well with the brain would help long-term viability and functionality. It has been shown that the material shape, size, and texture have little effect on glial scarring, though it still affects surgical insertion procedures[7].

Current state-of-the-art strategies for improving brain-electrode interface use passive protein coating to form a layer between the electrode and the immune system[11]. The brain immune reaction to chronic electrodes used the immune suppressing protein interleukin-1 (IL-1) receptor antagonist. Silicon electrode arrays were used and then protein-coated, leading to a significant reduction in amount of glial scarring.

Electrodes modified with conducting polymer coatings suggest an approach for improving the neural tissue-electrode interface. Nevertheless, there are still challenges in conducting polymers that include poor electroactive stability and mechanical properties[12].

A strategy to overcome loss of conductance uses a large number of electrodes to overcome the time limit that electrodes have in the brain. However, this is not applicable for deeper brain sites due to the excessive damage it causes. Therefore, it would be beneficial to develop a solution that not only maintains conductance over time, but also limits inflammation in the brain. Additionally, since signal clarity is dependent on proximity to the neural network, Kam et. al has shown that it can be beneficial to increase cell attachment to the surface of the electrode[13].

Proposed Technology Aims to Overcome Disadvantages

Given all the considerations detailed above, we propose to investigate the use of electrically conductive polymers for longer survival time of an implant. A low-resistance polymer coating on the metal electrodes may reduce the rate of corrosion and enable better integration between an implant and the native tissue. Such polymers include poly-3,4-ethylene dioxythiophene (PEDOT). Their electrical properties have been

documented, although their long-term interactions with live tissue still need to be established[12].

Long-term insertion of the implant causes glial scarring in which the scar encapsulates the electrode surface, preventing signal transduction between the brain implant and the surrounding neurons. While some have coated the electrode implant with anti-inflammatory agents[11], a polymer with a slow release mechanism would possibly be more effective at preventing chronic inflammation not only at the site of the implant but also the surrounding tissue. In addition, Arginylglycylaspartic acid (RGD) peptide, implicated in cell-attachment to mimic *in vivo* conditions, would increase cell adhesion and reduce the distance between the electrode implant and the cellular network, increasing long-term signal and stimulation efficacy for the BCI implant.

We propose to characterize a drug-eluting polymer coating implant for its long-term conductivity and ability to reduce inflammation in *in vitro* and *in vivo* models. We predict the the combination of a softer polymer material, active anti-inflammatory agents, and protein-coated surfaces will significantly reduce the body's chronic inflammatory response and increase longevity and functionality of the BCI implant.

Research Design and Methods

In Vitro Testing

The BCI implant will consist of a standard microelectrode array coated with a thin layer of PEDOT/PSS. This polymer layer will elute IL-1 antagonist at a controlled rate. Material properties such as bulk conductivity and diffusion characteristics will then be determined. In vitro tests will be carried out on rodent microglial cell cultures to analyze cytotoxicity, nitric oxide (NO) production, and other cell responses to the BCI implant. All methods are carried out with respect to the Environmental, Health, & Safety (EH&S) standards; no additional requirements are needed. These methods are documented below.

Fabrication and Characterization of PEDOT-coated microelectrode array:
Standard non-coated gold microelectrode arrays can be obtained as is (Brain Gate, braingate2.org) since the fabrication is a well-established process with the conductive layer residing in between two insulating dielectric layers of silicon nitride and silicon dioxide.[18] Three types of arrays will be constructed: (1) the standard non-coated arrays (control), (2) PEDOT/PSS/RGD coating (PEDOT), and (3) PEDOT/PSS/RGD coating with IL-1 antagonist surface treatment (PEDOT + IL-1).

PEDOT polymerization and polystyrene sulfonate (PSS) doping on the electrode surface will be accomplished simultaneously through electrochemical polymerization of EDOT monomer in the presence of 0.1 M PSSNa solution, in which an applied current to the solution will cause a thin deposition layer (<5 um) of PEDOT onto the BCI. To enhance better integration with the cell-BCI interface, adhesion biomolecules such as fibronectin and RGD peptide may be incorporated into the PEDOT/PSS coating by introducing these species with a concentration of 10 mg/mL during the electrochemical polymerization.[17] After the polymerization, IL-1 antagonist (500 ug/mL) will be evaporated on the surface of the PEDOT/PSS coating.[14]

Post-fabrication, the conductivity will be determined by measuring the impedance of the BCI implant. This will be accomplished with the 4-point method, in which the ratio of the change in potential to a constant driving current will be determined. From these measurements, the resistivity and hence conductivity will be known. A conductivity of 10^3 S/cm will be the minimum level for the BCI to be considered an electrical conductor.[16] In addition, the electrode will be mechanically tested for yield strength using compression. Prior to implantation, all BCI arrays will be sterilized by UV light exposure for a minimum of 30 minutes.[21]

Cell Cultures of Primary Microglia:

Microglial cells will be obtained using a procedure by Giulian and Baker. In additional, mixed glial cells will be obtained from the cerebral hemispheres of a Sprague Dawley rat[15]. The cells will be placed in 75 cm^2 poly-L-lysine coated tissue culture flasks in the culture medium that consists of DMEM-F12 media supplemented with 20% fetal bovine serum (FBS) and 1% penicillin/streptomycin.[15] After 7-10 days, flasks will be lightly

shaken to release microglial cells into the media supernatant. These floating microglia will be subsequently centrifuged into a pellet and re-suspended in 10% FBS-supplemented DMEM-F12 medium. The cells will then be seeded in a 96-well culture plate at a density of 2 X 10^4 cells per well.[14]

Twenty-four hours after seeding, the fabricated microelectrode arrays will be placed in direct contact with the microglial cell culture, for three types of cell cultures: (1) direct contact with the control electrode array, (2) with the PEDOT array, and (3) with the PEDOT + IL-1 array. The electrical activity can be assessed by energizing the microelectrode arrays and recording the cells' response with a neural acquisition board (Intan technologies). Inflammatory responses of the microglia will be assessed by measuring the level of nitric oxide (NO) production.

Analyze Nitric Oxide Production:
NO production indicates activation of microglia involved in an undesired inflammatory response. NO levels in the microglia culture will be measured with a commercially available electrochemical sensor (Thermo Fisher). Oxygen consumption will also be determined with a Clark electrode to assess the degree of cellular respiration inhibited by NO over time.[25]

Drug Loading and Diffusion Characterization:
The amount of drug loading in the PEDOT polymer coating will be determined by subtracting the unincorporated IL-1 antagonist from the total amount added to the solution during the electrochemical polymerization. This will lead to the in vitro IL-1 antagonist release assay. PEDOT polymer layers loaded with IL-1 antagonist will be incubated at 37^{o}C in PBS for quantification of IL-1 antagonist release, or in a microglial cell culture medium for bioactivity analysis with cell cultures. The release medium for the PBS and cell cultures will be changed every 24 hrs, and the amount of drug released every 24 hrs will be determined by subtraction of the UV absorption at 215 and 225 nm measured by a microplate reader.[14] The IL-1 antagonist containing microglial cell culture medium collected every 24 hr will be stored at -20^{o}C and then analyzed. Then the effects on the cell culture will be analyzed to understand the release kinetics.

In order to understand the release kinetics, diffusion experiments will be conducted similar to Varshosaz and Hajian.[22] The diffusion coefficient D_0 of the IL-1 antagonist can be determined with the Stokes-Einstein equation D0 = RT/6πηr NA, where R is ideal gas constant, T is absolute temperature (K), r is molecular radius (cm), η is the cell culture medium viscosity (g/cm-s), and NA is Avogadro's number. These diffusion experiments will be analyzed for pH 5 to 8 and temperature 35 C to 40 C to capture the range of possible in vivo conditions.

Surface Analysis by Scanning Electron Microscopy (SEM) and Profilometry:
The thickness of the electrode PEDOT coatings will be analyzed every 5 days for 30 days using a digital caliper (Caliper Corp). Measurements will be taken on the edges and the middle of each sample in order to assess any changes in bulk properties due to the release of IL-1 from the PEDOT coating. The decrease in thickness is expected to be less than 1 um; any more may indicate undesired degradation of the PEDOT layer.

Following the 30 days, the interface between the microglia cell culture and electrode surface will be imaged with Scanning Electron Microscopy (SEM). The electrodes will be taken out of the cell culture solution and vacuum coated with a thin gold or platinum film before analysis under a LVEM5 Multimode Electron Microscope (Delong LV-EM).

In Vivo **Testing**

The inflammatory response to the BCI implants will be tested using 24 rodent models with examination of inflammation occurring 1, 3, 6, and 12 weeks after implantation. Two rodents will be used for each of the time intervals for each of the following implants: (1) the standard non-coated arrays (control), (2) PEDOT/PSS/RGD coating (PEDOT), and (3) PEDOT/PSS/RGD coating with IL-1 antagonist surface treatment (PEDOT + IL-1).

Implantation

The BCI will be implanted into the right cerebral cortical hemisphere of Adult Wistar and Sprague Dawley male rats.[7] When handling animals, precautions should be taken to wear appropriate PPE and other biosafety precaution to avoid contamination. To allow accurate placement of the implants, the rat will be placed in a stereotaxic holder along with the forceps. The head will be shaved and the skin will be disinfected with isopropanol and butadiene.[21]

A hydraulic micropositioner will be used to drive the implant into the correct portion of the brain. The rat will be anesthetized via intramuscular injection of a combination of 40 mg/kg Ketamine, 8.0 mg/kg Xylazine, and 4.0 mg/kg Acepromazine anesthetized[24]. A midline incision in the skin will be made to drill a 2-mm craniotomy into the skull 3 mm lateral and 3 mm distal from the Bregma, an anatomical part of the skull. The chip will be implanted at a depth of 2 mm at a rate of 2 mm/s. The scalp incision will be closed via 5/0 sutures.[7,21]

Tissue Analysis

Immunochemical and pathological techniques will be used to examine the cranial tissue 1, 3, 6, and 12 weeks after implantation. For each of those time points, the implant and the surrounding tissue of six animals will be removed. The brain tissue will be post-fixed in 4% paraformaldehyde and stored, along with the device, in Hepes-buffered Hanks saline solution (HBHS) with sodium azide.[21] The tissue will be sectioned into 100-micrometer thick slices and stained for glial fibrillary acidic protein (GFAP). GFAP staining can determine the proliferation of astrocytes which is a significant component of the glial scar.[7]

The tissue slices will be treated with 5% sodium borohydride, mixed with 0.2% Triton X-100 in HBHS, and incubated with 0.1% bovine serum albumin (BSA). A GFAP antibody (Sigma, 1:100 dilution) will be utilized.[7]

The GFAP-stained tissue slices will be imaged with a confocal laser scanning microscope and examined for evidence of astrocyte reactivity and glial scarring, two strong indicators of inflammatory responses and degraded signal quality. The thickness

of the glial scar and the distance the scar is from the implant both indicate the severity of encapsulation.[7] The target goal would be minimal thickness and distance.

To quantify cytokine release, the electrodes will be placed in a tissue culture dish with 300 microliters of DMEM/F12 and 25 micrograms/milliliter gentamicin. After 24 hours of storage at 80 degrees celsius, the concentration of monocyte chemotactic protein-1 (MCP-1) will be determined via enzyme-linked immunosorbent assay (ELISA). An increased concentration of monocytes would correlate to an increase in inflammatory response in the rat model.[21]

Data Analysis

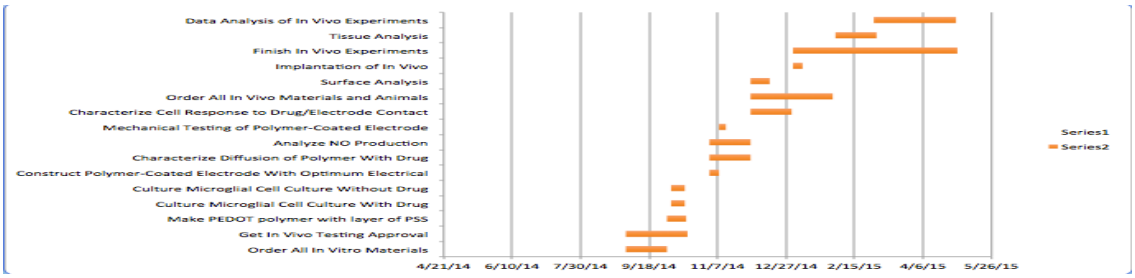
Data will be shown as the average value +/- the standard error of the mean (S.E.M). Results from all experiments will be analyzed with the Student t-test. To test the variance of each sample group Fisher's F test will be used with a $p < 0.05$ to indicate statistical significance. Different sample groups will be analyzed with ANOVA with a similar critical value $p < 0.05$. A significantly lower level of NO production for the PEDOT + IL-1 cell culture compared to the PEDOT and control will indicate the effectiveness of the IL-1 antagonist in suppressing the microglia inflammatory response. A significant difference in the concentration of cytokines and astrocytes as characterized by a p-value of < 0.05 will determine whether the design modifications made will correspond to a reduced inflammatory response and increased long-term functionality.

Limitations and Potential Results

Some potential limitations to our methods are the use of rat models, since the model may not be a clear indicator of human responses to the implant after implantation. This would also be the first time a conductive polymer is surface treated with a drug to perform controlled release and suppression of chronic inflammation. It remains to be seen whether the drug will affect the overall aim of maintaining conductivity of the electrode in vivo. Other drugs besides IL-1 antagonist that inhibit inflammatory responses may be investigated for future studies, as there have also been numerous experiments done that use other polymers to conduct drug release.

However, this is a pilot study to validate the potential that this proposed system has with loaded IL-1 antagonist. This construct can potentially lead to a longer life span of BCI implants and reduced inflammation, all while maintaining biocompatibility with the surrounding brain tissue.

Proposed Timeline



Vertebrate Animals

Wistar and Sprague Dawley rats will be used because of their availability, extensive use in medical research, and easiness to handle. Two animals will be used for each time interval as two is the minimum number needed to establish some statistical significance. All procedures involving animals will be taken to follow the *Guide for the Care and Use of Laboratory Animals* by the National Research Council.[23] Rats should be kept in environments with the temperature ranging from 20-26 degrees Celsius and humidity will be kept at an acceptable (30% - 70%) and without significant fluctuations.[23] Ventilation and preserving air quality are also incredibly important, especially if the animal is in an isolated setting. Typically, 10 to 15 fresh air changes per hour is conducted.[23] Recycled air should not be used because of the increased risk of spreading airborne pathogens. A time controlled lighting system should be used to ensure a nocturnal cycle.The intensity of light should be 325 lux at a 1 m distance to prevent phototoxic retinopathy.[23] Noise levels and vibration will be minimized. Other factors that will be taken into account are diet, nesting environment, and sanitation.[23]

Animals will be procured by approval of IACUC. The animals will be transported in accordance to the Animal Welfare Regulations as set by the USDA. Animal biosecurity measures will be taken to ensure to contain, prevent, and eradicate infections. Surgical techniques such gentle handling, minimal incisions, aseptic procedures, appropriate anesthesia, and sterilization will be used to minimize pain and distress of the animal. The anesthesia used will be a combination of 40 mg/kg Ketamine, 8.0 mg/kg Xylazine, and 4.0 mg/kg Acepromazine via intramuscular injection as according to IACUC regulation[24]. This anesthesia was used because of its efficacy and prior use in craniotomies for the purpose of brain implants in rodents.[21] Induction agents, while sometimes provide additional comfort to the rodent under anesthesia, will not be used because of the additional stress caused by a second injection.[24]

In summary, a number of steps will be taken in order to ensure that discomfort during car and the surgical procedure will be minimized.

Human Subjects

There will not be any human subjects in this study.

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