Master's Thesis
석사 학위논문

Optogenetic Neural Interface for Non-Human Primates

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Department of
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Co-advisor: Professor Jae-Woong Jeong

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A thesis submitted to the faculty of DGIST in partial fulfillment of the requirements for the degree of Master of Science in the Department of Energy Science & Engineering. The study was conducted in accordance with Code of Research Ethics[1]

12. 28. 2020

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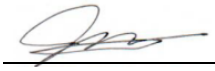
Professor Jae-Woong Jeong  (signature)
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Optogenetic Neural Interface for Non-Human Primates

Saehyuck Oh

Accepted in partial fulfillment of the requirements for the degree of Master of Science.

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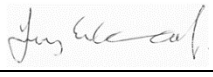
Committee Member  (signature)
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ABSTRACT

The non-human primate brain, which is similar to the human brain, plays a critical role in understanding of human brain circuits related to incurable diseases. Among the methods for studying the brain, optogenetics is widely used as a powerful way to reveal brain circuits by genetically coding certain parts of the brain and simultaneously performing optical brain stimulation and neural signal measurement. Although primate optogenetics in a free-moving state is important for complex brain related behavioral research, most of them were performed only under anesthesia due to engineering difficulties such as probe insertion method, full device implantation, wireless communication. To overcome the challenges, we fabricated a sucrose-coated long length flexible neural probe for insertion into the deep brain area with minimal invasion and also applied an optimized wireless communication protocol for fully implant operation. Finally, we developed a fully implantable wireless optogenetic neural interface for advanced research application such as behavior, cognition and emotional research in free-moving primates. Our integrated device shows the potential to research the control of higher behaviors leading to feeding behavior by regulating the LHA of primate's brain.

Keywords: Non-human primates, optogenetics, wireless, neural interface

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I. INTRODUCTION

The brain is an important center for determining all thoughts and actions of humans, and at the same time, there are many parts that humans do not know yet, and are considered the realm of God. As medicine advances, the causes of many diseases have been identified and treated, but in modern society, most incurable diseases (Alzheimer's, Parkinson's, dementia, etc.) are caused by the brain dysfunction. Brain scientists carefully study the brain in order to find the place where incurable diseases occur by classifying the connectivity of the cranial nerves and the roles that each part of the brain plays, and to provide appropriate treatment according to the cause. Amid these demands, brain engineers have developed engineering tools for brain research [6 - 45]. Among them, optogenetics, which modulates the activity of neurons by light by manipulating genes through viruses, occupies a major area in the field of brain research because of the advantage of selectively stimulating specific neurons [5].

Traditionally, a laser source connected with a wire was inserted into the brain and fixed with bio-cement to conduct optogenetics studies [8, 9, 44, 45]. Since then, for a little more freedom of research, brain engineers have integrated a light source for light stimulation into a thin and flexible neural probe to facilitate optogenetics research [27 - 42]. Furthermore, optogenetics researches have been done wirelessly by integrating electronic components that control light stimulation and measure and collect neural signals into a single device [43]. However, up to now, most optogenetic studies have been conducted in mice, which are relatively easy to experiment with animals [9, 15, 18, 21]. In the future, for advanced brain research such as analysis of the cause of incurable diseases, optogenetics research in the brain of primates that are almost similar to humans are essential. So for primate optogenetics, a fully implantable wireless neural interface that can control optical stimulation and simultaneously measure neural signal activity is required [46].

In this study, for the study of optogenetics related to advanced cognitive behavior, a flexible neural probe and sucrose coating that can be inserted into the deep brain have been developed. When inserted, the neural probe is hard and precisely positioned on the target and

after dissolved in the brain only the probe was left. The micro LED located at the tip of the probe performed photo-stimulation at the location where ChR2 was expressed by adjusting stimulation parameters such as frequency and duty ratio. In addition, the device measures the electrical neural signal changed by the photo-stimulation and transmitting data wirelessly at the same time. The performance of the developed device was verified through various in-vitro tests, and it was confirmed that it works well in an in-vivo environment through mouse opto-genetics experiment, showing the possibility as a wireless optogenetic neural interface for non-human primates.

II. Backgrounds

2.1 Need for Brain Research

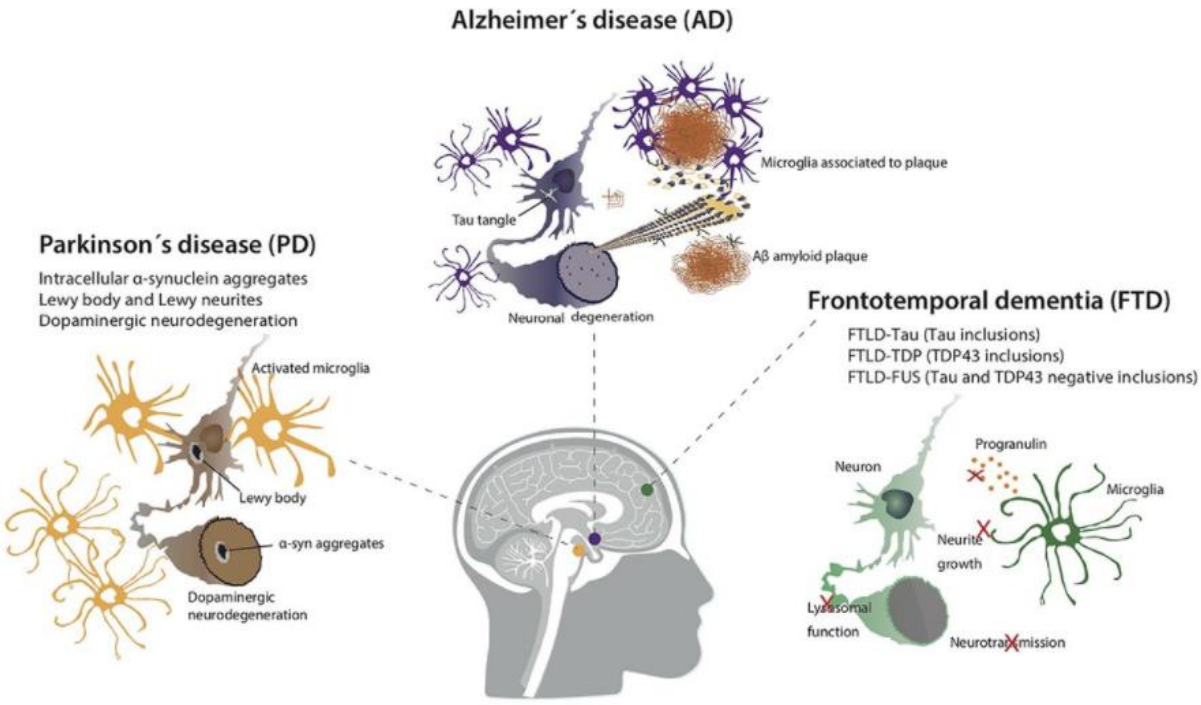


Figure 1. Brain induced incurable diseases

As society is getting older, many incurable diseases continue to increase. Parkinson, Alzheimer's, and dementia, which we commonly know, are representative incurable diseases, and many people still live in the pain of these incurable diseases. The reason why these diseases are difficult to treat is because they caused by the brain dysfunction. If the brain fails to function normally and performs abnormal functions, it can develop incurable diseases. Finding out the cause of brain-induced diseases in the brain and finding out exactly which parts have a problem is the beginning of preventing and treating these incurable diseases [1]. Therefore, many researchers are working hard to identify and classify the brain circuitry, the organic connection of the brain nerve, and each function of the brain region. With the increasing need for brain research, engineers are developing engineering tools for brain research in a variety of ways. It is called Brain Engineering.

For brain research, minimized sensors in millimeters to nanometers scale were developed to detect physical, electrical, chemical, and biological signals in the brain. Also, detected

small signals were amplified and tried to extract the meaning of the signal. Ultimately, brain engineering aims not only to identify the causes of incurable diseases from the brain, but also to engineer brain dysfunctions that cannot be restored biologically forever. In other words, engineers are trying to control brain diseases by engineering ways.

2.2 Brain Engineering : Chemical approach, Electrical approach

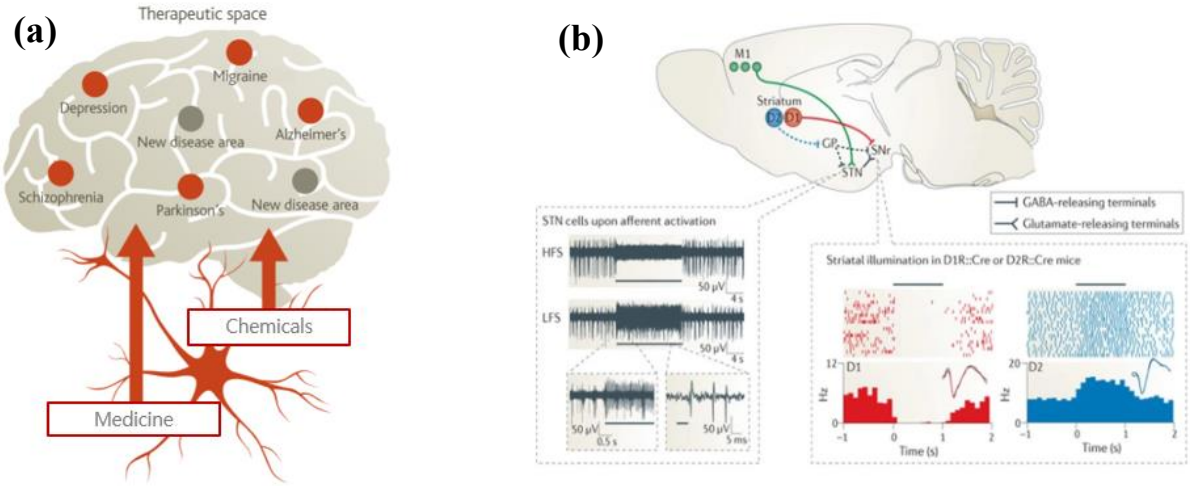


Figure 2. Types of brain engineering : (a) Chemical approach, (b) Electrical approach

The beginning of brain engineering started with chemicals. Traditionally, drugs are used to reduce and control negative symptoms caused by diseases of abnormal brain function. Eating or taking chemicals were used as a medium to compensate for abnormalities in the nerves and to control them. Recently, a method of injecting drugs directly into the brain, rather than taking drugs, is also being studied. Instead of chemical methods that are difficult to accurately target the exact brain area and control the amount of drugs, methods of electrically activating or inhibiting brain function have emerged. It is a method to restore brain function by electrical engineering and to check brain function by measuring the change of electrical neural signals of the brain nerve caused by stimulation. The electrical engineering approach has evolved in cooperation with the advancement of biotechnology to modulate the activity of neurons through light stimulation by modifying specific neurons in the brain to respond to light, which is called optogenetics [2, 5].

2.3 Closed-loop Control of Brain Engineering

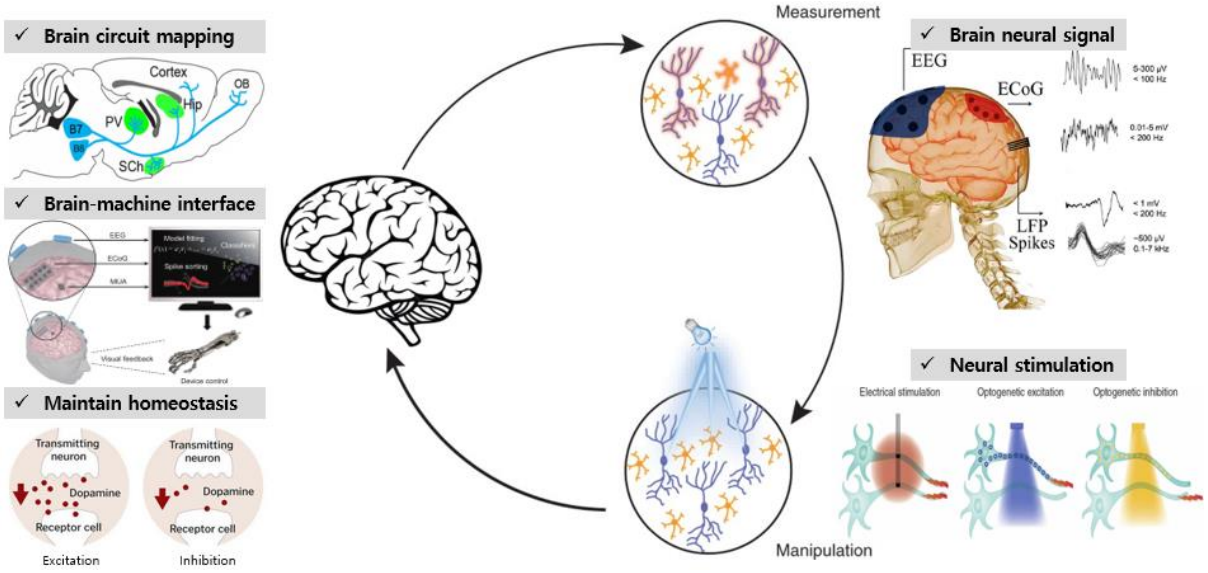


Figure 3. Illustration of closed-loop control process

Figure 3. shows a large circulatory process which is called closed-loop control in brain engineering. The process of closed-loop control includes measurement of brain signals and manipulation of brain functions through appropriate stimulation. The first is to measure brain signals like EEG, ECog, LFP, and APs from the brain in various ways, depending on the purpose. In terms of treatment, when an abnormality occurs in the detected signal, appropriate stimulation is applied to the cranial nerve. On the other hand, in brain research, cranial nerves are stimulated directly by electrical stimulation or indirectly using light by optogenetic technique [2]. Through this series of closed-loop control process, the organic function of brain nerves is identified and the brain nerve map is also created in the basic areas of brain research [3]. The formed brain map is used to analyze the causes of various diseases originating from the brain, or helps to understand the functions of the brain that are still unknown. Once the circuits responsible for the function of the brain are identified and the relationship between the cerebral nerve and the terminal is also revealed, closed-loop control is used to control practical behavior, such as BMI technology, which connects person's thoughts and behaviors [4]. Control of behavior through measurement of nerve signals in the brain can be applied to paralyzed patients. On the other hand, it is used to maintain homeostasis of various substances necessary for the body's condition and normal functions.

2.4 Recent Research of Brain Engineering

Figure 4. Recent research of brain engineering

Through these processes, brain engineering has made a lot of progress from neurons to humans in biological view, from stimulation to fully implant in engineering view. In terms of stimulation and measurement, despite differences in the degree of development of organisms, there have been many studies from neurons to humans [6 - 22]. This shows that brain research is in the process of expanding to humans, and engineering demands a higher level. In mice, closed-loop and fully-implant studies have been conducted, and many studies are still being published [9, 15, 18, 21]. Research has been conducted not only in mice, but also in human-like primates to the fully implant stage, and humans are also treated clinically using closed-loop technology in hospital [20, 22]. However, in the full-implant stage research, no final stage research has been published that satisfies all the conditions of stimulation, sensing, closed-loop, and full-implant. In this research trend, we intend to develop a wireless optogenetics tool for brain research in primates. This requires research in two major areas. In the Fabrication study, we need a flexible neural probe that performs measurements and stimuli with minimal invasion, and a flexible miniaturized thin circuit that can be inserted without physical pressure. The entire device should be packaged in biocompatible, soft material so that there is no negative impact on the organism. The circuit must implement closed-loop control and provide real-time wireless data transmission and system control with an optimized radio system. Finally, it is necessary to separate the measured brain neural signals by types of neurons and derive meaning

III. Materials and Methods

3.1 Concept of Wireless Optogenetic Neural Interface

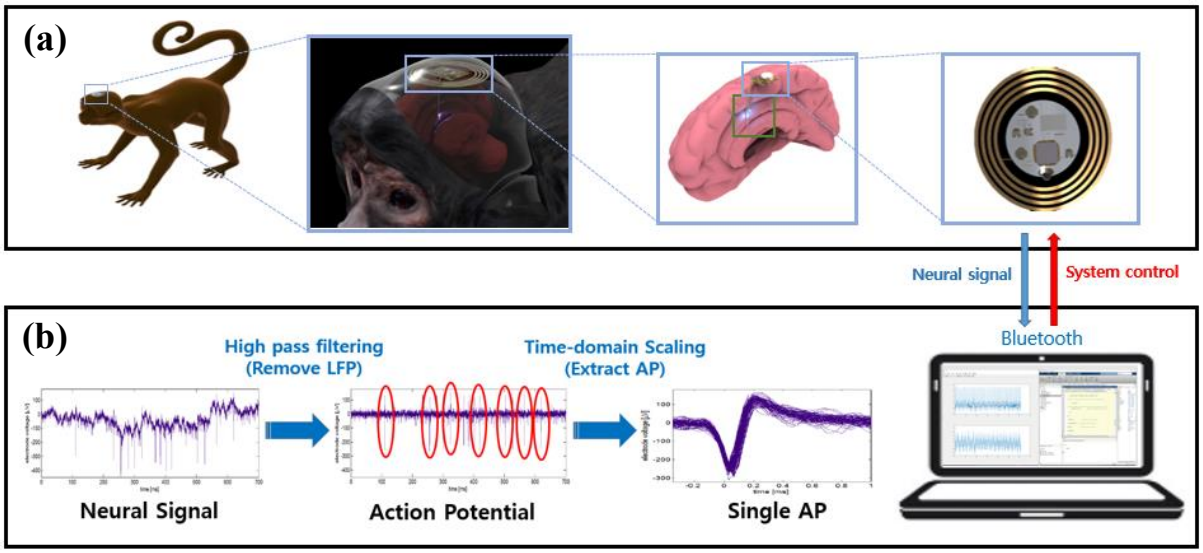


Figure 5. Concept of Wireless Optogenetic Neural Interface

(a) Fully implantable device (b) Signal processing

This is a schematic diagram of the wireless neural interface for non-human primates. In order to conduct animal experiments in a free-moving state, the device should be made in small size and placed under the scalp and above the skull for minimal invasion to brain. The device connected to the lithium-polymer battery for powering the printed circuit board and the circuit is used to amplify the electrical signal in micro voltage units obtained from the neural probe. Also, the power and stimulation parameters of the micro LED at the end of the neural probe are controlled via a button on the wireless receiver. The neural probe measures the change in the cranial nerve signal due to photo-stimulation, and transmits the amplified and filtered signal to an external receiver through optimized wireless communication protocol. The transmitted neural signal is processed in the frequency domain to separate the neural signals of each neuron to identify and classify what kind of neurons is activated. Based on these data, we assess behavior in freely moving state, and find out which part of brain is involved. After a detailed signal processing process, it can be applied to identify and classify the types of neurons that are related to a specific behavior.

3.2 Fabrication of Neural Probe

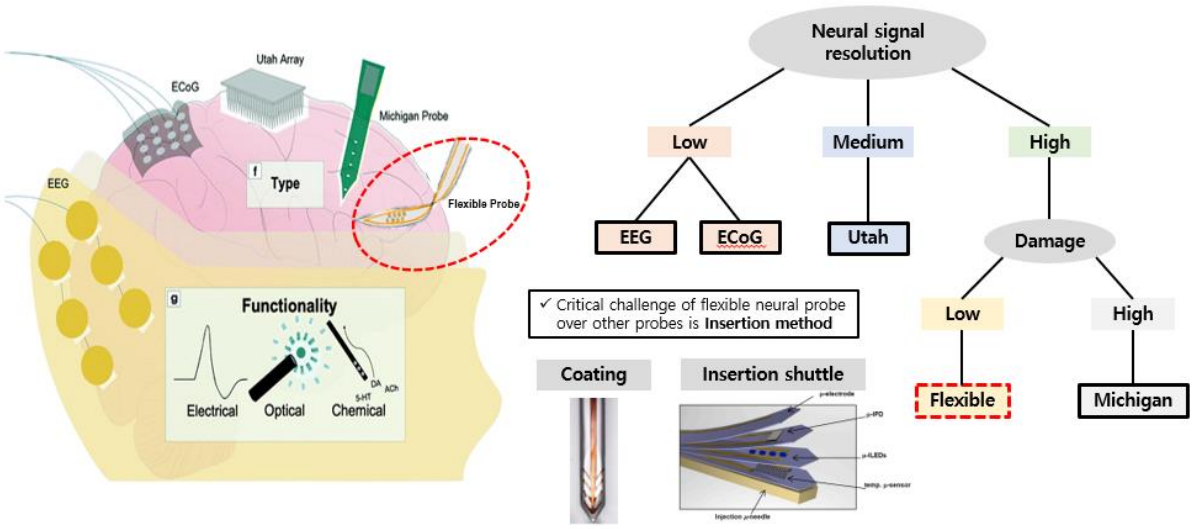


Figure 6. Classification of neural probe and types of insertion method

Figure 6 is the classification of the neural probe that collects the neural signal and the types of method of inserting neural probe into the target area of the brain. Electrode types of measuring neural signals are classified as EEG, ECoG, Utah, Michigan and Flexible [47]. Firstly, these types can be classified by neural signal resolution. Since EEG is a method of measuring brain nerve signals from the skin through the skull and scalp, the attenuation of the signal is large. ECoG is a method that measures on the surface of the brain and has a slightly higher resolution than EEG, but still has a lower resolution. Utah Array is a form of electrode first devised by Utah University. Needle-shaped probes are arranged in two dimensions, so that the distribution of neural signals in two dimensions can be known. However, due to its limited depth, it is difficult to know the neural signals in the deep brain, and it is severely invasive. Michigan and Flexible probes are classified again according to the level of tissue damage. The Michigan probe is a type of electrode first devised by Michigan University. A single needle-shaped probe is inserted deeply to measure the distribution of neural signals along the vertical axis. As a single thin probe is inserted, it is less invasive, but it is made of hard silicone, so that a barrier is formed around the insertion track, making it difficult to measure chronically.

On the other hand, flexible probes are very thin, not strong enough to damage tissue, so they can be inserted for a long time even in situations where animals are behaving freely.

However, the flexible probe is difficult to insert because it curls and bends when inserted into the brain. Many researchers have designed several methods so far and can be classified into two broad categories [48]. The first is a method of coating a neural probe using a mechanically transient material, which remains rigid when inserted, and dissolves and diffuses by biofluid after insertion. The second method is to use a hard shuttle on the probe and remove it after insertion.

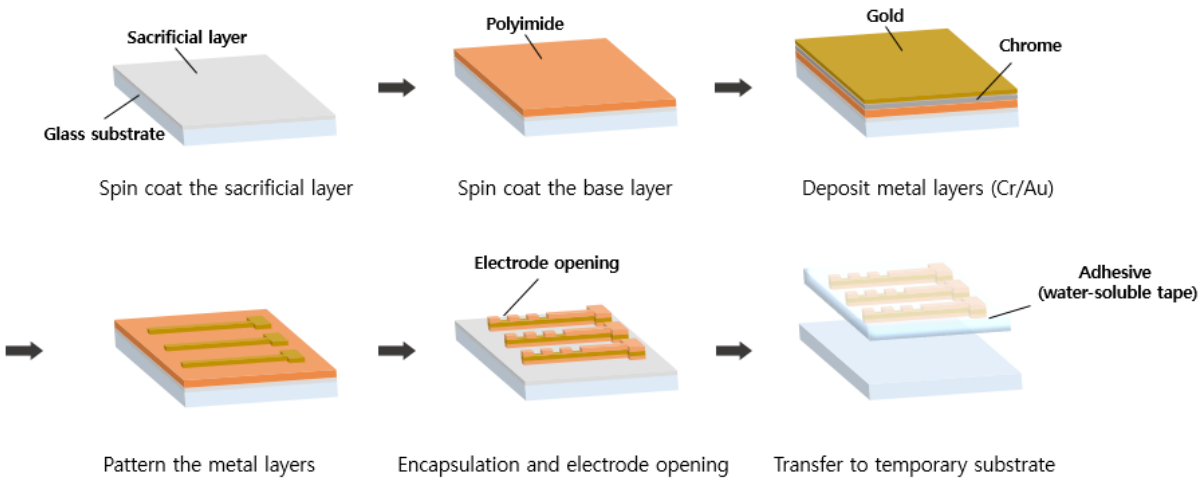


Figure 7. Fabrication process of flexible neural probe

Figure 7 shows how the flexible neural probe was fabricated. First, spin coat the sacrificial layer on the glass substrate to easily remove the substrate at the last process. Next, spin coat the biocompatible material PI. After depositing Cr and Au on the PI base layer by sputtering and they are patterned by photolithography. The patterned metal layer is encapsulated by PI again and electrodes and connection pads are opened by reactive ion etching process. After that, the sacrificial layer is removed and the probe was transferred to water-soluble tape. The thickness of the fabricated neural probe is 4~5um, which is very thin and soft, minimizing damage to brain tissue. The transferred neural probe was directly connected to the exposed pad of the PCB and the micro LED through low temperature soldering to minimize the interface resistance between the neural probe and the electric components.

3.3 Sucrose Coating for Insertion into Deep Brain Area

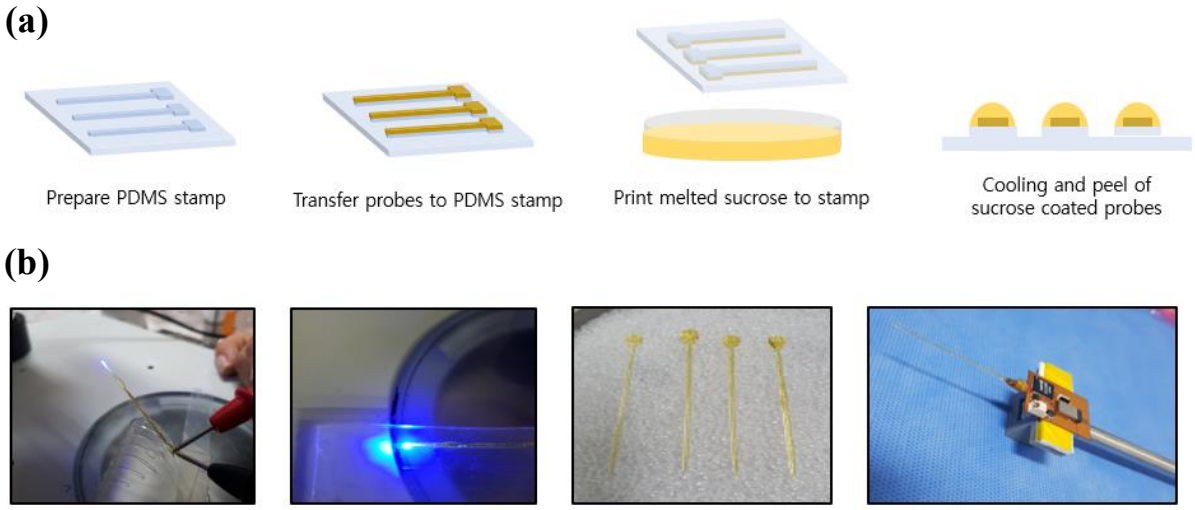


Figure 8. Sucrose coating

(a) Stamp printing process of sucrose coating (b) Images of sucrose coated probe

As explained earlier, the flexible probe had difficulty in insertion, so the probe had to be coated with a mechanically transient biocompatible material. We used sucrose, which is hard at the time of insertion, but completely melts within 10 minutes after insertion. Figure 8(a) is the sucrose coating method. Since melted sucrose has high surface energy, it is difficult to form elongated pattern, so we devised a quick and easy way to pattern sucrose on the one side of the neural probe. The PDMS stamp is made using a metal mold and the neural probe is transferred. The neural probe is so thin that all sides are stably attached to the embossed surface of the PDMS by Van der Waals force. Next, print the protruding part of the stamp on the completely melted sucrose. When sucrose is melted at a high temperature, it is sticky, so it is well coated on the PDMS surface to which the neural probe is attached. Finally, solidify the coated sucrose and remove it from the PDMS stamp. PDMS has a very physicochemical stable surface, and sucrose quickly hardens when exposed to room temperature, and when the PDMS is bent, the sucrose-coated probe falls from the PDMS. Figure 8(b) are photos of sucrose coated flexible neural probes and the final device attached to stereotaxic frame for insertion into brain.

3.4 Circuit Diagram of Wireless Neural Interface

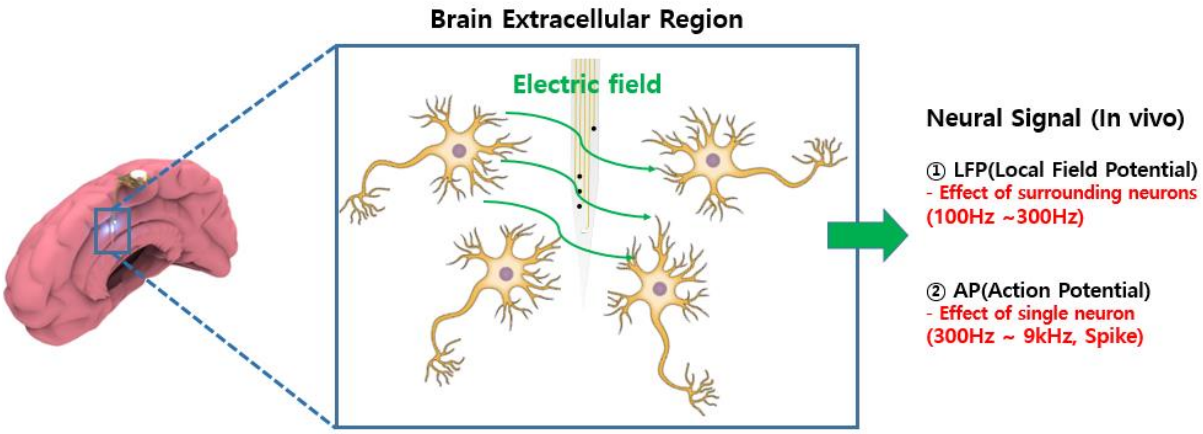


Figure 9. Fundamentals of signal acquisition of neural signal

The fabricated neural probe is inserted into the extracellular region of the brain. It detects electrical signals generated by nearby neurons. The neural signals in the brain can be classified into two categories. First is the slow-changing low-frequency LFP, which is the sum of the signals of several nearby neurons. Second is an action potential in the high frequency band caused by depolarization of one neuron. When optical stimulation is performed, nerves are activated in the target brain area. Then we observe the change of neural signal properties like firing rate or spike amplitude. This is the basic concept of stimulation evoked neural signal sensing (Figure 9).

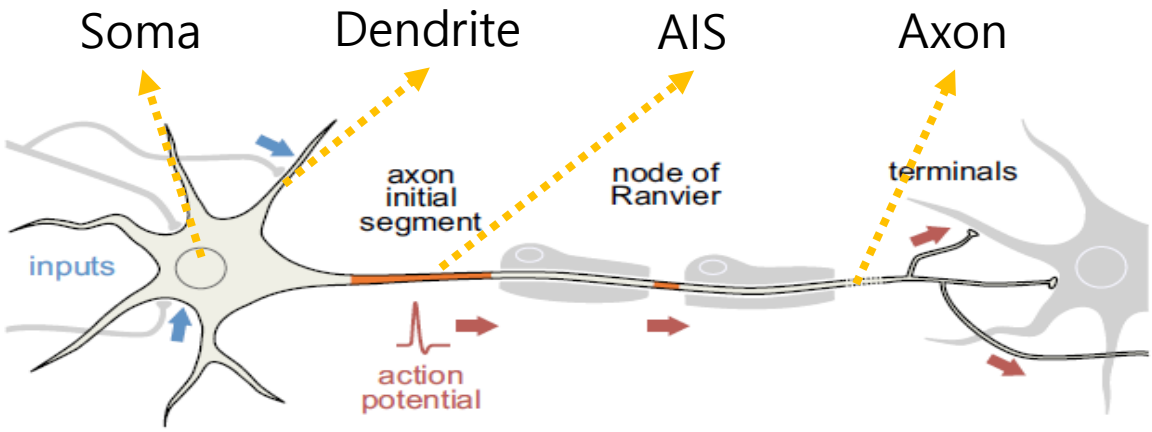


Figure 10. Neural signal propagation in a single neuron

As shown in the Figure 10, neuron can be classified into soma and dendrite, axon initial segment and axon from the perspective of transmission of neural signal in a single neuron. According to existing researches, when the size of the electrode increases, it is the same as the sum of multiple units of single unit size electrode, and the averaging effect increases. Accordingly, the magnitude of the measured signal decreases. On the other hand, when the size of the electrode decreases, the impedance of the electrode increases accordingly, and the magnitude of noise increases. As impedance and noise increase, the relative magnitude of the measured signal decreases. Due to the opposite effect of these two cases, the size of the electrode can be determined depending on the type of signal to be measured. When put together all the effects described before, it can be expressed as a signal to noise level. We focused on the large somatic and dendritic action potential signals rather than the axonal action potential from the surrounding multiple neurons. Accordingly, I set the electrode size to be a circular shape with a diameter of 20um and the electrode spacing to be 100um.

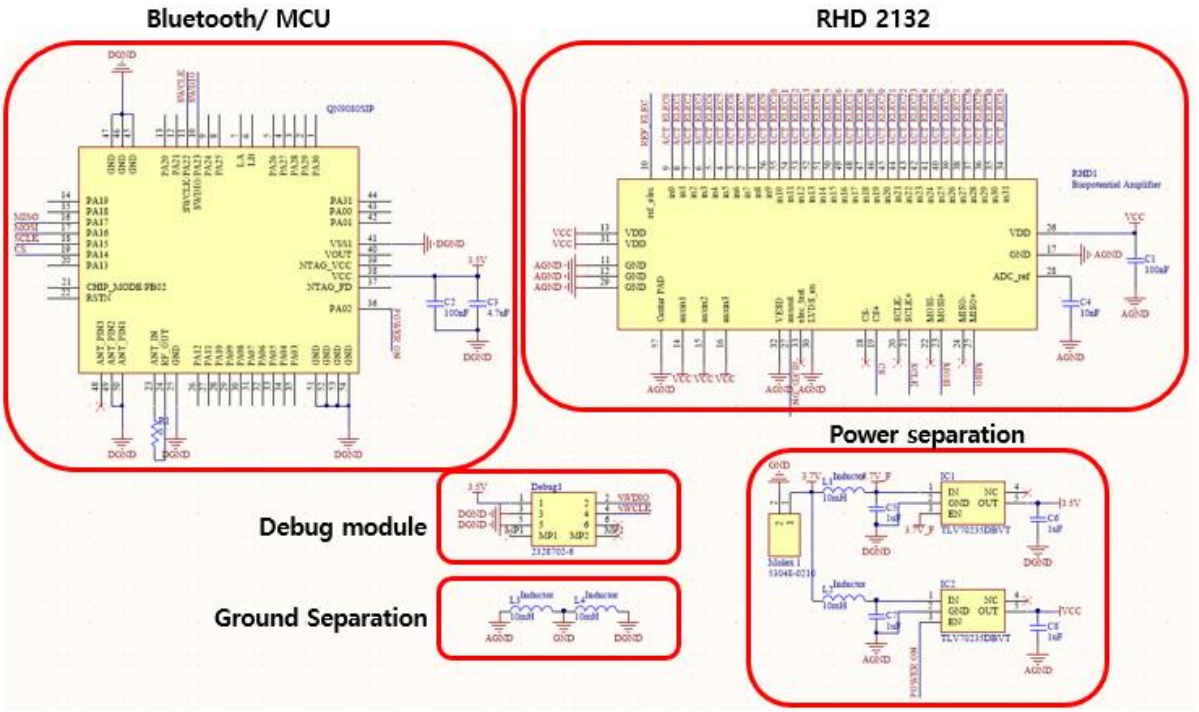


Figure 11. Schematic Diagram of Wireless Neural Interface Circuit

Figure 11. is a circuit diagram for amplifying the neural signal detected from the neural probe and transmitting data wireless. On the left is the MCU that controls the system and performs wireless communication, and on the right is a neural amplifier IC that can amplify a small neural signal in a microvolt unit, and performs an Analog to Digital conversion and frequency filtering. It can be programmed to set the lower and upper cutoff frequencies, so that the desired signal bandwidth can be adjusted. The bottom is a circuit that separates power and ground of analog and digital stages for low noise operation. Since RHD2132 uses SPI communication, spike noise is generated by the system clock, and because MCU uses wireless data transmission, RF noise by 2.4GHz flows into the PCB ground. Therefore, it is very important to separate the ground between the analog and digital stages in measuring neural signals vulnerable to noise. Also, when conducting an animal experiment, the influence of environmental noise (mainly 60Hz noise) is minimized by winding the reference and ground of the ADC around the screw embedded in the skull of the animal with a Pt wire and electrically connecting it to the surface of the brain.

3.5 System protocol of Firmware

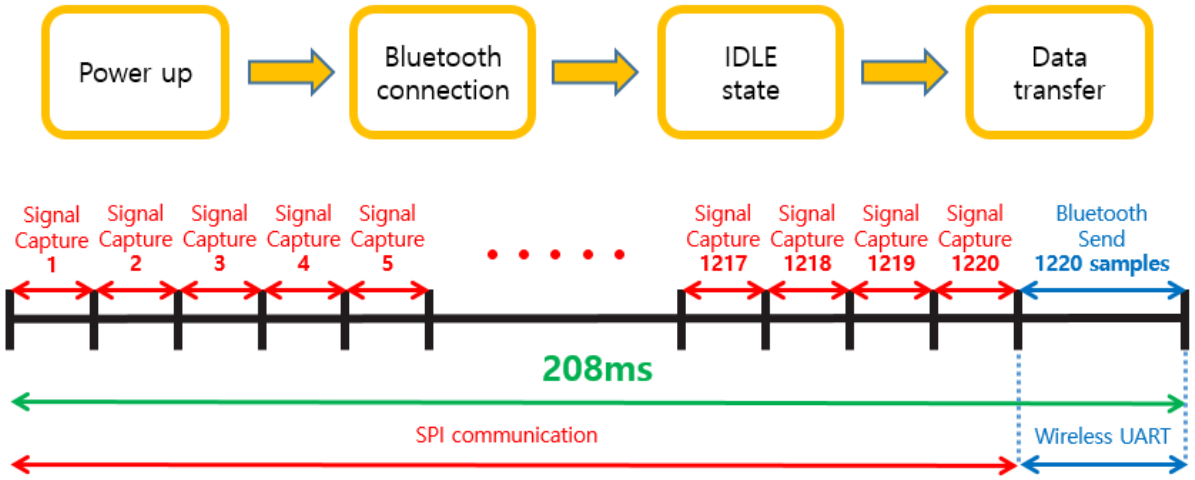


Figure 12. System protocol of firmware

Figure 12. illustrate the system protocol of firmware of the device. Optimized firmware has been built to allow the circuit to acquire signals at high sampling frequencies and transmit data in real time. After the power is supplied, wireless pairing is performed through the button on the receiver. After that, the system automatically enters the IDLE state and stays in a power saving mode. Next, data transmission starts through the control of the receiver. The signal is collected multiple times continuously and uniformly. The data rate of used wireless protocol is 1Mbps, but the actual data rate excluding the packet used in the protocol is about 500kbps at the maximum, so data loss occurs when a large amount of data is transmitted in real time operation. In other words, if the sampling rate is increased to capture a signal quickly, the amount of data to be transmitted wirelessly for the same amount of time increases, which can cause data errors. From the standpoint of measuring neural signals, a sampling frequency of 15kSa/s or more is required to extract the morphological features of the action potential of each neuron. However, considering the aforementioned data rate problem and ADC execution time, a speed of 15kSa/s or more is one of the engineering difficulties. Therefore, our protocol is optimized by measuring the signal at a high rate of 21kSa/s for a specific time to capture the action potential during that time and transmitting the collected large data at once.

IV. Results and Discussion

4.1 Electrochemical Impedance Measurement of Neural Probe

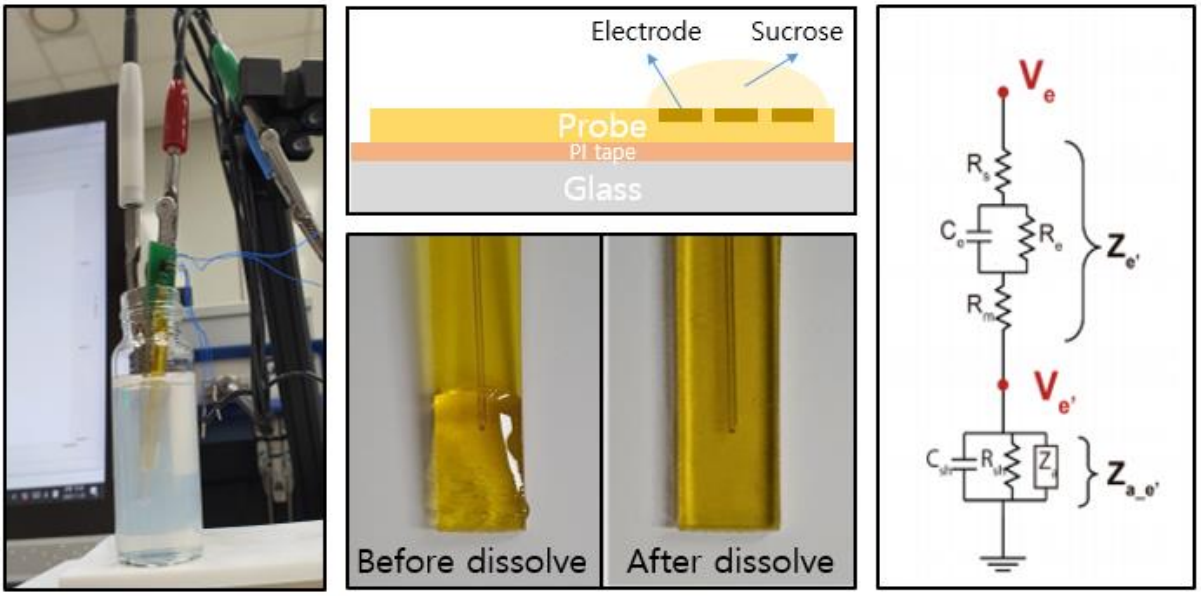


Figure 13. Measurement set-up of electrochemical impedance spectroscopy

(a) Experiment set-up (b) Sucrose coated probe (c) Equivalent circuit of impedance measurement

Figure 12. is the information about the impedance measurement of the probe electrode. This is to evaluate the performance of the probe electrode to be able to detect enough electrical brain signals through CSFs. The impedance of the probe was measured using Electrochemical Impedance Spectroscopy using a Potentiostat equipment with reference wire electrode(Ag/AgCl paste on the Ag wire) and counter wire electrode(Pt wire). Agarose gel was prepared in 1mM PBS with a pH of 7.4, and a probe was attached to the glass to measure how the impedance changes before and after the coated sucrose was dissolved.(Figure 12.(b)) Figure 12.(c) is an equivalent circuit for the electrode measuring neural signal and the amplifier. When effective electrode impedance is called Z_e' and input impedance of amplifier is Z_{ae}', the signal input to the amplifier is divided by the principle of voltage distribution. Therefore, since the input impedance of the used amplifier RHD2132 is 13M ohms, when the electrode impedance is 1M ohms, only 7% signal loss occurs in the neural signal, but when the electrode impedance

is 3M ohms, 19% signal loss occurs. Therefore, when the electrode impedance is about 1M ohm, sufficient performance can be achieved.

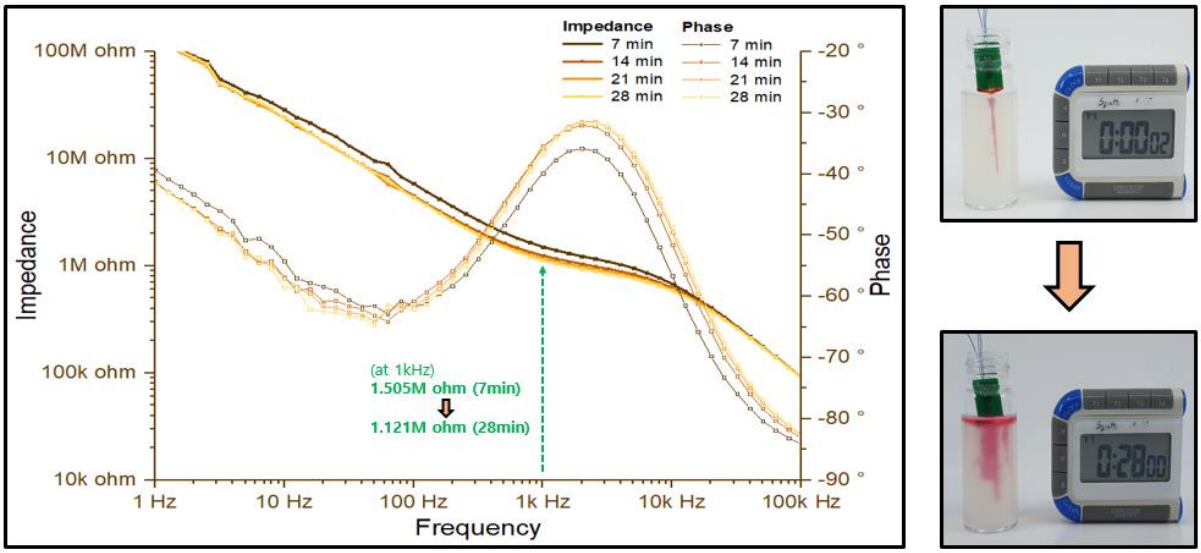


Figure 14. Electrochemical impedance of sucrose coated neural probe over time

Figure 13. is the experimental data of EIS measurement. After inserting the sucrose-coated probe into the agarose gel, as the time increased every 7 minutes from 7 to 28 minutes, it was confirmed that the coated sucrose that was blocking the electrode surface diffused around and the impedance of the electrode decreased. After the coated sucrose is sufficiently diffused, the impedance of the electrode is reduced from 1.505Mohm to 1.121Mohm. This is an enough impedance value to detect a neural signal according to the theory. As shown in the right picture, a sucrose coated neural probe containing a red pigment is inserted and after 28 minutes, it diffuses and spreads around. These results indicate that the sucrose coating does not lower the impedance performance of the electrode, and the manufactured neural probe can measure the electrophysiological signals of neurons.

4.2 Device Function : Frequency, Duty Variability

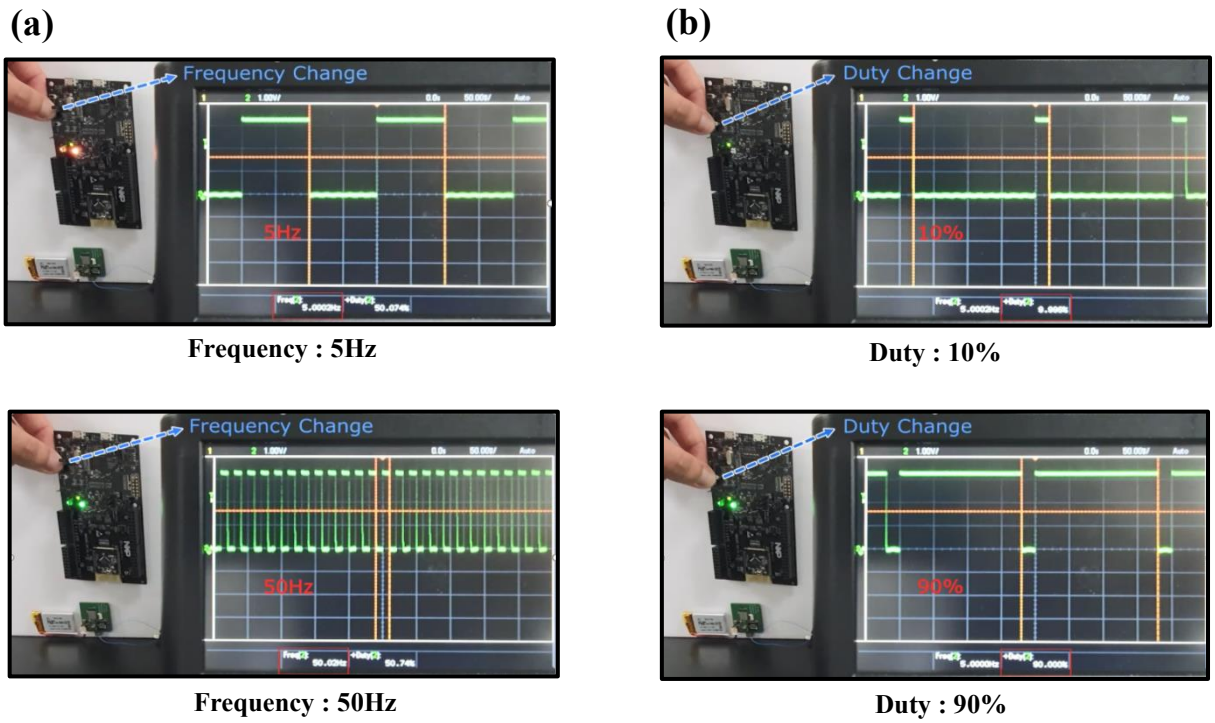


Figure 15. Optical stimulation parameter variability (a) Frequency (b) Duty

Commercial optogenetic devices and developed devices used in published papers can control the intensity, frequency, and duty ratio of the light source. As introduced in related papers, the activation of neurons by stimulation is related to the frequency of the stimulating light and the duration of the light pulse. In order to sufficiently activate or inhibit neurons in the target area, variability of the stimulating light parameters is necessary. Figure 14. is an oscilloscope waveform of changing the device's stimulation parameters wirelessly by modifying the firmware on the developed device. At the top, there is a receiver that acts as a remote control, and below is a developed optogenetic device. Pressing the center button on the receiver starts the wireless connection, and it can increase the frequency from 5Hz to 50Hz in 5Hz steps with the center button. Next, press the lower button to increase the duty ratio of the light pulse from 10% to 90% in 10% increments. In the case of changing the duty ratio, at the same frequency at 5Hz, only 10% of the time is turned on at first, and then 90% of the time is turned on at last. Through the duty ratio control, the amount of dose for the entire time of stimulation can be adjusted in order to sufficiently stimulate while reducing damage of neurons.

4.3 In-vitro Test for Verification of Device Performance

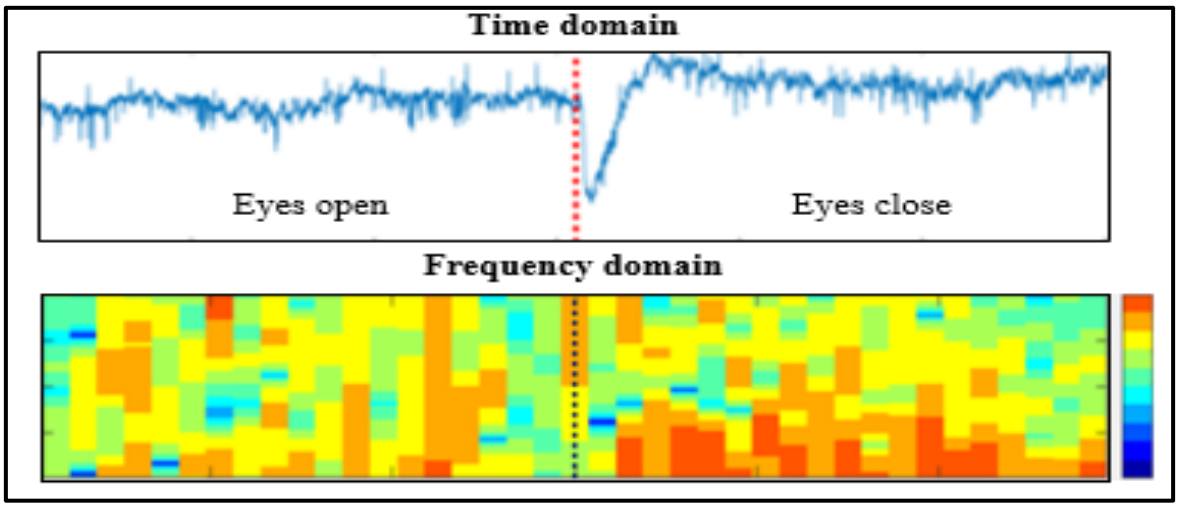
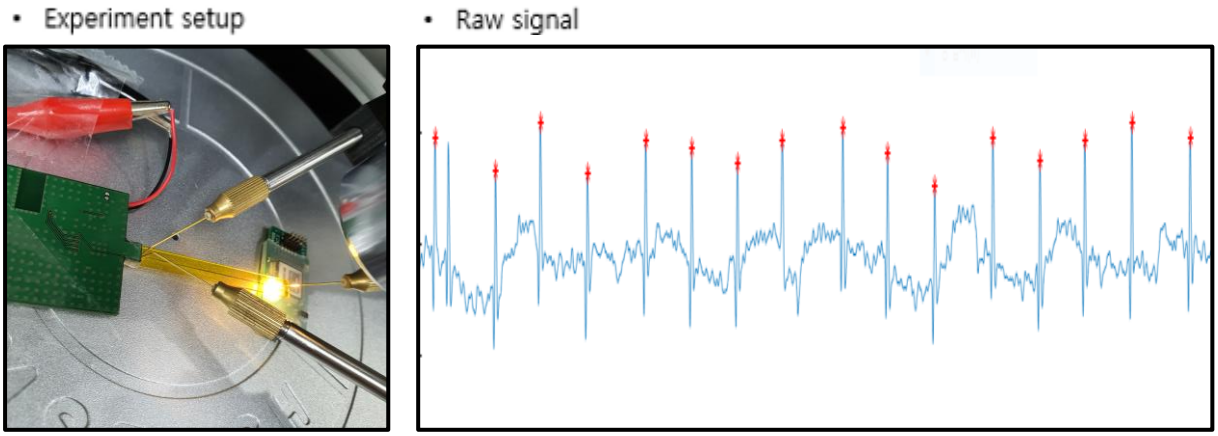


Figure 16. In-vitro test for verification of device performance

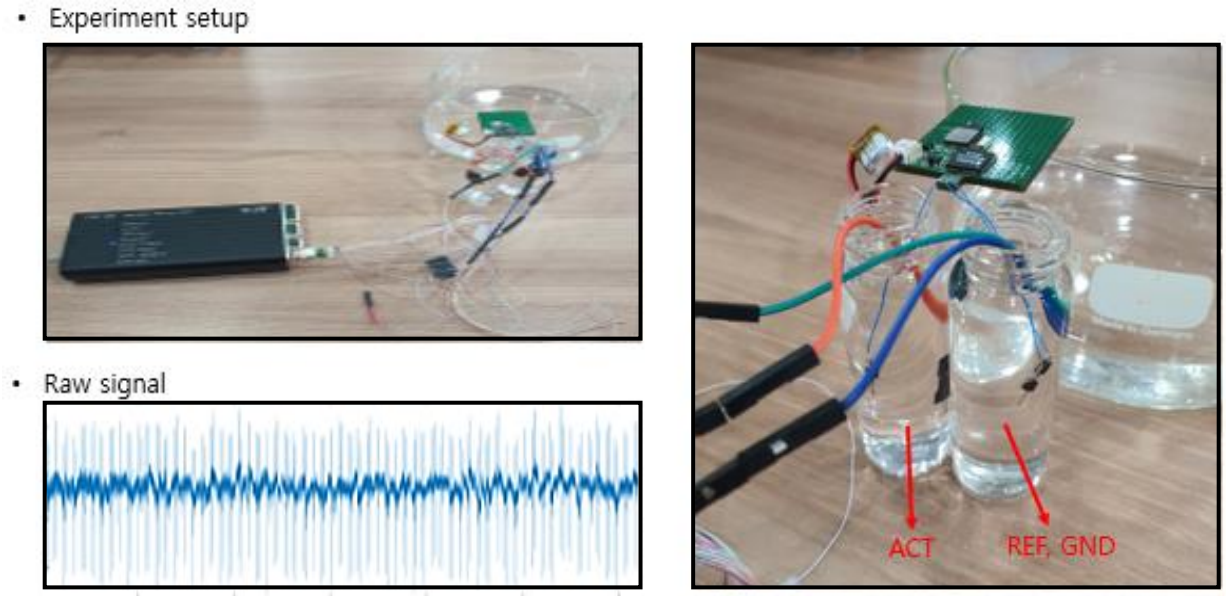
(a) EEG measurement

Based on the designed neural interface circuit, an in-vitro test was performed to see if the actual electrophysiological signal could be well measured. First, EEG was measured on the skin using a commercial Ag/AgCl electrode attached to the skin, excluding a neural probe, to verify the performance of whether the circuit could detect small amplitude brain nerve signal. EEG is an averaged electrical signal by summing the signals of nerve bundles generated in the brain, and a lot of attenuation occurs as it passes through the skull and skin. Therefore, the EEG signal has a frequency band of 50 Hz or less, and the amplitude of the signal does not change significantly when a change in thought or cognitive behavior occurs, so precise and minute measurements are required. Through EEG signal measurements, we validate that the circuit can measure changes in neural signals with sufficiently small frequencies and small amplitude. When the eyes are closed, a relatively large change in the signal occurs due to the movement of the eye muscles, and after that, it is not clearly visible on the time axis, but as a result of analysis in the frequency domain, a signal of 15 Hz or less was found to be prominent.



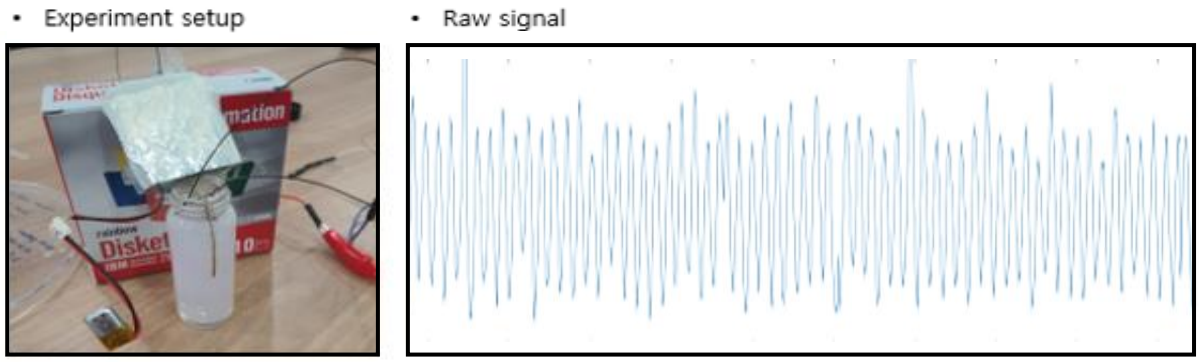
(b) Direct signal sensing test using probe station

Next, we tested whether the probe was able to carry signals from the brain to the circuit using the probe station. The fake brain device generates a periodic AP signal of 100Hz together with the LFP and transmits the signal to the electrode of the neural probe through the probe tip. By performing a test under the condition that there is no interface impedance due to metal contact between the gold electrode of the neural probe and the probe tip, only the performance of the neural probe can be verified. As shown in the measured raw signal, a periodic AP signal was measured along with the LFP and the peaks of each AP were also detected simultaneously. This measurement result means that the gold line of the fabricated neural probe has a low electrical resistance and soldering between the PCB and the neural probe is well performed to have a sufficiently low impedance. In addition, result showed that the neural probe and the circuit can cover the target frequency band of the neural signal because the device measured the LFP, which is a low frequency signal and the AP, which is a high frequency signal from the fake brain.



(c) Indirect signal sensing test in PBS solution

Third, we used PBS, which is the same as the electrolyte concentration in vivo, to confirm that the signal could be measured through body fluids. As in the previous test, a fake brain was used to generate periodic AP signals to check if the signal could be measured when the interface resistance was greater than the metal.



(d) Indirect signal sensing test in agarose gel

Finally, an artificial tissue was made with agarose gel to test whether the signal could be measured on the artificial tissue. Both PBS and Agarose gel provided an electrical environment similar to that of living tissue to observe the device performance. In both cases, there was a slight attenuation of the signal, but it was sufficient to measure the neural signal.

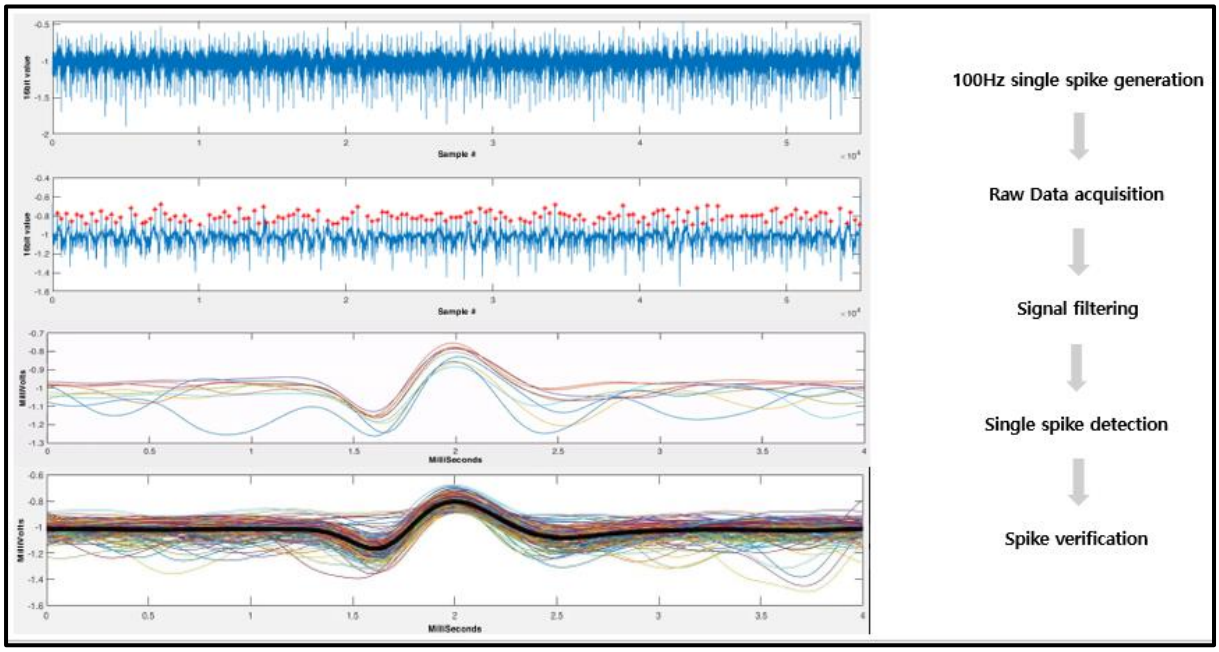


Figure 17. Spike detection of fake neural action potential

Figure 16. is the result of signal processing of the data obtained in the in-vitro test. Using a fake brain equipment that generates cranial neural signals, we detected periodic spikes and overlapped each spike to confirm that the signal originated from single neuron. Periodic AP signals are measured as raw signals along with LFP, and high pass filtering is performed to remove LFP. Among the filtered signals, spikes with high amplitude are sorted by detecting peaks of signals exceeding a specific threshold. After inferring one action potential by adding the time before and after the captured spikes, the waveforms are overlapped on the same time axis. The overlapped APs have similar timing and amplitude, so if the detected APs are averaged, it is determined what waveform the neuron's activation signal has. In the fake brain, only one type of neural signal can be generated, whereas many types of nerves are located in the actual brain, so an artificial intelligence technique such as CNN is used instead of the threshold technique.

4.4 In-vivo Test : Mouse

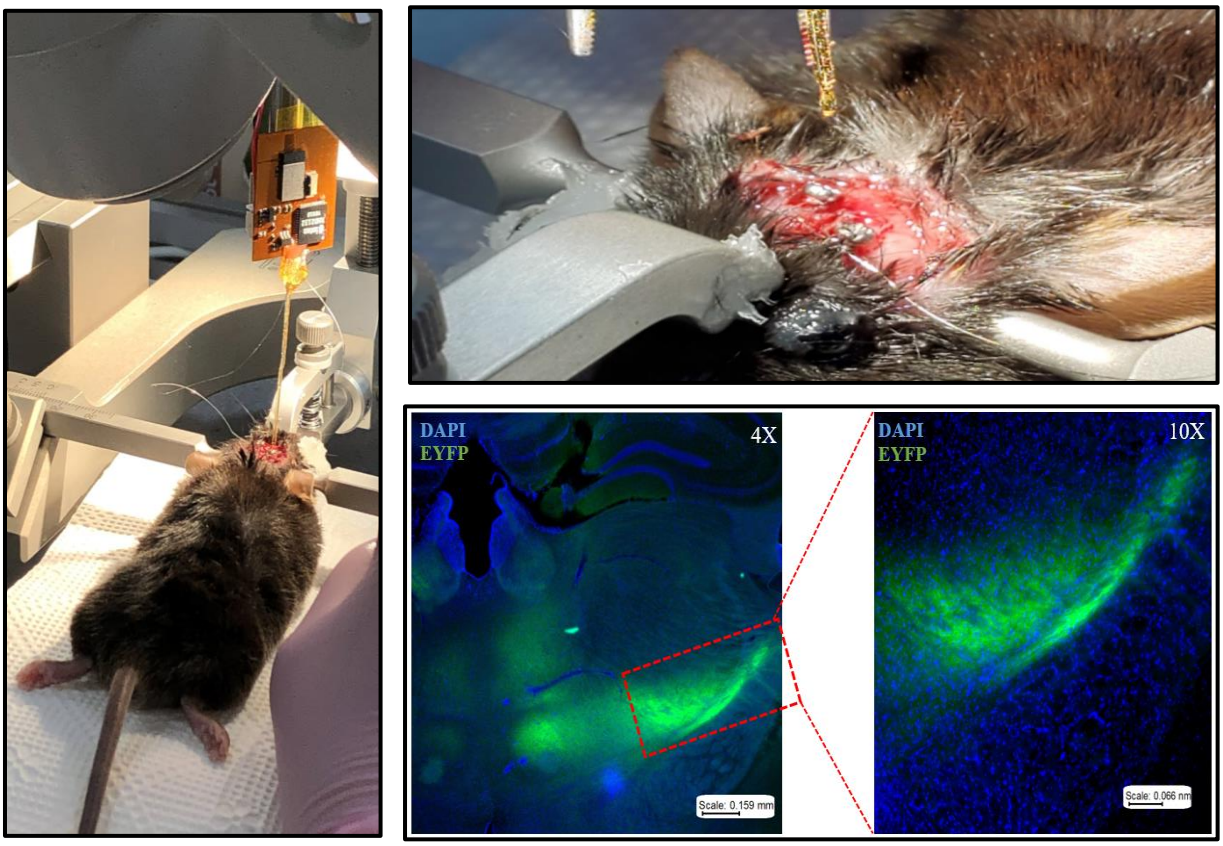


Figure 18. Optogenetic neural stimulation and sensing in LHA of mouse brain

After successful verification of the device through the in-vitro test, the experiment was performed in rodents, which relatively easily express channelrhodopsin before the primate experiment. Channelrhodopsin(ChR2), which is responsible for regulating neural activity in response to blue light, containing EYFP fluorescent protein was expressed in LHA responsible for feeding behavior and confirmed by fluorescence imaging. The micro LED attached to the probe is located right next to the electrode, so the leakage of current to drive the LED can affect the measured neural signal. Therefore, in actual animal experiments, stimulation was performed using an external laser light having the same wavelength as the micro LED. The laser and neural probe were inserted at the same location of the ChR2 expressed LHA, and stimulation and measurement were performed 1 hour after insertion until tissue recovery and anesthesia were released slightly.

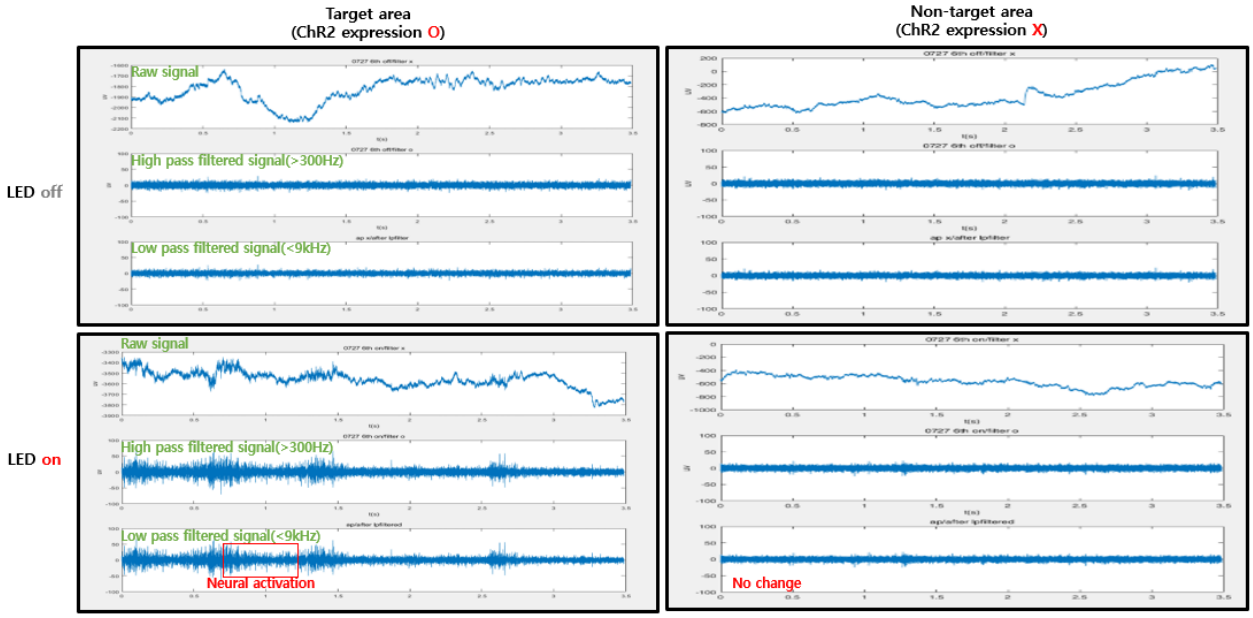


Figure 19. Signal analysis of optical stimulated neural activity

Figure 17. is a result of wirelessly measured neural signal activity by optical stimulation in the mouse. In order to confirm whether the nerve activity caused by the light stimulation occurred, the signal was analyzed by inserting a neural probe even where ChR2 was not expressed. In both cases, a low frequency and slowly changing LFP signal was measured in the raw signal. To detect the action potential, which means the activity of neuron cell, high pass filtering is performed at the boundary of 300Hz to keep the baseline constant. Then, a clean neural signal was obtained by removing high-frequency noise of 9 kHz or more outside the frequency band of the action potential. As shown in the upper two graphs, when the LED is not turned on, neuron activity is not observed. On the other hand, when the LED was turned on, the activity of neurons in the target area increased, and action potential with high frequency and large amplitude was measured. On the other hand, when the LED is turned on, action potentials with high frequency and large amplitude were measured in the target area, unlike the non-targer area. This result indicates that ChR2 expressed in LHA reacts to blue light to open a channel of membrane protein, thereby increasing the permeability of ions, increasing neuronal activity, resulting in a change in extracellular potential.

4.5 In-vivo Test : Primate

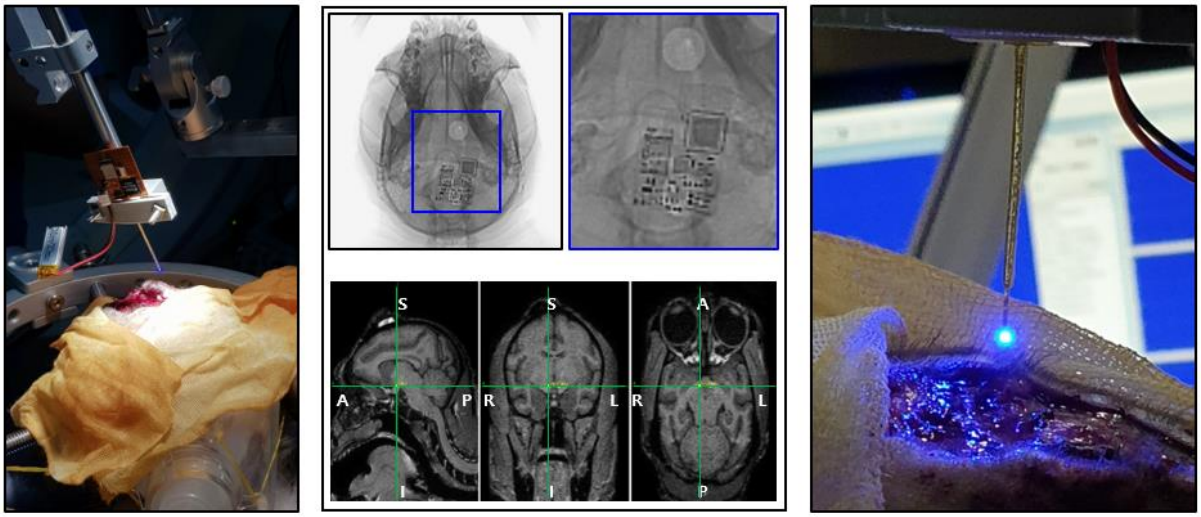


Figure 20. Photographs and XperCT image of implanted device in primate

Based on a successful rat experiment, optogenetic experiments in primates were performed. Figure 18. shows a neural probe inserted into the brain of a rhesus monkey 4 weeks after the virus was injected for successful ChR2 expression, and the device was placed on the skull. XperCT imaging shows that the probe is well inserted into the target area of the LHA and the device is well placed on the skull. Unfortunately, in primates, ChR2 was not expressed as in rats, so it was not possible to measure changes of neuronal activity by light stimulation. If ChR2 expression in primates is successful in the future, it is expected that behavioral experiments including regulation and measurement of neural circuits related to cognitive behavior that are difficult to perform in mice will be possible.

V. Conclusion

Recent researches in brain engineering aim to identify and treat the causes of diseases originated from the brain, and many brain engineers are trying to map the brain by finding out the functions of each part of the brain and identifying the connections of neural circuits. However, despite such efforts, a fully implantable wireless neural interface has not yet been developed that enables even behavioral analysis experiments in an environment where free movement is possible for brain research. In particular, primate experiments are essential for brain research related to higher cognitive behavior, and fully implantable devices are more needed in primates. In this study, we proposed a wireless optogenetic neural interface that enables free-behaving brain research in primates. The biocompatible and mechanically transient sucrose coating enables deep brain insertion of flexible neural probes. The fabricated flexible neural probe is very thin and is inserted into the brain with minimal invasion and has sufficient impedance to measure neural signals. The configured circuit and optimized firmware were shown to be capable of optical stimulation and wireless neural signal measurement in an in vivo environment through in-vitro testing. Finally, optogenetics experiments performed in a mouse showed that the device can control the neural activity of the brain with optical stimulation and transmit the measured neural signals wirelessly. The proposed device has potential as a wireless optogenetic neural interface for advanced brain research of non-human primates.

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요 약 문

영장류를 위한 광유전학 신경 인터페이스

인간의 뇌와 비슷한 영장류의 뇌는 불치병과 관련된 인간의 뇌 회로를 이해하는데 중요한 역할을 한다. 수많은 뇌 연구의 방법들 중에 광유전학은 뇌의 특정 부분을 유전적으로 감염시켜 빛 자극과 뇌 전기 신호 측정을 동시에 수행하여 뇌의 회로를 알아내는데 강력한 방법으로 널리 사용되었다. 뇌와 관련된 복잡한 행동 연구에 있어 자유롭게 움직이는 상태에서의 영장류 광유전학은 중요함에도 불구하고 프로브 삽입 방법, 디바이스 완전 삽입, 무선 통신과 같은 공학적인 어려움 때문에 대부분의 광유전학 연구는 마취 상태에서 수행되었다. 이러한 문제점들을 극복하기 위해 우리는 깊은 뇌의 영역에 적은 침습으로 프로브를 삽입하기 위해 설탕 코팅된 유연하고 긴 뉴럴 프로브를 제작하였고 디바이스의 완전 삽입이 가능하도록 무선통신 기술을 도입하였다. 최종적으로, 우리는 행동, 인지, 감정 연구와 같은 자유롭게 움직이는 영장류에서의 상위 연구를 위한 완전 삽입형 무선 광유전학 인터페이스를 개발하였다. 집적된 우리의 장치는 영장류의 시상하부 외측영역을 조절하여 섭식행동에 이르는 상위 행동의 제어 및 광유전학 연구 장비로서 활용될 잠재적인 가치가 있음을 보였다.

핵심어 : 영장류, 광유전학, 무선 통신, 신경 인터페이스