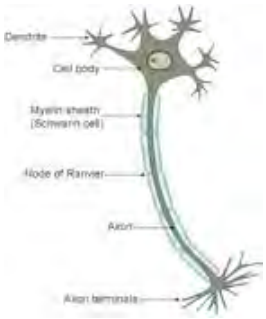
Silencing Your Cells

Often referred to as the 'big black box' of medical research, the brain and nervous system are among the least well understood of all physiological systems. We know that the basic building blocks of the nervous system are nerve cells or neurons . It is estimated that the human nervous system contains around 100 billion (1 x 10^{11}) neurons of many different specialized functions. Neurons are different from other types of cells in your body because they are able to send messages from one cell to the next. You can think of the brain as the control center for the whole operation. From the brain, the nervous system extends outwards to the extremities. Even a simple finger movement involves thousands of neurons that send messages from the brain to your finger and back. To see how this works, let's first take a look at individual neurons and how they function.



Each neuron consists of a cell body , which is similar to other types of cells in the body and contains many of the same sub-units, called organelles . But stemming from the cell body of the neuron are branches called axons and dendrites that make it possible to send messages from one cell to the next. Other types of cells simply don't have axons and dendrites. Generally a neuron will have only one axon but can have many dendrites. Incoming information will flow down the axon to the cell body and then back out to other cells through the dendrites. [See the YouTube video at http://www.youtube.com/watch?v=YwN9aCobCy8 for an illustration of this.]

You may be wondering what kind of information one neuron sends to the next. The "messages" neurons send are actually electrical impulses created by electrically charged atoms called ions . When an electrical impulse is "sent" from one neuron to the next, we say that the neuron "fired."

Action Potential

To understand what happens when a neuron fires, let's first look at a neuron before it is stimulated. A neuron at rest maintains a negative charge with respect to the outside of the cell. This means that there are more negatively charged particles inside the cell than outside. When a neuron at rest receives an electrical impulse from a nearby neuron, channels on the cell membrane are opened to allow for positive ions to enter the cell. This influx of positive ions creates an electrical current that is then passed to the next cell. Once the signal has been sent, the process is reversed and positive ions are moved to the outside of the cell to bring the neuron back to rest. This process of creating an electrical current through chemical signals is called an Action Potential. Neurons are able to communicate, then, through the creation of an action potential that sends electrical impulses from one cell to the next.

So we have a pretty good understanding of what goes on with neurons at the cellular level and the mechanisms that allow them to communicate. What we do not know much about, though, are the larger processes and patterns of neuron firing that allow for attention, memory, emotion, and almost every other function humans are capable of. One way to determine the function of a particular type of nerve cell would be to inactivate it (= turn it off). In the last decade or so a new scientific field has emerged known as optogenetics that combines optics and genetics to do just that. Optogenetics doesn't suppress neuron function permanently. Rather, it can be used to temporarily silence the action of specific neurons, allowing researchers to understand which neurons are involved in particular functions.

Opsins from Around the World

The key to optogenetics are light-activated proteins called opsins that convert light into electrical energy. Opsins are found in photosynthetic organisms, such as particular species of bacteria and fungi. While opsins have been known for a long time, their use has been limited by technology. A new breakthrough by researchers at the Massachusetts Institute of Technology, however, has renewed interest in these photosynthetic organisms.

Dr. Edward Boyden, of the Massachusetts Institute of Technology, has spent his career developing the techniques of optogenetics. Dr. Boyden began his work with opsins as a small

Dr. Boyden's lab team

Shown, right to left: Ed Boyden, Jiamin Zhuo, Masaaki Ogawa, Anthony Zorzos, Annabelle Singer, Daniel Schmidt, Amy Chuong, Nathan Klapoetke, Brian Allen, Giovanni Talei Franzesi, Christian Wentz, Patrick Monahan, Kyungman Kim, Aimei Yang, Alex Guerra, Mike Baratta, Claire Ahn, Jake Bernstein, Xue Han, Mike Henninger, Brian Chow

This YouTube video illustrates the movement of a message through a neuron:

In this 3-minute video Dr. Boyden offers an overview of his work and its goals:

Video courtesy of MIT

Student Worksheet
June 2010

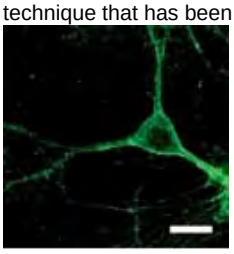
side project as a graduate student, and soon began spending all his time working on these molecules. He and his team have been surveying many different ecological niches around the world for organisms that exhibited photosynthetic properties, or the ability to turn light into electrical energy. In 2005, he and his collaborators showed that one of these molecules, channelrhodopsin-2 (ChR2) could, when expressed in neurons, enable them to be activated by pulses of blue light. Thus, blue light could enable information to be entered into specific circuits within the brain. This tool is in now use by hundreds of research groups around the world, who use it to understand how neural circuits compute. However, the ability to silence neurons with light remained a mystery. In 2007, Dr. Boyden's group published the use of a molecule, Halo, to enable neurons to be quieted by yellow light, but the effects were small, and the desire for a more powerful reagent remained.

Recently, Dr. Boyden and his colleagues found two proteins known by the nicknames "Arch" and "Mac". Arch is a protein from a type of archaebacterium , an ancient relative of bacteria found in the Dead Sea, that is stimulated by yellow light; Mac is a protein from a species of fungus stimulated by blue light.

How Do Arch and Mac Work?

Arch and Mac work by pumping protons, or hydrogen (H+) ions, out of the neurons. This means that Arch and Mac transport charge in the opposite direction of the neuronal action potentials. By moving positive charges out of neurons, the voltage of the neuron decreases, preventing the neuron from producing an action potential. A neuron that cannot produce an action potential cannot fire or transmit a signal to other cells. It is rendered temporarily silent. "The beauty of Arch and Mac is that they can be stimulated by light and once the light is turned off, the neuron returns to its normal state. So this response can be turned on and off very precisely," explains Dr. Boyden.

Dr. Boyden began by testing the effectiveness of each of these proteins in vitro using neurons in culture. The gene for each protein was inserted into a virus known as a lentivirus . Other viruses being used by Boyden such as adenoassociated-viruses or AAV are particularly useful for inserting foreign genetic material into cells because they can easily cross the cell membrane and permanently and safely deliver the genetic payload inside. The use of AAV is a safe and effective technique that has been employed extensively in many different areas of research in the last several years. Once the proteins had been inserted into the neurons, Dr. Boyden and his team used light to reduce neuronal activity. When the light was off, the neurons functioned normally and fired in response to sensory inputs or during behavior. When the yellow light was turned on, Arch was stimulated to pump protons out of the neuron, which silenced the neurons expressing it. When the blue light was turned on, Mac was stimulated and the cells expressing it were silenced. These initial results indicated that Arch and Mac were effective proteins for temporarily inhibiting neuron function. It is important that the two proteins respond to different colors of light because this enables researchers to examine two types of neurons at once.



The Next Step

Once Dr. Boyden and his team demonstrated that Arch and Mac worked in vitro, they were ready to see if this strategy could work inside the body. They developed the techniques needed to continue their experiments in vivo in mice. For the in vivo experiments , Dr. Boyden and his team developed a small light source to deliver beams of light to stimulate Arch. Dr. Boyden found that, when stimulated, Arch effectively (100%) silenced neurons and the neurons were able to recover very quickly.

"The applications for this new technology are endless," explains Dr. Boyden. "We can use this tool for basic science research to study how different parts of the brain function as well as, potentially down the road, clinically to treat diseases such as epilepsy or Parkinson's disease that result from erratic or overactive neuron firing."

> In epilepsy, there is an overactive area of the brain which stimulates action potentials that are out of synch with the rest of the brain, resulting in a seizure. If a sensor could detect changes in action potentials in different areas of the brain, Arch or Mac could then be used to quiet the overactive neurons and prevent the seizure. "These tools still need to be tested further before we can use them clinically, but my hope is that this technology will help lots of people in the future," remarks Dr. Boyden.

Opsins are also being used in research on mood, including anxiety and post-traumatic stress disorders . Initial experiments in mice are helping Dr. Boyden and his team to determine which areas of the brain are involved in expressing different emotions. "Once we understand what parts of the brain are involved in different functions, we can develop more precise drugs or other treatments to help people with anxiety or depression disorders," notes Dr. Boyden.

Teacher Guidance
June 2010

Sign Up for our Monthly Announcement!

...or subscribe to all of our stories!

What A Year! is a project of the Massachusetts Society for Medical Research.

And it is funded by a grant from **The William Townsend Porter Foundation**

Dr. Edward Boyden is a neuroengineer , an Assistant Professor at the Media Laboratory as well as a Joint Professor in the Departments of Biological Engineering and Brain and Cognitive Science at Massachusetts Institute of Technology. He received his undergraduate degree in physics and electrical engineering from Massachusetts Institute of Technology. He went to graduate school in Neuroscience at Stanford University. He views the brain as a computer and is able to combine his interests in engineering and neuroscience into a new field of neuroengineering. When not in the laboratory, Dr. Boyden enjoys writing nonfiction and fiction, as well as spending time with his family and traveling.

For more information:

1. Chow, BY, Han X, Dobry AS, Qian X, Chuong AS, Li M, Henninger MA, Belfort GM, Lin Y, Monahan PE, Boyden ES. (2010). “High-performance genetically targetable optical neural silencing by light-driven proton pumps.” Nature, 463: 98-102.

2. Popular descriptions of neural silencing: http://syntheticneurobiology.org/news_items

To Learn More:

1. Dr. Boyden’s webpage: www.syntheticneurobiology.org, and here is a sub-page with lots of articles and podcasts: http://syntheticneurobiology.org/publications/list_publications/40

2. Rewiring the Brain: Inside the Science of Neuroengineering. Available at: http://www.wired.com/science/discoveries/news/2009/03/neuroengineering1

3. Neuroscience at NIH (the National Institutes of Health). Available at: http://neuroscience.nih.gov/

4. Silencing Brain Cells with Yellow and Blue Light. Available at: http://www.nsf.gov /news/news_summ.jsp?cntn_id=116178&org=NSF&from=news

Rebecca Kranz with Andrea Gwosdow, Ph.D. Gwosdow Associates

Image Credits

Neuron schematic: http://scienceblogs.com/neurotopia/2006/07 /stem_cells_for_spinal_cord_inj.php

The Dead Sea: The Central Intelligence Agency World Factbook https://www.cia.gov/library/publications/the-world-factbook/geos/is.html

Neuron with the Arch gene:
Brian Chow, Xue Han, and Edward Boyden

Flight data recorder:
National Transportation Safety Board