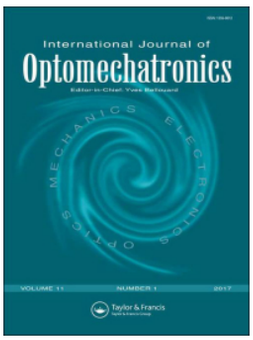

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# Mechanism of peripheral nerve modulation and recent applications

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**ABSTRACT**

Neuromodulation is a multi-interdisciplinary field of neuroscience, neural engineering, and medicine in a complex, but a way of understanding. Recently, the interest and researches in this field have been attracted due to its promising applications such as bionic limbs and bioelectronic medicine. For easier entry into this field, in this review, we approach the basic mechanism, methods, and applications of peripheral neuromodulation sequentially. Firstly, the overall structure and functions of the human nervous system are introduced, especially in the peripheral nervous system (PNS). Specifically, the fundamental neurophysiology regarding action potentials and neural signals is introduced to understand the communication between the neurons. Thereafter, two main methods for peripheral neuromodulation, which are electrical and optogenetic approaches, are introduced with the principles of the state-of-art devices. Finally, advanced applications of neuromodulation combined with the sensor, stimulator, and controller, called a closed-loop system are introduced with an example of bionic limbs.

**KEYWORDS**

Neuromodulation; peripheral nerve; electrical stimulation; optogenetics; bioelectronic medicine; bionic limb

## 1. Introduction

### *1.1. The human nervous system*

The human body is composed of various organ systems and organically related to each other. Among them, the nervous system takes the role of connecting organs to communicate with each other. The nervous system is largely divided into the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS includes the brain and spinal cord, and the PNS consists of nerves derived from the brain and spinal cord.[1]

The nerves of the PNS extend throughout the body like branches of a tree and are distributed in various tissues. By using the network, they exchange all information necessary for sensory, motor, and unconscious functions with the CNS. Nowadays, state-of-art researches on the CNS unveils the secrets of the nervous system and its function, showing promising future possibilities. For the PNS, the surgical risk is relatively lower and the accessibility of engineering intervention is higher than the CNS. So, it has the advantage of being able to proceed more easily with various engineering approaches to modulate bodily functions. In addition, the number and the density of the neuron are lower than that of the brain and having unique structures like a cable or a net,

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## Nomenclature

| | | | |
|---|---|---|---|
| PNS | Peripheral nervous system | SHP | Self-healing polymer |
| CNS | Central nervous system | CNT | Carbon nanotube |
| ANS | Autonomic nervous system | nXIIts | 12th cranial nerve nuclei |
| SNS | Somatic nervous system | TENGs | Triboelectric nanogenerators |
| Ach | Acetylcholine | ChR2 | Channelrhodopsin-2 |
| LIFE | Longitudinal intra-fascicular electrodes | LED | Light-emitting diode |
| TIME | Transverse intra-fascicular multichannel electrode | RVNx | Right vagotomy |
| | | $\mu$LED | MicroLED |
| A-SEE | Adaptive self-healing electronic epineurium | | |

which allows to easily access for modulating neurons. Also, the correlation and connection between the function of each neuron and each organ are clearer than the brain, which makes us easier to modulate the functions.

### 1.2. Classification of the peripheral nervous system

The PNS is divided into the autonomic nervous system (ANS) that handles involuntary movements (heart rate, breathing, digestion, etc.) and the somatic nervous system (SNS) that controls voluntary responses (muscle contraction, etc.). The autonomic nervous system is again divided into the sympathetic nervous system and the parasympathetic nervous system (and the enteric nervous system). Since the ANS regulates the functions of organs such as the small intestine, the large intestine, and the heart, it is being targeted for the treatment of various diseases by implanting bioelectronics into the relevant nerves (e.g., vagus nerve[2–4]). The SNS is classified into the sensory nervous system responsible for afferent signals and the motor nervous system responsible for efferent signals. In the case of the somatic nervous system, because it controls muscles for the movements of arms and legs, many researchers are targeting those nerves to improve the function of the bionic limbs,[5–7] as well as for therapeutic purposes such as muscle rehabilitation.[8,9]

### 1.3. Structure of nerve

The main structure of the PNS is a nerve that has an enclosed structure like a cable bundle in which neurons are gathered, playing the role of the passage for the electrochemical signals. As shown in Figure 1(*a*), a neuron consists of a cell body with the nucleus, a dendrite that receives nerve signals, generating an action potential when the signals exceed the threshold, and an axon that transmits the generated signals to an axon terminal to transfer the signal to another neuron. In some cases, this axon is covered with a myelin sheath, making the speed transmission is significantly faster compared to the unmyelinated neurons, which are covered with connective tissue called the endoneurium. In addition, the axon terminal forms a synapse with adjacent neurons, in which the electrical signal transmitted through the axon is converted into a chemical signal by releasing a molecule called a neurotransmitter that is a chemical messenger inhibiting or activating the neuron by influencing the receptor on the targeted neuron or organ. The aggregate of these nerve fibers is called a fascicle, and this fascicle is surrounded by connective tissue called the perineurium. Inside the fascicle, afferent fibers that send afferent (sensory) signals to the CNS and efferent fibers that send efferent (motor) signals from the CNS could be both located in a fascicle or a nerve which is called a mixed nerve fiber. The group of fascicles is called a nerve. A nerve is surrounded by epineurium, and it also consists of blood vessels that provide nutrients for the whole structure. (Figure 1(*b*)).[10]

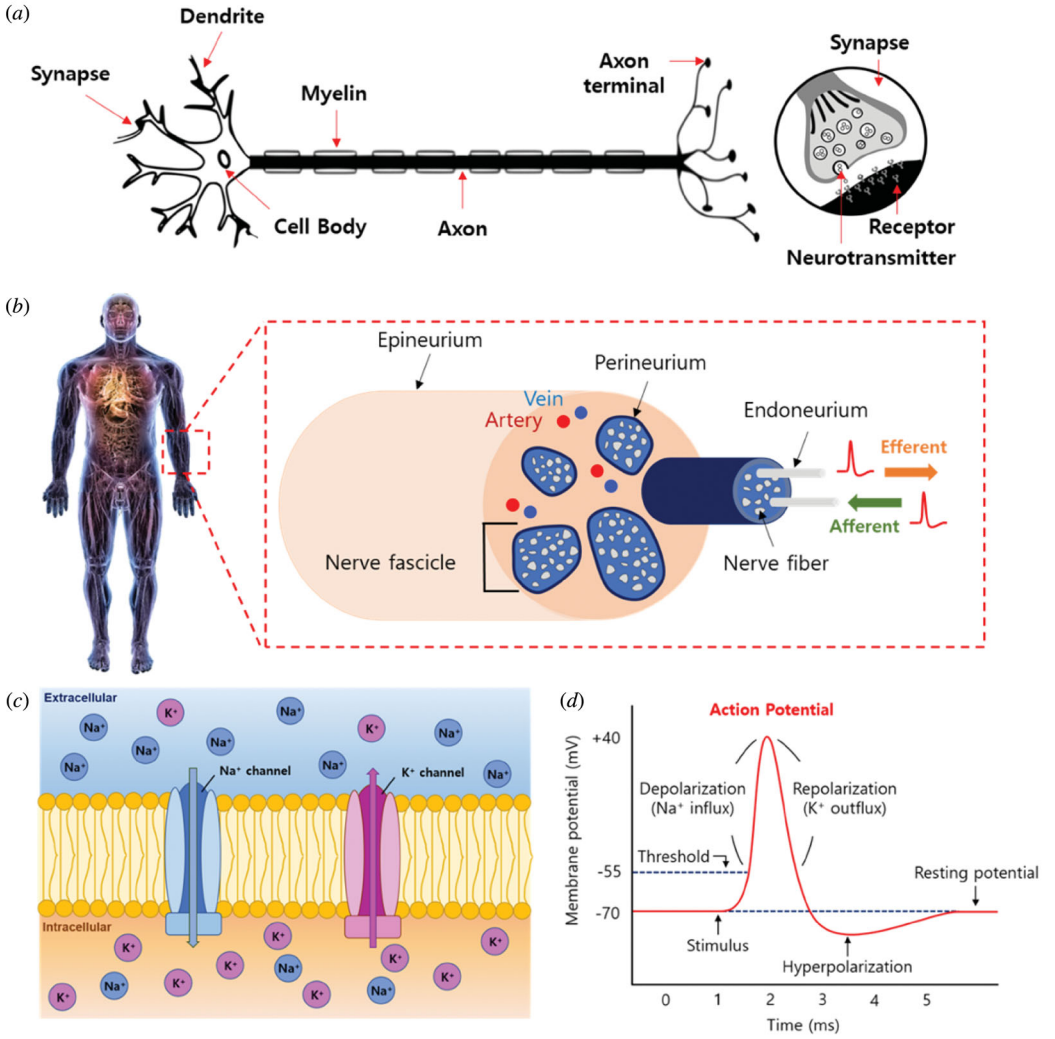


**Figure 1.** Overall view of anatomy and principles of the nervous system. (*a*) The structure of a general single neuron. (*b*) Schematic of the peripheral nervous system and anatomy of a single mixed nerve which both contains efferent and afferent neurons. Orange colored arrow indicates an efferent signal that comes from the CNS and green arrow indicates an afferent signal that goes to the CNS. (*c*) Schematic of the ion concentration gradient due to the ion channels at neuron membrane. (*d*) Graph of the action potential from a giant squid axon.

When a single neuron is zoomed, it can be seen that the cell membrane is formed of a lipid-bilayer like other cells inside the body. Various proteins are distributed in this membrane. Among them, proteins such as voltage-gated $Na^+$ channels and voltage-gated $K^+$ channels have an important role in the formation of action potentials, which will be discussed next section. (Figure 1(*c*)). Neurons in PNS have relatively long axons and have different morphological characteristics depending on the role of neurons. They are classified according to the presence or absence of a myelin sheath as discussed above, and also according to the diameter of the axon, which differs from the conduction velocity of axons. Depending on these characteristics, the type of information transmitted by each axon also varies. For example, the A fiber which has the highest speed of transmission due to its large diameter and myelin sheath, and it serves as a channel for signals related to proprioception. In the case of C fiber that has low conduction velocity and relatively small diameter in absence of myelin sheath, it serves as a path for sensory

information.[11] Therefore, selective stimulation for targeting specific fibers should be required to realize natural sensory feedback in bionic limb applications.[12]

### 1.4. Nerve signal transduction mechanism

Previously, it was explained that the neuron plays the role in the passage of electrochemical signals. After receiving information from the surrounding nerves, the neuron combines the information and decides what to transmit to the next nerve (or target organ) through its decision-making. To transmit the determined information, the neuron generates an action potential, which is an electrochemical signal, by itself, and this action potential goes to the nerve ending and is responsible for transmitting the information to the next nerve (or target organ). Since the purpose of neuromodulation is to modulate the information exchanged between neurons, it requires an overall understanding of the process of generating and transmitting the action potential, which is the subject of this information.

The most important role in generating action potentials inside neurons is the membrane permeability of membrane proteins such as ion channels and sodium-potassium pumps. The membrane permeability causes the concentration gradients of ions across the membrane between the inside of the cell (cytoplasm) and the outside of the cell, creating a membrane potential. In general, only the potential difference caused by $Na^+$ and $K^+$, which has the most influence on this, are considered. The voltage across the membrane in the absence of any stimulus is called the resting potential which has a value of about $-70$ mV.[13–15]

Neurons are activated by an external stimulus above the threshold or by commands from the brain or the spinal cord. And when activated, voltage-gated $Na^+$ channels located at the membrane are opened. In the case of $Na^+$, since the concentration outside the cell is higher than that inside the cell, the $Na^+$ ions rush into the cell as soon as the channel is opened, reducing the voltage polarity between inside and outside the cell. This process is called depolarization. When the depolarization process is finished, the voltage-gated $Na^+$ channel is closed again and the voltage-gated $K^+$ channel is opened. In the case of $K^+$, since the concentration inside the cell is higher than the concentration outside the cell, $K^+$ ions are released to the outside of the cell as soon as the channel is opened, and the potential within the cell gradually becomes relatively negative (repolarization), and later, a potential value lower than the resting membrane potential is obtained (hyperpolarization). After that, when the membrane potassium permeability returns to the resting state value, the membrane potential returns to the resting membrane potential value (Figure 1(*d*)). Due to the initial diffusion of $Na^+$, the voltage-gated $Na^+$ channel located next to it is also opened and the above process is repeated. Through this process, the action potential propagates along the entire axon, and at the end of the neuron (axon terminal), the specific type and amount of neurotransmitter are released based on the information of action potential.[16,17]

In the ANS, sensory information obtained from organs (pain, bladder distension, etc.) is sent to the brain using this action potential, and based on this information, action potentials are exchanged between neurons in the brain, and after the inside process, the final decision is made. Then, this decides the form of an action potential and sends it back to the organ or hormone system to modulate the organs (some signals return via the spinal cord). In the SNS, muscle cells and axon terminals (end of the neuron) are connected (neuromuscular junction). When an action potential arrives at the neuron terminal, acetylcholine (ACh), a neurotransmitter, is released, and ACh consequently activates muscle fibers through an internal process that causes muscle contraction.[18] If the action potential (information) is not properly generated or transmitted, incorrect information is sent to the function of a part of the body, which can lead to a malfunction of the organ and, in severe cases, cause disease.[19–22]

### 1.5. Necessity of peripheral nerve modulation

Peripheral neuromodulation artificially modulates the activation of peripheral nerves connected to various tissues. Using this technique, if it is able to send messages (signals) to organs (or tissues) on behalf of the brain, or conversely, if it is possible to send a desired message to the brain through peripheral nerves, it will bring great benefits from a medical and prosthetic perspective.[23] For example, let's make a case that a patient suffering from neurogenic bladder dysfunction due to a sensory nervous system problem does not know whether the bladder is inflated or not (does not know when to urinate).[24] If the relevant peripheral nerves can be artificially activated based on the data from any sensors detecting the degree of bladder expansion, it can be sent sensory signals of the bladder to the brain to determine the appropriate time to urinate by the patient himself. In another case, if the motor nervous system has a problem, the patient is unavailable to urinate by himself due to the lack of control of bladder-related muscles.[25] In this case, by giving the efferent signal to the motor nerves of the bladder, urination could be achieved.

For advanced prostheses, achieving the embodiment is one of the key elements. It means that one feels bionic limbs like one's own body parts by communicating with the user by sending sensory signals based on external information and reflecting the motor signals with the natural limb movement. This is one of the current challenges toward advanced bionic limbs.[26] An embodiment arises from the failure of the prosthesis to transmit sensation like a normal limb. Therefore, based on the various signals obtained from the prosthesis, if the C fiber, which is a sensory nerve, is stimulated to give sensory feedback, and information about proprioception is given to the wearer by stimulating the A fiber, the patient will feel that the prothesis is as a part of his body.

Various methods of artificially activating nerves have been studied, and examples are methods using electric, magnetic, optogenetic, and ultrasound, and each method is under active research.[27–30] Each method has its advantages and disadvantages and is used selectively (or in combination) depending on the purpose and situation. In this review paper, the method using electric and optogenetic will be examined.

## 2. Methods for neuromodulation

### 2.1. Electrical neuromodulation

#### 2.1.1. Electrical stimulation

Thanks to the experiment of L. Galvani, we now know that the nerve can be activated artificially using electricity.[31] Activation using electricity does not generate the action potential in the same way as the normal way, such as opening the mechanosensitive channel. It creates an action potential by activating the voltage-gated ion channels by artificially creating a potential difference inside and outside the cell by applying an external current directly into the axon.

Let's look deeper at the simple principle with an example of activating a single axon using a monopolar electrode and cathodic current (Figure 2(*a*)). When a cathodic current is emitted from a single electrode adjacent to the axon, the electrical potential outside the cell in the area adjacent to the electrode becomes negative, and thereby the inside of the cell in the adjacent area becomes relatively positive. By these phenomena, if the membrane potential becomes sufficient to exceed the threshold of the axon, the pores of the voltage-gated sodium channel open, and a series of processes for generating a general action potential is carried out.[32]

The above descriptions were about a single axon. However, in general, rather than stimulating a single axon, nerve or fascicle units are stimulated. As explained in the previous section, several fascicles are included in the nerve, and numerous axons are included in the fascicle. Axons have different morphological characteristics, and these morphological differences lead to electrophysiological differences that vary for each axon.[33,34] Therefore, when a nerve is stimulated, depending

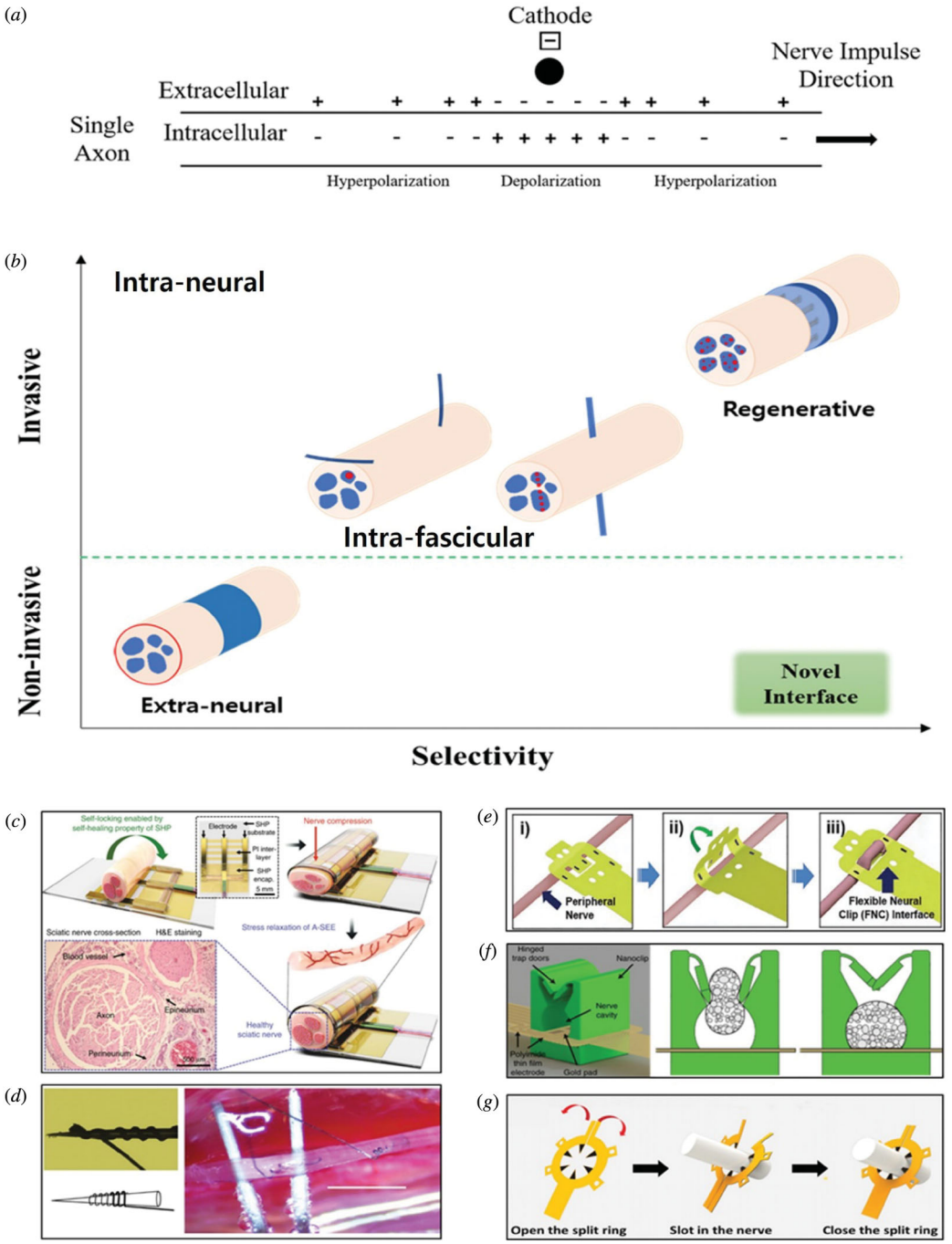


**Figure 2.** (*a*) Schematic of polarization of axon during monopolar cathodic current stimulation. (*b*) Trade-off graph of invasiveness and selectivity of the neural electrode. Red marked region indicates the place where activation occurs. (*c*) Adaptive self-healing electronic epineurium (A-SEE), using self-healing polymer.[45] (*d*) Carbon nanotube (CNT) yarn electrodes with tungsten microneedle.[46] (*e*) Schematic diagram of implanting a neural clip electrode on a peripheral nerve.[49] (*f*) 3D-printed nano clip electrode.[50] (*g*) Schematic diagram of implanting a split-ring electrode on a nerve.[51].

on the amount of current used, some axons are activated and some axons are not. Due to this characteristic of nerve stimulation using electricity, when a weak current is sent, only a part of the axon belonging to the nerve is activated. However, it should be kept in mind that if the strength of the current is increased too much, it may cause nerve damage and electrode damage. For more information on this phenomenon, refer to the Shannon model of neuronal damage.[35]

### 2.1.2. Electrode configuration and selectivity

The method of stimulation can be diversified through the number of electrodes used for stimulation. First, the method that uses a single electrode as described above is called monopolar configuration and the method that uses double electrodes is called bipolar configuration. In the case of monopolar stimulation, because the current spread out from the power source in all directions due to the absence of a returning electrode, it can affect not only the targeted tissue but also the surface of the electrode. This problem can be alleviated by increasing the number of electrodes used for the stimulation. In the case of the bipolar configuration using two electrodes, the current spread is lesser than the monopolar configuration method because the current returns to the other electrode. In addition, it is also possible to more precisely control the flow of current by using an additional electrode, such as in a tripolar configuration.[36,37]

The method can be divided not only by the number of poles but also by the position of the electrodes, and it is largely divided into a non-invasive method and an invasive method (Figure 2(*b*)). In a non-invasive method, the electrode is located outside the epineurium, which is the outermost layer of the nerve. This method has the advantage of causing relatively less physical damage to the nerve, but the selectiveness is lowered because a relatively wide range of fascicles is stimulated. The most common non-invasive electrode is the cuff electrode which fully covers the external circle of the nerve. The soft polymer is used for the substrate of the electrode, and thin metal film or metal wire is located on the inside of the substrate to contact the nerve. Due to the advantage of wrapping the nerve in a circle with a substrate (insulating layer) that also covers two side contacts, it shows effective suppression of noise.[38]

Contrary to the non-invasive method, the invasive method inflicts a lot of physical damage on the nerve because the electrode penetrates inside, but has higher selectiveness due to the advantage of the electrode being located close to the axon. Invasive electrodes are roughly classified into three main types, intra-fascicular, penetrating (not present in the figure), and regenerative electrodes. The most widely known intra-neural electrodes are longitudinal intra-fascicular electrodes (LIFE) and transverse intra-fascicular multichannel electrodes (TIME). Both LIFE and TIME are a flexible electrode that has several exposed (uninsulated) electrode sites. In the case of LIFE, the electrode is inserted into the nerve with a needle and pulled out of the nerve, and locates the exposed electrode sites in the middle of the nerve that makes the electrodes directly in contact with the fascicle or axon.[39] TIME, compared to the LIFE, is transversally inserted and pulled out of the nerve. This makes the electrodes address the fascicles and axons directly over the cross-view of the nerve.[40] The regenerative electrode is mainly used for nerve regeneration. Connecting the cut planes of the nerve with the electrode, the axons regenerate through the pores that have a metal contact layer, which makes higher selectivity compared with other types of electrodes.[41] Since the electrode at the invasive method is adjacent to the axon, it is possible to exceed the threshold value of the axon even using less current and has higher spatial selectiveness than the non-invasive method.[42,43]

This point is also closely related to long-term implantation. For instance, the invasive method has the advantage that it requires less energy to stimulate nerves increasing a battery lifetime, but higher invasiveness leads to more foreign body reactions caused by the immune response. Even though it is a non-invasive method, it does not mean that it does not damage nerves. The nerve tissue modulus is about 100 KPa, which is like a stretched rubber band. If the young's modulus of the electrode is higher than this value, a mechanical mismatch with the nerve occurs, and the

immune response of the nerve is activated to form scar tissue.[44] The generated scar tissue is not good for the nerve itself, but also increases the distance between the nerve and the electrode, reducing the contact area. This also reduces the performance of the electrode and has a fatal effect on long-term implantation. Recently, adaptive self-healing electronic epineurium (A-SEE), which self-adapts to the modulus of nerve tissue using self-healing polymer (SHP) to reduce mechanical mismatch and improve electrode performance, has successfully achieved a neural recording and stimulation in a rat sciatic nerve for 14 weeks have also been reported (Figure 2(*c*)).[45] In the case of the invasive method, a study of an electrode that is made with flexible, thin carbon nanotube (CNT) yarn using tungsten microneedle has successfully implanted into the vagus nerve of a rat for 16 weeks to maintain good performance even for a long-term implant was reported (Figure 2(*d*)).[46] As with these studies, various studies on changing design and material properties are still actively conducted.

Problems do not occur only after electrode implantation. Due to the small diameter of the nerve (rat sciatic nerve diameter: 0.8–1.0 mm), difficulties arise during the electrode implantation process. To overcome this point and make implantation quick and convenient, various designs of electrodes have been studied.[47,48] Among them, as an example, implantation into rat pelvic nerves (diameter: 250–300 μm) and wireless stimulation was conducted by simply clipping the nerve using "neural clip electrode" (Figure 2(*e*)).[49] Also, by using a "nano clip electrode," researchers successfully implanted the electrode into the tracheosyringeal part of the 12$^{th}$ cranial nerve nuclei (nXIIts) (diameter: 150–200 μm) of the zebra finch using 3D printing (Figure 2(*f*)).[50]

An ideal electrode should have high selectiveness while minimizing damage to nerves (right bottom of the graph). To fabricate an electrode that satisfies such conditions, a study of a split-ring electrode that increased selectivity by only activating the electrodes that are near to the targeted fascicle, has also been reported (Figure 2(*g*)).[51]

There are two main reasons for increasing selectiveness (it may be collectively referred to as one). There are several fascicles and numerous axons in the nerve, each performing different functions. Therefore, to obtain the desired response through nerve stimulation, only the fascicle (or axon) that causes or is related to the response should be stimulated. If an unrelated axon is activated, unexpected results may be obtained, and depending on the role of the activated axon, it may cause fatal side effects (pain induction, unwanted organ activation). However, due to the nature of the electrical stimulation, the current path crosses the nerve, and all axons that satisfy the threshold value on or around the path can be activated. As such, it is difficult to achieve complete selective stimulation because electrical stimulation also affects the axons located around the targeting axon. For these reasons, to modulate only a specific axon (or group of axons) which is difficult to achieve with electrical stimulation, a method using optogenetics is suggested depending on the purpose.

### 2.1.3. Electrical energy source

There are two methods of electrical stimulation using a "voltage source" and a "current source."[52,53] The above examples and explanations consist of contents using a current source. The reason is that the impedance between the nerve and the electrode varies depending on the state of the body and the nerve. Due to this, it is difficult to accurately control the amount of charge applied when using a voltage source. However, when using a current source, it is possible to precisely control the amount of charge applied regardless of the value of the impedance. However, in the case of medical equipment, there are many cases where a "voltage source" is used. The reason is that, in the case of voltage, the circuit can be implemented simply because it can use the energy directly from the battery. However, in the case of current, to change the form of the energy from the battery, a more complex circuit is required. So, research on a voltage source that can be manufactured relatively easily was actively conducted in the early days.

In the case of a monopolar phase current that is not in charge balance, it may cause an accumulation of electric charges in the tissue or a state of charge imbalance, which has the potential

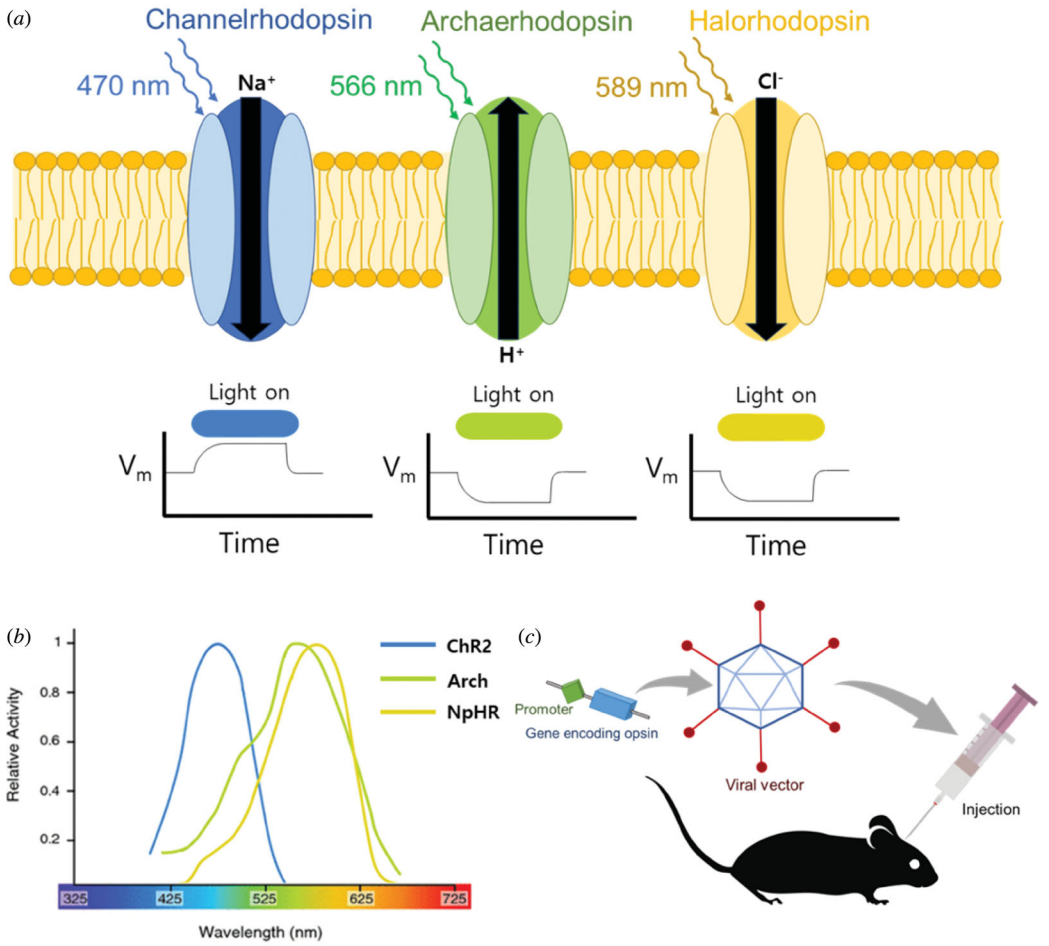


**Figure 3.** (*a*) Schematic of three kinds of opsins according to the wavelength range of light expressed.[66] Channelrhodopsin causes excitation, and archaerhodopsin and halorhodopsin cause inhibition. (*b*) Graph of relative activity on wavelength change according to opsin type.[67] (*c*) Schematic of the process by which opsin is transferred to the target neuron.[68].

to damage nerve tissue. Therefore, to prevent harm to nerves, bipolar phase current with charge balance is mainly used for stimulation.[54] Also, the cathodic first waveform is mainly used for nerve stimulation. This is because, as we saw earlier, depolarization is easier when a cathodic current source is used as both proven in the computational study and *in vivo* study.[55–57] Efficiency occupies an important part in devices that are implanted *in vivo*. This is because, since the volume and weight of the entire device that can be inserted into the body are limited, the size of the battery is limited too. So, even if using the same size battery, it can be used for a long time by consuming less energy.[58] In another direction, studies to stimulate nerves by triboelectric nanogenerators (TENGs) without using a battery are also being actively studied.[59] TENG produces electrical energy by the triboelectric effect and electrostatic induction according to the contact between the two materials. Since the current waveform generated at this time has a charge-balanced biphasic form, it is appropriate for nerve stimulation.[60,61]

## 2.2. Optogenetic neuromodulation

Optogenetic neuromodulation is a technology that has a higher selectiveness than electrical neuromodulation.[62] This technology modulates nerves using a photoreceptor protein called opsin,

which can open and close ion channels in cells according to specific wavelengths of light. There are different types of opsin that respond to specific wavelengths of light.[63–65] One of these opsins, channelrhodopsin is expressed in the sodium ion channel. When the blue light is irradiated, sodium ion channels are opened, allowing $Na^+$ ions to enter the cell and induce depolarization to cause excitation (Figure 3(*a*)). One of the types of Channelrhodopsin, channelrhodopsin-2 (ChR2) has the maximum relative activity at a wavelength of 470 nm.[66] Conversely, as opsins that cause inhibition rather than excitation, archaerhodopsin and halorhodopsin exist. ArchT1.0 and eArch3.0 of archaerhodopsin are expressed in the proton pump and when the green light is irradiated, the pump is activated to move the $H^+$ ions from inside to the outside of the cell, inducing hyperpolarization, which in turn causes inhibition. For ArchT1.0 and eArch3.0, the relative activity is maximized at 566 nm wavelengths, respectively. NpHR, a type of halorhodopsin, is expressed in the chloride ion channel and when the yellow light is irradiated, the chloride ion channel opens, and $Cl^-$ ions enter the inside of the cell and cause hyperpolarization. For NpHR, the relative activity is maximum at 589 nm. However, in the case of these opsins, since the wavelength range of the activated light overlaps (Figure 3(*b*)), there is a limitation that several types of opsins cannot be used in target neurons. To compensate for this limitation, research is underway on opsins whose wavelength ranges do not overlap, such as C1V1 and red-active ChR.[67]

To apply these optogenetic technologies to neuromodulation, opsin must be expressed in the target neurons. The most commonly known method is to attach the gene promoter to the opsin and carry it to the target via a vector such as a virus to express it genetically (Figure 3(*c*)). Promoters can only express in certain cells depending on the type, allowing them to express opsin only in the desired target neurons. There are many different types of vectors, including non-viral vectors for safety reasons.[68]

Light sources for expressing opsin mainly use light-emitting diode (LED) or laser.[69] LEDs have the advantages of relatively low price, good power efficiency, long lifetime, low heat generation, and above all, wide light spectrum width. Therefore, if a particular option is selected, appropriate LEDs can be found.[70] However, due to the relatively large degree of light emission, it is not appropriate for optogenetics with high intensity or local range. On the other hand, the laser has very high output power and low divergence, making it suitable for local optogenetics.[71] However, it is expensive compared to LEDs, has a narrow light spectrum width, and has a very high output power, which can damage nerve tissue, so it is important to control carefully. In general, LEDs are more useful in most optogenetics, laser is preferred in certain applications that require high intensity. Recently, a method of transmitting light to a specific nerve part with a cuff-type electrode through optic fiber, rather than simply inserting a light source, is being studied.[72]

Optogenetics can control ion channels at the cell level, and in the case of LED, on/off can be controlled in microsecond units, so very precise and selective modulation at an accurate time is possible. This has led to the mapping of the brain and peripheral nerves based on function. However, the stability of chronic opsin application has not yet been proven, and the vector targeting human should also be confirmed for stability and efficacy. In addition, in the case of a human with a more bulky and thick tissue, a light transmission technology that can deliver an appropriate intensity of light should be developed.

## 3. Application of electrical & optogenetics neuromodulation

### *3.1. Vagus nerve stimulation of optogenetics*

Vagus nerve, one of the cranial nerves, is an important component of the parasympathetic branch of the autonomic nervous system that controls involuntary movements of the heart, lungs, adrenal glands, and digestive tract.[73] Therefore, vagus nerve stimulation is effective in treating

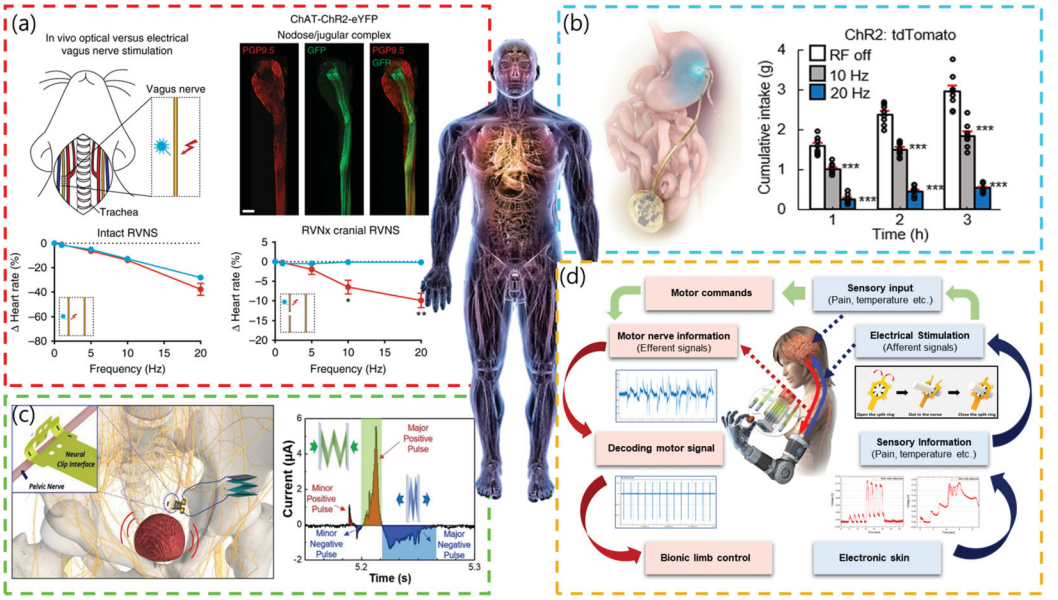


**Figure 4.** (*a*) Optogenetic vagus-efferent nerve stimulation and electrical vagus-mixed nerve stimulation.[77] (*b*) Schematic of optogenetic gastric smooth muscle stimulation for reducing food intake.[78] (*c*) Pelvic nerve stimulation for urination induction using TENG.[79] (*d*) Schematic diagram of the closed-loop system for the bionic-limb.[23].

and alleviating diseases such as heart failure, epilepsy, and depression as a bioelectronic medicine field.[74–76] However, since these various functions are regulated by the vagus nerve, selective nerve stimulation to produce only the desired effect is essential. As shown in Figure 4(*a*), optogenetic stimulation and electrical stimulation were applied to the right vagus nerve to compare the two stimulation methods.[77] The vagus nerve is a mixed nerve with efferent and afferent fibers. Because these researchers expressed the opsin ChR2 only in efferent fibers (GFP+), optogenetic stimulation stimulated only efferent fibers, whereas electrical stimulation stimulated not only efferent fibers, but also afferent fibers (PGP9.5+) using hook electrodes. The right vagus nerve controls function mainly related to the heart. Both optogenetic stimulation and electrical stimulation decreased heart rate during normal right vagus nerve stimulation, but electrical stimulation decreased heart rate only after right vagotomy (RVNx) since it also stimulated afferent fibers transmitting the signal to the brain. In this experiment, hook electrodes, which do not have selective stimulation capability, were used for stimulation afferent fiber, so, it cannot be concluded that optogenetic stimulation is superior to electrical stimulation. However, it has been shown that optogenetic stimulation with excellent selectivity is suitable for nerves containing fascicles responsible for multiple functions, such as the vagus nerve.

### 3.2. Gastric optogenetics

Optogenetic technology is not limited to neurons. As shown in Figure 4(*b*), food intake can be controlled by expressing opsin in smooth muscle cells of the stomach.[78] In this research, an attempt was made to figure out how certain components of the vagus nerve contribute to behavior and long-term physiological effects, and the target was to reveal the role of stomach vagal afferent endings in feeding behavior. To achieve the goal, the researchers chose the optogenetic method for selective stimulation and developed a wireless microLED (μLED) stimulator for long-term experiments and freely behaving conditions of rats that can be well operated inside harsh gastric conditions. For cell-type specificity, CalcaCre transgenic mice received a left nodose ganglion injection of AAV9-DIO-ChR2:tdTomato virus. By using the optoelectronic device, the

*Calca*+ vagal afferent chemosensitive endings in the stomach, that is a related neuron in the role, was selectively stimulated, and it turned out to conditioned mice to avoid the sucrose solution via a negative balance mechanism. These results indicate that the stomach mucosal *Calca*+ vagal afferent's role in appetite suppression and the mechanism of it.

### 3.3. Pelvic nerve stimulation using TENG

As briefly mentioned above, TENGs generate biphasic pulses with non-rectangular waveforms, which can be used for direct nerve stimulation. Figure 4(*c*) shows inducing urination by stimulating the pelvic nerve, which is a parasympathetic nerve that directly modulates bladder functions, using these TENGs.[79] They implemented the TENG and connected it to a neural clip electrode introduced in Section 2.1.2 to directly stimulate the pelvic nerve. The TENG was tapped at a rate of 50 BPM or higher to stimulate the pelvic nerve to induce urination. It generated an asymmetric charge-balanced biphasic current waveform, which is a favorable waveform for nerve stimulation. The amplitude of the major positive pulse exceeds the threshold for stimulation and has a short pulse width that makes the nerve stimulated only once during this phase. In the case of the major negative pulse, the amount of charge is the same as the major positive, however, has longer pulse width. Thanks to this fact, the amplitude does not exceed the threshold, avoiding stimulation of the nerve in this period, while achieving a charge balance. To generate this kind of waveform using commercial equipment requires a large stimulator or complicated circuits. On the other hand, TENG generated this waveform with a simple "tapping" operation and also achieved a charge value that was enough to stimulate the pelvic nerve and presented artificial urination.

## 4. Closed-loop system

Even if a great device that satisfies the conditions such as selectiveness, low volume, biocompatible, etc. is fabricated, if it cannot determine "when" (appropriate time to operate the device) and "how much" (how strong output it will produce), the value and effectiveness of the device will decrease.[80,81]

In order to determine "when" and "how much" and communicate with the device, information from outside (pressure, temperature, etc.) or inside the body (biomarker-electroneurogram, electromyogram, electrocardiogram, etc.) must be received, and the role of getting the information is played by the sensor. After the data is obtained from the sensor, it has to go through a process to be meaningful (usable) data to determine "when" and "how much" more specifically and accurately. The first process is to remove unnecessary data such as noise from the vast amount of data obtained through the sensor by using filters to leave only the desired data as much as possible. After that, it goes through a process of extracting useful information for decision-making through feature extraction.

Finally, the user empirically decides the value of "when" and "how much" of the device based on the extracted data and the response of the organ. By importing the procedures and the matched values to the algorithm of the controller, it makes the entire process can be performed automatically and quickly by matching the data obtained from the sensor and the "when" and "how much" value of the device with a small delay-time.[82]

### 4.1. Closed-loop system for bionic limb

For the case of electrical stimulation, recently there are studies about a closed-loop system for the bionic limb not only to realize the embodiment but also to be able to perform movements that better reflect the patient's intention.[83] Actually, the movement of the bionic limb itself has

already reached a certain level of maturity,[84] but further research is still needed to be able to produce natural movements that reflect the patient's intention, not rigid mechanical movements by continuously communicating with the patient.

As the conceptual image of this procedure (Figure 4(*d*)), the electronic skin acts as a sensor that receives external information.[85] Then, by using the microcontroller, the received signal is processed and then matched with the type and magnitude of the sensory information such as touch, pain, or temperature. Based on the matched sensory information, activating the proper afferent fibers from the nerve using peripheral interfaces that have high selectivity, the afferent signals are delivered to the brain then, feel senses. When the user responds, the brain transmits the motor commands through the efferent nerve. By receiving and decoding the efferent signals with peripheral interfaces, the signals are used to control the bionic limb. By simultaneously operating these two interactions (motor and sensory), advanced bionic limb performing natural movements based on the patient's intention just like a part of the body with realistic sensation could be achieved. A recent study showed that pulse modulation-based peripheral stimulation through peripheral interfaces delivered realistic sensation to a user who wears an upper limb prosthesis.[86] Also, Valle et al. showed that sensory feedback via the sciatic nerve resulted in functional and cognitive benefit in lower limb prostheses.[87]

## 5. Conclusion

The PNS plays an important role in transmitting sensory information obtained from organs to the CNS and transmitting commands from the CNS to organs or muscles. Since it has better accessibility compared to the CNS, research is being actively conducted to apply various types of neuromodulation to a variety of fields such as bioelectronic medicine and advanced bionic limbs. Among them, electrical neuromodulation is a conventional approach and is widely used for neuromodulation. We introduced the basic stimulation principles and method for this electrical neuromodulation and reviewed studies using various electrode designs in the direction of increasing selectiveness while inflicting less damage to nerves. In addition, voltage sources and current sources were discussed as electrical energy sources with pros and cons. Also, we introduced TENG, which can generate charge-balanced biphasic current waveform autonomously through energy harvesting. At the same time, we introduced that optogenetic neuromodulation can control the inherent limitations of selectivity of this electrical neuromodulation, and performs higher selectivity compared to the electrical modulation by controlling (exciting, inhibiting) ion channels in units of neurons. Although it is still necessary to prove the stability and efficacy of chronic opsin injection, it has shown that it is possible to perform precise selective stimulation through various applications and also can be applied to smooth muscle cells as well as neurons. Finally, the concept and importance of a closed-loop system were discussed with an example of bionic limbs. This closed-loop system is required not only for advanced bionic limbs but also for bioelectronic medicine. Various neuromodulation methods, which are developing in various directions, are expected to eventually be applied to a device with excellent selectiveness and a closed-loop system that can replace a broken body system.

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