Field of Life Sciences

Achievement

The development of methods that use genetically addressable light-sensitive membrane proteins to unravel neural circuit function

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Previous methods using electrical stimulation and drugs

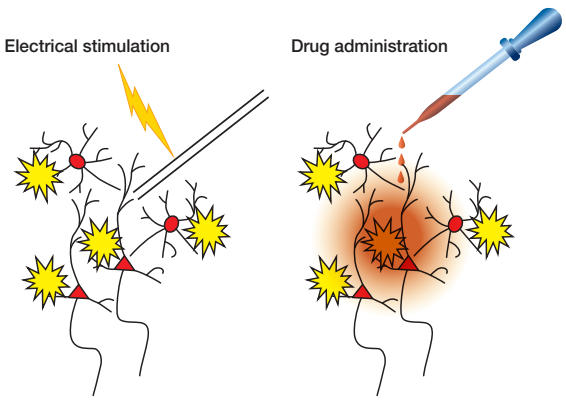
The brain is composed of an enormous number of nerve cells, or neurons, which form complex circuits that are used to exchange information. The reason humans are able to think and move is because the neurons responsible for controlling those functions are activated and thereby transmit information correctly.

Electrical stimulation and the administration of drugs have long been used to study the neural circuitry that controls behavior, thought, and other functions.

Electrical stimulation is applied by inserting a thin electrode into a specific region of the brain and passing an electrical current through it, which either activates or deactivates neurons in the area to study how behavior changes. However, this method changes the behavior not only of the targeted neurons but also any surrounding cells, which makes it difficult to precisely determine the role of a specific neuron.

The drug method involves local administration to the brain of a drug that activates or deactivates specific neurons, at which point subsequent behavioral changes are observed to understand the role of those neurons. However, neural activity moves at a rate measurable in milliseconds, so because it takes time for drugs to affect the targeted neurons, there is a limit to what can be gained from this method.

Figure 1: Conventional methods for investigating the role of neurons



Using conventional methods such as electrical stimulation (left) and drug administration (right) to manipulate neural activity results in other cells near the targeted neurons also being affected (yellow explosions in figure).

A new technology that controls neurons freely using light

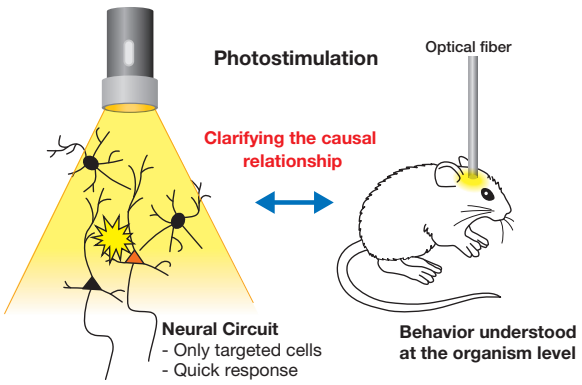
The method using light developed by Miesenböck and Deisseroth overcomes all the shortcomings of previous methods.

In 2002, Miesenböck developed a new technique that allowed him to use genetic manipulation to express photo-sensitive proteins in specific neurons, the activity of which could then be controlled through exposure to light. Then in 2005, Deisseroth used Chlamydomonas, a type of green algae that contains a photosensitive protein called channelrhodopsin, to improve this technology and make it more precise and easier to use, thereby making it possible to apply in a wide range of research fields.

This technology makes it possible to illuminate and control the activity of specific neurons in the brain of a living animal using optical fibers and other equipment, only changing the activity of the neurons being targeted. Furthermore, this technique allows neural activity to be freely turned on and off on a time scale of milliseconds or microseconds. For example, channelrhodopsin can be expressed in neurons in mice in the part of the brain called the amygdala, and they can then be illuminated with light using an optical fiber. Mice are normally uneasy in open spaces and so they tend to stay close to walls when they move, but after light is shone on specific neurons, they can temporarily be made to feel less anxious such that they can walk around far from walls without issue. In this way, the direct causal relationship between neuron activity and behavior can be made observable.

This light-based technology has revolutionized research in the field of neuroscience, and it continues to evolve today.

Figure 2: The award-winning technique



By expressing photosensitive proteins in specific nerve cells, it is possible to control the activity of target neurons through photostimulation.

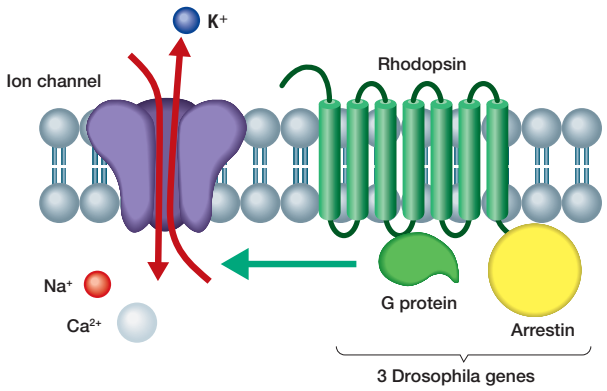
Developing the technique – Concepts and principles

Miesenböck was first to come up with the concept behind the technique and the principles upon it is based, and he was the first to demonstrate its utility. In 2002, Miesenböck took three photoreceptor genes from the eye of Drosophila, including a photosensitive protein called rhodopsin, and expressed them in hippocampal neurons of rats, whereupon he demonstrated that activity in these neurons could be freely controlled by illuminating them with light. This is possible because when rhodopsin senses light, the cell membrane opens something called ion channels, proteins that transport ions in and out of a cell. The flow of ions in and out of the cell produces an action potential, the mechanism by which neurons are activated.

Initial experiments were conducted in vitro, but the experiments were repeated in 2005 on living Drosophila. That experiment successfully induced escape behavior in the flies (flying and flapping wings) by using light to control activity in specific neurons. Moreover, in 2008, the same principles were used to identify the neural network in male Drosophila responsible for controlling courtship behavior.

These results had an immense impact as they demonstrated that photostimulation could be used to directly research the relationship between neural activity and behavior.

Figure 3: Illustration of one of Miesenböck's principles



Source : Miesenbock (Neuron 2002)

The structure of rhodopsin changes when exposed to light, activating G proteins and indirectly opening ion channels (green arrow.) When an ion channel is opened, Na^+, Ca^{2+}, and K^+ move in and out of the cell (red arrows) thereby transmitting information by inducing a large change in the membrane potential called an action potential.

Algae genes open the way to discovering the potential of the technique

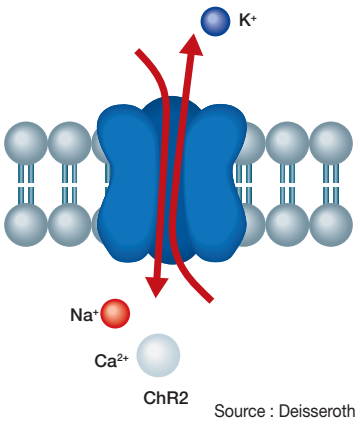
Deisseroth developed a technique for manipulating neural activity using light by focusing on the protein channelrhodopsin, or ChR2, a microbiological rhodopsin reported to be involved in photoaxis in green algae.

ChR2 is a protein that functions as a rhodopsin, which reacts to light, and as an ion channel, which allows ions to pass through the cell membrane, and one of its properties is the short time between it being exposed to light and the channel opening. In other words, using ChR2 has the benefit of making the process simpler because it involves the introduction of only one gene, and still allows for precise control of neural activity.

In 2005, Deisseroth demonstrated that photostimulation of ChR2 expressed in cultured hippocampal neurons from rats could affect neuronal activity within milliseconds. He later used the technique on live mice and successfully identified the neuronal population that produces gamma brain waves, and clarified the neural mechanism that controls social behavior and learning.

A variety of channelrhodopsins with different functions are currently being developed, and the technique is in wide use in everything from pure research to the development of treatments for neurological diseases. Algae genes opened the way to discovering the potential of this technique that has revolutionized neuroscience research.

Figure 4: Using the technique with channelrhodopsin (ChR2)



Source : Deisseroth (Nat Neurosci 2005)

The structure of ChR2 changes when exposed to light, opening ion channels contained with the protein itself. When an ion channel is opened, Na^+, Ca^{2+}, and K^+ move in and out of the cell (red arrows) thereby transmitting information by inducing a change in the membrane potential called an action potential.