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Investigating the Single Cell Chemistry of the Brain

Behaviors and Your Brain

Have you ever wondered what makes you behave the way you do? What gives you the ability to make memories and recall them later? How you can perform complicated tasks requiring coordination such as playing a sport or a musical instrument? Or even, how you can experience a wide range of emotions from fear and anxiety to love and happiness? What allows this variety of behaviors is the chemical communication that takes place in your brain. Chemicals called neurotransmitters are traded between brain cells, creating signals that tell your brain to perform certain behaviors. For many people, this communication is well controlled and regulated by the brain. However, for some people, these brain processes stop working correctly, causing a variety of medical problems. In order to understand these medical issues and create treatments to care for and cure them, we must first understand the basics of chemical communication and how it results in certain behaviors. By understanding normal, regulated brain processes we can determine what has changed in brains with diseases or illness. In this reading, we will briefly touch on the structure of the brain and how chemists utilize scientific tools to investigate the chemical makeup of the brain, with an emphasis on one method called single cell mass spectrometry.

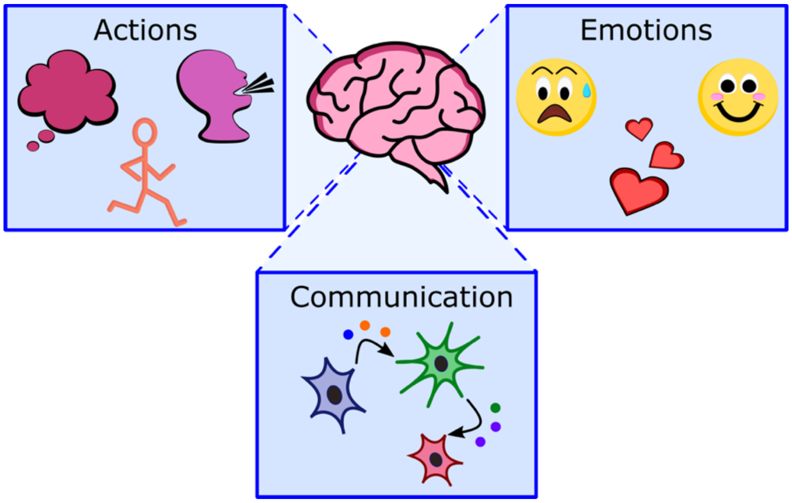

Figure 1. Your brain is responsible for how you interact with and react to the world around you. But how does it cause and control the behaviors you and I experience every day? The answer is chemical communication that occurs between specialized cells in your brain.

Structures of the Brain and Their Role in Behaviors

The passage of neurochemicals throughout your brain is controlled by a variety of structures within your brain. The images of brains most of us are familiar with seeing show a wrinkly, pink blob of squishy looking tissue. From the outside, the brain may look like one big mass, without much variation throughout. However, if we crack open the brain, we can see a large variety of structures and shapes throughout the brain. In fact, the brain has multiple levels of organization! The next level of organization down from the whole brain is what we will refer to as distinct brain regions. Brain regions are often named after their shape, location, or function. Two examples of brain regions are shown in figure 2: the hippocampus (left) and the cerebellum (right). The hippocampus (pronounced hip-puh-**kam**-puhs, derived from the Greek words for horse and sea monster) got its name due to its resemblance of a sea horse! Despite its odd shape, the hippocampus has many roles in forming and maintaining memories along with learning and spatial navigation. For the cerebellum (pronounced seh-ruh-**beh**-luhm, its name denotes its functions and shape since cerebellum means "little brain". The cerebellum is known to play a role in coordinating muscle movement and balance. Excitingly, researchers are currently exploring its role in social and emotional behavior making the cerebellum sound more and more like a smaller, secondary brain! These distinct regions of the brain also have their own unique, interior structures which help determine the possible functions of that brain region.

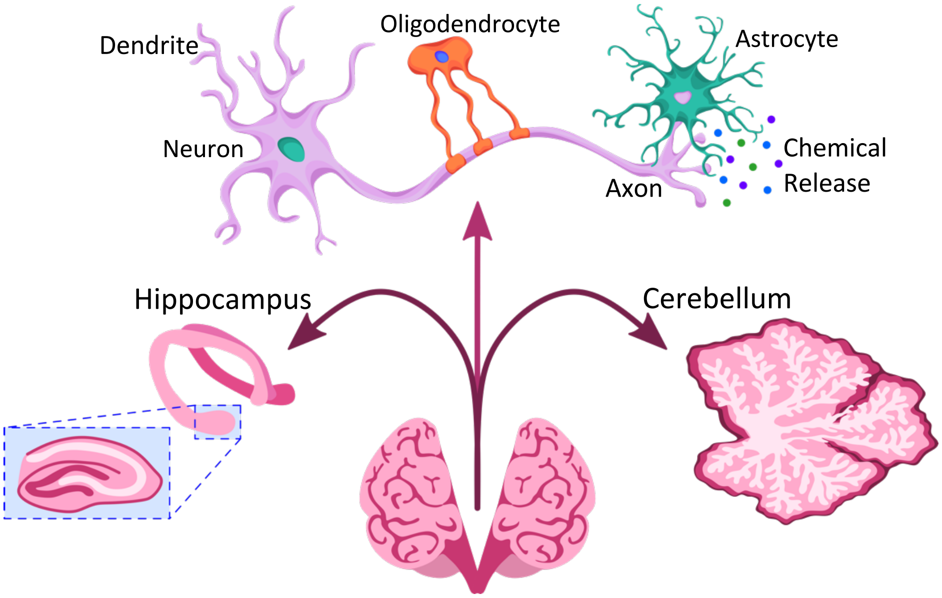

Figure 2. The brain is a complex organ made of a variety of structures that allow it to perform the amazing functions we rely on every day. Looking inside the brain, we first see major structures or distinct brain regions. Shown on the left-hand side is the hippocampus which plays an important role in memory. On the right-hand side is the cerebellum which helps coordinate your movements. Each brain region has its own unique, interior structure, with the hippocampus showing folded layers and the cerebellum showing complex branching. These interior structures are made up of many brain cells falling into two main categories: neurons and glial cells (ex. astrocytes, oligodendrocytes).

These interior structures are made possible by the layering and interaction of a variety of cells. Often referred to as the building blocks of biological tissues, cells provide structure, utilize nutrients, and different types of cells perform different types of tasks. In the brain, there are many different types of cells, but they can typically be grouped into two categories: 1) Neurons (pronounced **nur**-ahn) – the messengers of the brain, 2) Glia (pronounced **glee**-uh) – the support system of the brain. Neurons role as messengers means they are the cells responsible for the chemical communication introduced earlier. In addition to chemical messages, neurons can send electrical pulses (like a small electric shock). Together these chemical and electrical messages allow you to sense, interact, and react to the world around you. The structure of neurons allows them to both send and receive these messages. The main body of the cell, referred to as the soma, contains the nucleus which holds the cell's genetic material and makes decisions about what messages should be sent. From the soma, two types of structures branch off. The shorter, more numerous branches are referred to as dendrites. The dendrites receive signals from other neurons and relay the message to the soma. The longer branch is the axon. Each neuron has only one axon which is used to send messages to other neurons. During communication, one of neuron B's dendrites receives a message from neuron A's axon. The message is passed to neuron B's soma, where it is decided whether to pass the signal on further. If the signal needs to be relayed further, neuron B's axon will send the message to neuron C's dendrite where the process is repeated.

On the other hand, glial cells play a supporting role in this communication. Glia is the latin word for glue, but glial cells do far more than just glue together all the neurons! Glial cells play diverse rolls from clean-up of excess messenger neurochemicals to speeding up the messages. Some glial cells known as astrocytes (pronounced **a**-struh-site) are the clean-up crew at sites called synapses. The synapse is the space between the axon and receiving dendrite of neurons. When neurochemicals are released from the axon and flow towards the dendrite, not all are received and taken up by the receiver. Any of the excess chemical is cleaned up and recycled by the astrocytes. Astrocytes also help keep neurons healthy by releasing nutrients and playing a role in immune responses. Another type of glial cell, called an oligodendrocyte (pronounced oh-li-gow-**den**-druh-site), produce projections that wrap around the axons of neurons. This wrapping is a protective barrier made of lipids (fats) that speeds up message transmission.

Different types of neurons and glial cells are important for sending different types of messages and performing different tasks. To perform their respective functions, each type of cell needs a specific compliment of neurochemicals and cellular machinery. Knowing what those specific compliments of neurochemicals are provides an opportunity to better understand how behaviors and healthy brain functioning are controlled. Furthermore, knowing how those chemical compliments change during illness and disease allows us to determine how diseases affect the brain and hopefully how to treat them. Since there are many types of cells, each varying in abundance (some number in the billions while others may only be present in the hundreds), it is important to directly probe the heterogeneity (variability, diversity) of brain cells. To do so, chemists have produced many tools to determine the chemical makeup of individual cells!

How do Scientists Explore the Chemistry of the Brain?

A variety of tools and techniques have been designed by scientists to study the chemical heterogeneity of single cells. Spectroscopy (observation of light interacting with matter), electrochemistry (relationship between electricity and chemistry), RNA or DNA sequencing (decoding the order of amino acids that makeup genetic material), and mass spectrometry (determination of chemicals' identities based on measurement of their mass) are all popular tools for observing individual cells. Each of these tools have unique strengths and weaknesses along with providing different information. Because of this, one tool may be favored for a particular application, or a combination of many tools may provide more comprehensive answers to the questions that scientists are trying to answer. Some tools provide lots of chemical information but may cost lots of time or money to perform, limiting the number of cells that can be analyzed. Other tools may only provide information about a select few chemicals.

Mass spectrometry is a particularly interesting tool because it provides rich chemical information and is high throughput. This means scientists can analyze 1000s of single cells and obtain information about tens to hundreds of chemicals within them. At University of Illinois Urbana Champaign, the Sweedler lab is interested in doing just that. Within the Sweedler lab, we analyze 1000s of single cells using mass spectrometry to catalog the neurochemicals within them. We focus on studying the brains of animals such as sea slugs, rats, and fish that display interesting behaviors like humans such as learning, memory, and social interactions. For these studies we have developed a specialized technique to isolate single brain cells from our animals of interest and then measure their chemical content with mass spectrometry.

Single Cell Mass Spectrometry – Brain Sampling

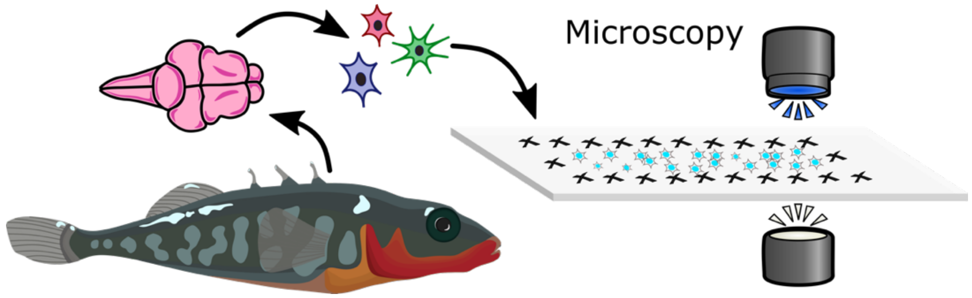

Figure 3. The brain of our animal model is isolated, and the desired brain region is broken down into individual cells. Those cells are dyed and imaged using a microscope.

To measure individual brain cells of different animals, we first isolate the brain structure of interest and separate all of the cells from each other. As stated earlier, the brain contains many types of cells that interact with each other. Because of these interactions the axons, dendrites, and other branches that project out of the cells are all intertwined. This intertwining means we must use force to break apart the cells, but we also must take care that we don’t break the cells. To do this, we use a process that first chemically breaks down many of the cells’ branches and then separates the remaining cell bodies from any debris. Typically, this process is done using only one, desired major structure of the brain. Breaking down the entire brain this way would produce more cells than we could analyze with most of our mass spectrometry methods. Furthermore, we are usually interested in the role that a specific brain region plays in behaviors of interest and so we only need to analyze cells from that structure.

Once the cells are separated and collected in a stabilizing liquid, we spread them out on a glass microscope slide. Since the cells we work with are often 10 – 100 micrometers large (about how thick one strand of hair is), we also dye them with a blue, fluorescent stain to make them easier to find. The fluorescent property of the dye means the cells will glow blue when a specific color of light shines on them. So, we can use a fluorescence microscope equipped with a colored lamp that when directed on the cells makes them glow blue. We use the fluorescence microscope to both capture an image of the entire microscope slide and make the cells glow in this image. Using the image of the entire microscope slide, we can determine the location of individual cells relative to each other and the edges of the slide. Using specialized computer software, we number each cell according to its location so we may analyze each cell individually.

Single Cell Mass Spectrometry – Analysis with MALDI Mass Spectrometry

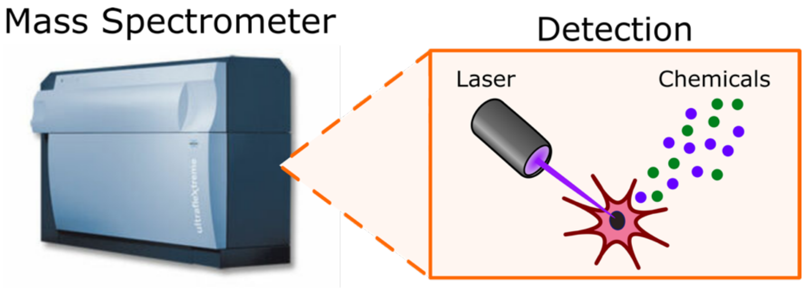

Figure 4. The slide containing our cells is placed into the mass spectrometer and a strong laser is directed at each individual cell, one after another. The laser causes a small explosion at the cell surface, releasing the chemical contents of the cell into the mass spectrometer. The chemicals are then detected and catalogued as a list of masses. This list of masses is then used to determine the identities of the chemicals that were inside the cell.

After exporting the locations of all the cells on the slide, we give that information to the mass spectrometer. Now the mass spectrometer knows the location of each cell on the slide. We then insert the slide into the mass spectrometer and start acquiring data. The method we use is called MALDI or matrix assisted laser desorption ionization. While the name is long, the main idea is simple. To perform MALDI, we cover our slide of cells with a light absorbing chemical and then shoot a high-powered laser at each cell. The laser interacts with the light absorbing chemical on the cell and causes a build-up of heat and energy. This build-up eventually becomes too intense, and the contents of the cell erupt from the surface of the slide! The chemicals that erupted from the cell are then collected and catalogued based on their mass. The mass spectrometer provides us with a list of the catalogued masses which we then use to determine what chemicals are present in the cells.

After determining what chemicals are present, we must relate them to the behavior of interest. Typically, we must compare two groups that show different behavioral norms, a control group and an experimental group. For example, our control group may be animals that perform behaviors that do not deviate from the expected norm. On the other hand, the experimental group will show behaviors that are not normal, either due to disease or some naturally occurring change in the chemicals used by the brain to control the interesting behavior. Our goal is to compare the lists of chemicals for each group and determine how those lists change in relation to changes in behavior. For example, one of our current goals is to determine how differences in parenting behaviors occur in a species of fish called the Three-Spined Stickleback (depicted in figure 3). One population referred to as common fish are very attentive parents, taking diligent care of their offspring. The other population referred to as white fish are the opposite, inattentive parents that do not take care of their offspring. In this case, the common fish are our control group, and the white fish are our experimental group showing a deviation in expected parenting behavior. Currently, we are working to determine what chemicals are responsible for these differences in behavior. Our hope is that we can relate our findings for all our animal behavioral studies to human behaviors so that we can better understand how our own brains work.

For further readings about many of the topics introduced in this primer on “Investigating the Single Cell Chemistry of the Brain,” see the following resources:

1) Anatomy of the Brain: https://www.hopkinsmedicine.org/health/conditions-and-diseases/anatomy-of-the-brain
2) The Hippocampus: https://www.britannica.com/science/hippocampus
3) The Cerebellum: https://www.britannica.com/science/cerebellum
4) What is a Cell: https://medlineplus.gov/genetics/understanding/basics/cell/
5) A Guide to Brain Cells: https://neurosurgerycnj.com/a-guide-to-brain-cells/

For those interested in introducing scientific articles to their reading lists, the following articles review chemical analysis of single cells and a couple specific applications of single cell mass spectrometry:

1) Review – Chemical Analysis of Single Cells: https://doi.org/10.1021/acs.analchem.0c04361
2) Single Cell Analysis – Neurons vs. Astrocytes: https://doi.org/10.1002/anie.201812892
3) Single Cell Analysis – Pancreatic Islets: https://doi.org/10.1021/acschembio.6b00602