Neuroscience: Canadian 3rd Edition

NEUROSCIENCE: CANADIAN 3RD EDITION

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Introduction

The writing of Neuroscience was motivated by our collective passions to further the understanding of the science in an accessible and engaging manner. We wrote this book as a window into the interesting world of neuroscience and to hopefully supplement your knowledge of the subject. Here, we discuss the fundamentals and basic themes of neuroscience, as well as newer developments and emerging topics. The topics range from neurodegeneration and common neuroscience lab techniques to the gut-brain-axis and crossovers with various other disciplines. The textbook is designed in a way to match typical neuroscience syllabi at the undergraduate level.

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FUNDAMENTALS OF NEUROSCIENCE

Santiago Ramón y Cajal is considered by many to be the father of modern neuroscience. What many do not know is that as a charismatic and fervent young man, his true passion was not for the sciences but rather for the arts. While familial pressure resulted in him entering the field of medicine, his romantic and poetic spirit never ceased. His journey towards birthing modern neuroscience began when he came across a 14 year old technique termed the Golgi (Silver Nitrate) stain in a textbook. This technique had revolutionized microscopic investigations of the nervous system, as it allowed for intricate staining of entire cell bodies as well as the fibrous branches which shot out from them. He was captivated, almost hypnotized, by what he saw. When he learnt how to set up the stain in his lab, he became consumed with the task of improving its resolution, and succeeded in doing so. Throughout this obsession, he created thousands of hand-made drawings depicting what he saw under the microscope. In his autobiography, he states "Look, here I am pursuing a goal of great interest to painters: appreciating line and color in the brain." He termed the cell bodies "mysterious butterflies of the soul" which reside in a "flower garden of grey matter."

With these images, Cajal was able to make one of the most foundational discoveries in the field of neuroscience. Up until that point, experts had postulated that the entire nervous system was a continuous network of fatty tissue; an uninterrupted web of nodes and wires, completely fused like one large pipe system. People knew that nerves conduct electricity, and at the time, the only known way to propagate such a spark was through continuous, uninterrupted copper wiring. With his imaging, Cajal was the first to suggest that there exists a tiny space between every one of the billions of neurons in this network, which we now know as the synapse. He inferred that electricity was somehow jumping past this space, from neuron to neuron, in chains of communication. Set in the 1800s, this theory he sketched was unbelievably bold, and completely counterintuitive to existing conjectures. However, it was empirically supported by his massive collection of drawings. Many years later, his ideas were confirmed by electron microscopy. This is a recurring theme throughout science; observation trumps intuition every day of the week.

While this discovery is his most famous, it is not the only contribution that Cajal made. He was the first to predict that these cells are "dynamically polarizable" and "adaptable", referring to the characteristic restructuring of neural connections with development and experience. This would later be termed plasticity. He was also able to infer that these electrical signals must travel in one way only, which was later confirmed, and will be discussed in great molecular detail in this chapter.

Cajal is an inspiration to neuroscientists around the world not just for his brilliance and incredible intuition, but also for the wonder, marvel, and passion that translated through each of his works. He cuts through the stereotype that science is only made for the rigorous and the quantitative. His devotion to the beauty of the brain is what allowed him to see so much where people saw so little. As Dr. Elle O'Brian puts it "Perhaps only

an artist's eye could have seen so much in a slice of the brain." As you mature into a neuroscientist, you will sometimes be bogged down by the molecular and anatomical details which have to be memorized. In these times, you must never forget that to study the brain is to study human nature. It is to inquire about what makes us think, behave and feel the way we do. It allows for discoveries regarding the basis of friendship, hatred, war, love, addiction, intelligence and so much more. It is awe striking that we are the only species with the capabilities of modelling ourselves. There is a saying that "if a tree fell in the middle of a forest and no one could hear it, did it really fall?". Dr. Jeff Hawkins extends this saying to the brain; "if a universe came in and out of existence but there was no brain to discover its wonders, then did that universe ever truly exist?"

In this unit, we will be introducing the structure and molecular function of these darkly stained cells, which we now call neurons. In addition, we will be discussing other types of cells, termed glial cells, which cannot be seen via silver stain but are extremely important nonetheless. Lastly, we will discuss how neurons conduct electrical signals, how these signals are converted into a chemical cascade within the synapse, and how this then allows that electrical signal to "jump" to the next neuron.

PART I

UNIT 1 – BUILDING BLOCKS OF THE BRAIN

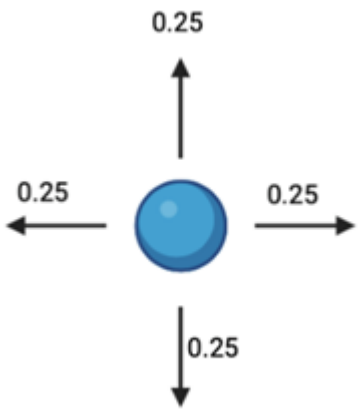
1.1 THE CHAOTIC LIFE OF AN AQUEOUS/ GASEOUS MOLECULE

You have probably been introduced to the notion of chemical gradients before. If two salt solutions with different concentrations are separated by a permeable membrane, then molecules travel from high to low concentration such that after a set amount of time, both solutions are of equal concentration. In other words, molecules tend to diffuse down their concentration gradient. While this notion serves as a foundational principle in the natural sciences, there are often considerable misconceptions surrounding its ontology.

The notion of a concentration gradient is predictive. It accurately describes the net movement of molecules and thus allows us to build models for much more complicated topics. These topics range from ocean currents and chemical reactions to the hardware that generates human behavior, intelligence, and dare I say, consciousness. One important caveat is that chemical gradients describe NET movement of molecules, but in reality, molecules are constantly being exchanged in a bidirectional manner across any semi-permeable membrane. This continues until equal concentrations are achieved by a balanced yet continuous exchange of molecules.

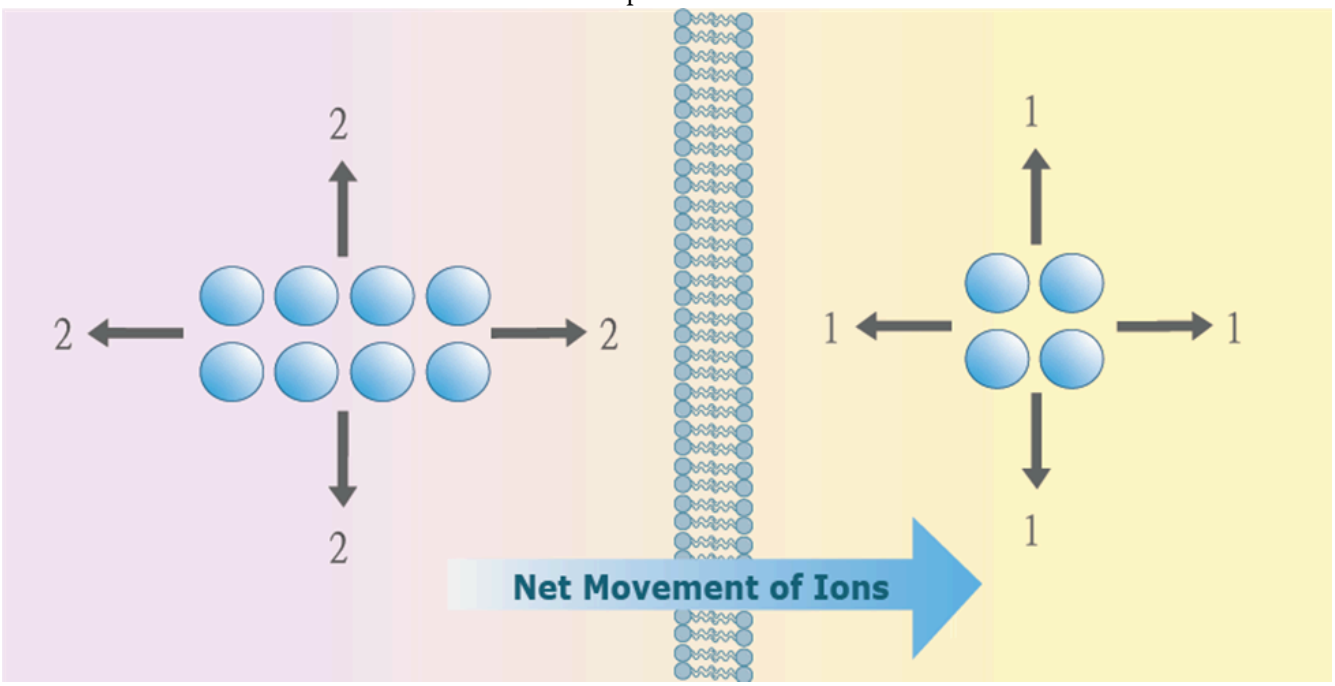
As the title suggests, aqueous and gaseous molecules live a chaotic life. Any one molecule, in an uncharged environment, does not know whether it is in a solution of high or low concentration. It simply moves randomly, purposelessly, changing direction only when it bumps into other molecules. This random behavior of particles is termed Brownian motion, and its considered to be one of the strongest supportive evidence for atomic theory. From the perspective of the atom, there is no such thing as a chemical gradient. There is no force which drives molecules down their concentration gradient like the force of gravity drives our feet to the ground. Diffusion gradients are not an innate property of nature in the way that electric forces and the existence of quarks are. Instead, the phenomenon of diffusion is simply an accidental consequence, an epiphenomena, of the random and chaotic motion of particles.

In an uncharged environment, molecules move randomly, full stop. How is it then, that a population of molecules have net movement down their concentration gradient? Why has this principle been so predictive and foundational for understanding the natural world. The answer is best described using illustration.

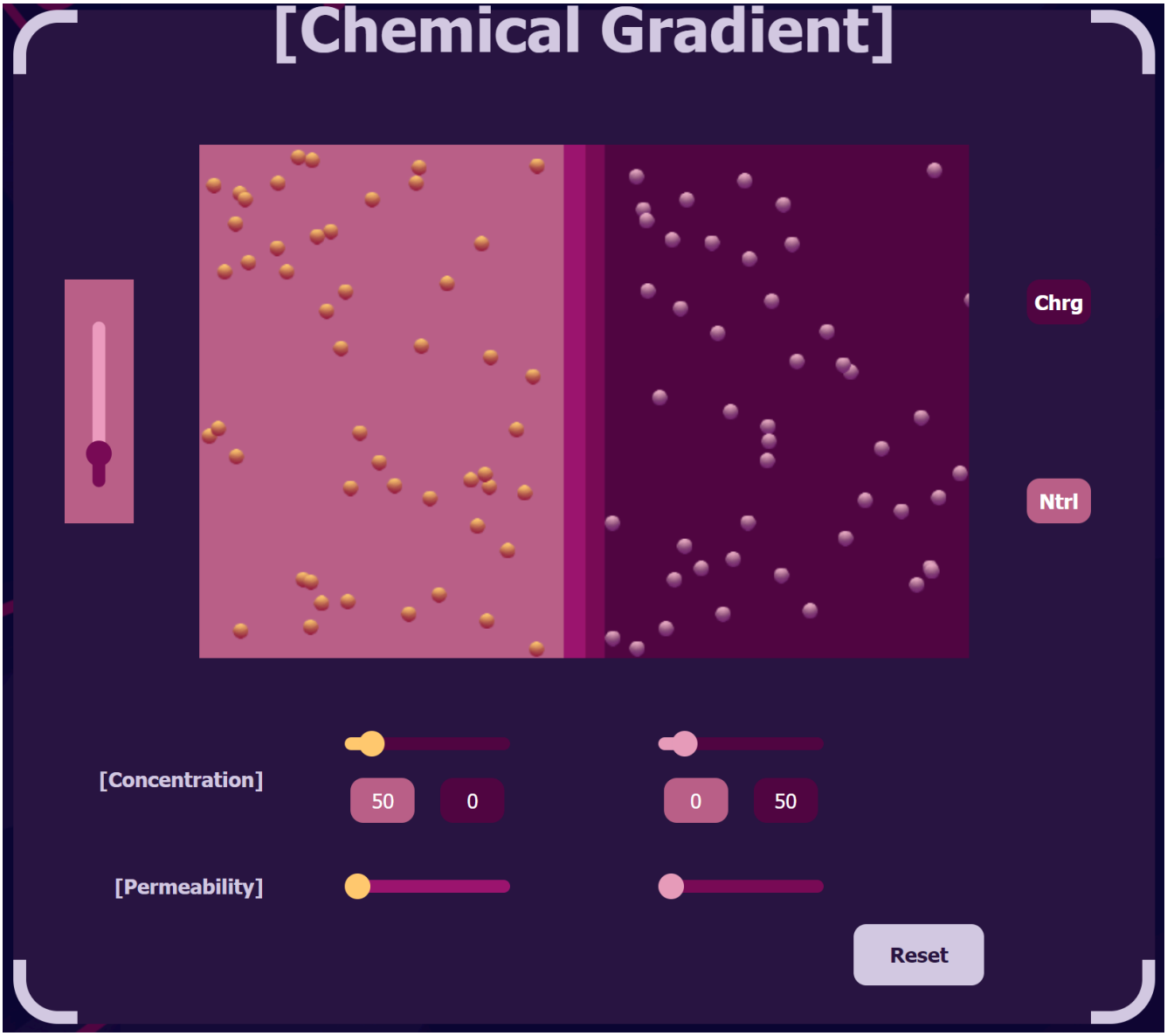


To the left, we have an image of a molecule. To simplify the conceptual process, let us assume that the molecule can only move in four directions. Whether or not it moves in any one direction is random, with a 25% probability per path.

Now, if these were four particles, then we would have, on average, one moving in each of the four directions. As can be seen in the figure below, if one solution has more particles in it, then it will simply have more molecules moving in all directions. This includes the direction that leads to the semi-permeable membrane, resulting in a net exchange of molecules until equal concentrations are achieved.



In short, movement down a chemical gradient is an extension of the second law of thermodynamics; universal entropy always increases and molecules move randomly over time. Increased chaos is the default state of the universe. Order, in the form of maintaining different concentrations across a membrane, would require energy to be put into the system. Indeed, neurons purposefully maintain a concentration difference across a membrane, but they can only maintain such order using ATP as well as the hydrophobic interactions which make a membrane not permeable to all ions. In fact, ⅓ of all ATP metabolized in humans everyday is used purely to maintain stable concentration gradients. We will come back to this point later in the chapter, when discussing action potentials, sodium/potassium pumps, and neuronal electrical propagation.



Click on the image above to experience a simulation of the Chemical Gradient on our website, www.neurocyte.ca

1.2 MEMBRANE POTENTIAL: ITS COMPONENTS AND MAINTENANCE

The plasma membrane surrounding every cell is selectively permeable. By itself, the lipid bilayer is not permeable to any ions. However, the introduction of channels and transporters allows for the exchange of some, but not all, ions. Channels are always selective for one to two ion types.

In a single ion system where the membrane is perfectly permeable to that ion, particles would exchange via channels until equilibrium is achieved. Given any concentration differential, an equilibrium potential that achieves equilibrium can be calculated. If the current charge differential across the membrane results in a membrane potential that deviates from the equilibrium potential, then ions would be exchanged in net until equilibrium is achieved.

In the brain, the concentration of ions surrounding a resting state neuron have already been characterized (Table 1). If a neuron was to be made perfectly permeable to only one ion, but perfectly impermeable to all other ions, then the Nernst equation could be used to calculate equilibrium potential. Ions would then be exchanged across the membrane to bring membrane potential closer to equilibrium. In this theoretical case, if one was to plug the numbers from Table 1 into the Nernst equation, they would calculate an equilibrium potential of +50mV for Na, -90mV for Potassium, +135mV for Calcium and -70mV for Chloride.

Ion	**Intracellular Concentration (mM)**	**Extracellular concentration (mM)**
K^+	140	4
Na^+	15	145
Cl^-	4	110
Ca^{2+}	0.0001	5

In reality, neurons exist as multi-ion systems, with varying membrane permeability for different ions. The plasma membrane surrounding a neuron is characterized as being selectively permeable. By itself, the lipid bilayer which forms the membrane is not permeable to any ions. However, the introduction of channels and transporters allows for the exchange of some, but not all, ions. Channels are always selective for one to two ion types. Therefore, if no channels exist to facilitate the transfer of one ion type, then the membrane is impermeable to it. If a cell has a low concentration of sodium channels but a high concentration of potassium channels, then it is permeable to both, but relatively much more permeable to potassium. There are two major types of passive transport channels; constitutive/leak channels and gated channels. The former are always open and facilitate the transfer of ions towards equilibrium constitutively. The latter are only opened after an event

has occurred, lifting the proverbial gates that otherwise keep the channel closed and unable to facilitate ion transfer. Gated channels exist in two major subtypes: ligand-gated and voltage-gated. The former is only open when chemically bound by a ligand, while the latter only opens at certain voltage thresholds.

In reality, neurons exist as multi-ion systems, with varying membrane permeability for different ions. As a consequence, the Nernst equation cannot be used to calculate an equilibrium potential. In response to this, mathematicians, physicists and chemists were able to derive a similar equation to the Nernst system which applies to multi-ion systems; The Goldman equation:

$$V_m \approx -60\,mV\ log_{10}\ \frac{P_K[K]_{in} + P_{Na}[Na]_{in} + P_{Cl}[Cl]_{out}}{P_K[K]_{out} + P_{Na}[Na]_{out} + P_{Cl}[Cl]_{in}}$$

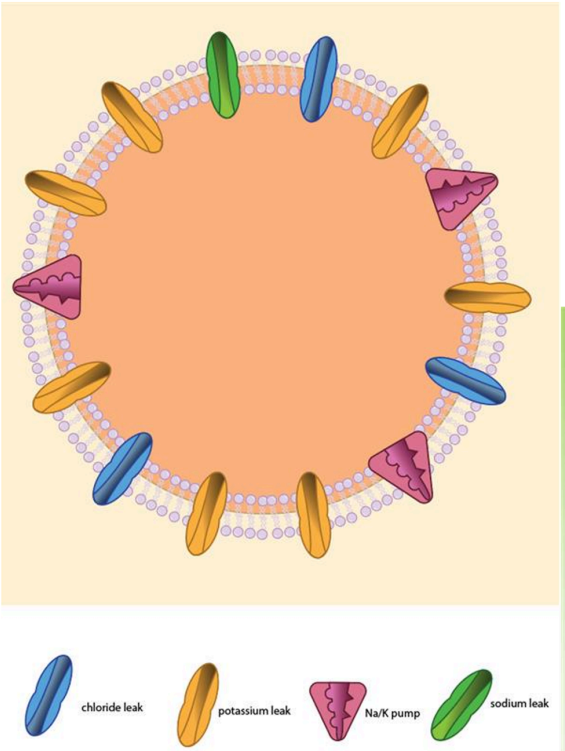
As can be seen above, the Goldman equation is very similar to the Nernst equation, the only difference being that it incorporates multiple ions, and introduces a new variable: . The value is a measure of the membrane's permeability to that specific ion. It is basically a proxy for the number of open channels found on the membrane that are selective to that specific ions. Remember, leak channels are always open, but gated channels open in response to a stimulus. Gated channels can thus transiently change the permeability to an ion i.e only when a ligand is bound. For calculations of resting state neuronal potential, we assume that all gated channels are closed.

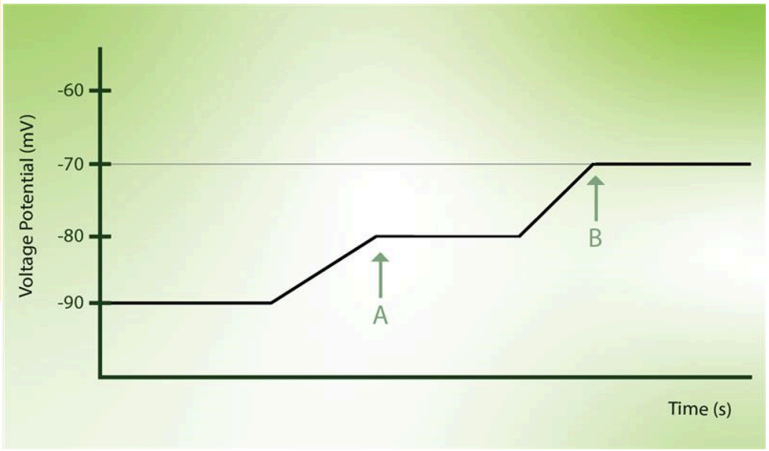
A neuron at resting state has an equilibrium potential of -70mV. There are three main components which contribute to this number; leak channels, Na/K pumps, and charged, non-permeable factors:

Leak channels:

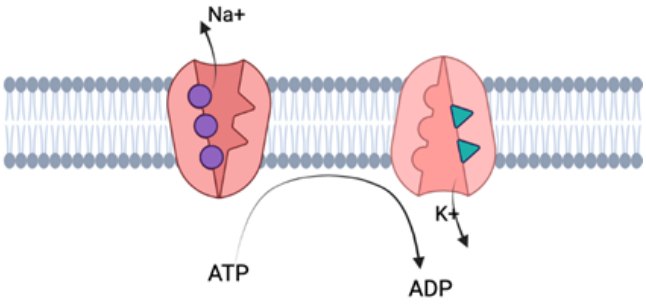
In the resting state, neurons contain constitutively/continuously open channels, termed "leak" channels. The majority of these are potassium leak channels, allowing for regular exchange of potassium ions. To a much lesser extent, there are few chloride leak channels, and very few sodium leak channels. These exist in a ratio of 100:45:4 (K:Cl:Na).

With 100 potassium leak channels alone, the membrane potential would be -90mV (potassium's equilibrium potential as calculated by the Nernst equation). However, the introduction of 45 chloride channels would raise that closer to -70mV, such that the membrane potential is somewhere between -90mV and -70mV. The third introduction of a very small number of sodium channels (i.e 4) is sufficient to then drag the average membrane potential up to around -70mV. This can be seen in the image below; while it initially starts with only potassium channels, the addition of chloride channels brings the membrane potential closer to -70, and the final introduction of sodium channels brings it all the way up to -70mV.





Na/K Pumps:



There is a third protein which actively aids in the maintenance of a resting membrane potential around -70mV: the Na+/K+ pump. This transport pump uses energy from ATP hydrolysis to move ions against their concentration gradient. Specifically, for every molecule of ATP that is hydrolyzed, 3 Na ions are pushed out into the extracellular space, and 2 K+ ions are brought into the intracellular space. This counteracts leak channels, which move these two ions in the opposite directions. It is the fact that this pump moves the ions in the direction opposing their chemical gradient that makes it necessitate the hydrolysis of ATP. This pump is very important for maintain membrane potential at resting levels of -70mV; it moves more positive charge out of the membrane, thus keeping the inside more negatively charged.

Non-permeable factors:

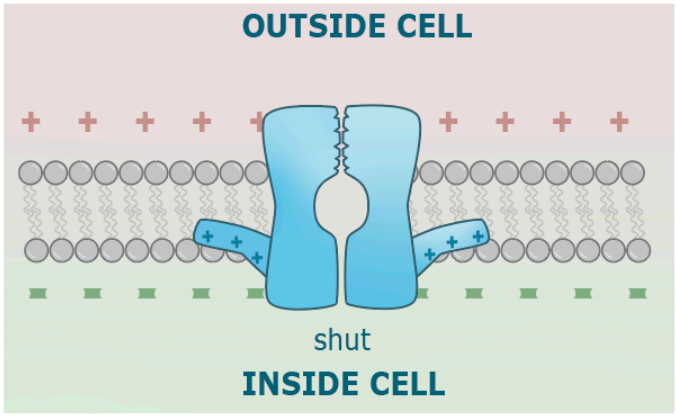
Neurons have additional charged molecules which contribute to the relatively negative intracellular charge differential. First of all, there is a high concentration of Calcium ions outside of the neuron. Since the resting

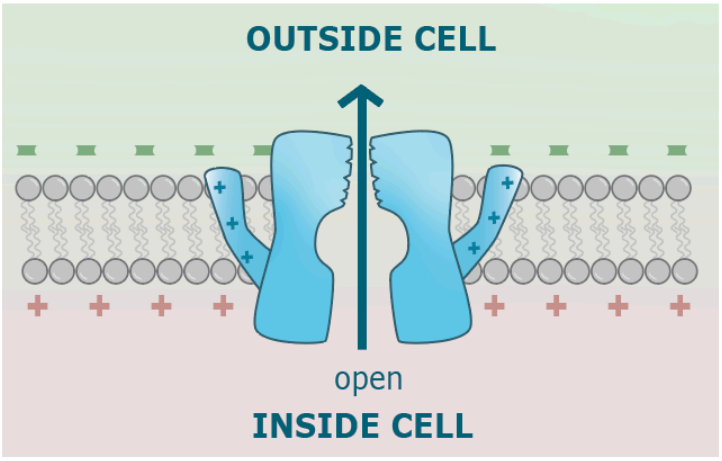
state neuron is completely impermeable to Calcium, these positive charges are trapped outside, contributing to the fact that the intracellular side is more negative than outside. Similarly, intracellular proteins, which cannot pass through the membrane, are net negative in a neuron. This traps additional negative charge intracellularly.

1.3 ACTION POTENTIAL FUNDAMENTALS

At a potential of -70mV, the membrane is said to be polarized. Polarized is a fancy word for different, and simply refers to the fact that there is a charge differential across the membrane. A resting state neuron is thus said to be polarized; a state that is maintained by the aforementioned pumps, channels, and non-permeable factors. Any event that brings membrane potential closer to 0 (zero charge difference across the membrane) is referred to as depolarization (making less polar).

While leak channels are constitutively open, and the Na/K pump is always active, most channels are gated. One subtype of gated channels are opened by the binding of a ligand to the extracellular portion of the channel, and promptly close after the separation of the ligand from its binding site. On the other hand, voltage-gated channels are opened at a voltage threshold; they remain open when voltage is at threshold or greater, and are closed at voltage's less than threshold. An image of a voltage gated channels is shown below. One of the peptides that composes the polypeptide, termed the S4 region of the protein, is made of many positively charged amino acids and is flexibly attached on a hinge structure near the base of the channel's intracellular portion. When the inside is negatively charged (-70mV relative to the outside), the S4 region is attracted to the intracellular portion and keeps the channel closed. However, when membrane potential is at -50mV or greater, this attraction becomes too weak to keep the channel closed. The S4 region changes it's position and with it, the conformation of the protein, resulting in an open channel.





Gated channels mediate activity-dependent changes in membrane potential, which cause a neuron to "fire", effectively sending out an electrical signal onto other neurons. "Firing" or sending out an electrical signal refers to the process of coordinated membrane depolarization, originating at the cell body and propagating along the axon until it reaches the axon terminals. While a polarized membrane has a voltage of -70mV, the depolarization process increases the voltage such that it becomes closer to 0 (hence less polar).

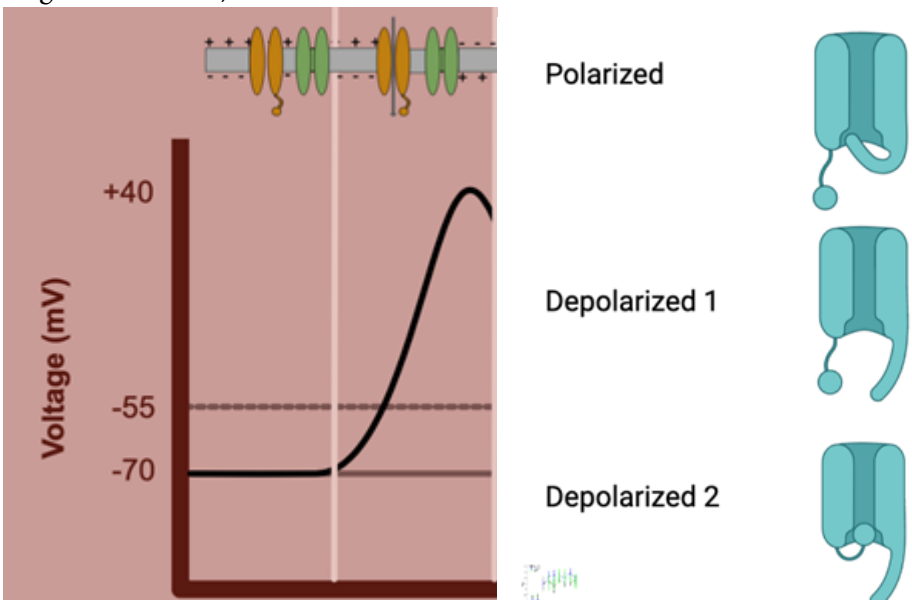
How does depolarization occur? Hypothetically, if gated sodium, calcium, and potassium channels open, then the membrane would suddenly become much more permeable to positively charged ions. These ions would chaotically enter the cell, rapidly altering the membrane potential in the process and bringing it to a more de-polar state. In this hypothetical scenario, Calcium and Sodium will rush in as they push the membrane potential closer to each ion's respective equilibrium potential (+135 and +50mV respectively), while potassium exits the cell in a similar attempt to push membrane potential closer to its equilibrium of -90mV. Depolarization can thus be thought of as the process of converting the potential energy (ie voltage) of the ions into kinetic energy (i.e current / velocity of ions) by making the membrane more permeable to more ions.

Neurons communicate in units of electrical activation which are termed action potentials. A neuron receives chemical input via its dendrites and computes this input in the cell body. If the input is sufficiently excitatory, then a cascade of coordinated chemo-electrical activity begins to create one or more action potentials. An

action potential is thought of as a depolarizing current which propagates from the axon hillock to the axon button, terminating on and sending a signal to a postsynaptic neuron. This starts at the axon hillock because voltage gated channels are only present in high concentrations throughout the axon, starting at the hillock. They are not found in abundance in the cell body and dendrites.

These cascades of depolarization are usually initiated by ligand-gated positive ion channels which open in response to a chemical signal. These are found in large concentrations at the dendrites. The rush of positive ions triggered by these channels would raise the voltage of a cell by making the intracellular region more positive. In this way, a neuron translates a chemical signal received from another cell into an electrical one. If excitatory enough, the rush of positive ions from ligand gated channels can result in membrane depolarization at the axon hillock which is greater than the threshold potential of voltage-gated channels (-50mV). This would result in the further opening of these latter channels. This then further depolarizes the membrane. It is this positive feedback that allows a membrane depolarization event to propagate across the whole cell axon's membrane.

Action potentials are tightly controlled within a neuron and can be codified into a series of steps. These are best represented using a graph with membrane voltage on the y axis and time on the x axis. Such a graph would be generated if one was to place a Voltmeter on the axon hillock during the production of an action potential.

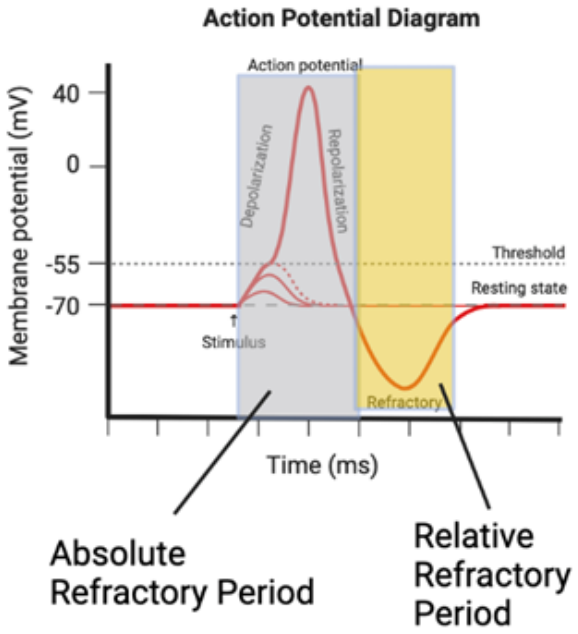
1. When an excitatory stimulus is received by the neuronal dendrites, it creates a positive ion current which makes its way to the axon hillock. The increase of intracellular positive ions caused by this current slightly depolarizes the membrane. However, if this depolarization does not reach a threshold of -50mV, then it will not be sufficient to generate an action potential. (Image will be added here when summation diagram is created).
2. 

When a cell is polarized, voltage gated sodium channels (VGNaC) remain closed. However, when the membrane becomes sufficiently depolarized (at least -50mV), then these voltage gated sodium channels open and increase the permeability of the membrane to sodium. If an excitatory stimulus received by the dendrites

is sufficiently strong enough, then this threshold will be reached. The opening of VGNaCs triggers flooding of sodium into the intracellular space, casing additional rapid depolarization as the membrane potential begins to parabolically rise. This is the first step of an action potential.
Voltage-gated sodium channels do not stay open until membrane potential returns under -50mV. They always close pretty rapidly after being opened. This is because VGNaCs have a self-stop mechanism; an additional, voltage-independent mechanism which closes the channel shortly after activation. This is termed a ball and chain mechanism. As can be seen in Images to the right VGNaCs have a literal ball hanging from a chain on their intracellular side. This ball quickly blocks the channels. Once that occurs, sodium ions stop flooding the cell and thus stop increasing the membrane potential. For this reason, an action potential always peaks at a membrane potential of +30mV (see images above), rather than increasing beyond that. The ball and chain mechanism is very important, as if it did not exist, the channel would stay open indefinitely. When the channel is open, it allows sodium to enter and in doing so, makes the membrane potential higher than threshold. If the channel was only closed when the membrane was polarized, then it would never close again after initially opening. It would be stuck in a positive feedback cycle where it increases potential and thus keeps itself open. The ball and chain mechanism allows the reduction of membrane potential back under threshold.

3. Voltage-gated potassium channels also open once the membrane potential is greater than -50mV. Due to the biochemical structure of these channels, they open more slowly than voltage gated sodium channels. This results in the very slightly delayed outflow of potassium to the extracellular space as they attempt to bring the membrane potential back down to -90mV. These channels have no additional gating mechanisms like the sodium channels, and are thus only dependent on voltage. This is not a problem since, unlike the sodium channels, voltage gated potassium channels cannot get stuck in a positive feedback cycle. Instead, their opening brings membrane potential back under threshold, thus closing itself in a negative feedback cycle. As can be seen in the images below, the process of bringing membrane potential back down is termed repolarization (this label should be added to the image). Similar to their slow opening kinetics, these channels can also be slow to close ,thus resulting in a temporary “hyperpolarization” where the membrane potential temporarily falls below resting potential (-70mV) down closer to -90mV.
4. Once all the voltage gated channels are closed, the Na/K pump, along with the leak channels, are responsible for returning the membrane potential back to resting membrane potential.

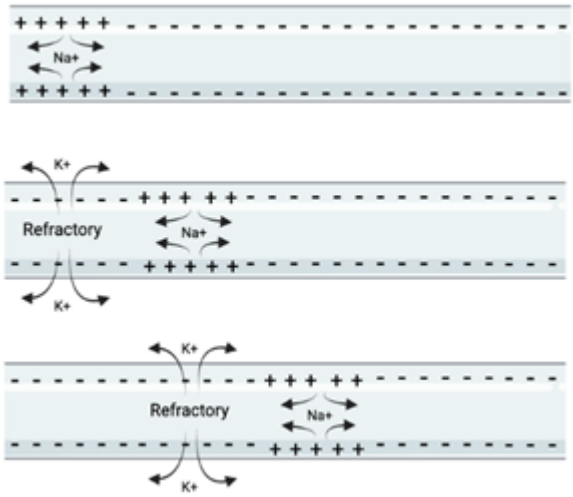
Refractory Periods and Action Potential Propagation:



The voltage gated sodium channel remains closed at all voltages under -50mV. Once membrane potential rises above that threshold, they open and allow sodium to enter. Very rapidly after opening, the ball and chain mechanism causes the channel to close, making it impermeable to sodium. During this time, even if the membrane potential is more than -50mV, they cannot open. This results in a refractory period; a time period after the initiation of an action potential where no additional action potentials can be created, due to the disengagement of VGNaCs. After a certain amount of time, the ball and chain return to their original position such that the channel is now capable of being opened if the membrane potential is past threshold.

When all voltage gated sodium channels are closed on a piece of membrane, this is termed an absolute refractory period. During this time, it is impossible to set off another action potential. However, some VGNaCs return to their original state faster then others. When only a percentage of VGNaCs are closed, then the axon membrane is said to be in a relatively refractory state. During this period, an action potential is technically possible, but the initiation of this second action potential would be much more difficult and would require a more excitatory stimulus to achieve.

Action Potential Propagation:



As aforementioned, the action potential starts at the axon hillock and propagates along the axon until reaching the axon terminals. When a dendritic signal is strong enough, it raises the concentration of positive ions in the cell body and the axon hillock. This causes local depolarization past threshold for strong excitatory signals. While the cell body does not have any voltage gated channels, the axon hillock and the entire rest of the axon is very dense with voltage gated sodium and potassium channels. At the axon hillock, the depolarization past threshold triggers the four steps of an action potential described above.

When sodium ions enter the axon hillock's intracellular cytoplasm during step 2 of the action potential, they diffuse to the right and left inside the axon. This then triggers the depolarization of the axonal membrane just to the right of the hillock past threshold. While the new piece of membrane is undergoing the steps of an action potential, the axon hillock would still be hyperpolarized and in a refractory period. This process repeats along the entire axon, resulting in t

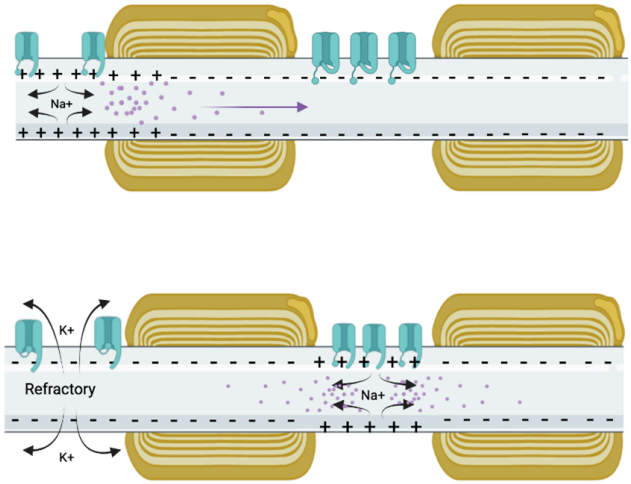
he propagation of the action potential towards the axon terminals. The refractory period thus prevents back-propagation of the action potential towards the cell body. This was one of Cajal's predictions; that an electrical nerve signal must in some way go in only one direction. It is only possible due to refractory periods.

Myelination and Saltatory Conduction:

No material is a perfect conductor of electricity or heat. As charge moves along a wire, a certain amount is always lost to the environment. This, along with safety reasons, are why copper wires used in our day to day lives are surrounded by plastic insulation. Since plastic is non-conductive, it limits the amount of electrons which leak out from the wire into the air and surrounding environment during electrical transmission, thus limiting loss of charge and signal.

In a similar fashion, ions that are near the membrane during an action potential can leak out as the signal is propagated across the axon. The leaking of ions outside the membrane slows down the current of a signal, delaying the amount of time it takes for the signal to reach the axon terminals. In order to speed up transmission by reducing leakiness, the nervous system has evolved its own insulation techniques similar to how we insulate our copper wiring. However, instead of plastic, we use biology's best non-conductive material: fat. Specialized glial cells wrap axons with a fatty substance termed myelin. This increases insulation and decreases the leaking of ions. In the central nervous system, these specialized cells are termed Oligodendrocytes,

whereas in the peripheral nervous system, they are of slightly different morphology, and are thus termed Schwann cells. Not all axons in the nervous system are myelinated, but the ones that are conduct messages at much greater speeds. For this reason, all axons that span a large distance are myelinated.



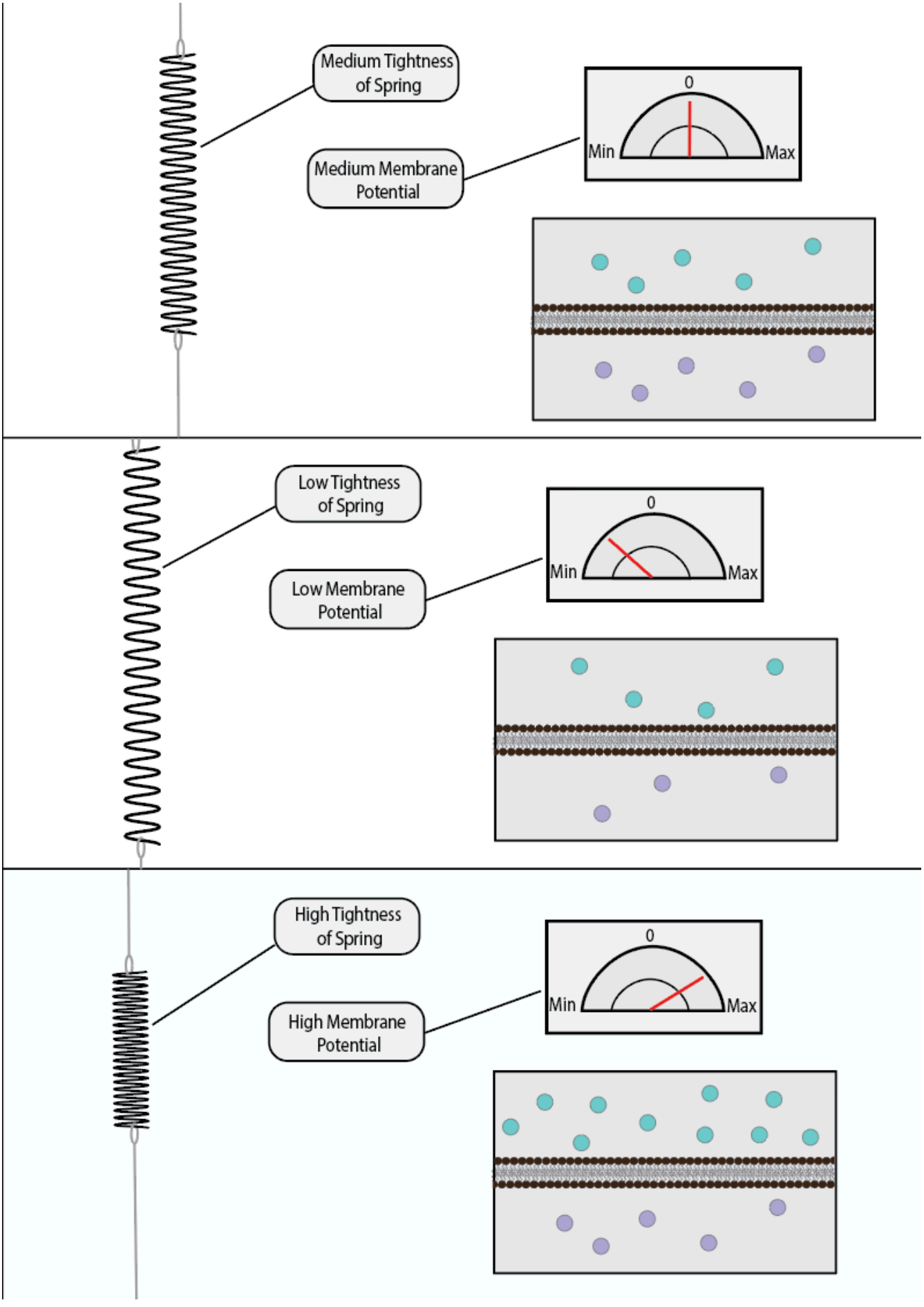
In a myelinated axon, regions surrounded by myelin sheaths cannot have any channels or receptors on their membrane. Instead, the fat takes up all of the space. Therefore, for a myelinated axon to still have the ability to conduction action potential, it must have small pockets of un-myelinated regions that are dense with voltage-gated channels. These small pockets are termed Nodes of Ranvier. This can be seen in the image above; at the axon hillock, an action potential triggers the opening of voltage gated sodium channels. This results in a high concentration of sodium (purple) at the first node, which by process of passive diffusion, eventually reach the second node of Ranvier. This then triggers the opening of voltage gated channels at the second node, while the first node is in a refractory period, repeating the cycle and passing the action potential onto the third node. Note that the action potential is continuously being regenerated as it moves down the axon, it does not simply passively drift along the axon to the terminals. Voltage gated sodium channels are always being opened to generate a spike in one node, setting the stage for voltage gated channels in the next node to be opened. This cannot back propagate as the previous node would become refractory.

In an un-myelinated axon, this regeneration of an action potential spike, via the continuous opening of new voltage gated sodium channels, occurs at each piece of membrane. This creates the image of one action potential travelling across the whole axon, even though in reality that action potential is constantly being regenerated. In myelinated axons, the action potential waveform is not seen at myelinated regions, only at nodes of Ranvier. This creates the image of an action potential "jumping" from node to node; a process termed Saltatory Conduction.

1.4 THE LIFE OF A MOLECULE IN A CHARGED ENVIRONMENT

What is voltage?

Potential energy is a quantitative measurement of a system's potential to do work. For example, if you hold a spring in a stretched position and keep it that way, one could say that the spring has high potential energy. It has the potential to violently compress back into its equilibrium length the second you let it go. When that occurs, one could say that the spring has realized its potential. Similarly, if you hold a ball high up in the air, it has high potential energy. It has the potential to fall and release its potential onto the world in the form of kinetic energy. The potential energy is realized when it is let go of.


Medium Tightness of Spring
Medium Membrane Potential
0
Min
Max
Low Tightness of Spring
Low Membrane Potential
0
Min
Max
High Tightness of Spring
High Membrane Potential
0
Min
Max

Voltage is considered a form of potential energy. It refers to the ability of a system to create electrochemical work, even though it is currently not doing any at that moment. As you have likely already encountered, like charges repel while opposite charges attract. When a negative charge accelerates towards a positive one, it is doing electrochemical work. However, if one was to place a non-permeable barrier between a negative particle and a positive one, then they would be unable to move towards each other and produce this work. They would still have the potential to accelerate towards each other, but this potential would not be realized unless barrier was lifted, or otherwise made permeable to ionically charged particles.

Electrochemical potential is termed voltage. Neurons always have a voltage across a membrane, as the inside of a neuron is more negatively charged than the outside. These negative charges would like to accelerate outside of the neuron, but they cannot because the lipid membrane is not permeable to such charges (unless it contains ion channels which allow them to pass). Theoretically, if there was no membrane, then these ions would exchange until they reach their equilibrium potential.

Voltage is measured in Volts; which is a fancy way of saying charge per meter. It denotes how much charge difference there is across a non-permeable membrane, and how far away these charges are from each other. The reference frame for the unit of Volts is completely arbitrary; one can decide to set the zero point anywhere they choose. Classically, the outside of the neuron is set as the zero point, making the inside of the neuron -70mV. However, one can easily set the zero point as the inside of the neuron, making the outside +70mV. Alternatively, the zero point can be a hallway through the membrane, making the outside +35mV and the inside -35mV. The only thing that matters is the difference between two points, which in this case, is always 70mV (between the outside and inside). Where exactly you start counting from does not matter, so long as you are communicating with people who are using the same reference point as you are.

What is current?

Current is simply the movement of charge over time. It is measured by Amperes, or how fast every charge moves per second. It is very analogous to velocity in that way. Voltage refers to the potential for creating current. When the barrier which creates voltage, i.e the lipid membrane, becomes permeable to certain ions, then these ions will suddenly become capable of moving towards opposite charges and away from like charges. That sudden movement is measured as a current. Thinking back to the example of a spring; if voltage is analogous to holding a spring in a stretched position, then current is analogous to the velocity at which the spring compresses back into its original size as soon as you let it go.

Molecules in Charged Environments

Unlike chemical gradients, which are a derivative of the law of entropy, the electrostatic force is itself a

fundamental law of nature. In fact, it is one of the three fundamental forces that explain all understood attributes of the physical universe (the other two being quantum forces). It causes like charges to repel and opposite charges to attract. Unlike uncharged environments, ions in charged environments do not move randomly, and would therefore not have an equal probability of moving in each possible direction. In such a system, there would be two factors to consider when predicting the net movement of molecules: chemical gradients and the electrostatic force.

Moving forward, it will be useful to think of chemical gradients as a "force", moving ions from high to low concentrations. While previous discussion on entropy is more accurate, thinking of chemical gradients as a force can help make this next section more intuitively understood.

If two people push a block in the same direction, it will move down that direction with more acceleration than if only one person was pushing it. However, if two people are pushing a block from opposite directions, the block may end up with 0 net movement. If one of them is stronger than the other, then maybe the block will slowly crawl towards one direction. One can extend this idea to ions separated by a membrane, treating the chemical and electrical forces as "different people".

In a membrane based environment with only one ion, and no other external charges, then ions will still move down their concentration gradient until equal concentrations are achieved on both sides in a dynamic equilibrium. Remember, dynamic equilibrium means ions are still being exchanged, just at a net of zero.

Now imagine that there are many negative ions on one side of the membrane, and these cannot permeate through the membrane. On the same side is a large concentration of positive ions, which are permeable to the membrane. The chemical gradient will "push" the positive ions towards the other side, but the electrostatic attraction to negative ions will "pull" them back. As a result, the equilibrium state of this system is not characterized by equal concentrations on both sides. Instead, there will always remain more positive ions in the side with the negative ions.

What if we continue to extend this idea? It is possible to completely nullify the "chemical force" if you create an equal but opposite electrostatic force. If one achieves this, then ion concentrations can remain stably asymmetric, such that at equilibrium, one side of the membrane has much higher concentration of a particular ion than the other side.

Not only is this possible, but chemists have derived an equation that calculates the exact voltage necessary to counteract any possible concentration gradient. We call this the "reversal potential". Remember, voltage/potential is just a fancy way to say that one side of the membrane is more charged than the other.

The equation used to calculate reversal potential is termed the Nernst equation, however, this equation is only applicable to single ion systems. For example, let's say there are roughly 20 potassium ions inside the cell for every one potassium ion outside the cell. The chemical "force" would like to push most of these potassium ions outside, until there are roughly 10.5 ions on each side. However, using the Nernst equation, a chemist has calculated that this system has a reversal potential of -90mV. If this chemist makes sure that there is 90mV more negative charge inside the cell than, then he/she can guarantee that there will be no net movement of potassium ions, and the 20:1 imbalance will remain stable. Chemists working on similar systems use batteries to apply

a voltage differential across membranes, but in biology, it is usually impermeable molecules that set a charge differential. Since impermeable molecules cannot pass through the membrane, they only create an electrostatic force without being ever influenced by chemical gradients.

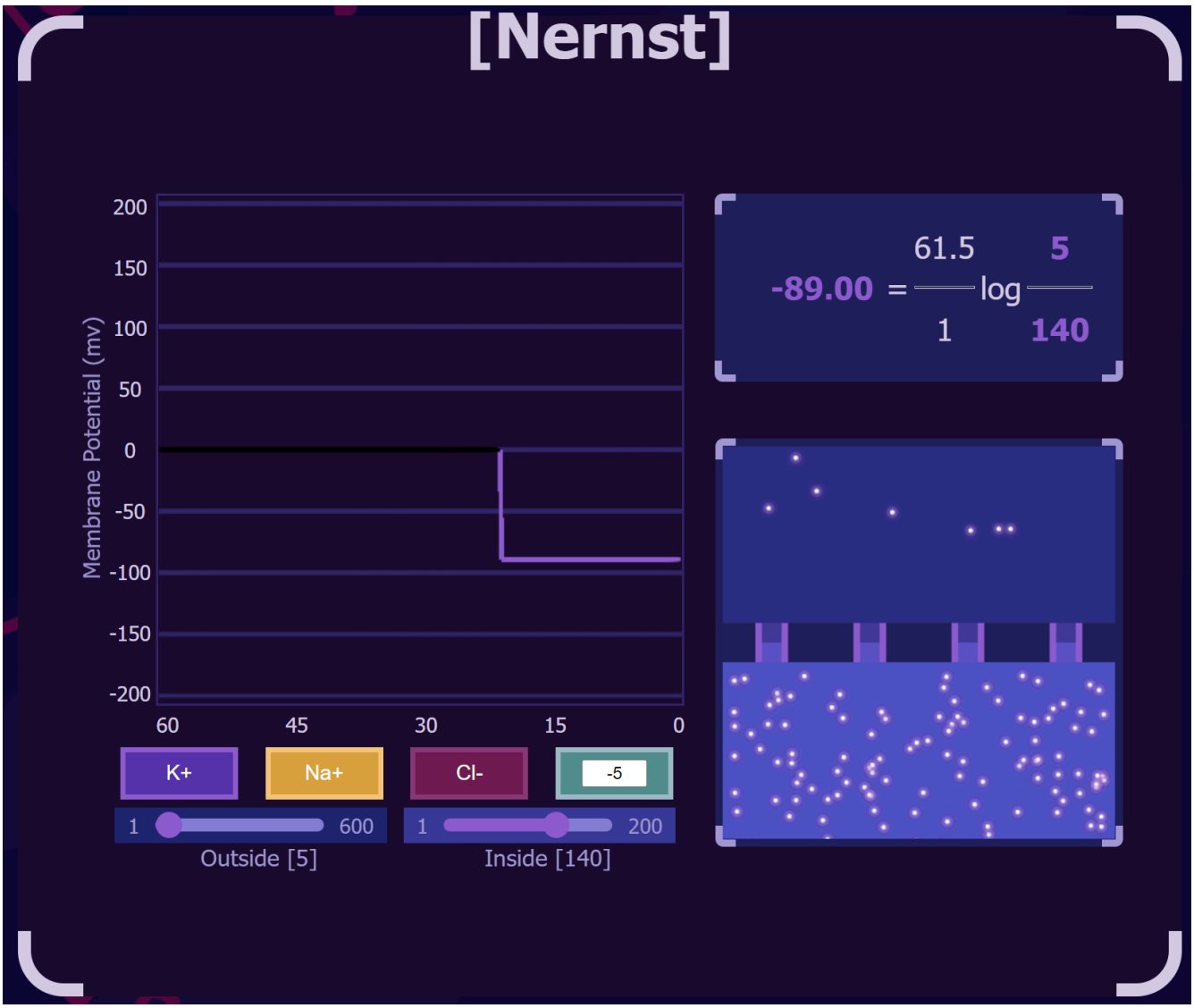
$$E = \frac{RT}{zF}\, ln\left(\frac{[X]_{out}}{[X]_{in}}\right)$$

Above is the Nernst equation. Both R and F are the universal gas constant and Faraday's constant, respectively. T represents temperature, z represents the charge of the ion that makes up the system (i.e +1 for potassium), and X represents concentration of an ion, inside or outside the membrane.

There are three intuitive patterns that can be extracted from this equation. The first and most obvious is that the larger the concentration gradient (i.e the greater the difference between Xout and Xin), the larger the reversal potential necessary to counteract it.

Second: since z is a denominator, the more charged an ion, the smaller the reversal potential. This is because highly charged molecules are relatively more sensitive to the electrostatic force, and can thus counteract the chemical "force" perfectly with small voltage magnitudes.

Third and maybe least intuitive: high temperatures increase the voltage necessary to counteract the chemical force. For this to be understood, it is best to analyze chemical gradients through the lens of entropy again. Molecules move down their gradients because of their intrinsically chaotic movement patterns. Temperature makes molecules move even faster and more chaotically. You can think of this as "increasing" the strength of the chemical "force", thus resulting in a need for higher reversal potentials to counteract it. Don't worry if this last point is confusing; physiological temperature remains within a narrow enough range that, at least in neuroscience, you will never really have to worry about the effects of temperature on reversal potentials and chemical gradients.



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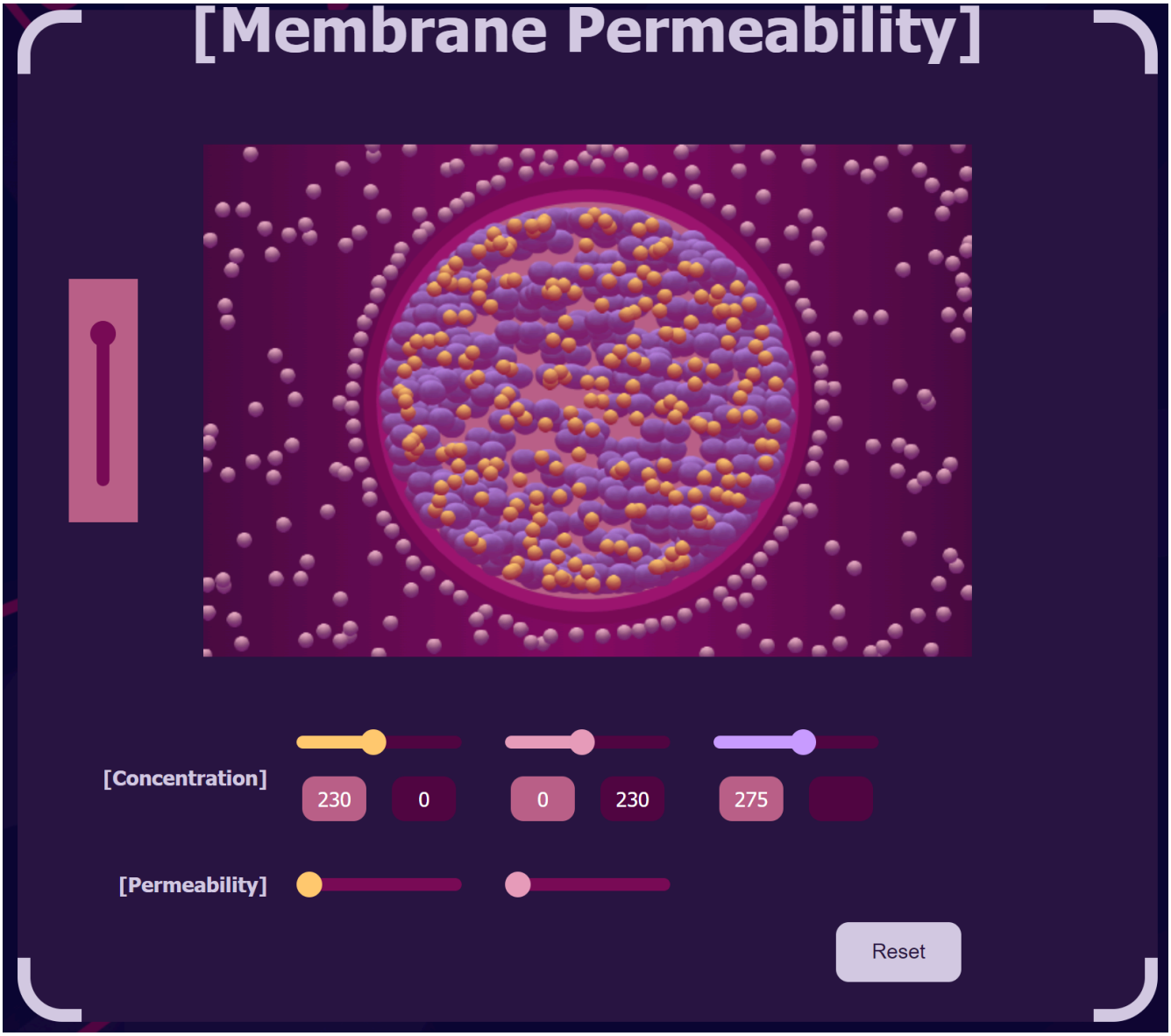

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Reversal Potentials in the Wild

While it would be an interesting scientific finding, there do not exist any chemists in your head that change the voltages of neurons to achieve reversal potentials. In fact, there are many cases in which the membrane voltage is not equal to the reversal potential. This would mean that the ion permeable to the membrane is at disequilibrium. Ions will thus move across the membrane until equilibrium is achieved.

Driving force can be thought of as an invisible "force" that exists in an electrochemical system at disequilibrium. It "pushes" ions towards the direction that will eventually result in equilibrium. Driving force is calculated as Membrane Voltage minus Reversal Voltage. It is obvious from this equation that when

membrane voltage equals the reversal potential, the system is at equilibrium, and driving force is 0. The larger the magnitude of the driving force, the farther the system is from equilibrium. This creates a greater "push" for ions to move towards equilibrium. The valence (i.e positive or negative) of driving force can be confusing, as it is influenced by the charge of the ions in the systems, and the direction they are moving. We will ignore valence for now and think only of the absolute value of driving force.

It is important to understand that these electrochemical systems are very dynamic. If a system is at disequilibrium, that means the membrane voltage is not equal to reversal potential. When ions consequentially move towards equilibrium, concentration gradients change. As a result, the voltage at equilibrium **will not** be equal to the reversal potential that was calculated when the system was in disequilibrium. In other textbooks, you might see the term reversal potential referred to as equilibrium potential. Note that this can be very confusing, and we purposefully avoided using the latter term in this textbook. Reversal potential is a much more accurate way to think about this.

To iron out this idea, let us take the previously elaborated potassium example. Under physiological conditions, where there are 20 ions inside the membrane for every 1 ion outside, potassium has a reversal potential of -90mV (calculated using Nernst). What if the membrane potential is -70mV, and no other ions are permeable to the membrane? Then there is not enough negative charge inside the membrane relative to outside to keep 20:1 ratio stalely. The lack of negative charge inside means that there is a driving force of 20mV, pushing potassium ions outside of the cell, decreasing the severity of the concentration gradient.

The very nature of pushing ions out results in a decrease in concentration gradient. This changes the calculated reversal potential (Nernst equation), making it smaller in magnitude (somewhere greater than -90mV but less than -70). At the same time, the movement of positive ions outside the cell results in less positive charge inside the cell. This changes the existing membrane potential, making it something less than -70mV but greater than -90mV. Eventually, equilibrium is ached when reversal potential equals membrane potential, and driving force is zero.

To summarize, the **incorrect** way of thinking about this is that when a system is in disequilibrium, the ions move and change membrane potential until it becomes equal to the reversal potential that was calculated at disequilibrium. This is a very common misconception because people use the work "equilibrium potential" instead of "reversal potential" commonly. **The correct** way of thinking about this is that when a system is in disequilibrium, ions move such that move both membrane potential and reversal potential dynamically change in value until they are equal. It is not only membrane potential's value that changes when ions are moving, but both.

PART II

UNIT 2 – BUILDING AND CHANGING HARDWARE OF THE BRAIN

2.1 SYNAPTIC MODIFICATIONS

Non-associative plasticity

Habituation & Sensitization

Non-associative learning is when a behaviour towards the same stimulus changes over time, without any link to outcome or any other stimuli. In non-associative learning, the properties of a single stimulus are learned. This type of learning is based on the frequency of the occurrence of the stimulus. There are two main types of non-associative learning: habituation and sensitization. Habituation occurs when there is a decreased behavioral response to a harmless stimulus that is repeatedly presented (Fig 1). Sensitization, on the other hand, occurs when there is an increased response to various stimuli following the presentation of a noxious stimulus.

An everyday example of this is when watching fireworks on a holiday. When you hear the first firework you may get startled, but as you hear more and more fireworks, you are no longer startled as you get habituated to the sound. After your startle response has weakened, if your friend were to pinch you, the next time you hear a firework, your startle response would be restored and you would show the same level of startle as you did to the first firework you heard. The pinch has caused you to become sensitized to an unrelated stimuli, the sound of the firework. (Kandel textbook)

Habituation & Dishabituation

In a neural circuit, a stimulus can act on sensory neurons in an organism and subsequently elicit a response in the form of behavioural output (Fig 2). Habituation occurs when repeated presentations of the same stimulus cause an output response that becomes weaker each presentation (Fig 3). The first response's magnitude can be considered the baseline level of response, and each response thereafter is weaker than the baseline response. Dishabition occurs when the output response returns back to the previous baseline level, usually after a stimulus free time interval (Fig 3). Both habituation and dishabituation have been extensively studied in the sea slug Aplysia californica.

Habituation experiments

The gill withdrawal reflex (GWR) in the aplysia is a well-studied example of habituation. This organism was chosen due to its large neurons and relatively simple nervous system. From an evolutionary perspective, the

gill withdrawal reflex is a defensive reflex that causes the delicate siphon and gill to become retracted when the animal is disturbed, thereby protecting it from potential threats (Fig 4).

The circuit involved is relatively simple. There is a siphon, which is used to expel seawater, that when a tactile stimulus comes into contact with, elicits the gill-withdrawal reflex. Mechanoreceptors in the abdominal ganglion innervate the siphon skin. These excitatory sensory neurons form synapses with the motor neurons that innervate the gill as well as on both inhibitory and excitatory interneurons that also synapse on the motor neurons. Repeated tactile stimulation of the siphon leads to a reduced response in the GWR (Fig 5).

This phenomenon has also been studied in rodents using the odour habituation test. In this task, a mouse is presented with one specific odour, where the amount of sniffing time directed at the odour is measured (Fig 6). The first time a mouse smells a particular odour, it will investigate it for a long period of time because it is curious about this novel smell. However, after repeatedly presenting the mouse with the same odour, the mouse will begin to sniff at the odour less and less, as it starts to become habituated to that odour. When the mouse is presented with a new odour, it will now become dishabituated and will begin sniffing longer towards this odor. Again, after multiple presentations, the mouse will become habituated to the new odour as well. This has also been studied in humans, where the human salivary response becomes habituated when participants are given the same flavour of juice, but becomes dishbautated when a different flavour of juice is given.

Aplysia Recordings

Since this is a relatively simple circuit from input to output, there are only a few potential synapses where this change could occur. The change could occur in the sensory neuron, in the motor neuron or at the gill itself (Fig 7). One potential way to find out where the changes happen is to record electrical activity from the potential areas of interest, and compare the electrical activity to the behavioural response. The magnitude of the behavioural response, in this case the GWR, should parallel the electrical activity of where the change is happening.

Stimulating the siphon while recording electrical activity of the sensory neuron and motor neuron and observing the gill response helps shed light on the mechanism. Repeated stimulation of the siphon elicits an action potential in the sensory neuron, where the action potential magnitude is the same each time the siphon is stimulated, even though the gill is moving less each time. This suggests the change underlying habituation of the GWR doesn't seem to be related to the sensory neuron's input changing. Recording electrical activity in the motor neuron shows that with repeated stimulation of the siphon, there is less electrical activity in the motor neuron with each stimulus. This reduction in EPSP amplitude seems to correlate quite well with the gill withdrawal. When the EPSP is high in the motor neuron, the gill withdraws a lot and when the motor neuron's EPSP is smaller, the gill withdraws less. This suggests that the change is happening somewhere after the sensory neuron fires but before the motor neuron does, since the change occurs in EPSP magnitude. The potential synapse involved therefore seems to be the sensory neuron to motor neuron.

Quantal analysis has shown that the mechanism behind this habituation is due to presynaptic depression, where less neurotransmitter is released from the presynaptic neuron for a given action potential (Fig 8a). Over the course of habituation, calcium channels are modified to allow less influx of calcium in response to an action potential. This results in less neurotransmitter being released from the presynaptic sensory neuron and therefore less depolarization of the postsynaptic membrane in the motor neuron, thereby leading to the weakened GWR (Fig 8b).

Short-term & Long-term Habituation

Habituation can be either short-lived or long-lasting, and can depend on both the frequency and intensity of the stimulus. Short-term habituation that lasts from seconds to minutes seems to be due to presynaptic depression. Long-term habituation, which generally lasts longer than 10 hours, cannot be accounted for by only presynaptic depression (Fig 9). Long-term habituation is both RNA and protein synthesis dependent and occurs by changes in both the presynaptic and postsynaptic neurons. These changes can include: a reduction in the number of presynaptic terminal varicosities, the number and area of active zones and number of presynaptic vesicles, as well as changes in postsynaptic AMPA/NMDA-type receptors and calcium signaling (Potential Figure x1).

SENSITIZATION

Sens/Desens

Sensitization is another form of non-associative learning involving a single noxious stimulus. Sensitization occurs when an animal encounters a harmful stimulus and the animal learns to respond more strongly to not only that stimulus but also other stimuli, including harmless ones (Potential Figure x2). In sensitization, applying a stimulus to one pathway can produce a change in the strength of another reflex pathway. Analogous to habituation, there is both short-term and long-term sensitization. A single aversive event can produce short-term habituation lasting only minutes, whereas multiple aversive events in succession can produce long-term sensitization that lasts from days to weeks.

In Aplysia, the previously habituated GWR response becomes very strong after a single electric shock to its tail (Fig 10). The noxious stimulus to the tail causes an enhancement of synaptic transmission in several areas of the neural circuit that support the GWR. The fact that this occurs in the same synapses that were depressed by habituation, instills the notion that a synapse can store more than one type of memory and can participate in multiple types of learning.

Just like in habituation, short-term sensitization involves transient changes in the amount of neurotransmitter released, whereas long-term sensitization involves larger synaptic reorganization. This

increased neurotransmitter release leads to an increase in postsynaptic motor output for the same presynaptic stimulus. There are however differences in the cellular mechanisms that produce these synaptic changes in the GWR. Habituation is homosynaptic, where decreased activity in the sensory neurons leads to the observed decrease in synaptic strength of this reflex path. Sensitization of the GWR is heterosynaptic, where modulatory interneurons that are activated by stimulation of the tail lead to the enhancement in synaptic strength.

Sensitization Experiments

The most widely-studied interneurons involved in the GWR are the ones that release serotonin (5-HT) (Fig 11). These interneurons form synapses on the siphon sensory neurons, as well as axo-axonic connections with their presynaptic terminals. Presynaptic facilitation occurs when these facilitating interneurons enhance neurotransmitter release from the sensory neuron via those axo-axonic connections. After a single tail shock, 5-HT is released from the interneuron, which can then bind to serotonin receptors to induce cellular changes. 5-HT can either bind to two different receptors engaging either the Gs protein pathway or the Go protein pathway. When 5-HT binds to initiate the Gs pathway, the activity of adenylyl cyclase (AC) is increased. AC converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which increases the amount of cAMP present in the terminal of the sensory neuron. cAMP then binds to the regulatory subunit of cAMP-dependent protein kinase A (PKA), thereby activating it by exposing its active catalytic subunit. PKA can then act via three different pathways. In the first pathway, vesicular release is enhanced where more neurotransmitter vesicles are moved to the active zone so that they are ready to be released. The machinery involved in exocytotic release is also enhanced and becomes more efficient. In the second pathway, PKA phosphorylates K+ channels, which causes a decrease in K+ current that leads to an elongated action potential. This then causes an increase in the Ca2+ influx, which subsequently causes more neurotransmitter vesicles to be released. The third pathway causes L-Type Ca2+ channels to become active. In the second 5-HT signalling point, the Go protein pathway, phospholipase C (PLC) becomes active, which then via diacylglycerol and protein kinase C (PKC) can act on both L-type Ca Channels as well as on the available pool of neutramistter vesicles. All these pathways converge to contribute to presynaptic facilitation, enhancing neurotransmitter release from the sensory neuron in the short term.

Sensitization has also been studied in rodents in the context of pain thresholds, where rodents with tissue injury start to withdraw their paws upon the presentation of a non-noxious stimulus, where normally they would not. (Fig 12). Spinal cord dorsal horn neurons show increased responsiveness to a given stimulus following injury. Before the injury, paw stimulation outside of the injured zone would not cause the neuron to become active, but after the injury, the neuron now responds to stimulation in the non-injured area. After the injury, in the injured zone, the amount of paw stimulation needed to cause paw withdrawal is much lower than before the injury. The non-injured area too has become behaviourally sensitized, and also requires less paw stimulation to cause a paw withdrawal. Central sensitization has also been documented in humans, where tissue injury results in increased pain sensation to mildly noxious stimuli or pain to non-noxious stimuli.

Aplysia: Short vs Long Mechanisms

The number of synapses has been shown to increase in GWR sensitization experiments (Fig 13A). Changes in the sensory neuron have also been shown (Fig 13B).

Short-term changes occur via presynaptic facilitation leading to enhanced vesicular release from the sensory neuron (Fig14). Long-term changes are supported by synaptic growth and require the synthesis of new proteins. PKA translocates to the nucleus and phosphorylates cAMP-response element binding 1 (CREB-1). CREB-1 can then bind to CREB Response Element (CRE) to active gene transduction pathways. This then leads to the growth of new synapses and increased activity of PKA. When PKA is translated to the nucleus, it activates another pathway, the MAPK pathway, where MAPK then phosphorylates CREB-2, which is an inhibitory repressor of transcription. Phosphorylation leads to less suppression by CREB-2, further enhancing these synaptic changes. Short-term sensitization can involve the activation of silent synaptic terminals and long-term sensitization can involve new synapse formation.

NON-ASSOCIATIVE SUMMARY

In a normal scenario, before any changes. A sensory neuron might synapse on a motor neuron like this: where it forms two synaptic connections (Fig15). A stimulus acting on the sensory neuron will have a stereotyped magnitude of response, as long as the connections don't change and assuming there are no short-term changes. This is the baseline response in the motor neuron output for a given sensory input.

In long-term habituation, where behaviourally there is a less pronounced response of the reflex, the relationship between the sensory and motor neuron might look something like this. Where now there is only one connection from sensory to motor neuron. Now the same stimulus can elicit a smaller response in the motor neuron, thereby driving behavioural habituation.

In long-term sensitization, where there is a more pronounced response of the reflex, the relationship might look like this: where new the sensory neuron forms many more connections, in this case four. Now the same stimulus input can elicit a larger response in the motor neuron, thereby driving the behavioural sensitization.

2.2 ENGRAMS

From Greek philosophers to modern-day neuroscientists, questions regarding the nature of human memory and experience have been at the forefront of great minds. While much remains a mystery, theoretical deductions and experimental setups over the last two centuries have elucidated [or shed light on] many aspects of the problem.

What is experience?

Human experience has a qualitative property that colours everyday life. If you were asked to imagine what it is like to be a baseball bat, you might not be able to imagine anything. We intuitively believe that a bat does not have that first-person, egocentric, qualitative characteristic to experience as a human does. Unlike the bat, there is such a thing as to be a human; we do not only interact with our environment, but we "feel" our environment. This is not just because humans are more complex than baseball bats; even other complex systems, which process inputs to produce behavioural or non-behavioural outputs, are intuitively thought to lack the privilege of "experiencing" events like a human. For example, a spectrometer can tell me what colour a beam of light is based on its wavelength, and so can a human. However, the human not only classifies the colour but "experiences" what red *looks like*. There is an ineffable quality that defines seeing the colour red. Trying to explain the experience of seeing red to a person who was born blind. It is impossible. Similarly, we do not simply "sense" that something is salty and then eat more of it for sodium homeostasis. We instead "taste" the saltiness, "enjoy" the flavour, and "want" more of it. Whether it is seeing colour, having an itch, or being in love, there is a subjective property to the human condition that can only be discovered through conscious experience.

Why is there such a feeling as to "be" in an experience? This mystery, dubbed the hard problem of consciousness, remains a much more mysterious phenomenon than memory is today. Throughout history, philosophers and theologians have pondered this question, putting forward numerous spiritual and materialistic models as solutions. Unfortunately, the heart of the issue is out of reach of the scientific method. It is impossible to test and measure subjectivity in an objective manner. The only way to proxy what someone is experiencing is to ask them and trust their answer. While not the best compromise, such methodologies have provided valuable empirical observations which intricately correlate the activities of the brain with conscious experience. These observations form theoretical constraints around the mystery of consciousness, pointing towards the fact that we do not experience the real world. Instead, we experience the internal representations of the world as simplified by the brain.

Humans report that they experience life as a series of multi-modal sensory episodes. "Multi-modal" is a

commonly used phrase in neuroscience to differentiate sensory modalities of an experience. For instance, if vision is a modality and sound is another, then a video is a two modality system. The human experience is inherently multi-modal, consisting not only of the five classical exteroceptive senses but also a host of interoceptive (internal) senses, including thought, emotion, position in space, and more. Through the combination of self-reporting and brain imaging, scientific studies have found that each aspect of experience correlates with neural activity in specific brain regions. For example, the occipital lobe is dubbed the visual cortex due to the fact that its activity strongly correlates with reported or deduced changes in visual state.

If you zoom into that brain region all the way to a cellular resolution, you will find that different constellations of neuronal activity consistently correlate with different characteristics of vision, such as colour and objects.

Every object can be represented by a unique neural code distributed across some region of the cortex, and correlational data suggest that it is. Every dimension and modality of experience has been found to have a neural correlation. An entire multi-modal experience can thus be thought of as the unique activation pattern across the whole brain, and so Theoretically, every experience can be represented in the brain by a completely unique neural code, which varies in a somewhat systematic way from experience to experience, and from person to person. If person A were to somehow have the ability to measure and translate person B's neural code, then they should be able to deduce every aspect of that person's experience perfectly. The data thus implies a necessary theoretical constraint regarding the hard problem of consciousness: it must somehow, intricately involve the brain. While this may seem obvious to the modern student, it diverges from historically non-materialistic approaches to the question; what is experience?

In pop culture, there is a famous idea termed "simulation theory." It puts forward the existential issue that we as humans have no way of knowing if we are living in the real world or in a simulation of a universe created by other beings. This was touched on in the movie "The Matrix," where the protagonist finds out that his life is a lie inside a simulation and that his real brain and body are in some chemical vat in the middle of nowhere, hooked up to computers to maintain this illusion. While at the surface level, these are fun possibilities to think about, they actually touch on a very important neuroscientific principle. Even though the movie protagonist, Neo, was in a chemical vat, his conscious experience was that of the everyman: working a job in a city. That's because the sensors hooked up to his brain were feeding his neural circuits that information. Based on these senses, his brain created an entire internal representation of a world, and it was inside that world that Neo lived, not inside the vat. In other words, the brain creates a model of the world based on its peripheral sensors and interacts with that model of the world. Experimental data strongly suggests that what we experience is much more correlated with the representational model created by the brain than by the true nature of real-world events themselves. For example, our retina is two-dimensional, and it sends two-dimensional input to the visual cortex, yet we experience space in 3 because the brain can infer the third dimension from many visual cues like shadows, relative size, previous knowledge, and more. It receives the compressed 2D data and tries to unpack that information back into three dimensions to the best of its abilities, sometimes making mistakes. Optical illusions are the best examples of how sometimes this process can be wrong, and when it is,

our experience is that of the incorrect mental representation, not the real world image. Other times, different brains can see the same stimulus and create different internal representations. For example, the famous blue/god dress was seen as blue by some and gold by others. People on each camp were so passionate that what they saw was correct because inside their head, in their world, it really was blue, while inside another person's head, it really was gold. Spectrometry reveals the dress to actually be blue in the real world, but that's not what many experienced seeing. Optical illusions are not the only times our internal representations diverge from what is happening in the real world. In each instance, human experience is more correlated with these internal models rather than its objectively real counterpart. Another famous example is phantom limbs, where people feel tingles or itches in amputated limbs that are long gone. While the amputated limb does not exist anymore, the brain region that used to receive information from that limb does. When someone reports feeling phantom limb pain, neuroimaging shows activity in the corresponding brain region, further enhancing the correlation between brain activity and experience while also strengthening the proposition that we live in a self-generated simulation.

While you may think you live in the real world, you really just live inside an intracranial simulation based on data from the real world. It is the same idea as is "The Matrix," except that we are not plugged into a computer; we are instead plugged into our peripheral sensors. The sensors are in the real world, but we are not.

If the brain is at the center of our experience, then memories of these experiences must also be intrinsic to the brain.

What is memory? A History of Theories

There is a dance between plasticity and memory/stability that every network must learn to do elegantly.

Memory is an integral part of nervous system functioning. It is the dynamic relationship between the synaptic malleability conferred by plasticity and the synaptic stability conferred by memory that makes the nervous system so effective at outputting adaptive behaviour. The former allows the incorporation of new relationships and information into our carbon software, while the latter allows us to use the past in service of the future and present. Memory is pivotal in defining who we are and how we react to situations. Even when these reactions seem instantaneous, our intuitions and personalities are rooted in past experiences. These past life events interact with our genetics to influence the strength of synapses in varying circuits, regions and networks which make us who we are.

Theories regarding the nature of memories have existed for a very long time. Many of these theories shared a theme, which was the idea that memory is stored as a long-lasting change in the same substance that produces perception and thought. As thoroughly discussed in the previous section, sensory details are analyzed by the brain to produce an internal representation of the world, and it is that internal world where our conscious experience resides. These internal representations are associated with a unique pattern of neuronal activity. The general idea is as follows: when a memory is remembered, for a flash second, the brain-wide neuronal activity pattern is identical to what it was like during the original experience being remembered. By internally

retrieving a memory, you are choosing to ignore your external sensory information for a moment and instead recreate the brain activity that was present in the past, going back in time to that "internal representation" or that simulation of the world and reliving it. If we live in a simulation generated by our brain's response to sensory stimuli, then think of memory as visiting and briefly living in a simulation that is not reliant so much on external senses but is internally generated retrieval of a simulation *previously* generated by external sensory information.

This idea goes back to the times of Plato and Aristotle but was most thoroughly characterized in 1904 by Dr. Richard Semon. In his theory, he introduced the term **engram** in order to describe the physical substrate of a memory in the brain. The basic idea was that since there are a group of cells activated at any singular time point in the brain of a human, and since these cells represent the experience that the person is having in an episodic, multi-sensory manner, then these same cells must later also represent the memory. In other words, the reactivation of the same cell population at future time points represents the experience where that cell pattern was first originally encoded. Richard Semon deduced that if this is the case, then it must mean that these cells undergo latent, off-line physical and molecular activity in order to become more connected to each other as a network. This leaves behind the engram, which we now know to be a network of neural units that have strengthened synapses amongst each other. Back at the time, Richard Semon was unaware of the process of LTP or LTD. He simply stated that there would be "primarily latent modification in the irritable substance produced by a stimulus" and that the cells would "form a connected simultaneous complex of excitations which, as such, act engraphically, that is to say, leaves behind it a connected, and to that extent, unified engram-complex." This makes his theoretical deductions all the more impressive. When asked about molecular underpinnings of his theory, he stated that "To follow this into the molecular field seems to me...a hopeless undertaking at the present stage of our knowledge and for my part, I renounce the task".

Due to these technological limitations, Semon's engram theory went unnoticed for a very long time. This was until Dr. Donald Hebb proposed his postulate regarding LTP and LTD. This idea introduced how "neural substrate holding a memory" can undergo "latent modifications" after initial activation, such as to stabilize the synapses connecting cells of a memory engram. As described in the previous section, when the activity of cell A precedes the activity of cell B in a highly temporally correlated manner, a molecular cascade is triggered that results in more ion receptors and eventually more synapses between those neurons. Donald Hebb did not just think about two neuron systems (i.e. neuron A and B) but extended his paradigm by contemplating its implications at the level of an entire network. He reasoned that synaptic modifications should facilitate the formation of entire cell assemblies; networks of cells commonly co-activated together. In his words, these assemblies are "simultaneously active reciprocally connected cells." These cell assemblies have certain properties; you only need to re-activate a proportion of them to activate the whole assembly, as the ones you directly activate will stimulate the rest via potentiated synapses. This property would be useful for memories, as one relatively small or discrete retrieval cue could trigger the retrieval of the entire memory representation. Similarly, the reciprocal nature of these assemblies means that the destruction or silencing of some of these cells would not cause catastrophic destruction of the entire memory representation. When Semon proposed

his engram theory, he also stated that engrams must have these properties if they were to support a memory effectively.

This touches on an extremely important concept in memory research; population codes. Real neuronal networks are much more intricately connected than two neuron systems, resulting in a complicated, web-like structure of interactions. In more complicated systems, it is extremely important that we approach memory from a population perspective as opposed to thinking from a single cell perspective. Different experiences and states of being are represented by a unique activity pattern across the whole population, termed a population code. Importantly, two different population codes might have some individual cells in common, even if they represent two widely different experiences.

Looking back through a historical lens, it is easy to see many similarities between Hebb's Cell assemblies theory and Richard Semon's theoretical coining of the term "engram." Today, a cell ensemble is used to generally refer to any co-active population of neurons, whereas a cell engram specifically refers to the population of cells that represent a specified memory. Importantly, an engram is not the memory itself but rather the physical substrate of memory. Experiments after Hebb's postulate elucidated the molecular and physiological details of synaptic potentiation and found these augmentations in molecular and physical factors to be critical and necessary for memory formation. Others found that enhancing them, i.e. via enhancing NMDA mediated LTP, can result in strengthened memory representations.

Modern Data and Perspective

While this evidence was important in asserting the role of LTP and LTD in memory, it remained circumstantial. It was not enough to reject or strongly support Semon/Hebb's theories with appropriate scientific rigour. Recently, advancements in molecular techniques have far increased the resolution with which scientists can manipulate the brain, allowing for manipulations at a cellular resolution like never before. A specific new technique, termed "IEG dependent Tet-off Cre-Lox systems," allowed for scientists to "tag" only the cells that were active during the completion of a certain task within a certain time window. In other words, one can make it such that if a mouse is completing a maze, then the experimenter can label only the cells active during maze completion, and no other cells, by making them express GFP and thus glow.

With this technology, you are able to "capture" cells that were active during an experience by molecularly tagging them. Using this technology, some recent experiments have provided extremely compelling evidence in support of engrams being both the cells active during an experience and the cells responsible for memory representations at future time points.

First, observational studies supported this claim. Specifically, experiments tagged cells active in the Lateral Amygdala of animals undergoing fear conditioning training in which a context was paired with an aversive stimulus, i.e. shock. This brain structure is responsible for learning to predict stressful and aversive relationships and is well known to be necessary for appropriate fear learning. Due to the implementation of this technology, cells active during the learning phase of the experiment were tagged with GFP and thus glowed

green under the microscope. Days later, experimenters allowed the mouse to explore the same context, which it got shocked in and labelled cells that were active during the second visit using a different colour. During this test session, the animal displayed behavioural signs that it remembers being shocked in this location (freezing). After sacrificing the animals and inspecting the brains, they found a very large degree of overlap between cells active during initial learning and during retrieval of the memory. However, in control mice, which visited a different location on the second day, there was little overlap. This suggests that a population of cells active during the first visit to context A were again active in the same context on a different day, correlating with memory retrieval (engram), but a different population of cells was active when the animal was placed in a different context B (control).

While suggestive, observation data was not sufficient to completely convince the masses. Later experiments manipulated "engram" cells and concluded that these cells are both sufficient and necessary for the representation of a memory. One experiment used a very similar methodology to tag the cells of the amygdala during fear conditioning training. However, instead of just making these cells glow a certain colour under the microscope, they instead made these cells express a toxin originally used by Corynebacterium diphtheriae, a pathogenic bacterium. This would functionally kill the cells active during training but otherwise cause no damage to neighbouring cells within the network. When placed in the same context where training originally took place a day later, the animals showed no behavioural sign of memory retrieval. Based on their freezing levels, it was as if they had absolutely no memory of ever being shocked by that experience. However, they had no problem remembering being shocked in another context and were also able to re-learn the shocking relationship within that original context once they were shocked again. This suggests that killing that small subpopulation within the amygdala had adverse general circuit effects that cause general loss of ability to learn fear relationships. Instead, it caused the very specific erasure of the memory and no other impairments, providing strong proof that engram cells are necessary for the expression of memory in service of the present.

On the other side of the same coin, gain of function manipulations have found engram activation to be sufficient for memory retrieval. In a similar experiment, scientists tagged cells active during fear conditioning training in context A. This tagging was special, as it caused these engram cells to express specialized sodium ion channels that the experimenter can trigger to open and thus cause the engram cell to fire whenever he wished (i.e. at the press of a button). Experimenters confirmed that the animal showed behavioural indications of having the memory as it froze in context A. In a different context B, where no fear learning had occurred, the animal did not freeze at baseline conditions. However, when the experiments pressed this button of theirs and activated all the engram cells that represent being shocked in context A, the mouse suddenly froze as if it was in context A. The experimenters concluded that activation of engram cells was sufficient to cause retrieval of the memory they represent. In science, showing that a variable is both necessary and sufficient for an effect to occur is the empirical gold standard for suggesting causality, as opposed to pure a correlation, between the variable and the effect.

Engram Allocation

Within a network of neurons, there tends to only be a subset active at any one time. Specifically, out of all of the principal neurons found in a network, roughly 30% tend to be sufficiently active during an experience such that they are incorporated into the memory engram of that experience. This is the case even though much more than 30% of the neurons in a circuit could theoretically be eligible to participate in a given memory trace. This is especially true in brain regions that are responsible for flexible learning and less true in primary somatosensory and motor areas.

This may be confusing at first. How are many neurons eligible to participate in the representation of an experience? Surely if the experience is unique, and every neuron in the brain has a predetermined meaning, then neural codes for different experiences must also be predetermined. However, while this may be intuitive, it is not the case. When someone experiences a completely novel experience, there is actually a competitive race amongst neurons in a brain region to participate in the representation of the experience. Some win and some lose, so at a population level, there are different neurons that represent different experiences. Once a neuron becomes active during the experience of a novel event, it then acquires the ability to represent the experience from scratch. In the future, the activation of this neuron in tandem with the rest of the sub-population active during the experience itself would trigger memory retrieval.

Why is this the case, and why does it fly against intuition? It is because the assumption that neurons have predetermined meaning is false. Empirical data suggests that neurons acquire meaning after an experience and represent a part of that experience. That neuron might become involved in the representation of multiple different experiences, but the total sub-population would be different for each experience.

This is not the case across the whole brain. In higher-order structures related to learning, such as the hippocampus, lateral amygdala, and prefrontal cortex, there are many eligible neurons that can participate in a memory engram. However, in more primary somatosensory and motor areas, there is much less variation. Neurons that represent your finger tend to be more or less consistent across all representations of your finger.

How and why do neurons in higher-order regions have this ability to acquire any new meaning at experience? It is because these neurons have a multitude of silent synapses with different primary sensory and primary motor neurons distributed across the cortex. For example, a neuron in the lateral amygdala, called neuron A, could have synapses connecting it to neurons in both the auditory cortex and to the visual cortex. Both are silent; i.e. there are no neurotransmitters at the pre-synaptic terminal. Now, if an animal undergoes a fear conditioning paradigm linking a sound to a shock, a race would start amongst neurons in the lateral amygdala. The first 30% to become active will win, after which network homeostasis will prevent any more neurons from activating, deeming the rest of the 70% as losers of the race. These 30% of neurons now represent the fear engram and will be termed fear engram cells. If neuron A was in the fear engram, then that means that it was active during the tone-shock pairing. That means at the same time that neuron A was active, tone representing cells in the auditory cortex were also active. Due to the mechanisms of LTD, these synapses will become unsilenced. Next time the animal hears the sound, because of synaptic strengthening, tone cells in

the auditory cortex will activate neuron A, which will activate other cells in the fear memory engram, which will cause a freezing response. Theoretically, if this fear conditioning paradigm replaced sound with light, it is possible that neuron A will have helped represent a light-shock parking instead of a tone-shock pairing. Neuron A only acquires its meaning at encoding and continues to represent that memory when activated with the rest of the engram. Neuron A can also become a part of different engrams in the future, so long as the rest of the representational population is different.

In some places, meaning is predetermined. For example, cells in your visual cortex that are attached to red perceiving cones have an anatomically predetermined meaning, which is to represent the colour red. In all the interesting brain regions, it is practically never predetermined.

It is thus empirically supported that higher-order neurons can change their synaptic weights and "learn" to represent many different things. If it wins the race for activation during the initial experience, then it will aid in representing the memory of that experience. What is this race exactly, though? What determines which of the eligible neurons become part of the 30% active at any one time point. The answer is intrinsic excitability.

Even amongst a population of neurons all at resting membrane potential, there exists variation in intrinsic excitability. Intrinsic excitability is the proclivity a neuron has to fire in response to a given excitatory input. In other words, if all neurons were to receive the same excitatory input, some will fire an action potential while others will not. This is because of variation in intracellular proteins and transcripts, which result in different metabolic states. Neurons with the greatest intrinsic excitability at any one time point will be highly biased to become a part of a memory engram representing that specific time point. A landmark experiment found that neurons with high CREB levels have higher intrinsic excitability. Afterwards, by artificially creating a subpopulation of neurons that over-express CREB, they found that these neurons were highly biased to become a part of a fear memory engram relative to chance levels. Conversely, experiments inhibiting CREB in a subpopulation of cells found that these neurons were excluded from the memory engram at a far greater rate than would be predicted by chance.

Meaning is created, not predetermined: An intuitive example

Have you ever looked at your lectures notes after a class and thought to yourself, "it would be tough for somebody else to understand my notes if I were to just hand it over to them"? When writing our notes, we tend to take shortcuts in writing them, and despite the logical leaps that are a part of these shortcuts, we are confident that we will understand them in the future because we have the initial memory of writing it in the first place. This is analogous to how a code (in this case, your scribbled down notes) might be difficult to extract meaning from intrinsically but becomes much more meaningful after you have **experienced** creating the code. This is analogous to how a neural code is consolidated with meaning after an experience and becomes meaningful only after the experience.

Another example: let's say Steve is trying to memorize the first seven cranial nerves. He creates an acronym:

Ooo, Tim Tom Ate Five Cakes! During the test, If Steve sees this acronym, it will help him help retrieve the seven cranial nerves.

However, let's say two weeks before Steve took HMB200, someone gave him a piece of paper with this acronym written on it. It will mean nothing to him. It is the exact same code, but without experiencing the encoding process, the code means nothing. Similarly, a neuron code might activate and mean nothing. Two weeks later, the exact same neural code might activate and have a very robust meaning. The difference is that some form of encoding happened in the middle, by which what was for a long time simply a random subset of neurons became an engram population.

You can really trace this back in time indefinitely. Even the very letters that you are reading only have meaning because you stared at very similar shapes while someone uttered the sounds that the letter represents systematically for hundreds of thousands of hours across your lifetime. Language is different across cultures precisely because meaning is created as humans experience life. Very little is predetermined.

Changing Intrinsic Excitability

The intrinsic excitability of a network is not consistent; it is always changing. At any one time point, a neuronal network has a very specific pattern of excitability, which is different later on. There are two main factors that cause intrinsic excitability to change; 1) time and 2) past experience.

The more hours that pass, the more different an excitability pattern becomes. Some neurons become less excitable, while others become more excitable. While the exact mechanism as to how this time-dependent effect appears remains elusive, the fact that it exists is apparent. As a result of this, engrams for two experiences that occur very close in time are largely overlapping. That is to say, many neurons that represent experience 1 will also represent experience 2. Experiences close in time thus have similar memory traces. Theorists pose that this could facilitate the emergence of a mental timeline within our memories. Since memories close in time have overlapping representations, this can be used as a code to represent the close temporal proximity of these events.

After a neuron has been activated, it remains in a slightly more excitable state for multiple hours. Remember from the previous section that the opening of NMDA receptors in a neuron triggers increased CREB, which then maintains increased excitability of the cell in parallel to its roles in LTP. For these hours in which CREB is over-expressed in that recently active neuron, it is more excitable. A compelling experiment was done where scientists picked a random subpopulation of cells within a network and electrically stimulated them. A couple of minutes later, they trained the mouse under a novel paradigm. They found that the memory trace/engram representing that training episode was largely populated by cells that they stimulated minutes ago, even though they picked those cells randomly. This is because their past stimulation made them more excitable for a prolonged period of time, increasing their probability of being incorporated into new memory engrams.

Why is this beneficial? Theorists suggest that it might be pivotal in our formation of semantic networks. For example, imagine that you are taking an HMB lecture and learning about LTP but only finish half of the

subject matter. A certain population of cells is active during this learning and eventually encodes your memory of LTP as you had learnt it. Next week, you come back to class to learn the rest of LTP. As you go through the lecture, you must build on what you have previously learnt so you recall many of the facts that were taught to you last lecture. During these recollections, you re-activate cells from last week that represented the last lecture you learned. As a result of this, these re-activated cells now have greater intrinsic excitability and are more likely to become a part of these lecture's engram. Even though the two lectures were separated by one whole week, the resultant lecture 2 memory engram has many cells that overlap the lecture 1 engram, merging these concepts within the same interconnected web. Now when you retrieve your knowledge of LTP, you will retrieve information from lecture 1 and lecture 2 in tandem, resulting in a more holistic knowledge base and improving your ability to answer questions on the topic.

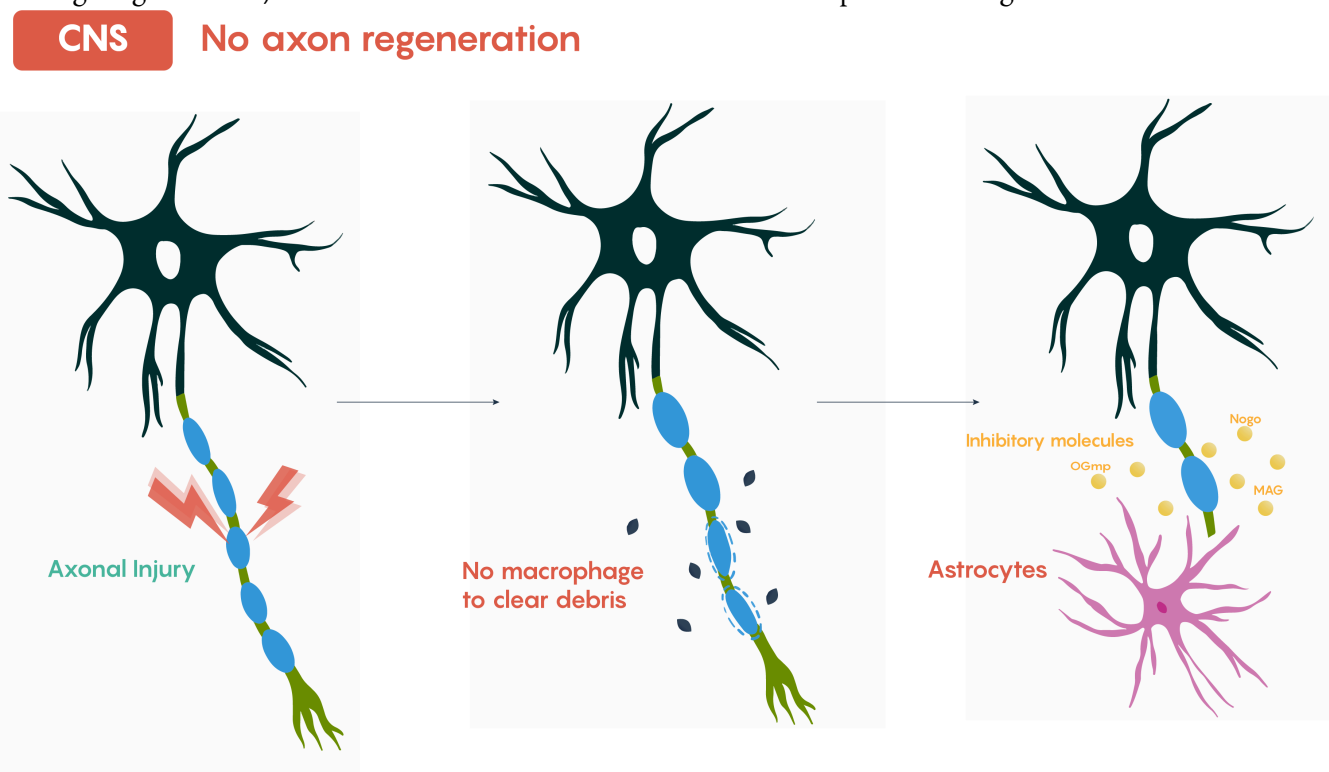
PART III

UNIT 3 – NEURODEGENERATION

3.1 ACUTE PHYSICAL DAMAGE TO THE NERVOUS SYSTEM

Axonal Injury in the PNS and the CNS

The previous chapter dealt with the impact that micro-organisms had on the health of neurons. This chapter outlines the differing roles of the PNS and CNS following injury and during recovery. It has long been known that following damage to axons, that the neurons within the PNS can undergo outgrowth and can recover (i.e. undergo regeneration) while neurons within the CNS do not seem capable of doing so.



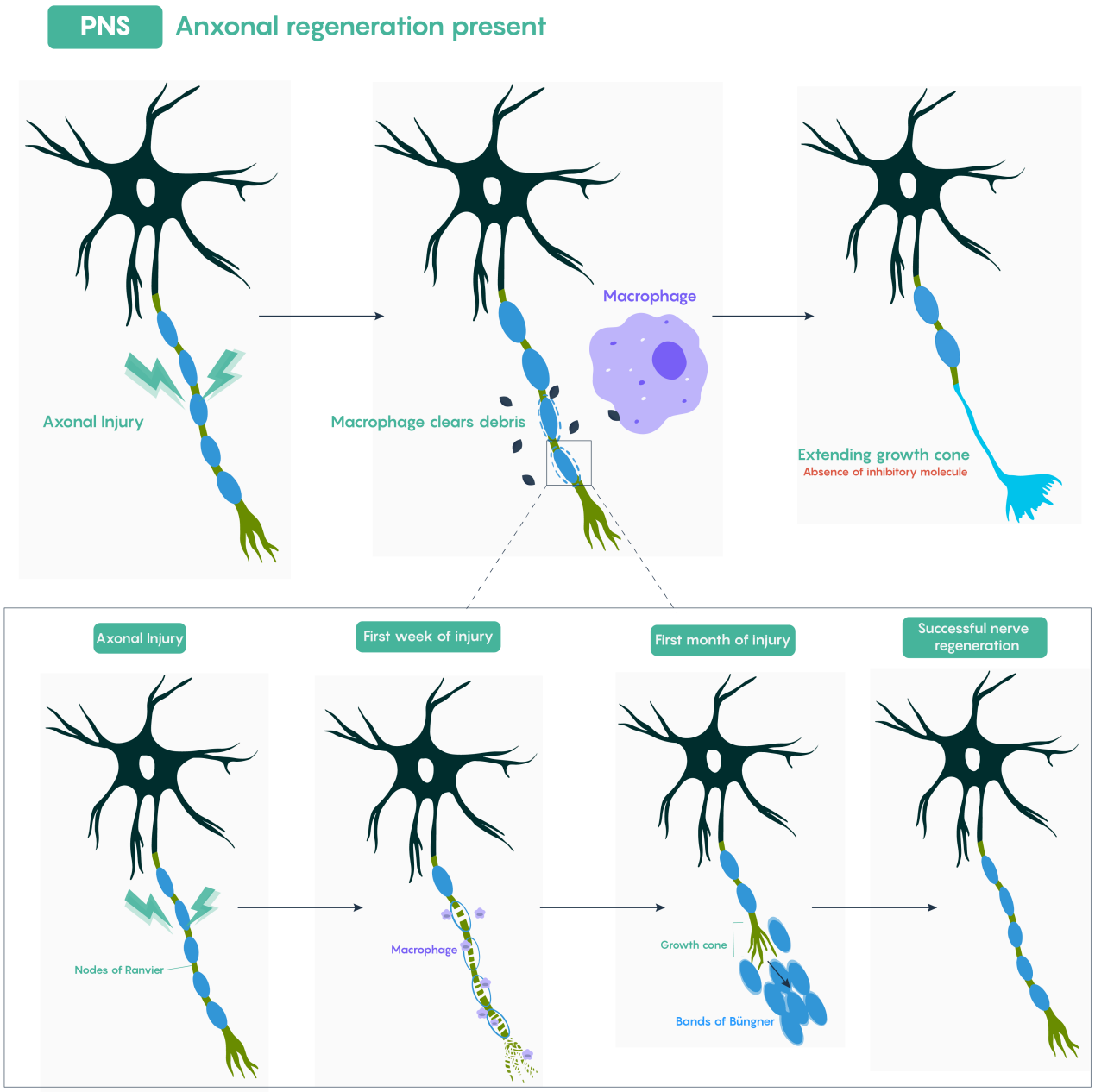


Figure 1. *Differences in neuronal regeneration and outgrowth in the CNS (top) and PNS (bottom panel) following damage to axons.*

What are the key differences?

Although it is possible that there are inherent properties that make neurons in the CNS somehow different from those in the PNS, it is more likely that external factors and cells account for the differences in their abilities to regenerate following damage. As shown in Figure 1. above, within the CNS, following axonal injury, the site of damage distal to injury (i.e. further away from damage) often undergoes degeneration and the distal axon

degenerates and eventually disappears. Although there are several reasons that this is thought to occur, most notably this is believed to involve a lack of macrophage clearing of damaged myelin as well as the formation of a glial scar via activated astrocytes that cause a physical barrier to their re-growth. Additionally, molecules that are unique to the CNS (see the chapter in this Unit on Multiple Sclerosis) also are believed to be up-regulated within the CNS following damage that prevents regeneration. These molecules are associated with the CNS specific glial cells, oligodendrocytes that provide myelination, and these cells increase the production of Nogo, MAG (Myelin Associated Glycoprotein) and OGmp (Oligodendroctye myelin glycoprotein) among others.

In contrast, PNS neurons (bottom panel of figure 1) show significant clearing of damaged axons via macrophages (not observed in the CNS) and then axonal outgrowth and repair across the bridge of Schwann cells that occurs following a process known as Wallerian degeneration (the loss of the axon distal to the injury).

Pathophysiological implications – TBI (Traumatic Brain Injury)

You might be asking yourself, where do these types of axonal injuries occur with the brain and CNS? Increasingly researchers and clinicians are finding that there is a specific axonal injury that could occur following traumatic brain injury (TBI) or concussion-related injuries. One of the ways in which axons within the brain become damaged includes the process of coup/contrecoup where the brain (and its neurons) are first compressed (coup) and then stretched (contrecoup). The mechanical forces on the neuron on the brain may result in varying degrees of damage to the neurons (Figure 2.).

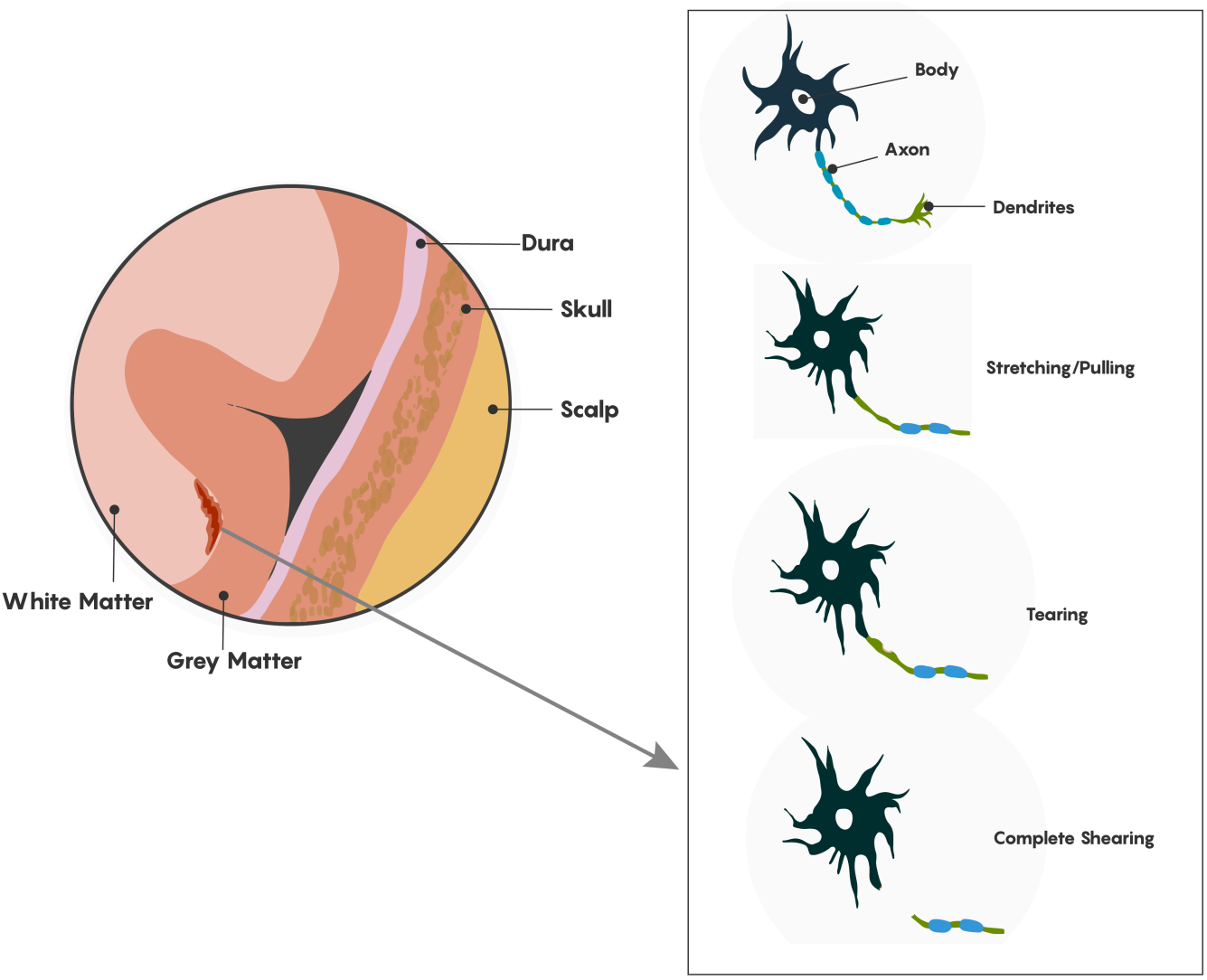


Figure 2. *Schematic diagram of diffuse axonal injury following TBI/concussion.*

As illustrated in Figure 2., depending on the area of the brain is impacted, a number of different types of damage can occur along the length of the axon including stretching/pulling of axons which may affect myelination and localization of axonal channel proteins, and both tearing and shearing which will cause loss of axonal integrity. This type of diffuse axonal injury will have effects on both the structure of the grey matter of the brain and the white matter axonal tracts. Ultimately these types of injuries often go undetected and the most common methods used are clinical assessments using variations of the Glasgow Coma Scale (GCS) that examine the severity of the loss of consciousness as these injuries, although structural are undetectable by either MRI or CT-scans.

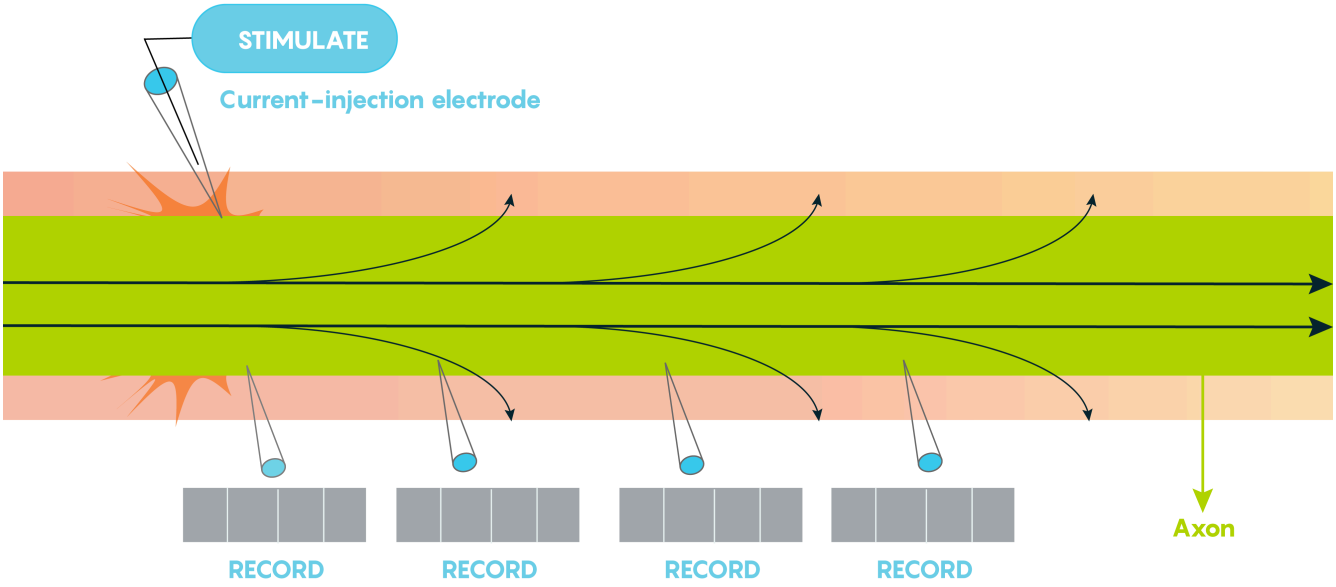


Figure 3.

Did you know?

Did you know that there is still controversy between TBI/concussions and the development of a neurodegenerative disorder known as Chronic Traumatic Encephalopahty (CTE) which is thought to cause behavioural changes in athletes in sports with contact/impact?

Although there are likely *structural* changes as indicated above, most often the diagnosis of post-concussion or TBI related disturbances occurs because of *functional* changes in cognitive function and possible mood disorder changes (depression etc.). Although there are likely *structural* changes as indicated above, most often the diagnosis of post-concussion or TBI related disturbances occurs because of *functional* changes in cognitive function and possible mood disorder changes (depression etc.).

Did you know that one of the chief complaints following TBI/concussions is the inability to concentrate and focus on computer/TV screens? Individuals with concussion injuries require not just physical rest but cognitive rest, and typical LCD screens refresh at a 60 Hz rate. Most individuals are capable of processing and fusing these high-frequency images without a cognitive load, but following concussions, individuals will have higher critical flicker frequencies

such that staring at a screen will increase cognitive fatigue and eyestrain. We don't understand why this happens yet but perhaps there is a Nobel Prize in it for an aspiring neurobiologist!

Chapter Checkpoint: Complete the following quiz

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://ecampusontario.pressbooks.pub/neurosciencecdn3/?p=236#h5p-6

3.2 STROKE AND LOSS OF BLOOD FLOW AS AN ACUTE INJURY TO THE BRAIN

Introduction

The lack of blood flow to an area of the brain known as *ischemia*. This is particularly dangerous in the brain, as a lack of circulating blood deprives neurons of oxygen and nourishment. Stroke is the condition of ischemia specific to the brain and can occur in 2 different ways: *hemorrhage* or *blockage*. In the case of hemorrhage, the release of blood from blood vessels after the vessel rupture causes damage by cutting off connecting pathways, resulting in local or generalized pressure injury as well as impaired blood flow to the brain. The blockage of a blood vessel causes a disruption of blood flow to that region of the brain as shown in Figure 1. This blockage can be either thrombotic or embolic.

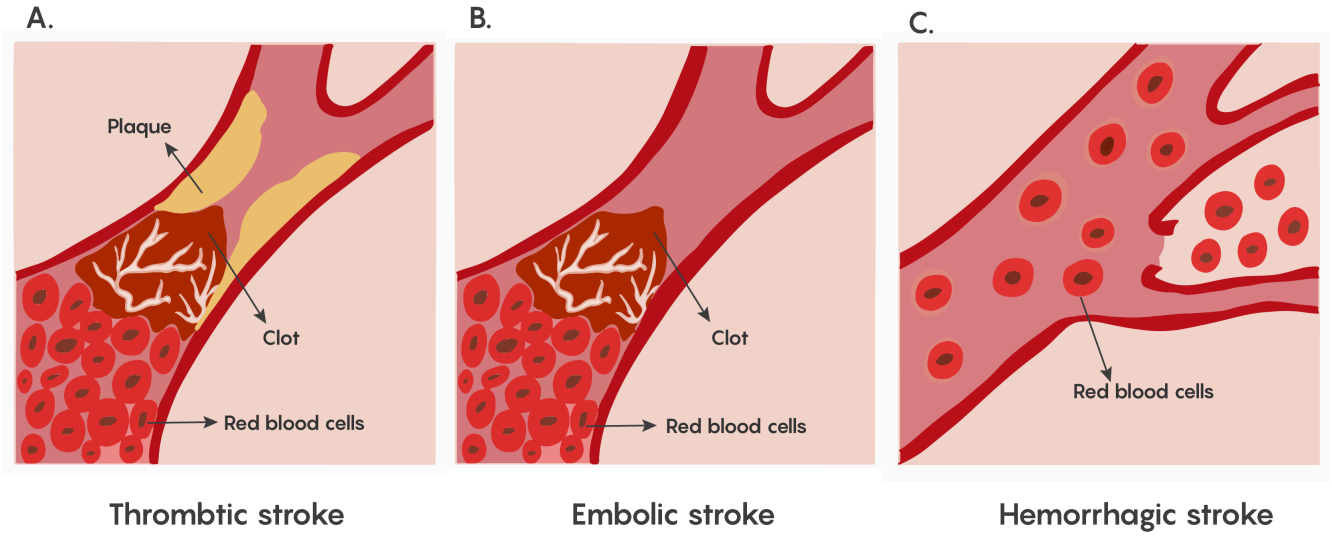


Figure 1. *Showing the major types of ischemia (lack of blood flow) in stroke. Stroke symptoms depend on the region of the brain where the damage has occurred. These symptoms may include deficits to movement, sensation, emotion, or speech. The loss of function varies with the location and extent of the damage.*

Most forms of ischemia in stroke involve the thrombotic or embolic types of blockage, and a much smaller percentage involve hemorrhage. Although both are serious, all strokes have a therapeutic window that allows for some neurons to be rescued.

Cellular Mechanisms underlying ischemia-induced cell

death

The brain requires a continuous supply of oxygen and glucose to maintain normal function. If cerebral blood flow is interrupted, then neuronal metabolism can be effected within 30 seconds and will completely stop within 2 minutes of deprivation. If left unchecked, neuronal cell death occurs in 5 minutes. During this time period, these neurons will not be able to maintain their resting membrane potential. Upon death, they will release potassium ions causing other nearby cells to depolarize. In addition, Na+/K+ exchanger pumps will no longer have ATP production to drive their activity, causing neurons to depolarize and fire inappropriately. This is the basis of the glutamate excitotoxicity theory proposed by John Olney and may explain why pyramidal neurons in the hippocampus and Purkinje cells in the cerebellum that rely on glutamate neurotransmission are particularly vulnerable to ischemia.

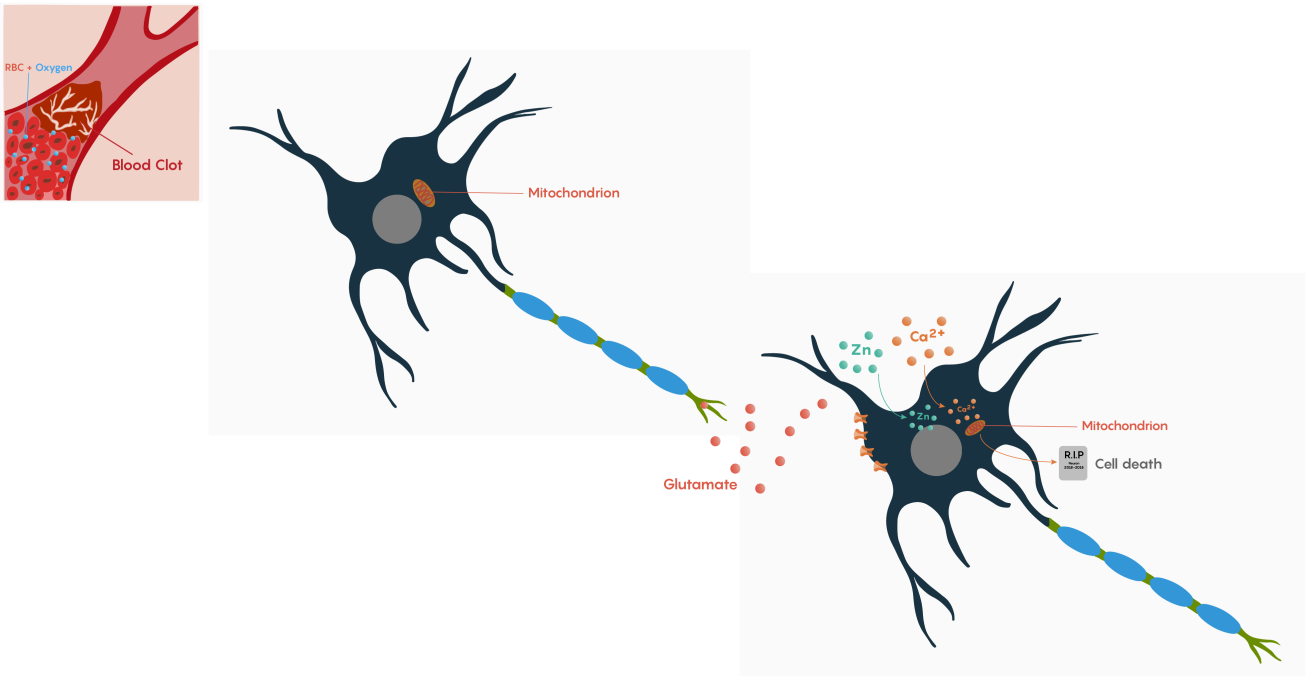


Figure 2.

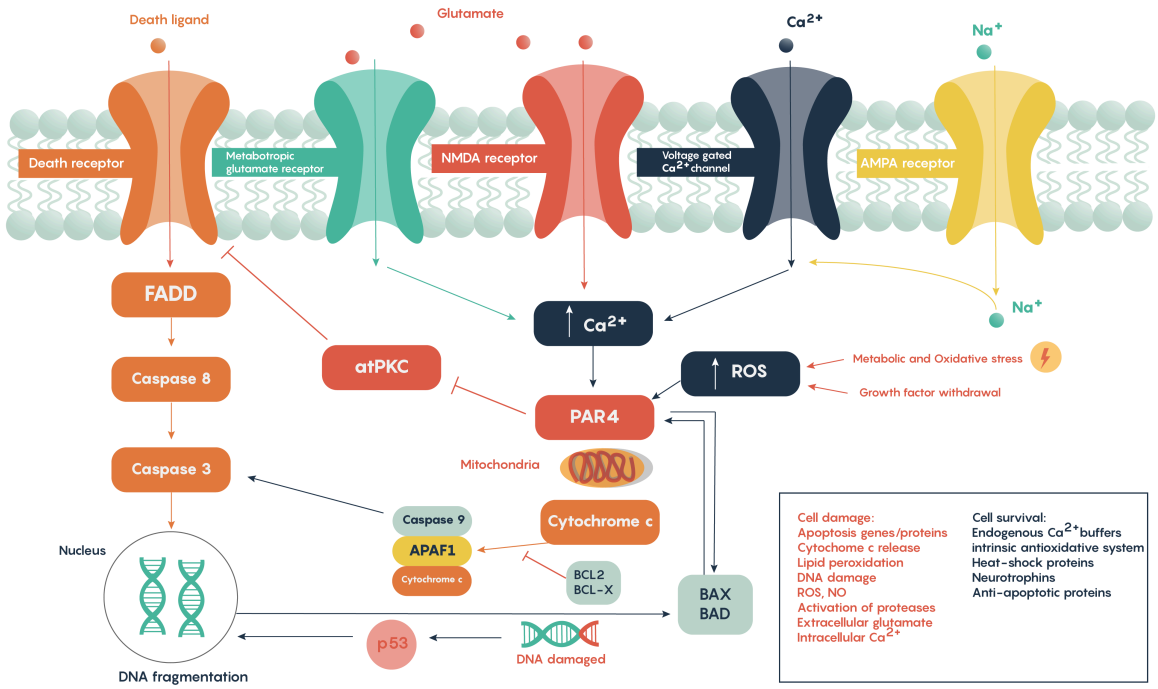


Figure 3. *Molecular pathways outlining the role of glutamate in excitotoxicity following stroke.*

Following ischemia to a region of neurons, the increased release of glutamate and its decreased uptake without ATP production (Figure 2.) produces an excess of glutamate which impacts downstream neurons. Glutamate will impact a number of glutamate specific receptors such as the AMPA, NMDA and metabotropic type receptors which in turn will cause activation of voltage-gated Ca2+ channels, all of which result in the increase in intracellular Ca2+ levels. In turn, this will activate the protease-activated receptor PAR4 which will activate the pro-apoptotic proteins BAX/BAD that will then form a complex with the mitochondrial factor cytochrome c which is released from the mitochondrial matrix as the intracellular ATP stores fail. This complex, in turn, will then activate caspase-9 and the downstream and ultimate factor caspase-3 which is involved in the apoptotic DNA fragmentation and eventual death of the neuron. As this is a programmable/ apoptotic form of cell death, there are a number of therapeutic interventions that may prevent this stroke and ischemia-induced form of cell death.

Animal models of stroke

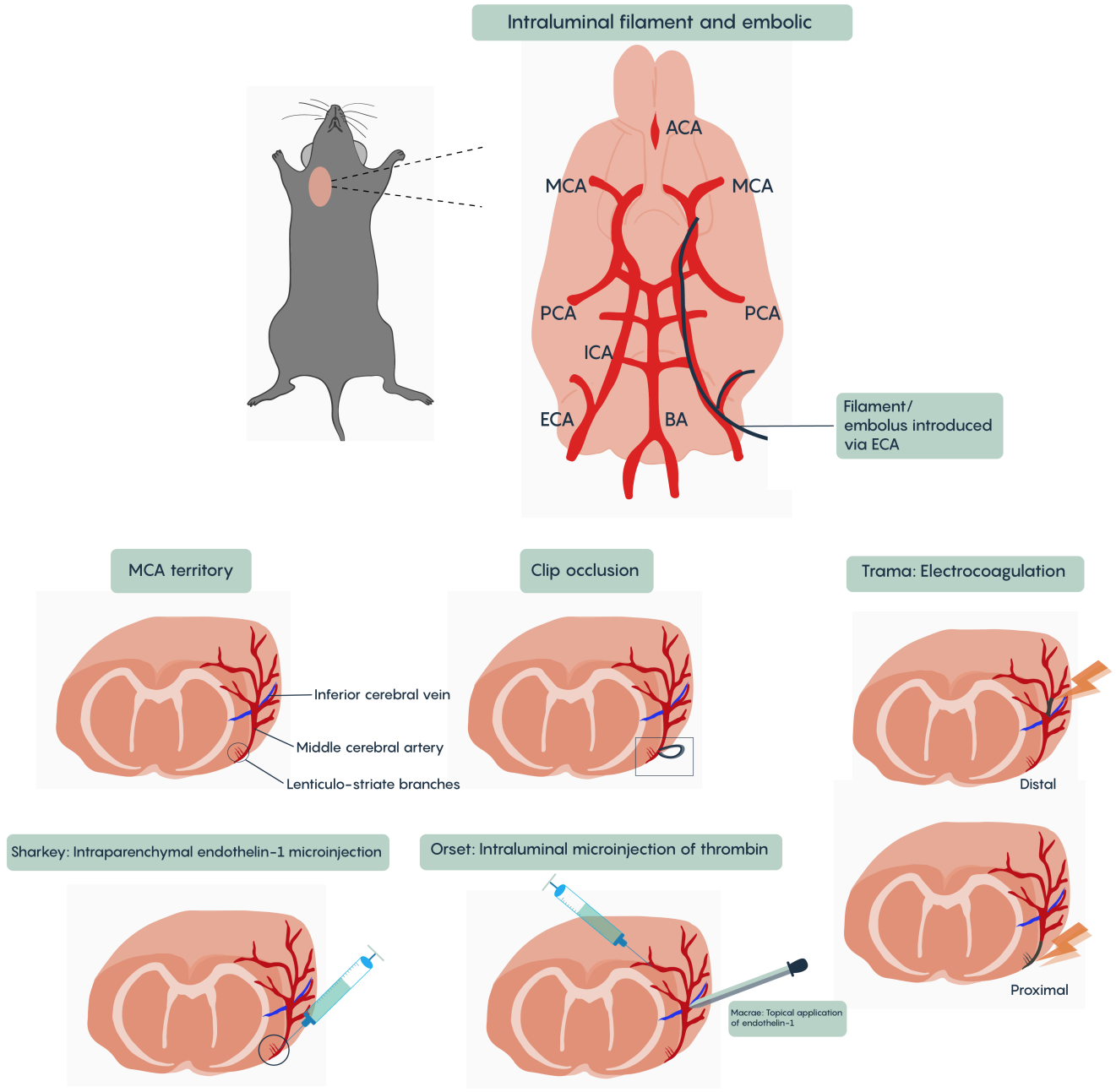


Figure 4. *Various animal models of ischemia and stroke.*

A number of different animal models of stroke have been developed. Most involve interruptions in blood flow such as the intraparenchymal (i.e. directly into the brain tissue) injection of drugs such as endothelin-1 (Et-1) which induces vasospasms of the blood vessels creating ischemia, of the introduction of coagulants such as thrombin injection that will block blood vessels (Figure 4. Animal models of stroke). Most methods will involve blockade of normal blood flow to an area of the brain to produce ischemia whether through occlusion of a blood vessel such as the middle cerebral artery (MCA) or the chemical methods described here. These

animal models have proved invaluable in understanding the role of different factors such as cytochrome c or BAD/BAX in excitotoxicity and hold the promise of developing therapeutic interventions.

Stem Cells in the Recovery of Strokes

A key player in stroke recovery is adult stem cells, also known as somatic stem cells. These cells are undifferentiated with a non-specific function. Stem cells are located in specific areas known as germinal niches, within a tissue. Within these areas, the cells remain in a quiescent, or dormant, state until a signal such as a disease or tissue damage is received. In the brain, the germinal niches primarily include the subventricular zone (SVZ) and dentate gyrus of the hippocampus where neural progenitor cells (NPCs) reside. The SVZ is composed of four main types of cells which include proliferating neuroblasts (Type A), slowly proliferating stem cells (Type B), transient amplifying stem cells (Type C) and ciliated ependymal cells (Type E). Once dividing, the stem cells may produce additional stem cells (self-renewal), or they may differentiate into specific cell types with a specific function.

Tissue injury, such as brain ischemia, can trigger the proliferation of NPCs in the anterior aspect of the SVZ. At the site of injury, NPCs will undergo neurogenesis in which they will differentiate into mature neurons. Before reaching their final destination, the precursor cells will first need to travel through the rostral migratory stream (RMS). The RMS is a route directly connecting the SVZ to the forebrain, allowing NPCs to easily access ischemic regions. Interestingly after an ischemic stroke, researchers have noted increased amounts of Type A and C cells, as well as a temporary increase in Type B and E cells. This surge in proliferation has shown to peak approximately a week following the stroke. However, after three to five weeks, the mitotic activity declines to basal levels. As a result, it is believed NPCs may be responsible for promoting neural remodelling and repair. Although this process is still not well understood, it remains to be a promising therapeutic approach to treat a variety of brain injuries such as ischemic strokes.

Chapter Checkpoint: Complete the following quiz

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3.3 DEMYELINATING DISEASES WITH AN EMPHASIS ON MULTIPLE SCLEROSIS

Myelin is produced from the cell membranes of either Schwann cells (for neurons in the PNS) or oligodendroglia/oligodendrocytes (for neurons in the CNS). As such, myelin is enriched with membrane lipids and proteins that are found within these cells. Normally myelin functions to increase nerve cell conduction by increasing the biophysical property of the membrane resistance (i.e. a neuron cell membrane's leakiness). Myelin reduces membrane leakiness by preventing open channels and as a result, increasing how far a single electrical impulse within the axon will travel. Importantly this also increases how quickly an action potential will travel down an axon – myelin greatly increases the conduction velocity of a neuron (Figure 1.).

Myelin helps neurons to cheat

Various factors help to determine how fast an action potential travels down an axon (called the conduction velocity). The biophysical features of a neuron including axonal diameter and membrane leakiness, help scientists to determine what is known as the length or space constant (lambda or λ) for each neuron. Why is this λ value important? λ is directly proportional to the conduction velocity – so the factors that determine λ also determine a neuron's conduction velocity. Let's not forget –speed thrills within the CNS and brain, and the faster a signal gets there, the more the brain likes it.

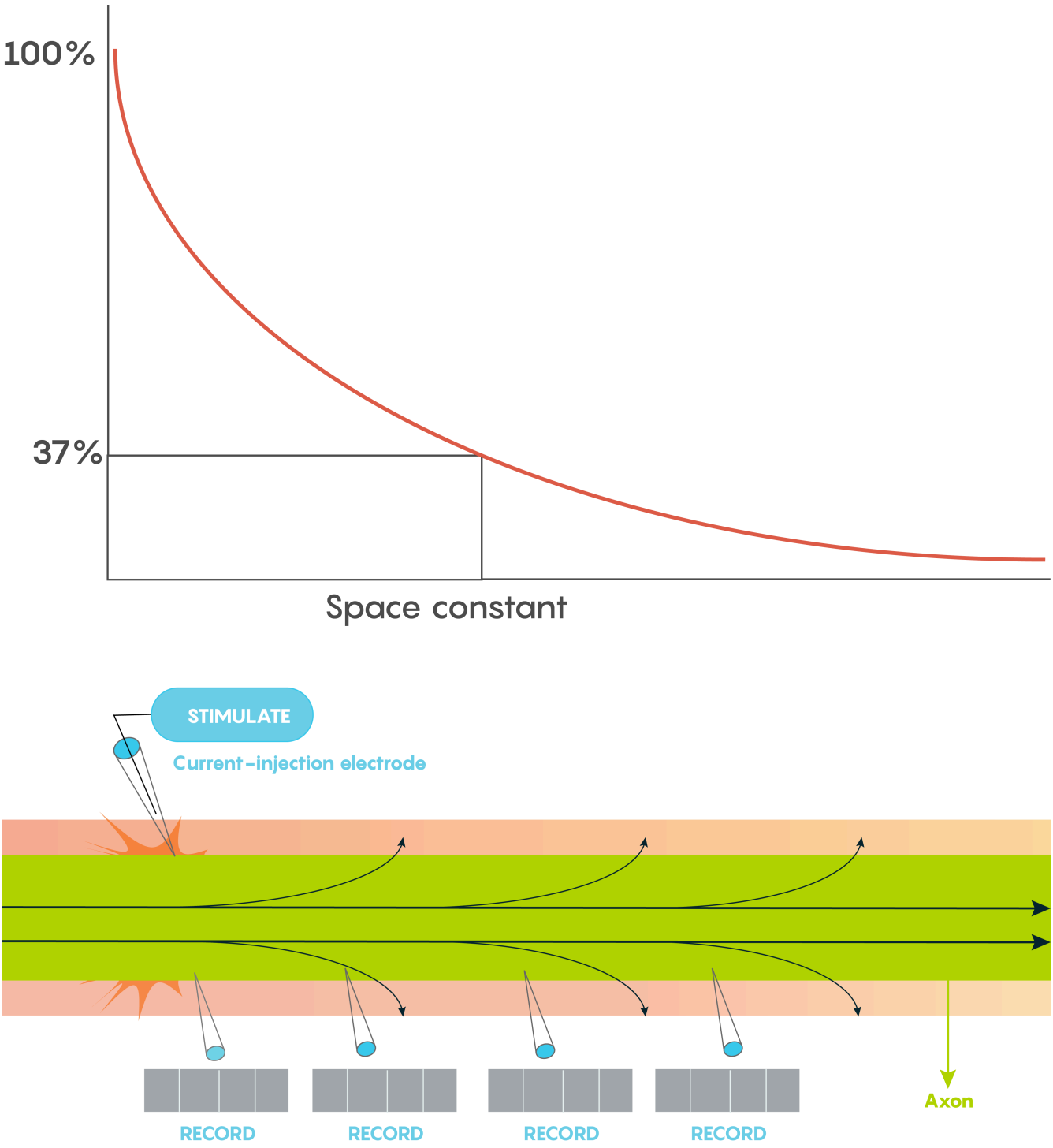

Figure 1. *Showing the biophysical variables that determine the length constant including membrane leakiness (R_m) and internal diameter (R_{axial}). Myelin increases Rm to increase both the length constant and therefore speed of conduction.*

Another reason for myelination includes having a lot of neurons so space is a premium. Although the length constant is inversely related to the axonal diameter (i.e. bigger diameter axons have bigger length constants), we need to pack as many neurons into a small space as possible. So within the brain, faster communication means myelination is key.

Large vertebrate nerve fibres are wrapped in myelin sheaths formed by central oligodendroglial cells or Schwann cells in the periphery but the myelin only wraps the axon at specific locations. Myelin is interrupted at regularly spaced intervals or nodes. This helps the neuron because during action potential propagation the excitatory signal jumps from one node to the next, and we call this *saltatory conduction which is much faster than passive electronic spread.* In addition, myelin also helps Rm (leakiness or membrane resistance) because it only directs voltage-sensitive Na^+ channels to highly concentrated areas known as the nodes of Ranvier (Figure 2.). So in many ways, myelin helps the neuron "cheat' by limiting leakiness (i.e. open channels to specific areas). As shown in Figure 2., Before glial ensheathment, sodium channels are distributed uniformly, and at low density. At the time of glial ensheathment but before the formation of compact myelin, loose clusters of sodium channels develop at sites that will eventually become nodes. Following the formation of compact myelin and mature paranodal axon-glial cell junctions, well-defined nodal clusters of voltage-gated sodium channels are established, and sodium channels are eliminated from the axon membrane beneath the myelin sheath.

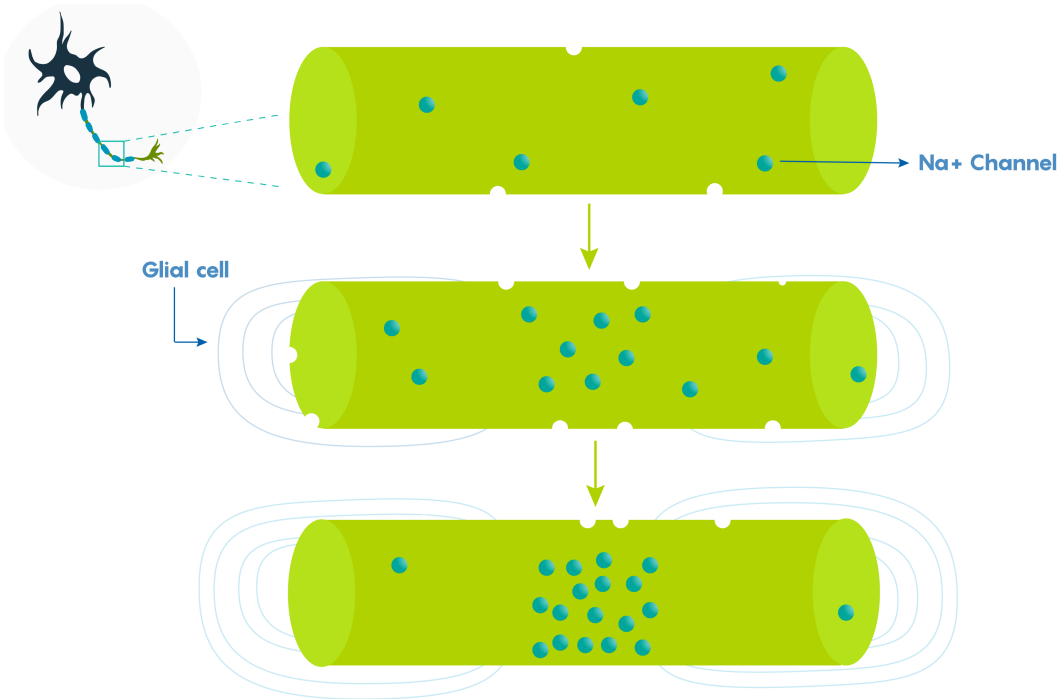

Figure 2. *Myelination directs leakiness to Nodes of Ranvier. During development voltage-dependent Na+ channels are found along the entire length of the axon. As myelination occurs, these voltage-gated channels (and hence leakiness) are localized to only the node (part c).*

Demyelinating diseases are prevalent – and in Canada, Multiple Sclerosis affects many

Demyelinating diseases remain one of the most common disorders where 1:500 to 1:1000 adult individuals in North America are affected, but these diseases tend to affect women more frequently than males. As the name suggests, within these diseases, the myelin described above is lost and the axons which previously have been myelinated no longer have myelin, changing their conduction velocities.

Multiple Sclerosis: Overview

Within this disease, the myelin is destructively removed from around the axon which slows down nerve impulses. As axons are demyelinated, these result in inflammatory patches called lesions and it is thought that this disorder is an autoimmune disease. As the disease progresses, oligodendrocytes and, ultimately, the axons themselves are destroyed. There is very compelling evidence that the destruction is caused by selective activation of the cellular immune system and inflammatory molecules.

Potential causes and theories

Currently, there is no known cause for multiple sclerosis although many different hypotheses have been put forward. Surprisingly, there are no known associative genes that have been implicated in causing multiple sclerosis and scientists believe there might be a complex interaction between genes and environmental factors to produce demyelination observed in multiple sclerosis. The most common theories about the cause(s) of multiple sclerosis include:

1. Viral infection and resulting in autoimmune reaction where evidence from Experimental allergic encephalomyelitis mouse models suggest a strong link
2. Genetic factors: inherited predisposition possibly through the immune system, although a mutation in a gene has not been discovered
3. Environmental factor(s) which might work with genetics such as low vitamin D levels or smoking.

Although this is a well-characterized disorder, we still don't know much about its causes.

Diagnosis, loss of function, and pathology

Typically an individual with demyelination in multiple sclerosis may have functional deficits that result from a reduced speed of conduction which might include loss of vision, different atypical sensations in the periphery and findings on an MRI that include hyperintensities, bright patches on structural MRIs (Figure 3.), around sites like the lateral ventricle, optic nerve, brainstem, spinal cord, cerebellum and other areas.

Multiple sclerosis also presents itself differently including having acute phases where the condition has been associated with relapsing/remitting where symptoms may not appear for periods of time and a chronic phase which is associated with progressive forms of multiple sclerosis where the individual progressively becomes worse and the symptoms become more severe.

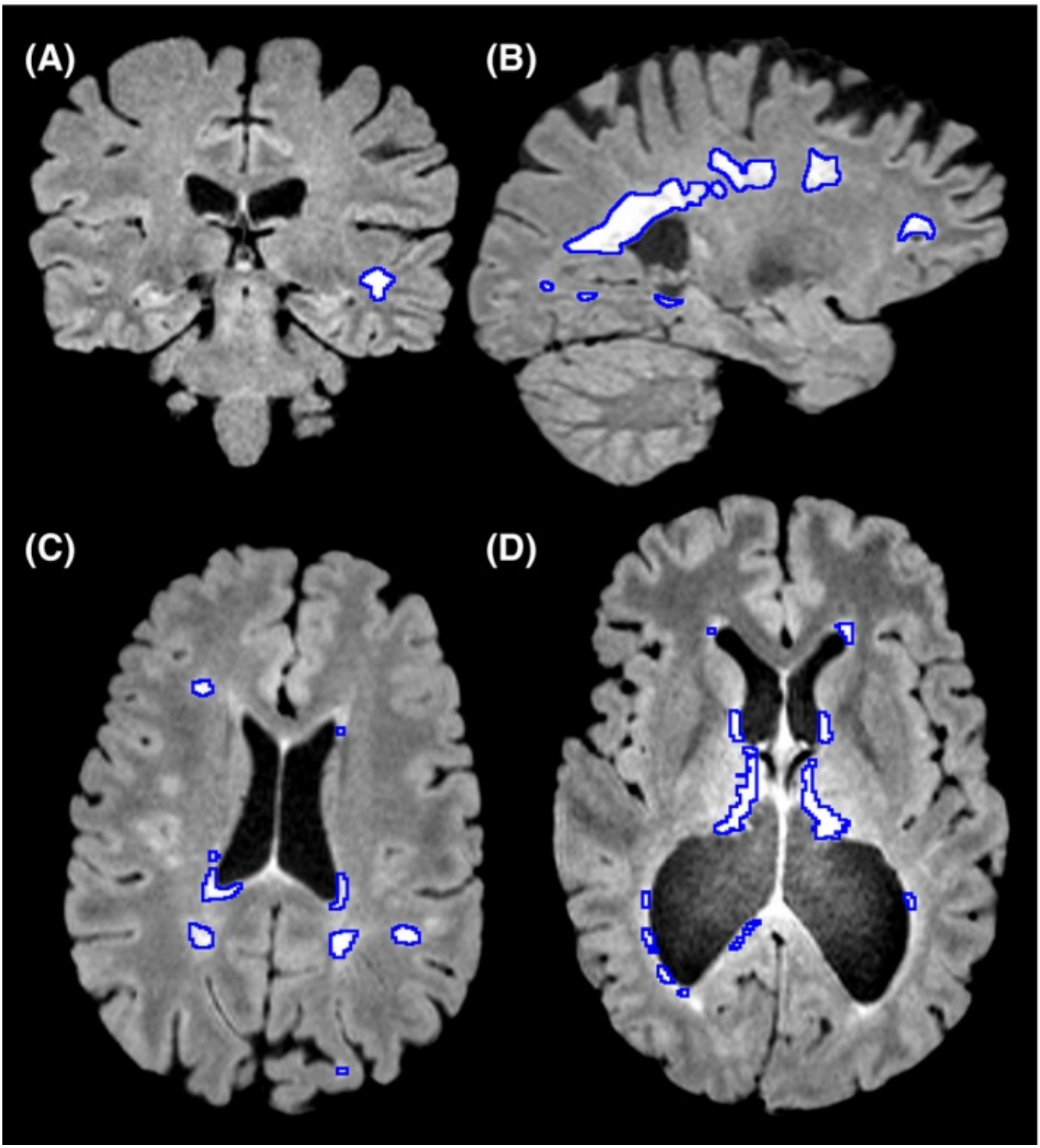

Figure 3. *MRI images showing white hyperintensities in different areas of the brain of an individual experiencing symptoms of multiple sclerosis. (A) A coronal slice showing a lesion in peripheral white matter. (B) A sagittal slice showing large periventricular lesions. (C) An axial slice showing both periventricular and peripheral white matter lesions. (D) A case where severe atrophy caused midline false positives to not be removed, as they were further from midline than expected. Image from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4731385/ Under CC by 4.0.*

Animal models of multiple sclerosis

Experimental autoimmune encephalomyelitis (EAE): one of the best-studied models of autoimmune diseases. This model produces inflammation of both the brain and spinal cord. This is a good validity model for multiple sclerosis, as EAE is believed to be mediated by T cells and can be induced in many animal models following immunization with a myelin-specific protein such as myelin basic protein (MBP) or proteolipid protein (PLP) in Freund's complete adjuvant (or CFA which helps to produce a specific immune response). Within weeks animals develop cellular inflammatory cell infiltration of the myelin sheaths of the central nervous system which then results in demyelination or paralysis (Figure 4.).

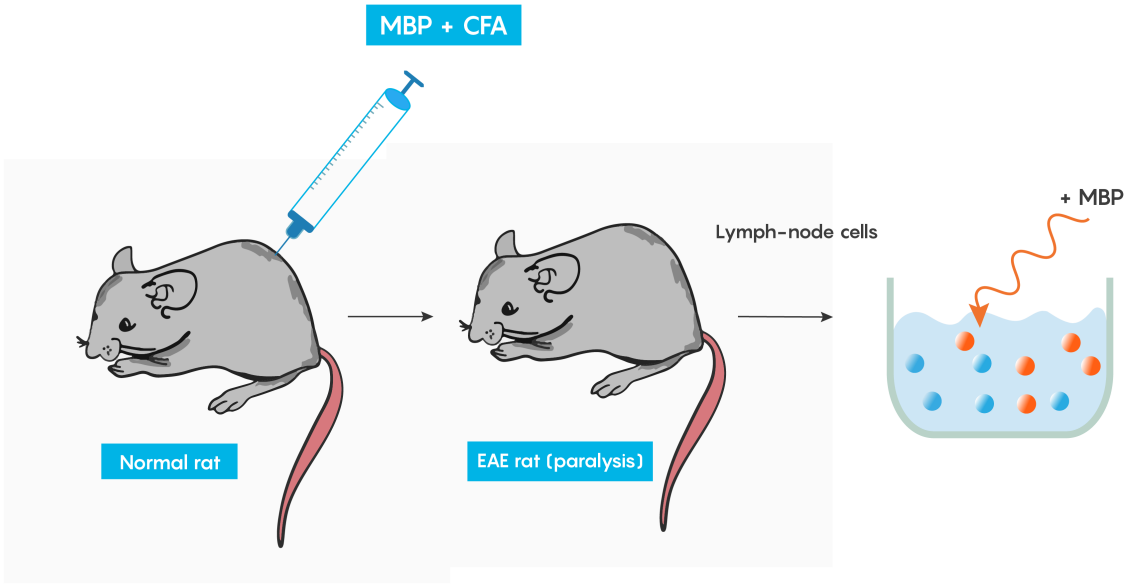

Figure 4. *Inducing an inflammatory response to produce EAE as a model of multiple sclerosis.*

The cuprizone model of multiple sclerosis

Cuprizone treatment is one of the most frequently used toxin-induced multiple sclerosis models. Other toxins such as lysolecithin or ethidium bromide require stereotaxic microinjections into brain areas and result in only localized focal demyelination but cuprizone models are advantageous in that oral administration of cuprizone produces global damage. Following cuprizone ingestion, the mouse often exhibits impaired divalent ion (Cu^{2+}, Zn^{2+}) homeostasis and results in the loss of Cu-Zn superoxide dismutase activity within the brain's tanycytes resulting in the production of reactive oxygen species (ROS) which activate M1 macrophages in the brain to increase the production and release of pro-inflammatory molecules as highlighted in Figure 5.

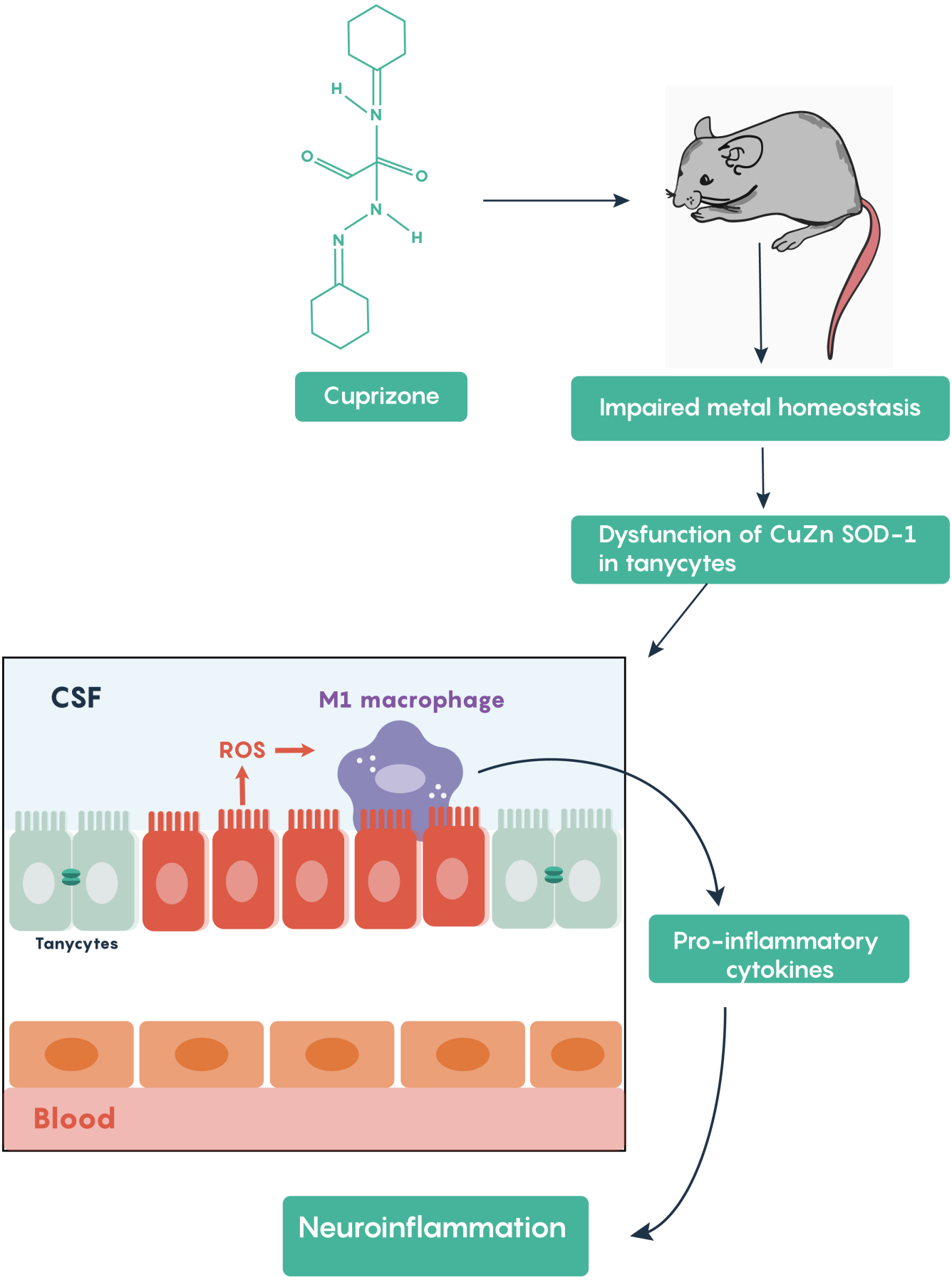
Cuprizone
Impaired metal homeostasis
Dysfunction of CuZn SOD-1 in tanycytes
CSF
M1 macrophage
ROS
Tanycytes
Blood
Pro-inflammatory cytokines
Neuroinflammation

Figure 5. *Mouse model of demyelination in multiple sclerosis highlighting the activation of the inflammatory process.*

Chapter Checkpoint: Complete the following quiz

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3.4 CHRONIC NEURODEGENERATIVE DISEASES

Alpha-Synucleinopathies and Parkinson's Disease

The current model for most neurodegenerative conditions is that neurons die via apoptosis or a programmed form of cell death. This has very important clinical implications as turning on or turning off specific genes/proteins is a method to stop or possibly reverse neurodegeneration. Neurodegenerative diseases such as Parkinson's Disease and Alzheimer's Disease and others are all believed to use apoptotic pathways and additionally suggest that there is a component protein that is aberrantly folded to produce apoptosis.

Parkinson's Disease: Overview

Most cases of this disorder appear to be sporadic (i.e. non-genetic in origin). A very few cases seem to have a genetic origin (for example the genes most often associated with *DJ-1*, *Parkin* (*Ubiquitin E3 ligase*) and *alpha-synuclein* genes). Parkinson's Disease is most often associated with the loss of "pigmented" nuclei in the brain and typically involves the loss of a group of neurons found in the Substantia Nigra (Figure 1.). The substantia nigra neurons are dopaminergic and are pigmented because they contain the protein *melanin*.

The typical onset for Parkinson's Disease is middle to later stages of life (i.e. 50 and beyond) although a small percentage of individuals who have known genetic mutations in *Parkin* or *alpha-synuclein* develop symptoms earlier.

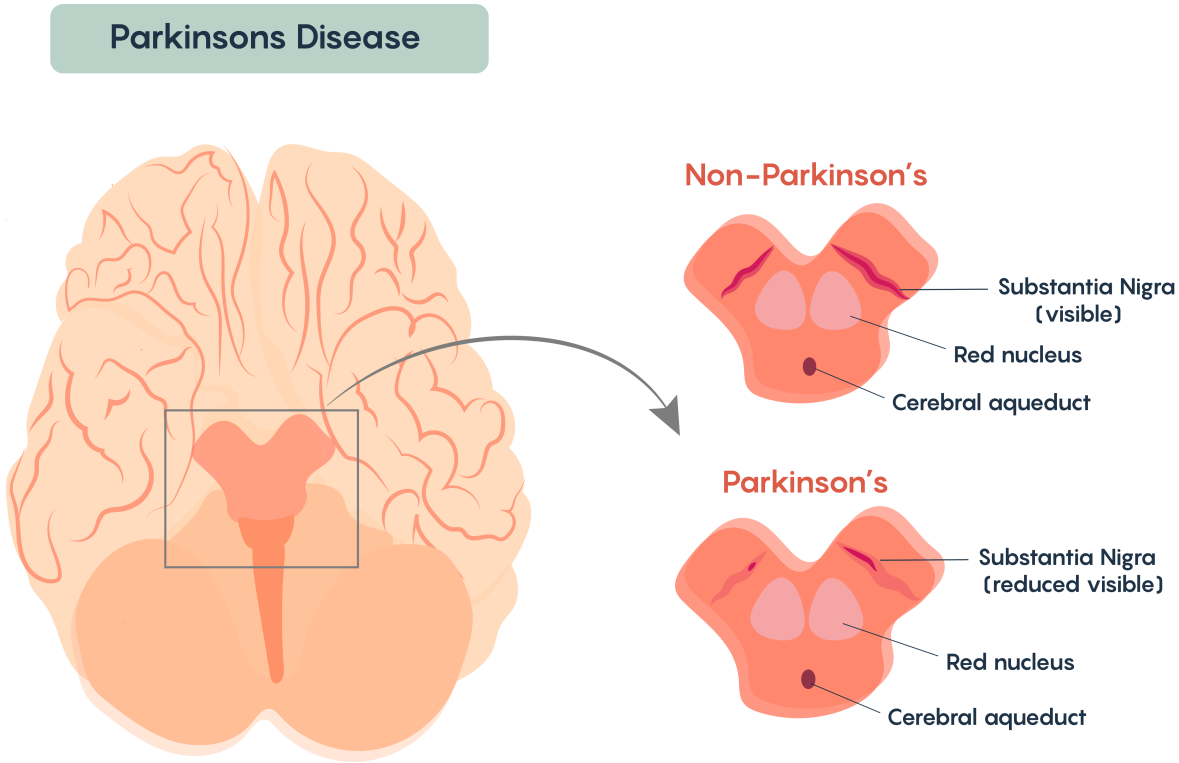

Figure 1. *Loss of the pigmented dopaminergic neurons within the Substantia Nigra of individuals with Parkinson's Disease.*

Loss of these cells affects the processing and execution of voluntary movement in individuals with Parkinson's Disease. Similar to Multiple Sclerosis, once diagnosed, the symptoms become continuous and progressive – i.e. the symptoms worsen over time. Again, there are many similarities with Multiple Sclerosis, and there is no known cure for Parkinson's Disease and the disease remains idiopathic although there are some known causes of Parkinson's Disease including loss of movement following cerebral atherosclerosis, viral encephalitis, and as the result of side effects from drugs such as phenothiazides and reserpine.

"Classic" symptoms associated with Parkinson's Disease

- **Bradykinesia:** Slowness in Initiation and Execution of Voluntary Movements
- **Rigidity:** Increase Muscle Tone and Increase Resistance to Movement (Arms and Legs Stiff) – as severity increases produce cogwheel rigidity
- **Tremor:** Usually Tremor at Rest; When a person sits, arm shakes; Tremor Stops when a person attempts to grab something (pill-rolling tremor)
- **Postural Instability:** abnormal fixation of posture (stoop when standing), problems with equilibrium, and righting reflex
- **Gait Disturbance:** Shuffling feet
- **Orthostatic Hypotension**

- **Dementia** (in some instances)
- **Dystonia** (inappropriate and continuous muscle contraction)
- **Ophthalmoplegia** (weakness in eye muscles)
- **Affective Mood Disorders** (such as major depression)

Lewy bodies and alpha-synuclein – hallmark features of Parkinson's Disease

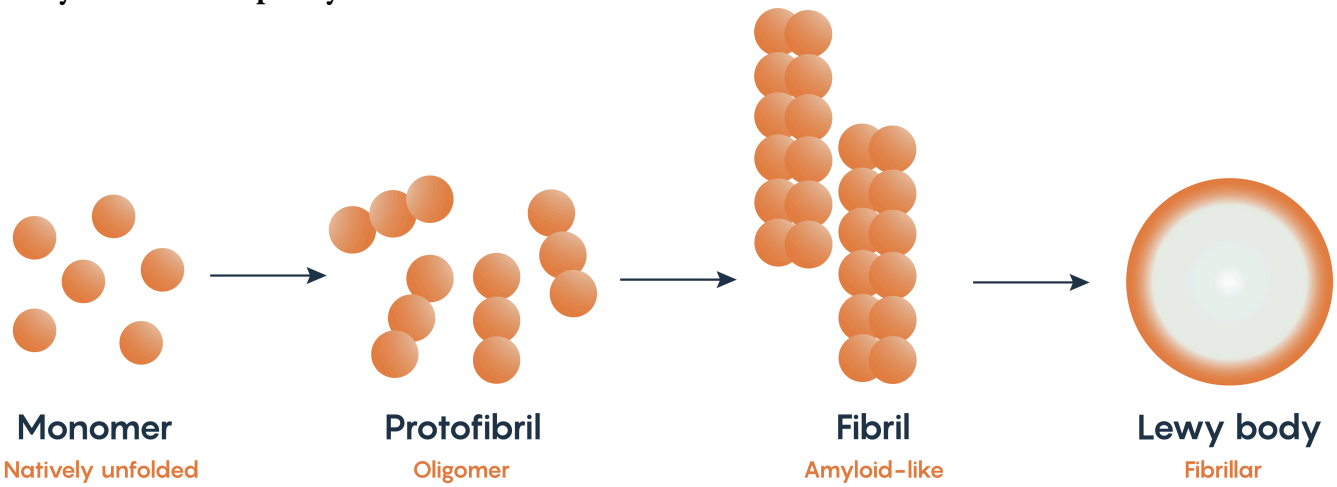

Figure 2. *Misfolding and subsequent accumulation of alpha-synuclein.*

Alpha-synuclein is a naturally occurring protein within neurons. Mutations in the PARK1 and PARK4 genes which normally encodes for alpha-synuclein have been associated with Parkinson's Disease. As such, many animal models of Parkinson's Disease look for the production of the fibrillary form of alpha-synuclein as it misfolds and then accumulates within the substantia nigral neurons known as Lewy bodies (Figure 2.). The misfolding and accumulation of alpha-synuclein has been hypothesized to be the reason that neurons undergo apoptosis, although the exact mechanism for how this occurs remains to be elucidated.

Animal Models of Parkinson's Disease

The lack of candidate genes (except for alpha-synuclein, Parkin and DJ-1) has meant that most scientists have looked at toxin models. Most of the toxins that produce features that resemble changes in movement as well as Lewy-body like formation use catecholaminergic destruction. In fact, most of these toxins are incredibly powerful and dangerous mitochondrial complex inhibitors such as reserpine, MPTP, methamphetamine, 6-OH-dopamine, rotenone, and paraquat). In fact one toxin, MPTP is a by-product of synthetic heroin production suggesting that there may be a synthetic substance that causes Parkinson's Disease and some epidemiological studies show a correlation with pesticide (such as paraquat and rotenone) usage and Parkinson's Disease.

Alzheimer's Disease: Overview and General Pathology

Another neurodegenerative condition is Alzheimer's Disease (AD). This neurodegenerative condition is a form of dementia characterized by the most common symptoms associated with Alzheimer's Disease notably memory loss, problems with communication and difficulty finding words, attention problems, confusion and spatial disorientation, decreased or poor judgement, changes in mood and personality.

Affected areas explain the AD pathology, which is characterized by memory loss due to shrinkage of the hippocampus, and other problems which include higher-level thinking and performance which are controlled by the cortex. Brain atrophy typically begins in the medial temporal lobe (i.e. hippocampus), moves on to the association cortices and therefore affects sensory and motor areas. It also affects the Nucleus Basalis of Meynert which has multiple cholinergic projections to the cortex (and is thought to be responsible for the control of sleep, attention, and consciousness) as highlighted in Figure 3.

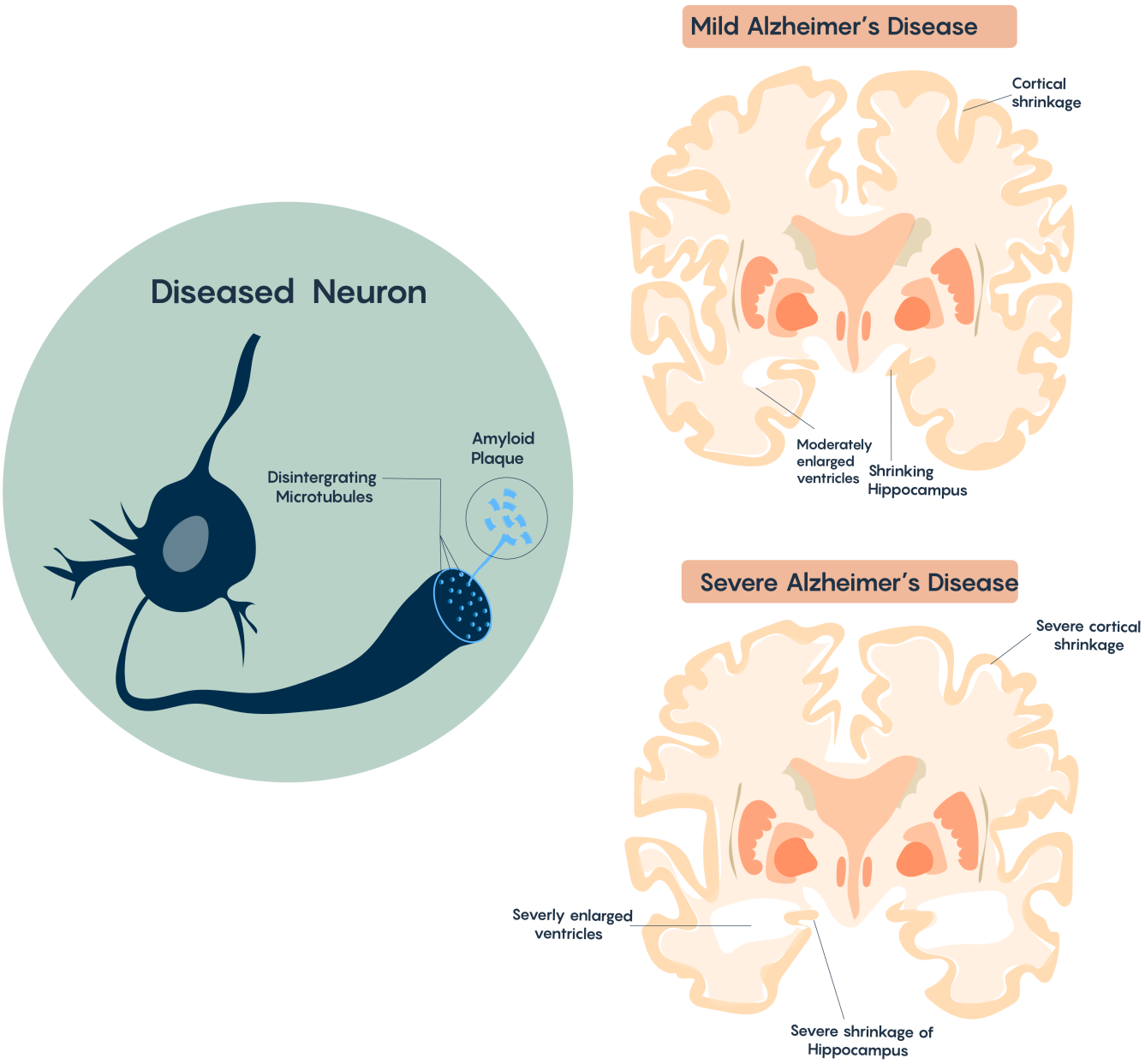

Figure 3. *Shrinkage and loss of specific regions of the brain.*

Cellular Pathology

The typical AD brain is characterized by the loss of neurons, progressive loss of synapses and cholinergic projections, the accumulation of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). With increased time and pathology this will often correlate with the formation of a glial scar (involving reactive astrocytes). There is also very strong evidence that this disorder may also have a strong immune component as there is often the infiltration of microglial cells in the Alzheimer's Disease brain.

β-amyloid (Aβ) plaques: βA Cascade hypothesis

One of the hallmark histopathological and molecular features of Alzheimer's Disease is the presence of extracellular aggregates that include β-amyloid plaques. These plaques are believed to induce cytotoxicity by disrupting normal neuronal functions, ion concentrations, action potential generation. As with the modern theory of neurodegeneration, these plaques are composed of the cleaved form of **amyloid precursor protein (APP)**.

APP can be processed via two different pathways:

- **Non-amyloidogenic pathway** (cleaved by α- and γ-secretases)
- **Amyloidogenic pathway** (cleaved by β- and γ-secretases)

APP Processing

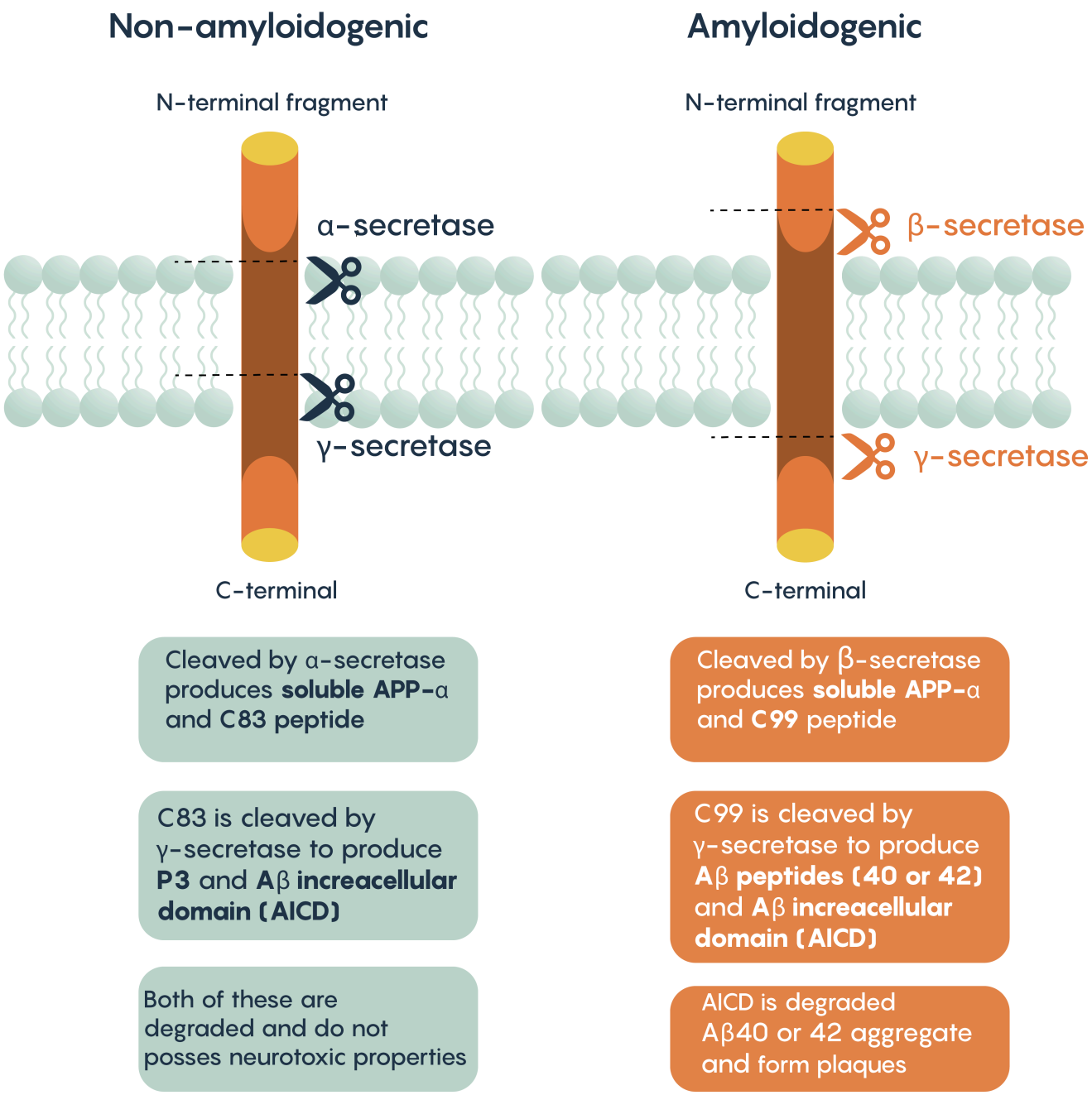

Figure 4. *APP processing pathways leading to the production and aggregation of β-Amyloid.*

Neurofibrillary Tangles (NFTs)

Another feature of the Alzheimer's Diseased brain is the appearance of neurofibrillary tangles (NFTs) that are composed of hyper-phosphorylated tau protein. Normally within neurons (and in fact most cells), tau binds to and stabilizes microtubules (MTs), however, when this is phosphorylated by kinases, tau loses its affinity to MTs and dissociates from the MT complex. This causes the microtubules, in turn, to disassemble which interferes with proper axonal transport and eventually leads to loss of neuronal integrity. As such the Phospho-

tau proteins form intracellular aggregates (NFTs) that become another defining feature of this disease as well as a potential target for therapeutic intervention.

Genetics of Alzheimer's Disease

While sporadic AD cases account for the majority of AD patients, familial AD (FAD) accounts for only 5% of them. The familial form, FAD manifests earlier (around 40-50 years of age) compared to >65 years of age in sporadic cases. Despite this drastic difference in disease onset, symptoms are identical. Only 50% of FAD cases can be explained by known mutations in genes encoding **APP** and **presenilins 1 and 2**.

APP mutations are relatively rare and is characterized by individuals showing symptoms with an average age onset in their early 50's. The conclusion that APP mutations might cause Alzheimer's Disease is based on the observation that most Down Syndrome patients also develop AD after age 40 since they have an extra copy of Ch21 (which codes for APP). However, most inherited forms of APP mutations alter APP processing to:

- increase cleavage via the β-secretase pathway
- increase the Aβ42/40 ratio
- therefore, lead to the production of peptides with higher fibrillogenic potential (Figure 2.)

Chapter Checkpoint: Complete the following quiz

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PART IV

UNIT 4 – FUNDAMENTAL NEUROSCIENCE TECHNIQUES (AND WHEN TO USE THEM)

4.1 PATCH-CLAMP ELECTROPHYSIOLOGY

Overview of Cellular Methods

Since we have learned that neurons contain channels, receptors and transporters in Unit 1, one of the most useful parameters that neuroscientists examine involves determining the movement of ions and the resultant modulation of neuronal membrane potential. Specifically, electrophysiological techniques used in excitable tissues rely on the ionic conductance of ion-channels and how these influence changes in the membrane potential of the cell being examined. Various electrophysiology techniques have been developed to detect and manipulate ion-channel function and/or action potential generation. Determining when to use each electrophysiological technique depends on many different factors; these include the biophysical properties of the recorded cell, the type of tissue being examined, the use of current- and/or voltage-clamp, whether the intra- and/or extracellular environments will be modulated in the experiments, and most importantly whether a single channel or several ion channels will be recorded.

Patch-clamp Electrophysiology

The most common method used to assess ion-channel function is known as the patch-clamp electrophysiological technique that was developed in the 1970s by Nobel Prize Laureates Erwin Neher and Bert Sakmann. The patch-clamp technique allows a researcher to measure the biophysical properties of ion-channels on millisecond timescales. Patch-clamp requires the initial formation of a Giga-ohm (GΩ) seal between the plasma membrane and the blunt tip (0.5–2 μm in diameter) of a heat-polished glass or quartz micropipette (electrode). Once a Giga-ohm seal has been created, this *cell-attached configuration* (Figure 1.) maintains the integrity of the plasma membrane (i.e. the membrane is not ruptured) preventing the intracellular solution inside the micropipette from dialyzing into the cell. However, this also restricts electrical access to the cell intracellular space resulting in an inability to control the membrane potential of the cell. In this configuration, only the patch membrane potential relative to the cell's resting potential can be directly controlled. Through the alteration of the magnitude of the seal resistance (a *loose seal* vs. *tight seal*) and/or whether the recording electrode is current- *vs*. voltage-clamped, the cell-attached configuration can be used to measure single-channel currents, spontaneous neuronal cell firing and synaptic potentials as well as evoked action potentials within the cell. Although the configuration is limited as described above, a major advantage is that this configuration is the starting point for the majority of the types of patch-clamp recordings.

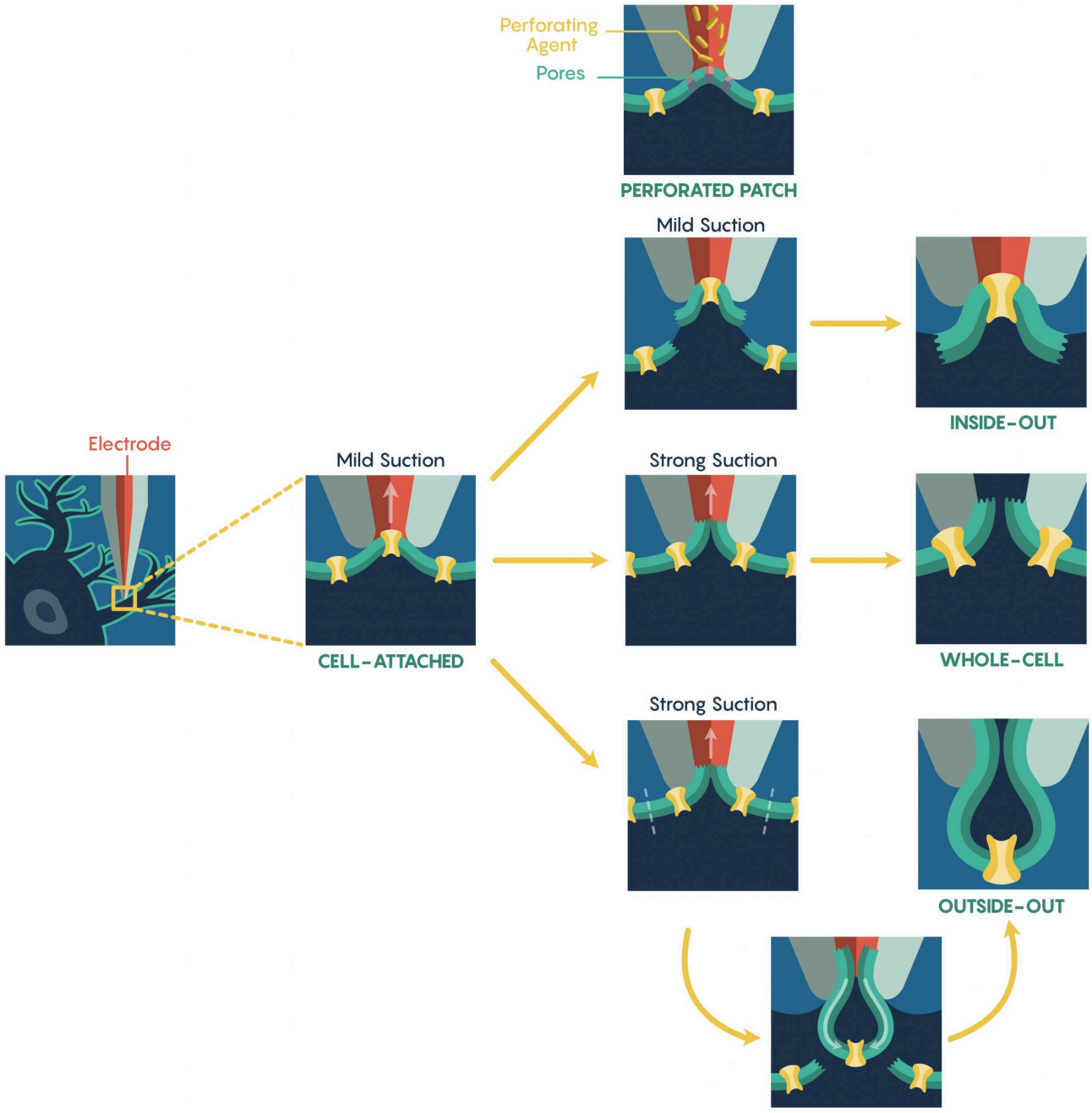

Figure 1. *Electrophysiological methods. The cell-attached patch-clamp method. The perforated patch, outside-out and inside-out configurations. To show potential dialysis, the pipette lumen and cytoplasm are represented by red and navy blue, respectively.*

To increase electrical access to the cell interior, two different methods are used. First, the internal pipette solution contains antibiotics or antifungal agents (e.g., nystatin, gramicidin, amphotericin-B), these agents form small, monovalent ion-permeable pores that 'perforate' (Figure 1.) the membrane allowing access to the entire cell. Importantly, these pores do not allow passage of proteins thus ensuring that the intracellular contents remain intact, preserving intracellular signalling pathways. However, this perforated patch technique

has several limitations including higher electrical noise, loss of single-channel resolution and patch instability. Additionally, the creation of a perforated patch requires a significantly long period of time.

An alternative approach to the perforated patch technique is to apply a strong suction, or brief voltage transient, after Giga-ohm seal formation in order to rupture the intact plasma membrane. Upon rupture, a low-resistance electrical and physical continuity is established between the pipette and the cell interior and this new configuration is known as the *whole-cell configuration* (Figure 1.). Accordingly, this configuration permits direct measurements of the cell's membrane potential (via current-clamp) and its manipulation (via voltage-clamp). Due to the physical continuity between the cell interior and the pipette solution, the cytosolic contents can be reasonably controlled. Furthermore, unlike the perforated patch, pharmacological or ionic manipulations of both the intracellular and extracellular environment can lead to the elucidation of individual ion-currents. However, this physical continuity between the pipette lumen and cytosol may also dialyze out and/or alter the activity of endogenous second messenger systems. Thus, whole-cell recordings are vulnerable to this limitation and it is critical to assess the current 'rundown' of the system and cells within these types of whole-cell recording systems.

It is also possible to create 'Cell-free' variations of patch-clamp techniques also exist. For instance, upon giga-seal formation, the electrode can be gently retracted pulling the membrane patch into the bath solution. This arrangement, known as *inside-out configuration* (Figure 1.), enables the complete manipulation of the cytoplasmic face of the plasma membrane via the bath perfusion – a feature not possible in the cell-attached configuration. As follows, the extracellular solution can be manipulated via the pipette solution. Unfortunately, this arrangement suffers from the loss of intracellular signalling pathways acting on the ion-channels following patch excision; a particularly important consideration when examining altered channel activity.

Similarly, an *outside-out configuration* (Figure 1.) also requires the gentle retraction of the patch electrode from the whole-cell configuration. However, in this situation the pipette retraction forces the plasma membrane surrounding the electrode tip to detach from the cell and reseal forming a cell-independent patch whose extracellular membrane is facing the bathing solution. This allows an experimenter to have complete control over the intracellular environment via the pipette solution and can rapidly exchange different external physiological or pharmacologic drugs over the same patch.

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4.2 MOLECULAR TOOLBOX – NEURAL CIRCUITS: THE BASICS

Key Takeaways

- Precision targeting of neurons
- Optogenetics the basics
- Channelrhodopsins
- Inhibitory Opsins

Precision Targeting of Neurons

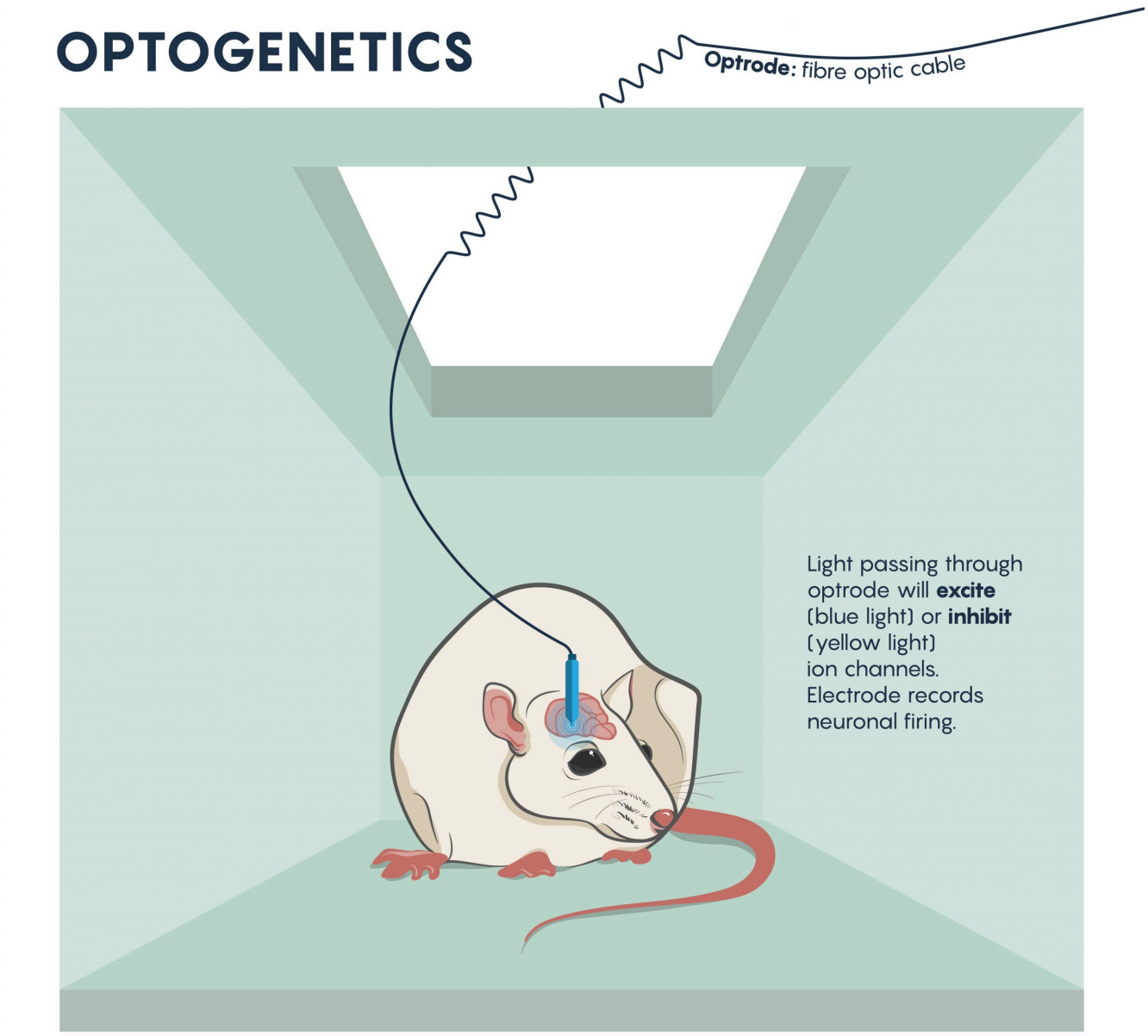

Figure 1. *The beginnings of brain circuit studies in mice using optrodes to excite or inhibit ion channels. Adapted from Nature 2016 (open access cc by 4).*

Neurons do not work in isolation, they typically make synaptic connections that resemble circuits. Most investigations of behaviour and development have relied on mapping the neural circuits within the brain and CNS. Understanding the connectivity of these circuits allow neuroscientists to understand behaviour and pathologies (Figure 1.). In the past, this has meant lesioning or destroying parts of these circuits, or

electrophysiologically stimulating these circuits and examining the resulting behaviour. These older methods have allowed us to dissect the detailed workings of the neural circuits underlying natural behaviour, and have also enabled us to understand how some neural circuits become dysfunctional in disease states such as Parkinson's disease or epilepsy.

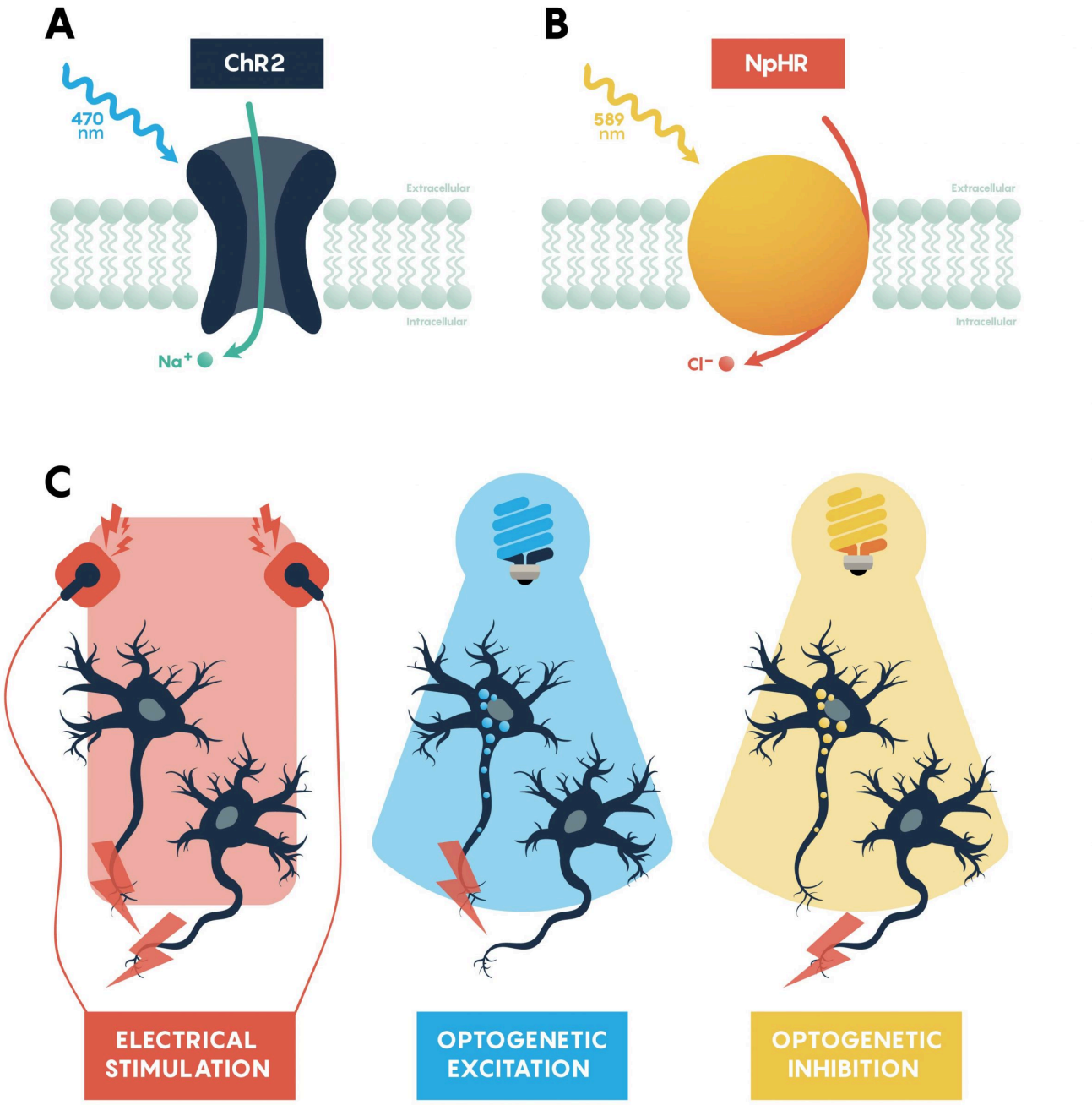

Figure 2. *Panels summarizing the features of excitatory, ChR2, (A) and inhibitory, NpHR, (B) opsins. Panel C showing that all neurons would be excited using a standard electrode. However, optogenetic excitation (blue light) and inhibition (yellow light) only work on specific neurons that express the appropriate opsin.*

The vertebrate brain (mice, rats, primates and humans), contains many different cell types with distinct molecular expression patterns, physiological activity, and topological connectivity, which are intermingled in a highly heterogeneous network. Studying specific groups of neurons in this milieu becomes very challenging and scientists using lesion, stimulation and tracing studies were never sure about the spatial (i.e. were only specific neurons affected) and temporal (i.e. can the lesion be reversed, allowing for reversal of behaviour).

The questions around specificity and temporal reversibility changed with the introduction of optogenetics. Since the late 2000s, optogenetics has ushered in a new era of potent and targeted control over multiple aspects of neural function. Genetic and optical methods applied together allow tight spatial and temporal control of the activity of specific kinds of neurons in the living brain, a revolutionary advance that will allow us to achieve an unprecedented understanding of neural circuit function in behaving animals. Using this technique, neurons are first genetically engineered (using a variety of mechanisms, described later) to express light-sensitive proteins (opsins). When these neurons are illuminated with a specific frequency of light, they will be transiently activated or inhibited or their signalling pathways will be modulated, depending on the particular kind of opsin that was chosen for expression. Cell type–specific expression is typically achieved with transgenic animals, viral vectors, or a combination, and spatially restricted light application allows for further refinement in targeting to specific brain regions. Light can be applied in a variety of temporal patterns in order to optimally influence neuronal function (permitting experimental control of spike frequency and burstiness, among other parameters), and may be restricted to specific short behavioural periods of examination.

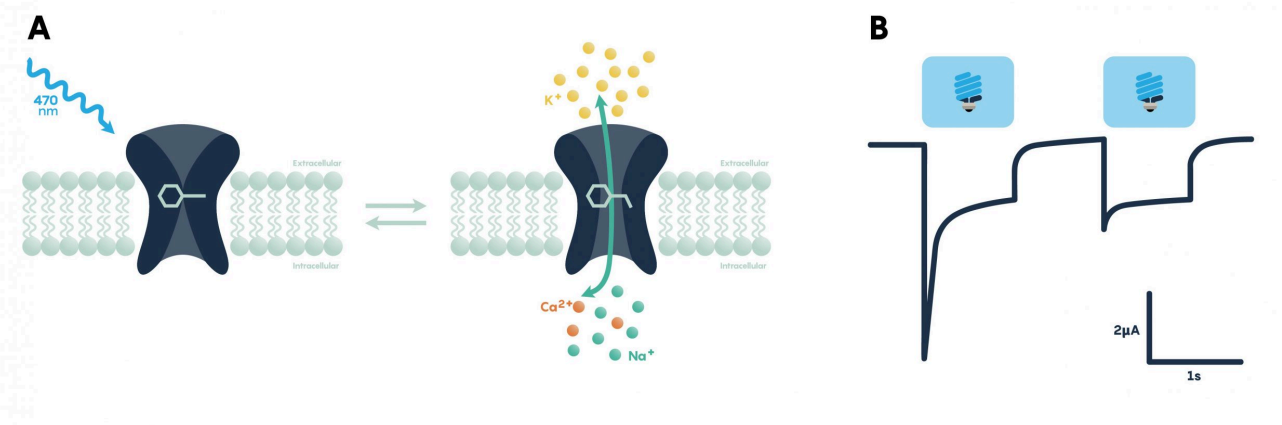

Figure 3. *Panel A showing the excitatory effect of blue light on a membrane-bound opsin functioning as a channel. Panel B showing action potentials in response to blue (excitatory) light.*

Optogenics: The Basics

What Are Optogenetic Actuators?

Optogenetic actuators are proteins that modify the activity of the cell in which they are expressed when

that cell is exposed to light (Figure 3.). These actuators can be used to induce single or multiple action potentials (which can be organized into regular spike trains or which can be pseudo-random at a user-controlled rate), suppress neural activity, or modify biochemical signalling pathways, with millisecond control over the timing of events. The most powerful and widely used actuators are opsins—naturally occurring light-sensitive transmembrane proteins. They are found in a variety of organisms ranging from microbes to primates, and that can be used as found in nature or engineered to optimize functioning. Naturally occurring opsins can be broadly categorized into two major classes: microbial opsins (Type I) and vertebrate opsins (Type II). Type I opsins are found in prokaryotic and eukaryotic microbial organisms, including bacteria, archaea, and algae, and are composed of a single membrane-bound protein component that functions as a pump or channel. These opsins are used by their host microorganisms for a variety of functions, including navigation towards sources of energy and away from hazardous environments, and control the intracellular concentrations of a variety of ions and the beating of flagella.

Type I opsins was used in the first optogenetics experiments to control neuronal function, due to the ease of genetic engineering using a single component protein, and because of their faster kinetics, they remain the primary (but not exclusive) source for new natural and engineered opsins.

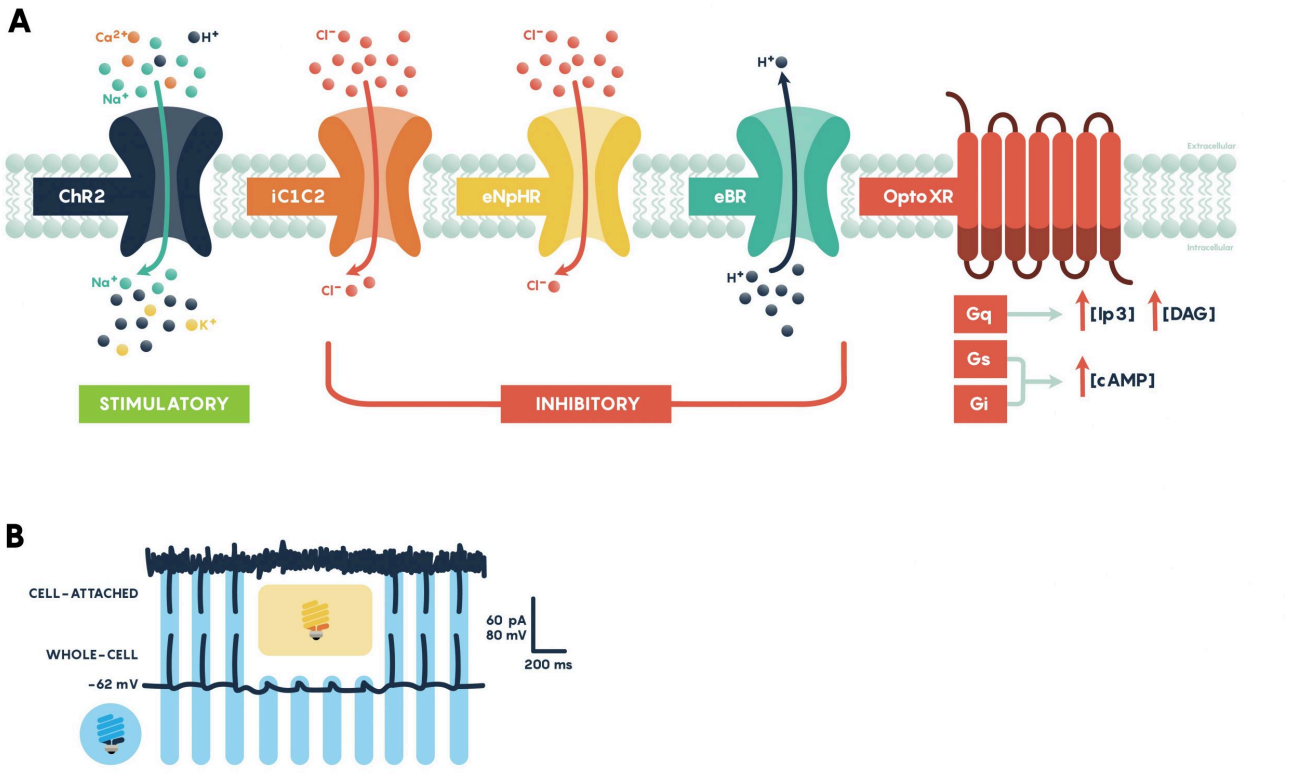

Figure 4. *Opsins are membrane-bound proteins that are activated with light, which results in cell activation (depolarization), inhibition (hyperpolarization), or modulation of intracellular signalling cascades (panel A). Illustrated here are ChR2 (a cation channel used to stimulate neural activity), iC1C2 (a newly developed chloride channel used to inhibit neural activity), eNpHR3.0 (a chloride pump used to inhibit neural activity), eBR (a proton pump used to inhibit neural activity), and OptoXR (a G protein-coupled receptor used to modulate*

intracellular signalling cascades). (Panel B) Cell-attached and whole-cell recordings from a neuron expressing both ChR2 and NpHR. Note that individual spikes can be elicited with a short pulse of blue light (which activates ChR2) and that these spikes can be blocked with continuous yellow light (which activates NpHR). Panel A adapted with permission from Fenno et al., 2011, and panel B adapted with permission from Zhang et al., 2007.

How Do Optogenetic Actuators Work?

Opsins require retinal, a form of vitamin A that isomerizes upon absorption of a photon, in order to function. When retinal binds to the opsin the retinal-opsin complex becomes light-sensitive, and if a photon strikes the retinal in this state its resulting photoisomerization will induce a conformational change in the opsin. This leads to channel opening or pumps activation, a change in membrane potential, and ultimately the activation or inhibition of neuronal activity. Therefore, retinal must be present in order for optogenetic actuators to function. Fortunately, particularly for the early proof-of-principle experiments, retinal is already present in sufficient quantities in mammalian neural tissue to permit the use of optogenetic tools without exogenous retinal supplementation. However, invertebrate model systems such as *Drosophila* do need retinal supplementation through their diet in order for optogenetic effectors to function. Here we review the different classes of optogenetic actuators, grouped by their effect on neural activity or signalling.

Optogenetic Stimulation of Neural Activity: How to Turn Neurons "On"

Channelrhodopsins

Channelrhodopsins (ChRs) are light-gated ion channels discovered in *Chlamydomonas reinhardtii,* a unicellular green alga. The first use of a microbial opsin to control the spiking activity of neurons utilized Channelrhodopsin-2 (ChR2), one of two channelrhodopsins expressed by this organism. ChR2 is a light-gated nonspecific cation channel which, when illuminated with blue light, opens and allows the passage of cations and the subsequent depolarization of the cell. In 2005, ChR2 was introduced into cultured hippocampal neurons and successfully used to control spiking activity with fine temporal precision. As demonstrated by this pioneering paper, very brief (millisecond) pulses of blue light can be used to induce single action potentials in ChR2-expressing neurons, and spiking activity driven by the activation of this opsin can be controlled with high precision at frequencies approaching 30 spikes per second.

Optogenetic Inhibition of Neuronal Activity: How to

Turn Neurons "Off"

Chloride Pumps

Inhibiting neuronal activity in neural circuits can complement excitatory tools by allowing investigators to test the role of individual neuronal circuit components. One of the most efficient and widely used optogenetic inhibitory opsins, NpHR, is a halorhodopsin from the archaeon *Natronomonas pharaonis*. NpHR pumps chloride ions into the cell upon long-wavelength light activation, resulting in hyperpolarization. Genetic engineering has led to a series of revisions producing eNpHR3.0, an opsin with improved surface membrane localization and a larger photocurrent. With an excitation maximum at 590nm, eNpHR3.0 can be driven by green, yellow, or red wavelengths of light, enabling the use of less expensive laser systems.

Proton Pumps

Proton pumps can also be used to inhibit neurons through hyperpolarization, by pumping protons out of the cell. They have some features that make them desirable alternatives to chloride pumps, which include fast recovery from inactivation and larger sized currents following activation. Arch (archaerhodopsin-3 from *Halorubrum sodomense*), Mac (from the fungus *Leptosphaeria maculans*), ArchT (an archaerhodopsin from *Halorubrum* strain TP009), and eBR (an enhanced version of bacteriorhodopsin from *Halobacterium salinarum*) are proton pumps that show robust efficiency in inhibition. Recent work has demonstrated that inhibition of eNpHR3.0-expressing neurons may render the inhibited neuron transiently more excitable due to a chloride-driven shift in the type-A γ-aminobutyric acid (GABAA) receptor reversal potential which may point towards a proton pump inhibitor as the opsin of choice for some experiments especially involving this system.

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4.4 CHEMOGENETIC METHODS TO EXAMINE THE BRAIN AND BEHAVIOUR

Introduction

Although optogenetics provides a superior level of temporal control (it's quick – flash on pulses of light to elicit immediate activity) and spatial control of a neuronal circuit, it requires specialized fibre optic cables (also called optrodes), lasers and actuators. As such, this can lead to higher than expected costs as well as a significant investment of time and training.

Another precision method for examining the function of specific groups of neurons within a known circuit to alter behaviour is known as chemogenetics or pharmacogenetics. Instead of using light, unique and specific drugs can activate exogenously expressed receptors. The most popular of these precision pharmacogenetic techniques is known as DREADDs. This is an acronym for **D**esigner **R**eceptor **E**xclusively **A**ctivated by a **D**esigner **D**rug and as mentioned above this requires the incorporation of a synthetic receptor (from the hMxDx family from the Acetylcholinergic muscarinic receptor) into an animal's genome and the neuronal function is controlled by the synthetic ligand clozapine-N-oxide (CNO). As with optogenetics, the experimenter decides which neurons (or other cells within the nervous system) have the machinery to express the receptor and once expressed, DREADDs may be used to tightly control neuronal activity.

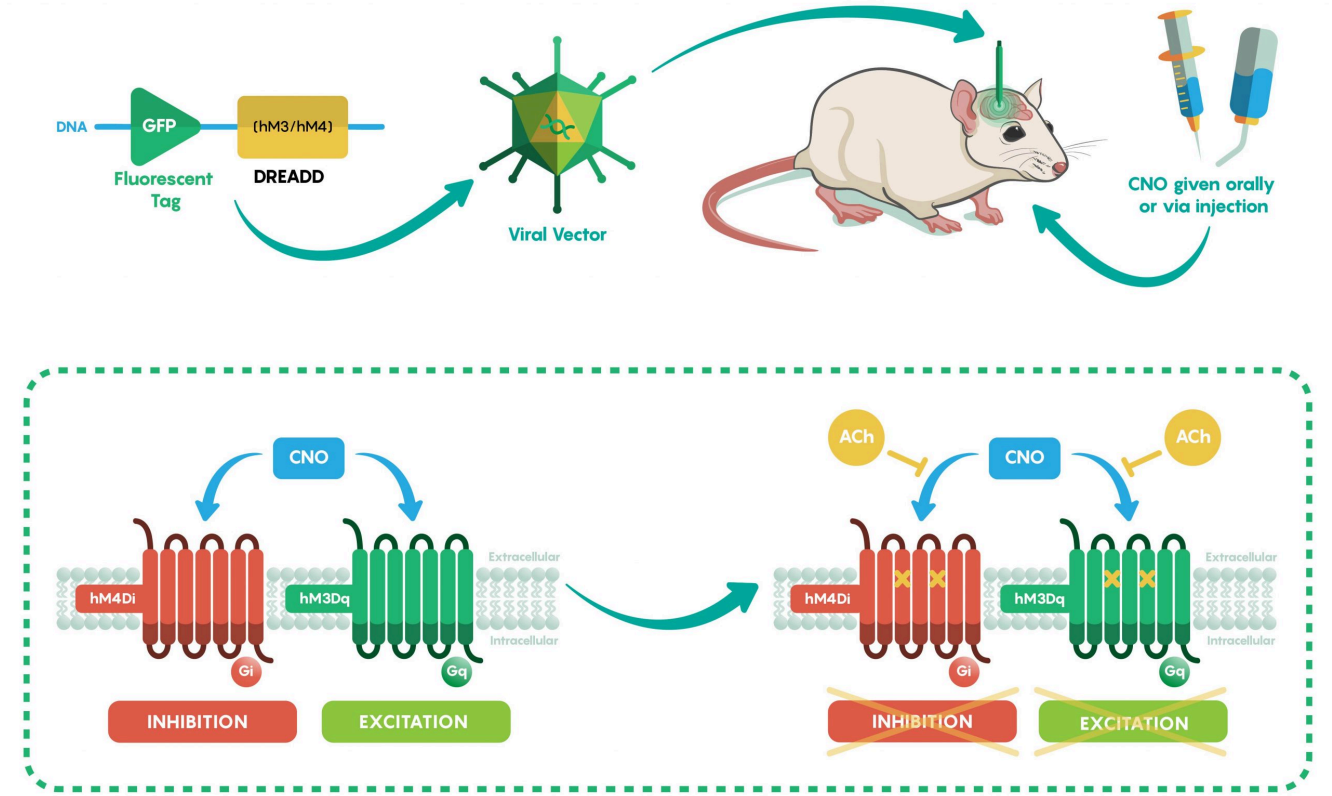

Figure 1. *Schematic overview of how different variants of DREADDs (hM3Dq and hM4Di) can be used to activate and also inhibit groups of neurons using CNO. The figure also shows achetylcholine's (ACh) inhibitory effects on CNO.*

Similar to optogenetics, different DREADDs, once expressed on the appropriate groups of neurons can cause excitation (HM3D) of the neurons or their inhibition (HM4D) in the presence of CNO. Importantly, CNO can be injected into the test animal or be added to their drinking water so there is no requirement for any additional equipment. As CNO is a compound that is designed to work specifically on DREADDs, it was thought that there would be no activation of any endogenous receptors that were not genetically engineered.

However, a recent report in 2017 by Gomez et al., in the journal Science (ref), suggested that CNO was metabolized in peripheral tissues and formed the metabolite clozapine. Clozapine can bind to a number of different serotonergic, dopaminergic and adrenergic receptors within the brain, and it was suggested by Gomez et al., that care should be taken when interpreting behavioural data using the CNO-DREADD system.

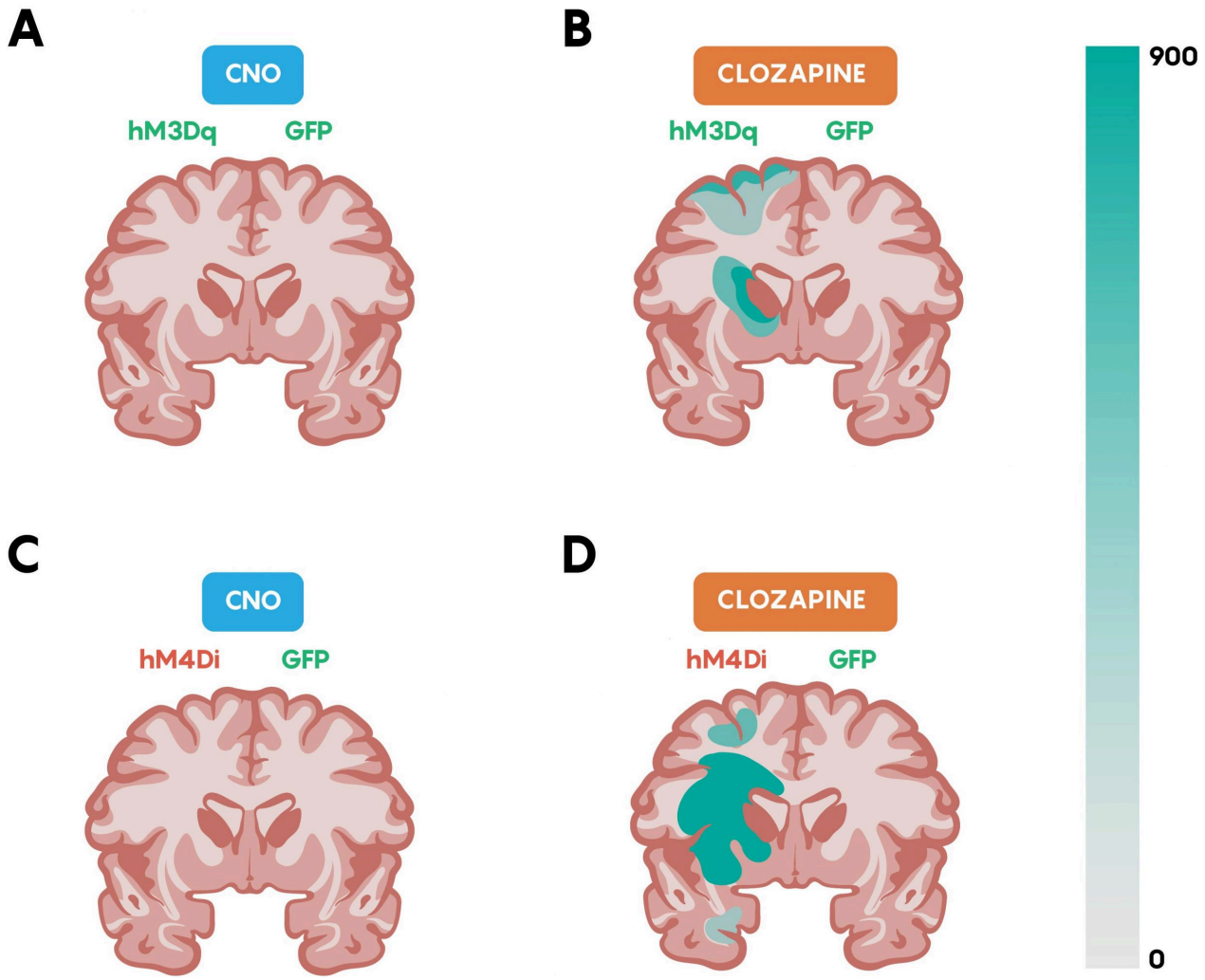

Figure 2. *Figure adapted from Gomez et al., showing the lack of relative binding of the ligand CNO (A and C) to the DREADDs versus the metabolite clozapine which shows significant binding to the same DREADDs (B and D) as indicated by the darker intensity stains.*

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4.5 CRE-LOX, DRIVER LINES, AND NEXT ORDER SPECIFICITY

Key Takeaways

- What is the Cre-Lox system?
- What is the advantage of mouse Cre-driver lines?
- Examples of Cre-driver Lines used in neuroscience.

What is the Cre-lox system and why do we need it?

In the previous chapters, we have examined the use of recombinant molecular techniques that help neurobiologists to understand the role of specific neurons within a brain circuit in complex behaviour. However, we have yet to examine how to ensure delivery to specific neurons to ensure that the construct (i.e.ChR2, hM3DR or other constructs) is not inappropriately expressed in other cell types nor other neurons. Instead of relying only on the cells' internal machinery being driven by cell-specific gene promoters, the Cre-lox system is often used in neuroscience. This is a powerful method to selectively manipulate gene expression in specific subsets of tissues. There are two elements required to make this system work effectively; loxP sequences that are created de novo and the presence of Cre-recombinase (an exogenous nuclease). Importantly, neither the nuclease enzyme Cre-recombinase nor loxP sequences are normally found in mammal systems, so their introduction has no functional consequences to the DNA nor to the neurons. Genetically engineered loxP sites are 34 bp DNA sequences that bind to the protein Cre-recombinase (and the nuclease always needs to bind to 2 loxP sites). Interestingly, as shown in Figure 1, when loxP sites are in the same orientation (5' to 3') and Cre-recombinase binds to both, it causes the deletion of the DNA sequence between the loxP sites while leaving one loxP. This type of "floxing" reaction is known as excision. If the loxP sites are in the opposite orientation then the sequence between them is inverted or "flipped".

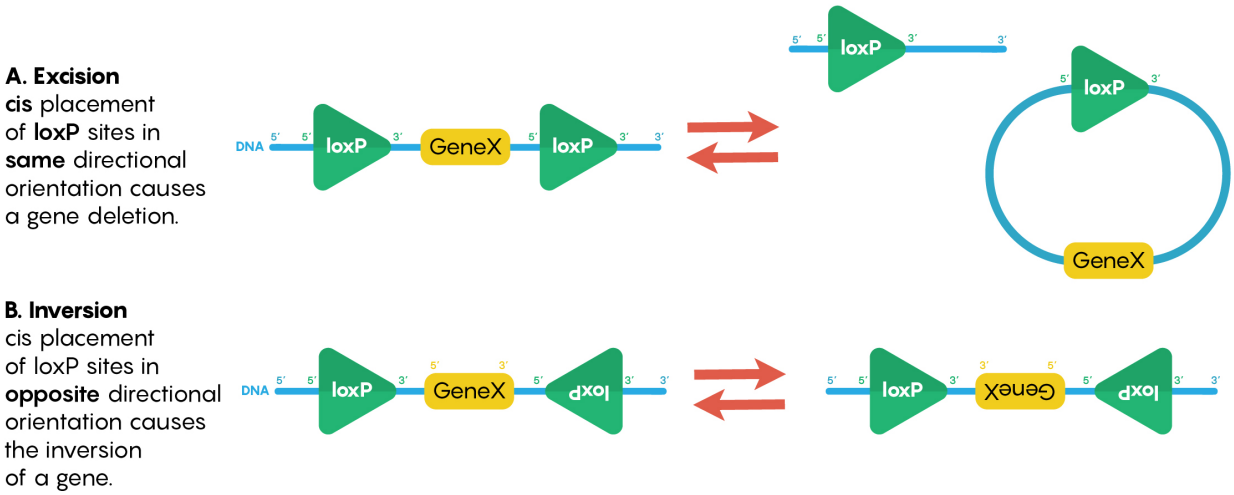
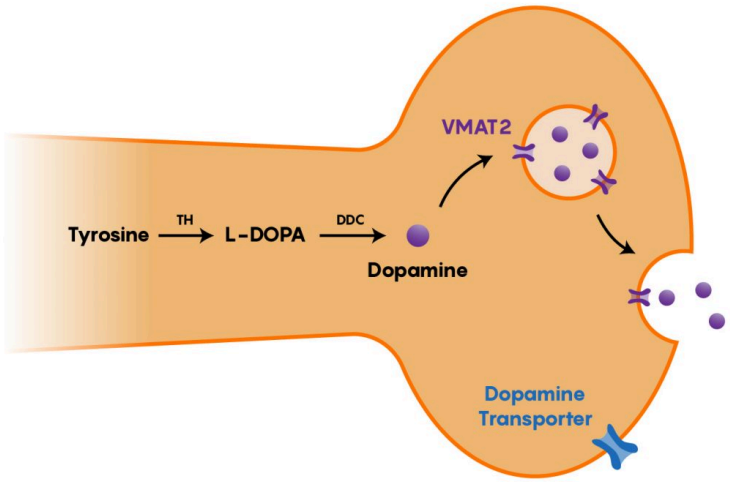


Figure 1. *Different orientations of the loxP sites around the DNA sequence of interest will cause either excision (removal of the DNA) or inversion of the DNA sequence.*

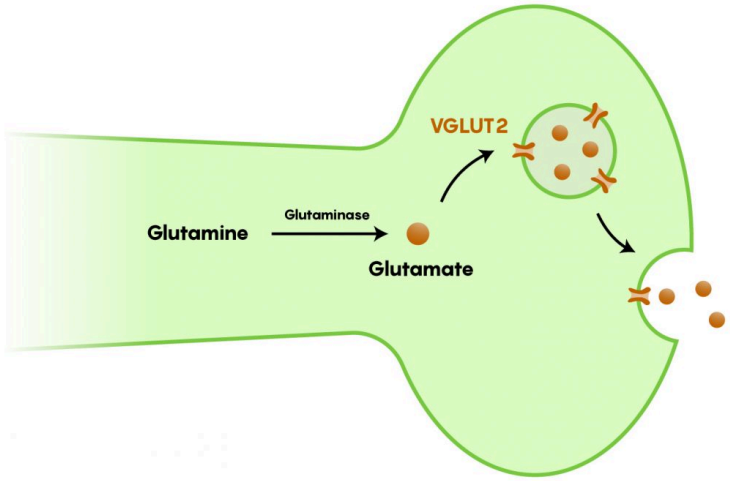
Cre-driver lines: A special type of transgenic mouse

Since Cre-recombinase has no effect on DNA except for those that contain loxP sites (which are never found in mammalian DNA), generating transgenic Cre-recombinase mouse models was a logical step in examining the specific expression of genetic tools (optogenetic, chemogenetic or other) in neurons. As different neurons have different cellular machinery to activate and express certain genes, Cre-recombinase was targeted to specific neurons (or other brain cell types) with this in mind. As the Cre-recombinase expression was being "driven" by the machinery specific to a cell type, the term Cre-driver lines have become embedded within neurobiology. Figure 2 shows that different types of neurons have proteins that are unique to only a specific subset of neurons. These genes would, therefore, allow for the specific expression of the exogenous Cre-recombinase. For example, a TH::IRES-Cre-recombinase mouse only expresses Cre-recombinase within dopaminergic neurons as TH (Tyrosine Hydroxylase) is the synthetic enzyme that converts the amino acid tyrosine to dopamine in neurons. In the same vein, VGAT::IRES-Cre-Recombinase would be a transgenic mouse line that would be used to investigate inhibitory GABAergic neurons. A list of different genes/protein products is found in both Figure 2 and the following table.

DOPAMINERGIC DRIVERS



EXCITATORY/GLUTAMATERGIC DRIVERS



INHIBITORY/GAGAERGIC DRIVERS

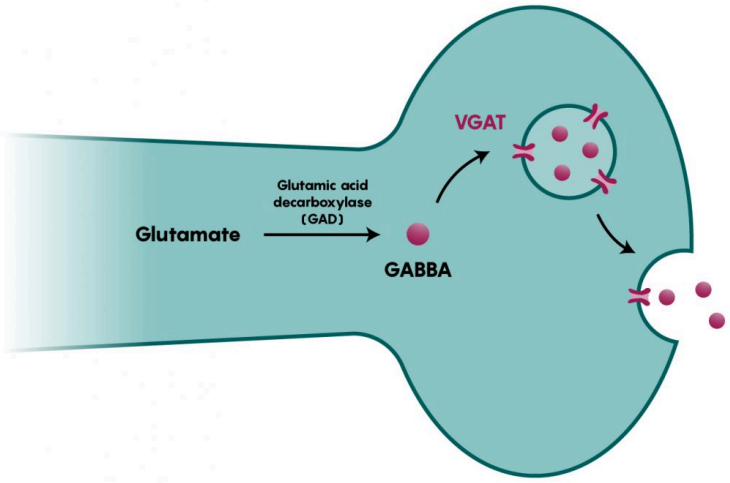


Figure 2. *Differential expression of proteins in specific groups of neurons including dopaminergic, excitatory glutamatergic and inhibitory GABAergic types that allow for the directed expression of Cre-recombinase in specific subsets of neurons.*

Examples of cells targeted with Cre-Recombinase	
Driver line	**Specific cell types – Cre-Recombinase**
GFAP-Cre	Astrocytes or neuronal stem cells
Nestin-Cre	Neuronal stem cells or precursor cells
CAG-Cre	General promoter: Ubiquitous expression in all cells of the brain
Synapsin I-Cre	All types of neurons
EF1a-Cre	General promoter: Ubiquitous expression in all cells of the brain

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4.6 VIRAL MEDIATED DELIVERY OF GENES TO NEURONS

There are a number of ways to deliver DNA (or other genetic information) to neurons including transfection, electroporation, biolistic delivery, and nanoparticles, but within an intact biological system like the brain is challenging. Luckily, neurons are very easily infected by viruses (see Unit 1). Neurobiologists will, therefore, package genetic material that they want to be expressed within specific groups of neurons inside different types of viruses (Figure 1.).

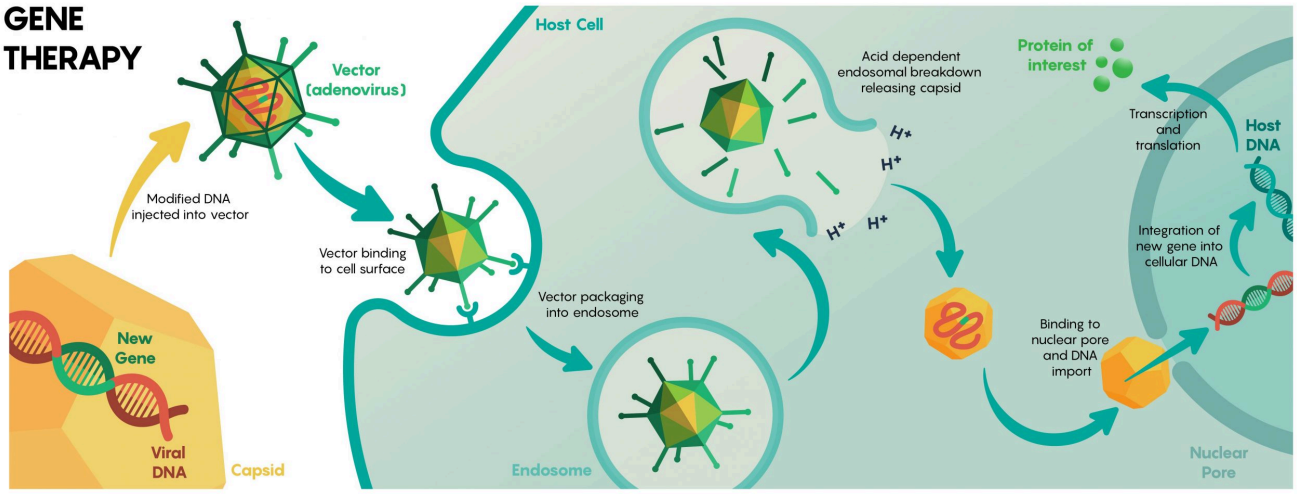

Figure 1. *Schematic overview of how viruses (i.e. adenovirus) can be used to deliver recombinant genetic material to neurons, or other cell types within the nervous system. Steps include viral binding to the cell membrane, endosomal packaging and breakdown, delivery and integration of the vector into host DNA, and ending with the expression of the desired protein.*

In fact, as molecular neurobiologists, we often have a number of different viral vectors that we can use to our benefit. Some viruses for example package only a small amount of genetic material (ie. AAV) while others like HSV will be able to package up to 150 kb worth of genetic material (and this is a lot) into the virus. Speed of expression and how long the expression of the recombinant genetic material will persist for, are often considerations on the choice of a viral vector (see Table 1).

Properties of recombinant viral vectors useful for gene delivery in the adult nervous system

	Adeno-Associated Virus (AAV)	Lentivirus	Herpes Simplex Virus (HSV) Amplicon
Genetic material	Single-stranded DNA	RNA	Double-stranded DNA
Capacity for genetic material	~5 kilobases	~8 kilobases	~150 kilobases
Speed of expression	Weeks	Weeks	Days
Duration of expression	Years	Years	Weeks to months, but elements can be added for persistent expression

One technical consideration that is often overlooked – how are viruses containing the gene of interest that we hope to express in neurons generated? If viruses aren't truly alive, then how does this work? We often use a cell line, like the HEK293 cell, which is a non-neuronal cell line. We transfect three separate plasmids into the HEK293 cells in culture. Two of these plasmids are viral (pVSV-G and packaging plasmids for example for lentiviruses), and the last one is the construct that we want to specifically deliver. Figure 2 shows the workflow for the production of lentiviruses (upper panel) and AAV viruses (lower panel). In both cases, viral particles are released from the transfected HEK293 cells and the virus is collected from the cells and spun on a gradient using ultracentrifugation and a final purification step. The purified fluid now contains a live virus that can infect neurons.

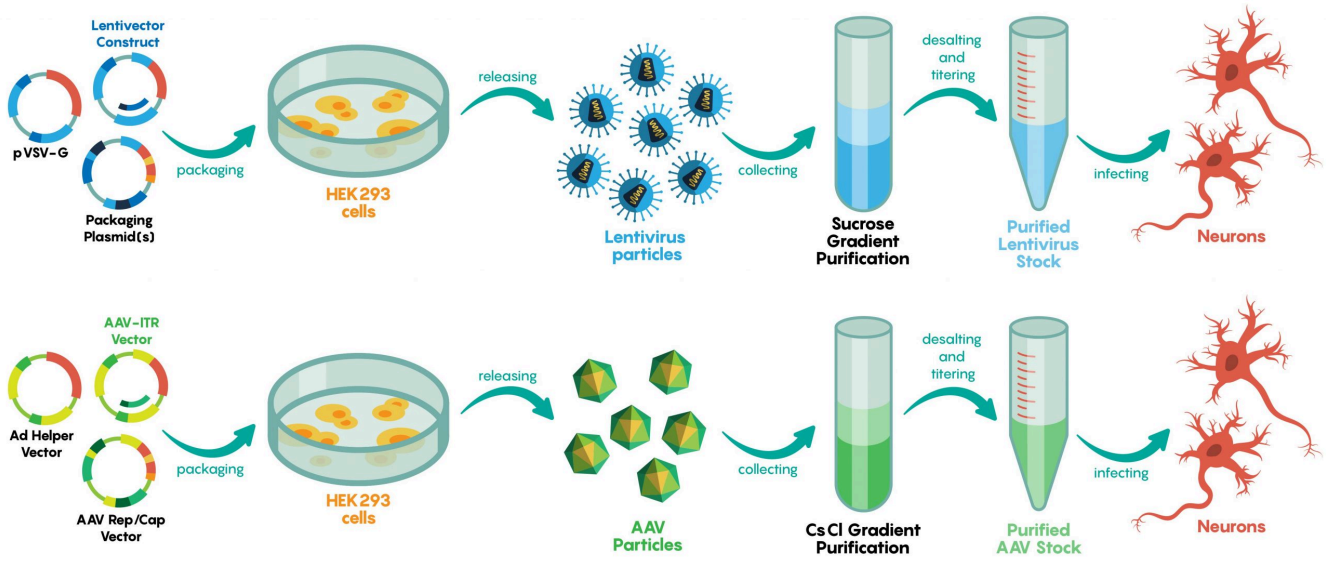

Figure 2.Lentivirus *(Upper panel) – Part of the retroviridae family (viral sizes are 80-100 nm diameter) derived from HIV-1 virus. To produce lentiviruses with the gene of interest as the lentiviral DNA construct, first, transfect cells with a packaging plasmid and the envelope vector (VSVG).* ***Adeno Associated Virus (AAV)*** *(lower panel) – Member of the parvoviridae family (20 nm diameter). To produce AAV, package a gene of interest into the AAV-ITR vector and transfect cells with a Helper vector and the Rep/Cap DNA integration vector.*

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4.7 A VERY INTERESTING TRANSGENIC MOUSE FOR NEUROSCIENCE

One of the very exciting aspects of neuroscience is the use of transgenic mice to aid in our understanding of behaviour. One particular mouse, not originally intended for use in neuroscience was the Rosa or iDTR mouse. The Rosa or iDTR (inducible Diphtheria Toxin Receptor) mouse utilizes a transgene very similar to the Cre-driver lines. In this mouse (shown in Figure 1) all cells throughout the mouse's body carry the DNA sequence for the Diphtheria Toxin Receptor (DTR). Note that this DTR DNA sequence is preceded by a STOP codon that is flanked by loxP sites. If Cre-recombinase was introduced by vector injection then the STOP codon is removed and the DTR is now transcribed and translated in the area or tissue where the Cre-recombinase was injected. Normally, this does not present a problem as the mouse has had no exposure to diphtheria toxin. However, we can inject the toxin systemically into the mouse and only those cells expressing the receptor will undergo apoptotic cell death.

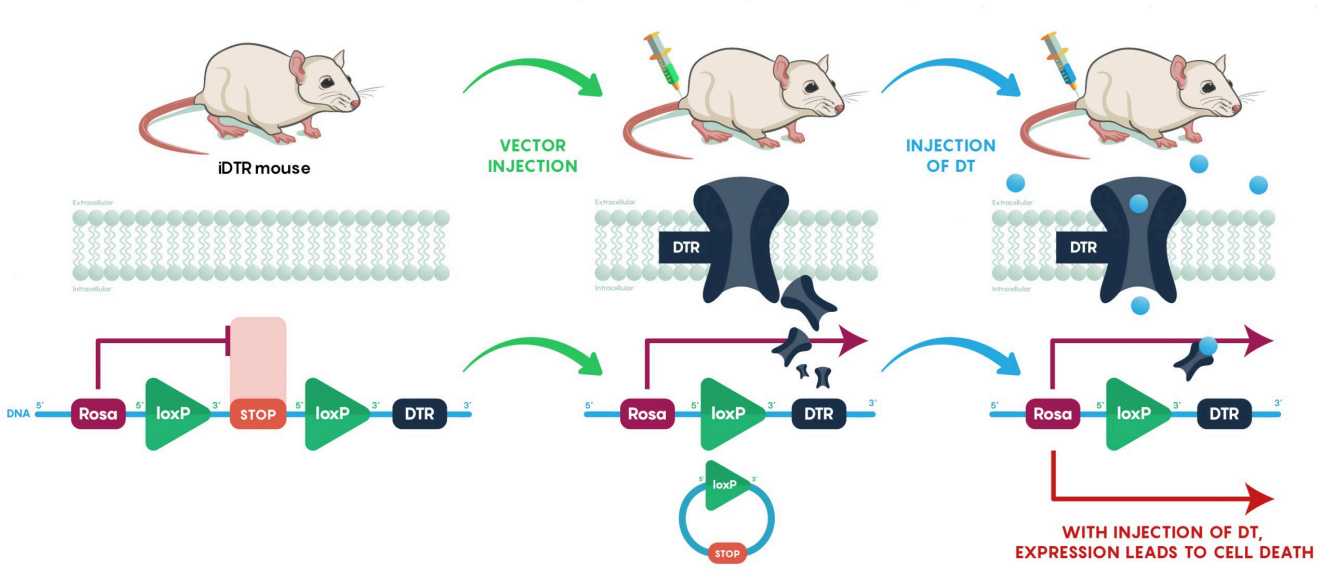

Figure 1. *Schematic diagram illustrating the concept of using the Rosa/iDTR mouse to eliminate specific groups of cells. Much like the Cre-Lox system, there is a reliance on driver lines. All cells in the mouse's body carry the DNA sequence for Diptheria Toxin Receptor(DTR). However, due to the STOP codon being upstream of the DTR, the DTR is never transcribed. However, when a vector is injected into the mouse, the LoxPs excise the STOP codon and thus DTR is able to be transcribed and translated in the tissue where Cre-recombinase was injected. When injected with the Diptheria toxin, the cells are able to bind it with the expressed DTR and thus undergo apoptosis.*

4.8 MOLECULAR BIOLOGICAL MEASURES OF NEURONAL ACTIVITY: "CATFISH"

What is CatFISH?

Although electrophysiological techniques allow researchers to examine the activity of neurons, it is often challenging to know a priori which neurons will respond during a particular behaviour. At the cellular level, neuronal activity can now be visualized using compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH), which can map the distribution of neurons activated during two discrete behaviours by visualizing the sub-cellular localization of various immediate-early gene (IEG) mRNA. The catFISH method examines the activity history of neurons at two different time points and estimates the numbers of neurons active based on the staining of particular IEG mRNAs during a distinct behavioural episode. This makes the technique similar to electrophysiological estimates under comparable conditions (i.e. before and after stimulation) and researchers typically measure "active" neurons by looking for the expression of ***c-fos*** or ***Arc mRNA*** before and after stimulation/behaviour etc.

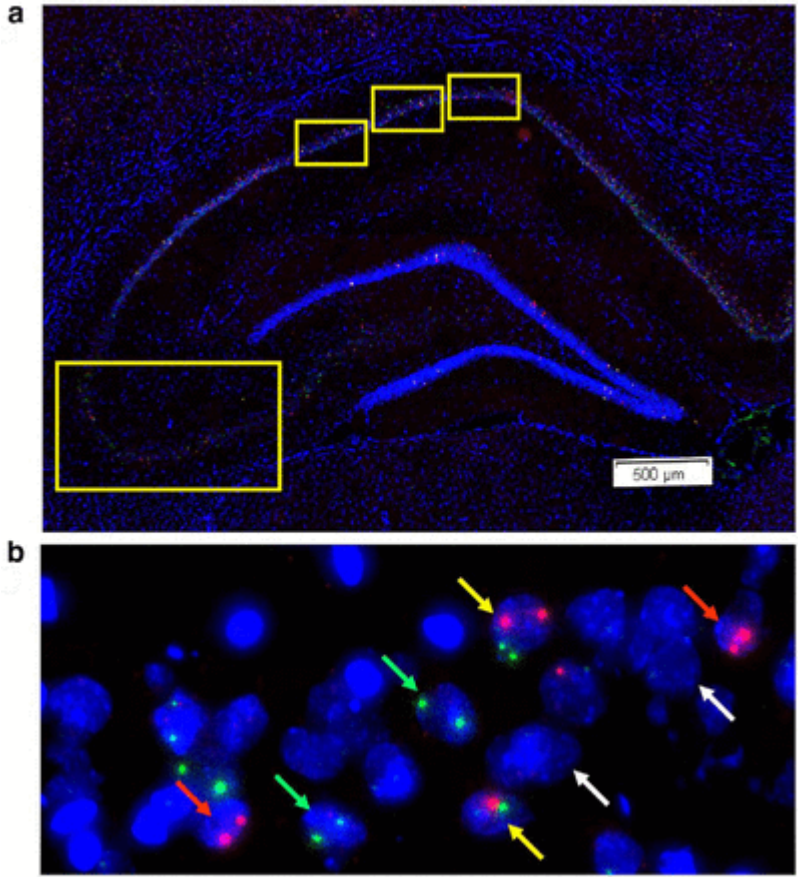

Figure 1. *IEG expression in the dorsal hippocampus. (a) Low-magnification image of DAPI-stained dorsal hippocampus indicating the fields imaged for CA1 and CA3. The relative positions analyzed for CA1 (top) and CA3 (bottom, left) are indicated by yellow boxes. (b) Representative 20× projection image of CA3 from rat trained in context discrimination conditioning, showing Arc+ (red arrows), H1a+ (green arrows), Arc/H1a+ (yellow arrows), and negative (white arrows) neurons. Image from: https://doi.org/10.1523/JNEUROSCI.0542-14.2014. Under CC by 4.0.*

References

From Acute Neuroinflammation Impairs Context Discrimination Memory and Disrupts Pattern Separation Processes in Hippocampus

Jennifer Czerniawski and John F. Guzowski

Journal of Neuroscience 10 September 2014, 34 (37) 12470-12480; DOI: https://doi.org/10.1523/JNEUROSCI.0542-14.2014

(cc by 4.0 by website to sFN 6 months after publication)

Front. Neurosci., 05 April 2011 | https://doi.org/10.3389/fnins.2011.00048

4.9 GENETIC APPROACHES TO EXAMINE THE INTACT AND LIVING BRAIN

Labelling the Living Brain

Owing to the complexity of the mammalian brain, it has remained a major challenge to decipher the patterns of connectivity made onto and by newborn neurons as they integrate into circuits of the adult brain. With major advances in both molecular genetics and light microscopy, our ability to query not only neuronal morphologies but also the molecular and cellular composition of individual neurons and their associated synaptic networks has become possible.

Arguably, one of the most influential contributions to contemporary neuroscience has been the use of fluorescent proteins (FPs) and their targeted expression in living neurons of the mammalian brain tissue. The wide array of FPs available provides an ever-expanding toolbox of vital reporters and gene expression tags. Applications for these proteins range from vital reporters expressed throughout the cytoplasm to subcellular protein fusion tags, which together can be used to monitor the process of circuit integration *in vivo* using both electrophysiological methods and fluorescent imaging.

Beyond merely marking cells for identification, a number of other methods have been developed to exploit the vital properties of FPs to investigate neuronal properties. For example, super-ecliptic pHluorin fluoresces at neutral pH but is quenched at acidic pH, and thus can be used to monitor the trafficking and exchange of intracellular compartments within neurons. This variant allows direct imaging of membrane dynamics, exocytosis and endocytosis of synaptic receptors, and neurotransmitter release *in vitro* and *in vivo*. More recently, a new method termed GFP reconstitution across synaptic partners (GRASP) shows promise for revealing synaptic interactions between contacting neurons. By tethering split GFP fragments to separate pre- and post-synaptic proteins, the reconstitution of GFP fluorescence can be observed when genetically targeted cells form synaptic pairs. Although this technology has been successfully applied to reveal invertebrate synapses, it has yet to be demonstrated in rodents (Gordon and Scott, 2009).

The range of FP reporters for visualizing neuronal morphologies, cellular dynamics, and synapse function continue to expand. However, perhaps the single most limiting factor for using FPs in neuroscience is our incomplete knowledge of neuronal gene regulation. Often transgenic reporters fail to recapitulate endogenous patterns of gene expression or such patterns are too broad to identify neuronal subtypes with cellular precision.

Trans-Synaptic Circuit Tracing

A major goal toward understanding mechanisms of neuronal development, synapse formation, and circuit wiring has been to elucidate nodes and patterns of synaptic connectivity. A creative angle to address this challenge has been the incorporation of genes encoding FPs and FP-fusion proteins into neurotropic viral vectors, which show the innate ability to infect neurons and trans-synaptically spread throughout the nervous system (Kuypers and Ugolini, 1990; Callaway, 2008).

Two types of viruses that have been broadly employed for this purpose include rabies and herpes. Herpesviruses belong to a family of double-stranded DNA viruses, while rabies belongs to a family of negative-strand RNA viruses (Voyles, 1993). Although evolutionarily different, they are both endowed with the unique ability to bind to and infect neuronal cells. This cell-type-specific infectivity is conferred to the viruses via their mature enveloped coat particles, which are made of both host membrane and virally encoded glycoproteins. The composite envelope proteins are the determinants that mediate neuronal membrane recognition and subsequent neuron-to-neuron infection by binding to membrane surface receptors.

Herpesviruses have been used to label neural circuits for years. Two common tracing strains are herpes simplex virus-1 (HSV-1; Lilley et al., 2001) and pseudorabies virus (PRV; Enquist, 2002). Both of these variants predominantly spread in a retrograde direction, and each has been effectively applied to dissect synapse and circuit connections in the rodent brain (Callaway, 2008). However, one limitation of using the herpes viruses for circuit analysis is polysynaptic spreading. Due to the vast cohort of cell types within brain tissue, the number of synapses formed on each of those cells, and the high degree of interconnectivity in intact neural circuits, this approach still poses a challenge to dissect precise patterns of neural connectivity. To simplify trans-synaptic circuit analysis, Wickersham et al. (2007b) devised a clever coat protein complementation strategy that allows for monosynaptic tracing of neuronal connections using a pseudotyped rabies virus (RV). Not to be confused with PRV (which as stated above is actually a herpes virus), pseudotyping a viral particle refers to synthetically modifying the viral envelope to recognize a foreign receptor not normally present on the membranes of mammalian neurons. The strategy will be briefly discussed below, and for further reference also see Wickersham et al. (2007a), Arenkiel and Ehlers (2009), Hasenstaub and Callaway (2010).

The RV gene encoding its glycoprotein (termed G) has been the primary target for genetic modification and RV vector engineering. Removal of G from the RV genome renders the virus both incapable of generating infective particles and replication-incompetent. However, even in the absence of the native glycoprotein gene, RV is still capable of expressing its genome. Thus, G can be replaced with sequences encoding FPs or FP-tagged biomolecules to generate RV vectors for vital reporter expression (Wickersham et al., 2007a). To make these replication-incompetent viruses useful for circuit tracing studies, they must be "armed" by providing an envelope *in trans* by propagating and packaging the particles *in vitro* using cell lines engineered to synthesize the required glycoprotein.

To perform monosynaptic circuit tracing and target FP-expressing RV to desired neuronal subsets, the particles can first be pseudotyped with the foreign coat protein EnvA from avian sarcoma leukosis virus, which

specifically binds to a class of avian membrane proteins called TVA receptors (Barnard et al., 2006). Genetic targeting of neuronal subsets for TVA expression directs RV infection to only those neurons. To facilitate monosynaptic tracing, Wickersham et al. (2007a) added a clever twist on this approach. By introducing a plasmid that encodes the wildtype RV G-protein, the disarmed EGFP-expressing virus is now able to undergo one round of subsequent infection to presynaptic partners of TVA-targeted neurons. Since only the initially infected neuron contains G, viral spread ceases after one round of monosynaptic jumping. Including a plasmid encoding, a red FP allows the cell originally targeted for infection to be identified amongst the monosynaptic network of GFP labelled cells (Figure 1). Of course, it must be considered that true monosynaptic tracing is dependent on targeting individual neurons for the expression of G. If, for example, synaptically coupled cells both harbour G, but only one of them serves as the primary source cell of TVA-mediated infection, then viral spread can become multi-synaptic through subsequent rounds of viral packaging in presynaptic partners. Monosynaptic tracing control thus depends directly upon the precision of neuronal targeting for the RV tracing components.

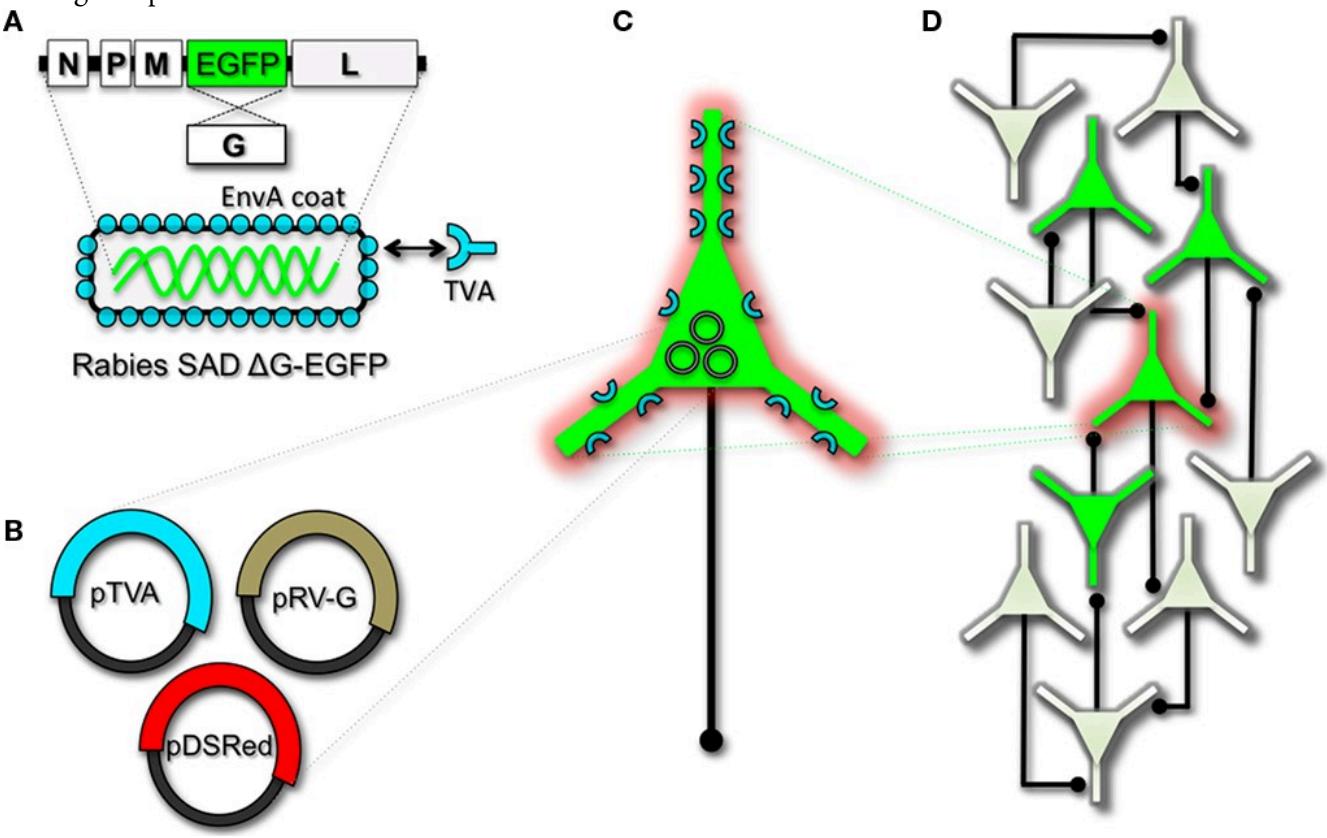

Figure 1. *Engineering and pseudotyping rabies virus (RV) for transneuronal tracing.* ***(A)*** *RV can be genetically engineered to express EGFP by replacing the genomic sequence encoding the G coat protein. The genetically modified G-deletion mutant RV must be propagated in vitro to supply a coat protein. The particle can thus be pseudotyped by providing a foreign coat protein such as EnvA, which originates from the avian leukosis virus and binds specifically to it its cognate receptor TVA. EnvA pseudotyped RV can be used to selectively infect neurons that have been genetically targeted for TVA expression. By including additional constructs that encode the wildtype G-capsid protein and a red-coloured "cell fill"* ***(B)****, the modified RV can be genetically targeted to*

individual neurons for restricted circuit mapping and monosynaptic tracing ***(C).*** *Since no endogenous receptors exist in the mammalian brain for EnvA, only neurons that are programmed to express TVA are capable of being infected by the EnvA pseudotyped virions. Because the wildtype G-protein sequence has been deleted from the RV genome, G must be supplied by complementation to allow trans-synaptic spread from the neurons targeted for infection.* ***(D)*** *Viral spread ceases monosynaptically due to the absence of G in unmodified neuronal populations.*

With this new technology, it is now feasible to dissect complicated patterns of neuronal connectivity with synaptic precision (Stepien et al., 2010; Weible et al., 2010). Targeting adult-born neurons for monosynaptic circuit tracing holds certain promise toward elucidating the numbers, types, and synaptic inputs that might usher and/or promote the formation and maintenance of functional circuit integration. Unfortunately, there still remains much to be learned about the viral mechanisms of infectivity, trans-synaptic propagation, and replication to make viral tracing methods broadly applicable for detailed circuit analysis throughout the nervous system. For example, one major limitation to viral-mediated circuit tracing using either HSV or RV type vectors is the inevitable deterioration of neuronal cell health with time (Callaway, 2008). While the HSV particles show rapid and high levels of expression within 1–2 days, they also show a lytic-type phase of replication that induces neuronal loss within 1–2 weeks. Although most neurons appear to tolerate RV infection for longer periods of time, they too eventually show signs of dysfunction and poor health beyond 2 weeks. In addition, not much is known regarding the exact tropism for the various viruses to infect particular subtypes of neurons. Although it is clear that viral particles can cross axodendritic, dendro-dendritic, glutamatergic, and GABAergic synapses (Willhite et al., 2006; Wickersham et al., 2007b; Stepien et al., 2010; Miyamichi et al., 2011; Rancz et al., 2011), the different efficacies of transfer have not been determined. Preferential binding of viral particles to different types of presynaptic proteins must exist, which would ultimately result in a more efficient transfer of viruses between certain synaptic pairs. This information is currently unknown, thus it remains a challenge to reliably perform unbiased quantitative circuit analysis using viruses over extended periods of time.

Although current trans-synaptic circuit tracing methods are in their infancy, with further understanding of the viral mechanisms, and a subsequent "re-tooling" of existing vectors, one can easily imagine that this experimental avenue for intact circuit mapping will become indispensable. Moreover, this methodology holds definite promise to address outstanding questions in adult neurogenesis, ranging from identification of the types of connections that are dynamically made and broken during circuit development, to exposing the complete cohort of input types that are observed in mature circuits within the intact brain.

Manipulating Cell and Circuit Activity

Earmarking neuronal subsets and their associated networks for has been invaluable toward our current understanding of neuronal morphologies and circuit architecture. However, to fully understand the cellular and molecular mechanisms that guide adult-born neuron synapse formation and circuit integration, we must

be able to probe neuronal connectivity. Recent advances in genetically encoded actuators now provide this possibility. Technologies, such as heterologous receptor expression, optogenetics, and genetically encoded synaptic toxins, are beginning to allow functional circuit mapping with synaptic precision (Luo et al., 2008; Arenkiel and Ehlers, 2009; Figure 3). By targeting pre- or post-synaptic cell types for activity manipulations, coupled with functional imaging and/or electrophysiological recordings, it is now possible to genetically dissect circuit nodes by monitoring evoked synthetic output responses. Some of the earliest efforts to genetically control neuronal output relied on engineered expression of heterologous receptors in neurons that normally do not show their presence. For example, the expression of modified opiate receptors in the brains of transgenic mice showed that introducing synthetic exogenous ligands could activate neuronal subsets (Zhao et al., 2003). To date, numerous variations on this theme have proven effective for both driving neuronal excitability and inhibition. Complementary strategies to these methods have been to genetically express small-molecules for inactivation of synaptic transmission (Karpova et al., 2005), or toxins that disrupt synaptic transmission (Harms et al., 2005; Ehlers et al., 2007).

Genetic strategies to mark and manipulate neurons and circuits

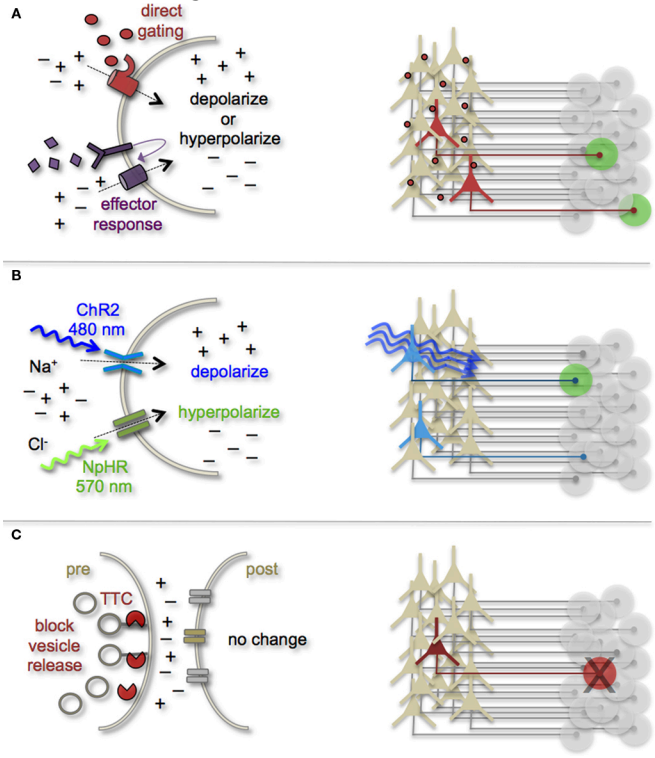

Figure 3. (A) *Neurons can be targeted for heterologous receptor expression. These foreign receptors can be directly or indirectly gated by application of exogenous ligands (depicted as red ovals and purple diamonds). Left: heterologous receptor activation via application of synthetic ligands can be used to change a neuron's ionic equilibrium and thus firing properties. Right: depending on molecular properties, exogenous ligands spread variably throughout brain tissue. All neurons expressing the heterologous receptors are capable of being activated and driving target cell responses (represented as green circles).* ***(B)*** *Expression of light-gated channels can be used to modulate neuronal firing with photons. Left: ChR2 is a non-selective cation channel that responds optimally to*

blue light. Photostimulating this channel results in positive inward currents, depolarization, and neuronal firing. NpHR is a photoactive chloride pump that responds optimally to greenish-yellow light. Photostimulating this pump protein results in negative inward currents, hyperpolarization, and neuronal silencing. Right: FP-fusion reporters can be used to identify cells that express photoresponsive proteins (represented by blue colouring). Only neurons expressing the photoresponsive channels and receive photons show light-activated modulation, whereas downstream circuit targets can be monitored for post-synaptic photoresponses (green colouring). ***(C)*** *Targeted expression of synaptic toxins in neurons can be used to block synaptic vesicle release and inhibit neurotransmission to post-synaptic targets. TTC, tetanus toxin.*

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4.10 – IN VITRO NEURONAL MODELS

A variety of *in vitro* models can be used to examine circuits as well as to test many of the techniques mentioned in this unit and to help understand the development of the nervous system. Often these *in vitro* models have been developed to provide a simplified understanding of the much more complex *in vivo* condition. These reduced complexity models allow for a simplified approach to studying key neuronal processes on both the cellular and molecular level. At the same time, some tissue preparations suffer several limitations due to their simplicity, reducing direct *in vivo* comparisons.

Expression systems and immortalized cell lines

The simplest *in vitro* electrophysiological models include heterologous and recombinant expression systems which are cells/cell lines that can be maintained in culture for an extended period of time. The cells/cell lines typically used as heterologous (*e.g.*, *Xenopus* oocytes; or recombinant expression systems (*e.g.*, human embryonic kidney 293 (HEK-293) cells, Chinese hamster ovary (CHO) cells)) that are easily maintained, allow for manual and automated electrophysiological techniques and express high levels of desired protein within a short period of time that can be consistently observed. As such, these systems have been used extensively to evaluate the pharmacological properties and structure-function relationships of multiple neuron ion-channels. However, despite their simplicity and ubiquitous use, these cells lack many of the complexities associated with a neuronal function within the intact brain (e.g. network associations, glial interactions, and developmental regulation) — a disadvantage when modelling the brain. Furthermore, these cells are typical of a non-neuronal origin and thus lack the same sophisticated level of cellular architecture, sub-cellular organization or biochemistry associated with native neuronal preparations.

Early efforts to address these non-neuronal concerns focused on neuronal cells derived from mouse neuroblastoma C-1300 tumour (e.g. N1E-115)) or the human SH-SY5Y neuroblastoma cell line. However, subsequent advances in molecular biology enable the use of neural stem cells (NSCs). NSCs are uncommitted cells with self-renewal potential and the ability to differentiate into cells of all neural lineages. These cells can be derived from several sources such as pluripotent embryonic stem cells isolated from the blastocyst, human umbilical cord blood, induced pluripotent stem cells and multipotent somatic progenitors derived from several tissues including the CNS. Electrophysiologically, these cells possess Na^+, K^+ and Ca^{2+} currents that resemble the known patterns described for their *in vivo* neuronal counterparts, even at early stages of differentiation. Furthermore, these cells are also capable of forming rudimentary, yet functional, glutamatergic and GABAergic synapses in culture. Limitations in the use of cells obtained from adults offer limited neural lineage potential and senesce after only a few passages (Jakel, Schneider, & Svendsen, 2004). Moreover, NSC

cultures may possess mixtures of both undifferentiated and differentiated neurons, for which some neurons are developmentally immature, and thus hinder extrapolation of data to the adult *in vivo* condition.

Dissociated neuronal primary cultures

Increasing in complexity, dissociated neuronal primary cultures represent another common tissue preparation. These cultures are mechanically and enzymatically dissociated from various brain regions (*e.g.*, hippocampus, cortex, cerebellum, striatum, midbrain, superior cervical ganglion, etc.) and consist of either: one predominant neuronal cell type, a co-mixture of different neuronal populations or mixed neuronal-glial cultures. Dissociated neurons and astrocytes retain much of their functional capacity *in vitro* enabling these preparations to address many important processes observed in the *in vivo* conditions such as network dynamics and neuronal-glial interactions. However, dissociated neurons cannot be maintained in culture for extended periods of time and are thus required to be freshly isolated and grown regularly.

Three-dimensional (3D) neuronal organoid models

The 3D neuronal model represents the next level of complexity for CNS *in vitro* models. Like the two-dimensional (2D) preparations discussed above, 3D brain cell cultures can consist of a co-mixture of different neuronal and non-neuronal populations. Interestingly, instead of being cultured in a traditional planar monolayer, 3D brain cultures are created up to 10 cell diameters thick within reaggregate or spherical cultures (i.e. spheroids), hydrogel/scaffold cultures or rotary bioreactor cultures with cell aggregates or microcarriers. When grown in a 3D environment, neural cells demonstrate better survivability and behave differently when compared to traditional 2D-models. As such, these models promote better development of native voltage-gated ion-channel functionality, resting membrane potentials, intracellular Ca^{2+} dynamics, Na^{+}/H^{+} exchange, enhanced neurogenesis and differentiation, synapse formation, neuronal mobility and axon myelination (Lancaster & Knoblich, 2014; Lancaster et al., 2013; LaPlaca et al., 2010; van Vliet et al., 2007).

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PART V

UNIT 5 – EMERGENT TOPICS IN NEUROSCIENCE

As a discipline, neuroscience encompasses the entire range of biology from genetics and molecules to system-level approaches to understanding neurons and the brain. Often this means that neuroscientists are finding new and previously unsuspected influences on how neurons function, and therefore affect behaviour. This unit highlights some of the exciting areas of neuroscience that have emerged over the past decade that promise to give the discipline new avenues of research for the next generation of neuroscientists.

5.1 THE GUT MICROBIOME AND ITS IMPACT ON THE BRAIN

Introduction

Previously it was believed that micro-organisms were solely responsible for the production of disease/ pathology. However, more recently many different researchers and scientists have begun to re-evaluate this concept that microbes were ***only*** present or only useful in studying diseases.

Scientists have known for a very long time of the ***microbiota,*** which is an individual-specific micro-environment that consists of all of the bacteria, viruses and eukaryotes (i.e. fungi) that either grow in and or on the body (1014 bacteria internally; 1010 bacteria on skin; and an estimate quadrillion viruses). Since the microbiota is present on humans throughout almost our entire lifetime, current theories now highlight the possibility that the microbiota might also play a role in health, not only in disease. Conceptually, the genes encoded by all of the microbiota are known collectively as the **microbiome**. As the number of bacteria within the gut far exceeds all of the other microbiota, most research has started to investigate the role that the gut microbiome might have in health and disease, and more importantly in brain health and disease.

Specifically, within all fields of biology, there is an emerging theme that an organ (such as skin) can be colonized by different types of microbes and this colonization does not have to be homogeneous across a particular organ. Although many of these gut bacteria can be treated pharmacologically in order for them to be eliminated, this may not always be the best treatment. From a research perspective then, one key question that dominates: can control our gut microbiome produce changes in our biology, physiology and even behaviour?

What factors affect the gut microbiome?

Many factors have now been identified to affect the gut microbiome. As shown in Figure 1, an individual's genetics, the types of drugs or antibiotics they are taking, hygiene, physical activity and other factors, all play a role in either maintaining or, if changed, altering the gut microbiome.

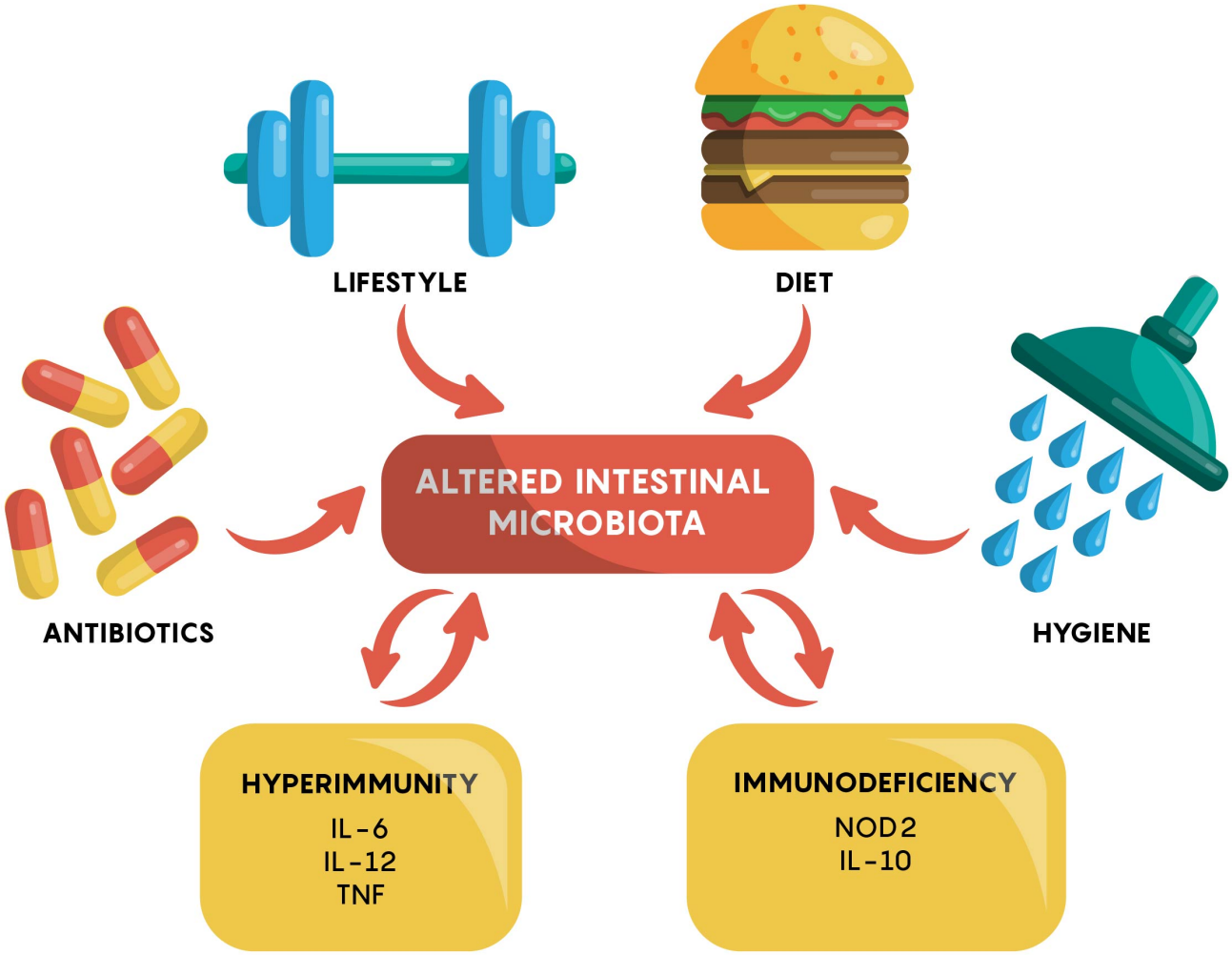

Figure 1.

The "Normal" Gut Microbiome

As the intestine is an anaerobic environment, the majority of the gut bacteria species have been found to be anaerobic. Between 500-1000 different bacterial species have been identified in the gut through genetic sequencing, although these species belong to a very small number of phyla. In most healthy adult individuals, the most abundant phyla include ***Bacteriodetes (Gram(-) rods)*** and ***Firmicutes (Gram(+))*** although other phyla such as *Proteobacteria*, *Verrumicrobia*, and *Actinobacteria* have also been identified.

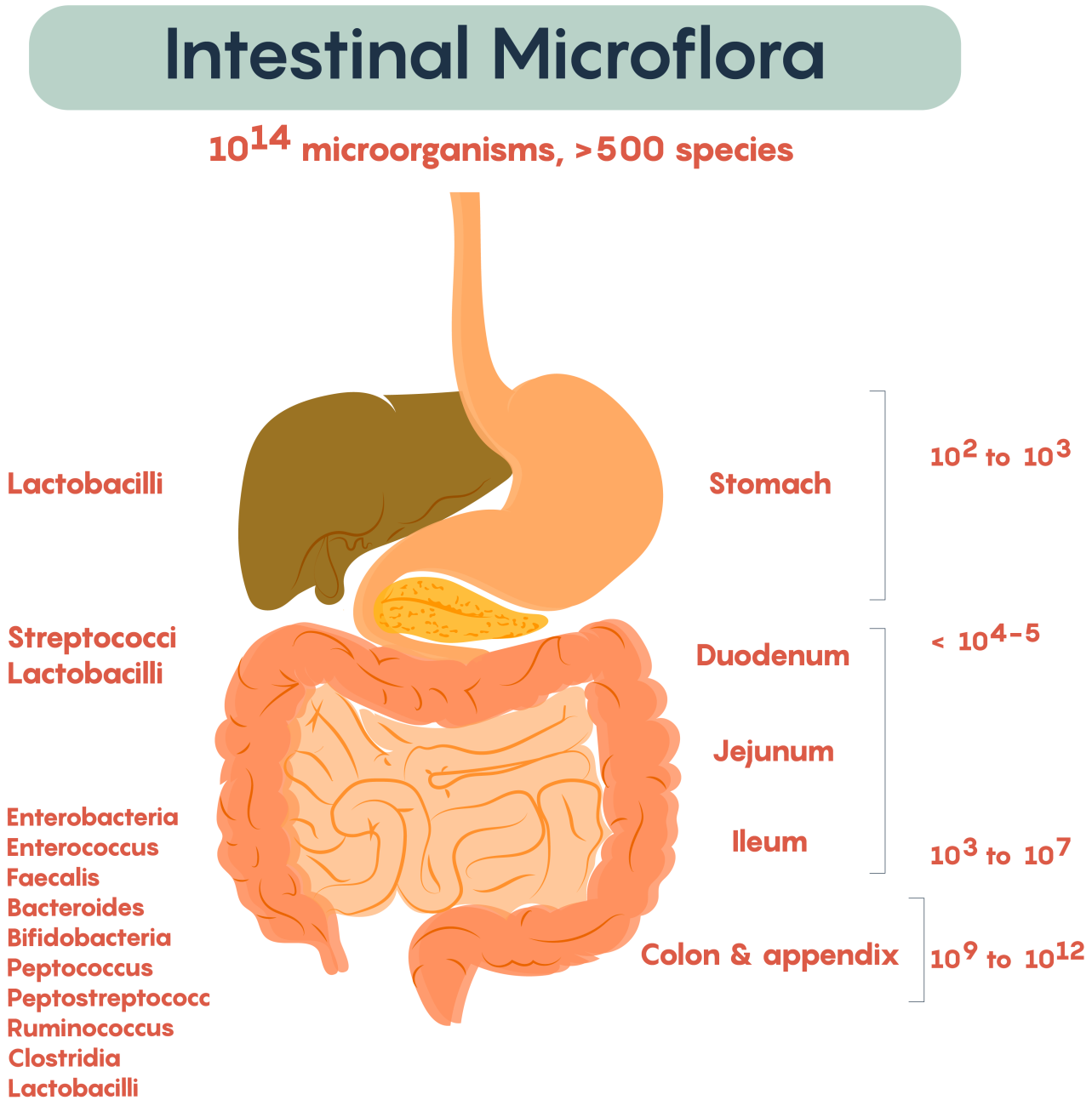

Figure 2. *Microbial density in the gut. Overall, there are 10^{14} microorganisms residing in the human gut, with over 500 unique species. In the stomach, the majority are the Lactobacilli, making up about 10^2 to 10^3. In the Duodenum, there are Streptococci and Lactobacilli, with a concentration of 10^4 or 10^5. As we descend past the jejunum and into the ileum, the concentration of bacteria increases dramatically – up to ten million bacteria reside here. Bacteria such as Enterobacteria, Enterococcus, Faecalis, Bacteroides, Bifidobacteria, Peptococcus, Peptosteptococc, Ruminococcus, Clostridia, and Lactobacilli. These bacteria are also common in the colon and appendix, but the microorganism concentrations increase yet again, to 10^9 to 10^{12}. There is a general trend that complexity and concentration of bacteria increase as we descend the GI tract.*

Frequently, researchers not only measure the typical bacteria living in various parts of the gastrointestinal (GI)

tract but also measure the microbial density within various regions of the gut. As shown in Figure 2., the types of resident or commensal bacteria found in the lumen of the gut regions, as well as the density of bacteria (numbers on the right), show the density. Not surprisingly, the numbers, as well as the complexity of bacteria, increases distally down the length of the GI tract. This complexity started at birth and continues throughout development into adulthood.

But what types of bacteria dominate?

The gut microbiota is dominated by bacteria of which the major divisions of bacteria consist primarily of ***Bacteriodetes*** and ***Firmicutes***. As mentioned previously there seems to be variation in bacteria between individuals although there is a suggestion that individuals normally have gut microbiota that dominates (the dominating bacterial fingerprint of each individual is referred to as an ***enterotype***). The dominating bacterial enterotypes in a healthy individual typically consist of the genera of ***Bacteroides***, ***Prevotella*** or ***Ruminococcus*** (everyone has all 3 bacteria genera but the ratios between each individual will differ from having more or less of each genus).

Changes to the gut microbiota and the gut microbiome

Interestingly, although everyone has a common "core" group of bacteria as outlined in the previous section, lifestyle effects on microbiota (i.e. changes in diet and physiological status) seem to have the ability to quickly modify the types and numbers of bacteria living in the gut. This offers both a therapeutic opportunity by introducing new bacteria that may be beneficial through probiotics that contain bacteria capable of colonizing the gut.

From the earliest developmental age, our but show large changes in the bacterial genera and their numbers 2 during development, changes again depending on lifestyle changes and ageing. The stability of the gut microbiota and the changes in the types of bacteria are emerging as an important factor that has increasingly been implicated in impacting our health status and may be contributing to neurodegenerative processes (and other changes to the brain) as shown in Chapter 2.

Developmental Changes

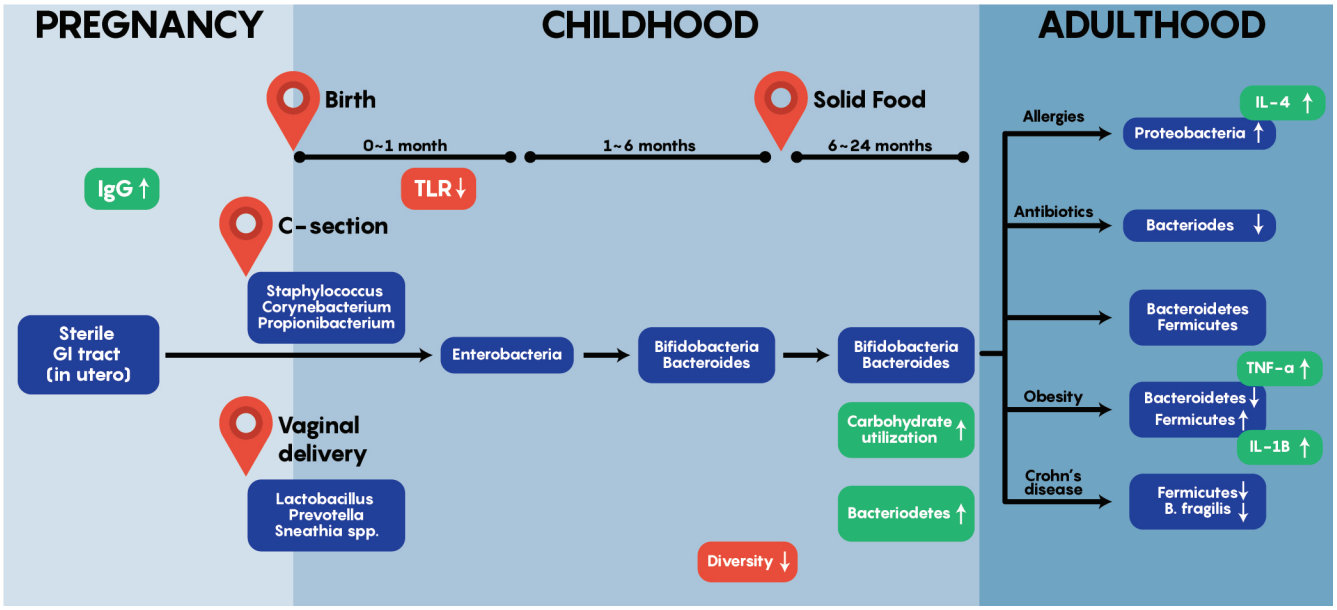

Figure 3. *An illustration of the typical developmental colonization of the gut by bacteria. The initial colonies of bacteria that settle depend on the delivery method. In the first week of life, TLR is reduced, which may allow for the formation of stable bacterial colonies in the gut. During the first 6 months, as children are subjected to solid foods, the diversity of microbiota increases. The immune system is able to differentiate the difference between pathogenic and helpful bacteria. The disease appears to correlate with bacteria concentration and composition.*

As shown in Figure 3. above, early bacterial gut colonizers such as *Lactobacilli* etc. are aerobic and most likely come from maternal sources – these are almost entirely unique and different by the first year of life. *Bacteroides* and *Enterobacteria* are anaerobic bacteria and tend to dominate later between 1-6 months of age. One of the most interesting aspects of gut colonization is that the source of bacteria is dependent on the environment as babies born via C-section tend to have colonization by *Staphylococcus*, *Corynebacterium*, *Propionibacterium spp* etc. which are normally found on the skin. This differs from the bacteria found in the gut of babies born through vaginal delivery as *Lactobacilli*, *Prevotella* and *Sneathia* dominate early on. Note that regardless of origin and initial colonization, that stability within the gut is reached around 1-2 years of age and is maintained into adulthood – where a unique balance between *Bacterioidetes* and *Firmicutes* exist while an individual stays healthy but can be altered by allergies, obesity, drugs and others to change this balance as shown in Figure 3.

Dysbiosis: The Basics

Dysbiosis represents an imbalance in the normal microbial community within your body. Experiments show that in adults, the bacteria, viruses and eukaryotes populations stay relatively stable. However, this stability occurs only in healthy individuals and these populations can change with diet, disease, treatment and environmental effects. For example, gut viromes (an extension of viral genomes) have been shown to have

>95% stability in their sequences over the course of a year in healthy individuals – however, these changes with alterations in an individual's diet.

The effect of diet on the gut microbiome

- **In mice models –** shifting diet from low-fat, plant-polysaccharide rich to high-fat, high sugar (Westernized) diet able to change the bacterial content within a day (Turnbaugh et al., 2009 Science Translational Medicine)
- ***In human studies*** – similar changes in gut microbiota observed within 24 hours (Wu et al, 2011 Science).
- This study showed that diet correlates with changing bacteria – high-fat diet ***Bacteroides*** dominate, carbohydrate-rich diet ***Prevotella*** dominate

In diet-induced animal models of obesity, there is a shift in dominant gut phyla with a decrease in *Bacteroidetes* and an increase in *Firmicutes*. Similar results have been shown in human twin studies with a decrease in *Bacteroidetes* but an increase in *Actinobacteria*. The shift between these two phyla results in increased capacity for increasing energy from food but also results in inflammatory responses. By altering the gut microbiota in animal models, this seems to be sufficient to induce obesity as a phenotype (Turnbaugh et al., 2006 and 2008). These data suggest that the gut microbiota is capable of not only responding to changes in the diet but can also induce phenotypes such as obesity.

5.2 GUT MICROBIOME AND THE BRAIN

Dysbiosis

As previously outlined in Chapter 1, a state of microbial imbalance inside the body known as dysbiosis, has recently been linked to the development of many diseases and disorders. These include obesity, colorectal cancer, and cardiovascular disease. There is, therefore, increasing and substantial evidence that homeostasis of the gut microbiota is essential to human health. Exercise, diet, antibiotic use, and personal hygiene are all important factors in maintaining this balance.

There are several ways in which the gut microbiota interacts with the brain. Components of bacteria, such as lipopolysaccharides, activate the innate immune system. In dysbiosis, the innate immune system is overactive, which may result in inflammation of the central nervous system. Certain bacterially-derived metabolites, such as D-lactic acid and ammonia, have also been found to have neurotoxic effects. In addition to these metabolites, many gut bacteria interact with the brain through the production of neurotransmitters, such as serotonin and dopamine. Finally, the gut microbiota communicates with the brain through the vagus nerve, which connects the brainstem to the heart, lungs, and digestive tract (Galland, 2014).

Dysbiosis has also been found to play a role in several neurological and psychiatric disorders, such as Major Depressive Disorder, Parkinson's Disease, and Alzheimer's Disease. Many of these diseases and disorders are often comorbid with gastrointestinal disorders. In some cases (e.g. Parkinson's Disease), it is possible to induce disease in a healthy animal by exposing it to the gut microbiota of a diseased human. The gut and the brain have been shown to communicate bidirectionally, but this link and the mechanism behind it are not fully understood.

Case study: Gut Microbiome, the Brain and Parkinson's Disease

Evidence for interactions between the gut and brain

Traditionally, neurological diseases have been studied solely through the lens of issues occurring within the CNS, although taking into account that peripheral influences have been implicated in the onset and/or progression of diseases that impact the brain. One of these peripheral influences that have been gaining increasing interested in the impact that the gut microbiome has on the brain (Dinan and Cryan, 2015). Emerging data suggest bidirectional communication

between the gut and the brain in anxiety, depression, nociception, and autism spectrum disorder (ASD).

Gastrointestinal (GI) physiology and motility are influenced by signals arising both locally within the gut and from the CNS suggesting that such a synergistic interplay could exist. Neurotransmitters, immune signalling, hormones, and neuropeptides produced within the gut may, in turn, impact the brain (Selkrig et al., 2014, Wall et al., 2014) but still remain largely unknown.

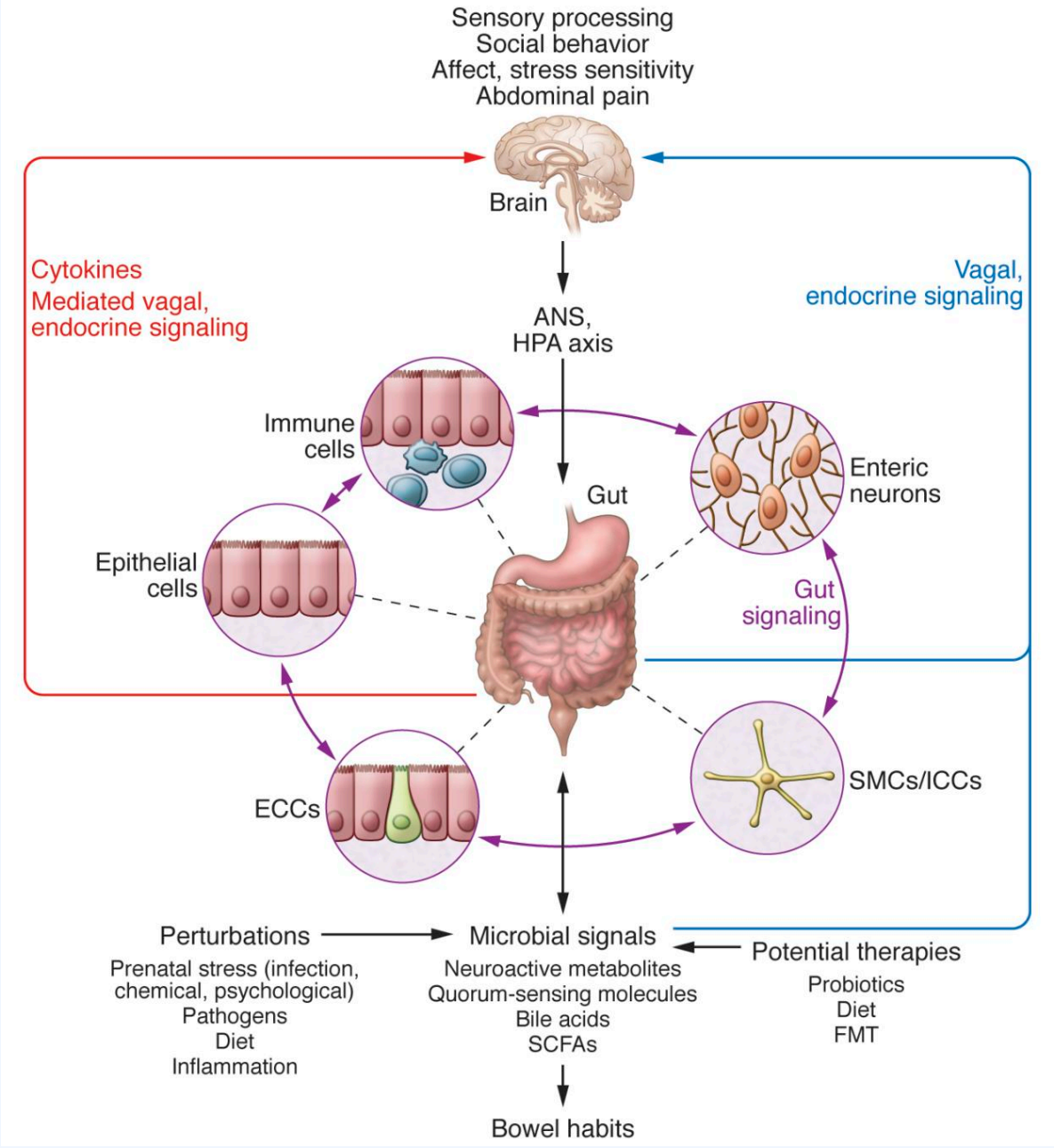

Figure 1. *(taken from opensource https://www.jci.org/articles/view/76304/figure/3) A network of specialized target/transducer cells in the gut wall functions as an interface between the microbiota and the host lumen. In response to external and bodily demands, the brain modulates these specialized cells within this network via the branches of the ANS (sympathetic and parasympathetic/vagal efferents) and the HPA axis. Such modulation can be transient, such as in response to transient perturbations, or long-lasting, such as in response to chronically altered brain output. The microbiota is in constant bidirectional communication with this interface via multiple microbial signalling pathways, and this communication is modulated in response to perturbations of the microbiota or the brain. The integrated output of the gut microbial–brain interface is transmitted back to the brain via multiple afferent signalling pathways, including endocrine (metabolites, cytokines, and microbial signalling molecules) and neurocrine (vagal and spinal afferents). While acute alterations in this interoceptive feedback can result in transient functional brain changes (GI infections), chronic alterations are associated with neuroplastic brain changes. Potential therapies aim to normalize altered microbiota signalling to the ENS and central nervous system. FMT, fecal microbial transplant; ICC, interstitial cell of Cajal.*

Research is beginning to uncover the profound impacts that microbiota can have on neurodevelopment and the CNS (Sharon et al., 2016). Germ-free (GF) mice and antibiotic-treated specific-pathogen-free (SPF) mice exhibit altered in hippocampal neurogenesis, resulting in impaired spatial and object recognition (Möhle et al., 2016). The gut microbiota has been shown to regulate expression of the 5-hydroxytryptamine receptor (5-HT1A), brain-derived neurotrophic factor (BDNF), and NMDA receptor subunit 2 (NR2A) (Bercik et al., 2011, Diaz Heijtz et al., 2011, Sudo et al., 2004). The microbiota promotes enteric and circulating serotonin production in mice (Yano et al., 2015) and affects anxiety, hyperactivity, and cognition (Clarke et al., 2013, Diaz Heijtz et al., 2011, Neufeld et al., 2011, Selkrig et al., 2014).

Dysbiosis in Neurodegeneration

Dysbiosis of the human microbiome has been reported in subjects diagnosed with several neurological diseases. For example, fecal and mucosa-associated gut microbes have been found to be different between individuals with Parkinson's Disease (PD) and healthy controls although it is not clear what this link is.

Individuals with PD also tend to exhibit intestinal inflammation, and GI abnormalities such as constipation which often precede clinical motor defects by many years (Braak et al., 2003, Verbaan et al., 2007). One theory (Braak's hypothesis) has suggested that aberrant αSyn accumulation initiates first within the gut and then propagates via the vagus nerve to the brain in a mechanism that resembles prion diseases where infectious

proteins spread from one area to the other (Del Tredici and Braak, 2008). A hallmark feature of PD involves αSyn (alpha-Synuclein) inclusions, and these appear early in the enteric nervous system (ENS) and the glossopharyngeal and vagal nerves (Braak et al., 2003, Shannon et al., 2012). Interestingly, vagotomized individuals (where the vagus nerve has been surgical cut) are at reduced risk for PD (Svensson et al., 2015). This suggests that there is evidence that in PD an alteration in the gut microbiome might somehow produce neurodegeneration in the brain, and that this factor travels along the vagus nerve.

How would you prove this? What would a researcher need to do?

- Can gut bacteria regulate the hallmark motor deficits and pathophysiology of synucleinopathies such as PD?
- Are changes in the gut microbiota necessary to promote αSyn pathology, neuroinflammation, and characteristic motor features in a proper mouse model?
- Try to prove that gut microbes may play a critical and functional role in the pathogenesis of synucleinopathies such as PD

Recent papers that answered all of the above lines of evidence used germ-free mice to examine the effects of the gut microbiota on microglial activation in the brain. Furthermore it was found that the gut bacteria modulate microglia activation during viral infection through the production of microbial metabolites, namely short-chain fatty acids (SCFAs). To address whether SCFAs impact neuroimmune responses in a mouse model of PD, animals were treated with a mixture of the SCFAs acetate, propionate, and butyrate (while the animals remained microbiologically sterile). These results demonstrated that the gut microbiome and changes to the gut microbiome could alter the production of SCFAs which in turn could signal to alter brain microglia and that this might be the mechanistic link between gut microbiomes, immune system activation and damage to the brain.

The main effect of the gut microbiota perturbations on the brain may occur at times of lower diversity and instability of the gut microbiota (infants and the elderly) and during brain development (perinatal and infant period). During the prenatal period, the developing brain is first exposed to maternal gut-derived metabolites and may be exposed to intrauterine microbes. During birth, the newborn's gut microbiota is shaped by the maternal vaginal (or skin) microbiota (reviewed earlier). Even though the possibility that pre- and postnatal influences on the microbiota can affect brain development is intriguing, there has not been any research in humans characterizing the effect of maternal microbiota modulation on fetal brain development and adult sequelae of such modulation as might be suspected in autism spectrum disease. Again, it is intriguing

to consider the possibilities associated with elderly changes in the gut microbiome and the onset of neurodegenerative conditions but further research is needed in this exciting new area of neuroscience research.

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5.3 DYSBIOSIS AND DEPRESSION

Hannan Algitami

Introduction

As discussed in earlier chapters, the bidirectional relationship between the gut and the brain, or the **gut-brain-axis**, raises many questions about how brain function is affected by the gut and vice versa. More specifically, the degree to which the gut microbiome is implicated in mental illness has only recently been explored.

Mood disorders such as **MDD (Major Depressive Disorder)** are idiopathic and have not had a successful targeted treatment mainly because they affect many areas. One of the speculated targets of these disorders is the gut due to the high rates of comorbidity between mental illness and gastrointestinal illness. For instance, many people suffering from IBS (Irritable Bowel Syndrome) also suffer from MDD, with antidepressants being one of the only medications successful in treating IBS. Therefore, there is a clear connection between the gut and mental illness; however, which system is affected first and hence which imbalance leads to the other is yet to be elucidated (Rogers et al., 2016).

To solve this conundrum, researchers from far and wide have attempted to find a relationship between gastrointestinal imbalance and MDD. What was known thus far was that MDD is comorbid with many gastrointestinal disorders and that antidepressants somehow affect the gut and serve as treatments for these disorders. They hence proposed that the gut microbiota must be affected by MDD, which is why antidepressants, acting in most cases to reverse the condition, also bring the MDD-induced changes in the gut back to homeostasis. All of these were mere conjectures and therefore had to be followed up by more investigation.

Dysbiosis and depression

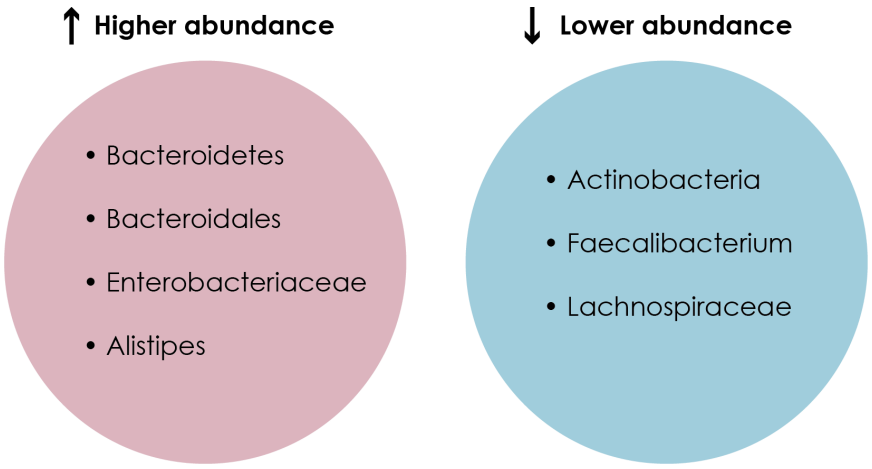

Figure 1. The relative abundance of gut microbial populations in MDD patients compared to healthy controls.

The first area that needed to be addressed was the gut microbial profiles of MDD patients and healthy controls. Comparative analyses using techniques such as 16S rRNA sequencing revealed a significant difference between the gut microbial populations of the two groups (See Figure 1). For example, one study found that MDD patients had a higher relative abundance of Bacteroidetes and a lower relative abundance of Actinobacteria compared to healthy controls (see Chapter 4.1 for details on bacterial phyla) (Zheng et al., 2016a). In addition, another study found that patients with MDD had higher levels of Enterobacteriaceae and Alistipes but lower levels of Faecalibacterium compared to healthy controls (Jiang et al., 2015; Liu, 2017). Moreover, a third study noted higher levels of Bacteroidales but lower levels of Lachnospiraceae in MDD patients compared to healthy controls (Liu, 2017; Naseribafrouei et al., 2014). Hence, there is indeed a relationship between the MDD pathology and the gut microbiome, but the direction in which this relationship goes is unknown.

To attempt to find out the direction of this complex relationship, researchers manipulated the gut microbiome composition in a number of ways and observed the behavioural and physiological changes that occurred, if any. Wiping out the microbiome completely using antibiotics has been used as a way to gauge the importance of gut bacteria on behaviour; however, this has come under much scrutiny especially after many studies have shown antibiotics to have vastly acting effects, introducing many confounds. Specifically, antibiotics are able to alter the microbiome by either depleting it, increasing the number of antibiotic-resistant bacteria or affecting the host tissue directly. Antibiotic-induced effects on host tissue may extend to the CNS (Central Nervous System), potentially having a neuroactive effect. All of these may separately affect mental health and therefore make it difficult to attribute results merely to microbiota (Champagne-Jorgensen et al., 2019; Flux & Lowry, 2020; Morgun et al., 2015).

Psychobiotics

Enriching the gut microbiome using agents like prebiotics and probiotics has also been widely explored. **Probiotics** are live microorganisms found in fermented foods that, when ingested, are believed to enrich the gut microbiome. They have been proposed as a treatment for depression due to their supposed ability to improve gut health. Even though they have shown promise as therapeutic agents or as accompaniments to traditional antidepressants by alleviating symptoms of those with depression, studies have shown mixed results and a definitive claim about their efficacy cannot yet be made (Flux & Lowry, 2020; Nadeem et al., 2019). Although studies on probiotics have often focused on individuals with pre-existing pathologies, supplements given to healthy adults were shown to have only a transient effect on the gut microbiome. Hence, their lack of universal effect calls to question their mode of action and necessitates that more studies be done on more diverse samples in order to elucidate whether they can be used as a treatment for different pathologies affecting the gut, such as depression (Khalesi et al., 2018).

Furthermore, **Prebiotics** are substances that can be broken down by the gut microbiota for which humans do not have the enzymes to metabolize (Flux & Lowry, 2020; Holscher, 2017). An experiment done on chronic-stress mice showed that prebiotic administration affected SCFA (Short Chain Fatty Acid) levels in ways that are correlated with a positive behavioural state. It also reduced stress hormone levels, reduced pro-inflammatory cytokine levels and had anti-depressant and anxiolytic effects (Burokas et al., 2017; Flux & Lowry, 2020; Kao et al., 2016; Louis et al., 2016). These results show that prebiotic treatment may have a positive effect on depression; however, as with probiotics, more studies need to be performed in order to make a strong claim for its effectiveness.

Prebiotics and Probiotics can be combined together under the more modern term **"psychobiotics"** which has been recently coined to refer to any bacterial agent that affects mental health. However, there are often mixed results about how these nutrients affect mental health and behaviour. Therefore, further studies using larger samples and more human trials need to be done to clarify this idea (Flux & Lowry, 2020; Sarkar et al., 2016).

Microbiota Transfer

Another technique that has been used to study the relationship between dysbiosis and depression is **FMT (Fecal Microbiota Transfer)**. In this technique, fecal samples are extracted from a donor and administered to a recipient in various forms. In gut microbiome research, FMT is done by transferring human fecal samples to GF (Germ-Free) or SPF (Specific Pathogen Free) mice in order to create disease models. A number of studies have used this technique to examine the gut-related effects of various disorders, including MDD. In one study, fecal samples were collected from either MDD patients or healthy controls and administered to healthy GF mice. The results showed that mice receiving MDD-derived microbiota showed depressive-like behaviours, such as higher immobility time in locomotor tests as well as disturbances in metabolic activity

among other behavioural and physiological symptoms of the MDD pathology (Zheng et al., 2016b). This shows that microbiota transfer alone can induce depression in otherwise healthy mice.

Since FMT has been shown to induce depression, the question of whether it can be used to reverse the condition arises. If this technique is effective in altering gut microbiota composition, depression-induced dysbiosis should be attenuated with a healthy microbiota transfer; however, this has not yet been tested. Moreover, although FMT has been viewed as a potential treatment for many pathologies such as ASD (Autism Spectrum Disorder), it is still in its infancy and has not yet been widely tested in humans (Kang et al., 2017). In addition to this, the natural variation in the human gut microbiome makes it difficult to standardize a singular sample of healthy microbiota to be used for transplantation (Flux & Lowry, 2020; Human Microbiome Project Consortium, 2012). Hence, whether FMT can be used as a potential treatment for MDD and other such conditions is still in question and whether it will be viable and generalizable to humans is another point to consider.

A Multifaceted disorder

Like the gut-brain axis, a similar highway known as the **microbiome-gut-brain-axis** is thought to be a bidirectional form of communication between the microbiota and the CNS, ultimately influencing behaviour. Although the research that has been outlined above shows a very strong connection between depression and dysbiosis, a definitive order in which the imbalances occur is difficult to be understood in isolation. Rather, the interplay between the gut microbiome and depression should be thought of as a two-way communication that is dependent on many other systems and factors along the way. Hence, the gut microbiome may influence the CNS directly by way of its metabolites such as SCFAs and cytokines travelling through the vagus nerve, or indirectly by way of the enteric system. Conversely, the CNS may release factors that influence the gut, intestinal permeability and microbiota composition (See Figures 2&3). This entire system and the communication between these two components, the gut microbiome and the brain, allow for a nuanced manifestation of depression that cannot be meaningfully understood unless a comprehensive outlook is taken (Flux & Lowry, 2020).

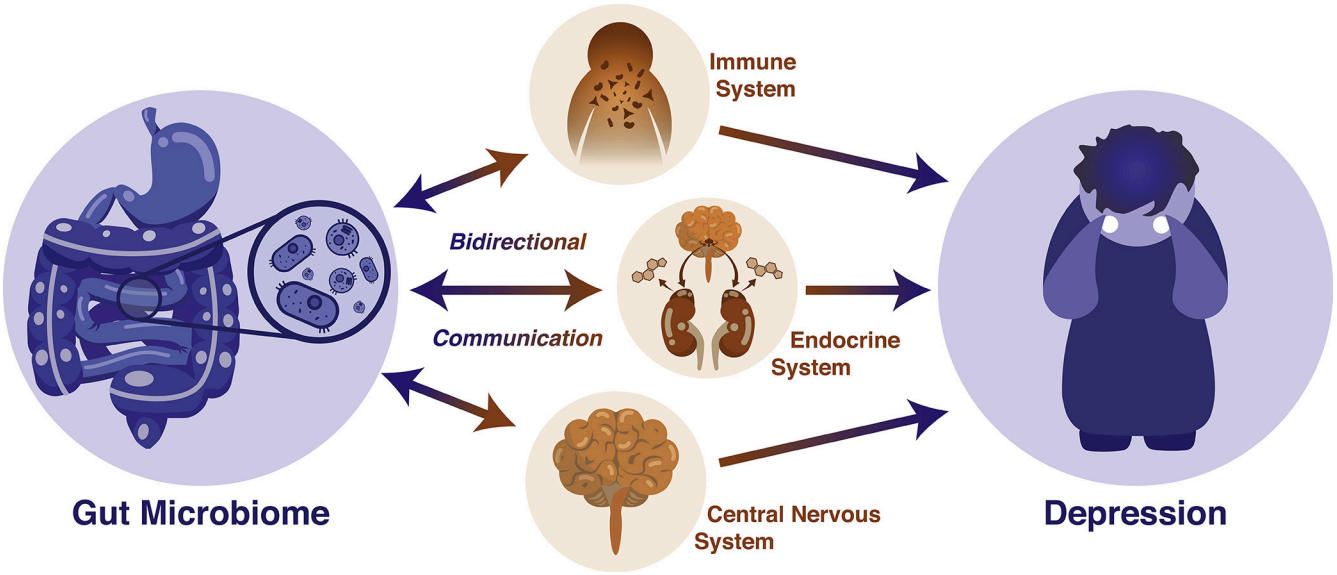

Figure 2. An illustration of the interdependent communication between the different body systems and the CNS to produce depression as well as the subsequent effects of depression on these systems. Adapted from "Finding intestinal fortitude: Integrating the microbiome into a holistic view of depression mechanisms", treatment, and resilience. Flux, M. C., & Lowry, C. A 2020, Neurobiology of Disease, 135(104578).

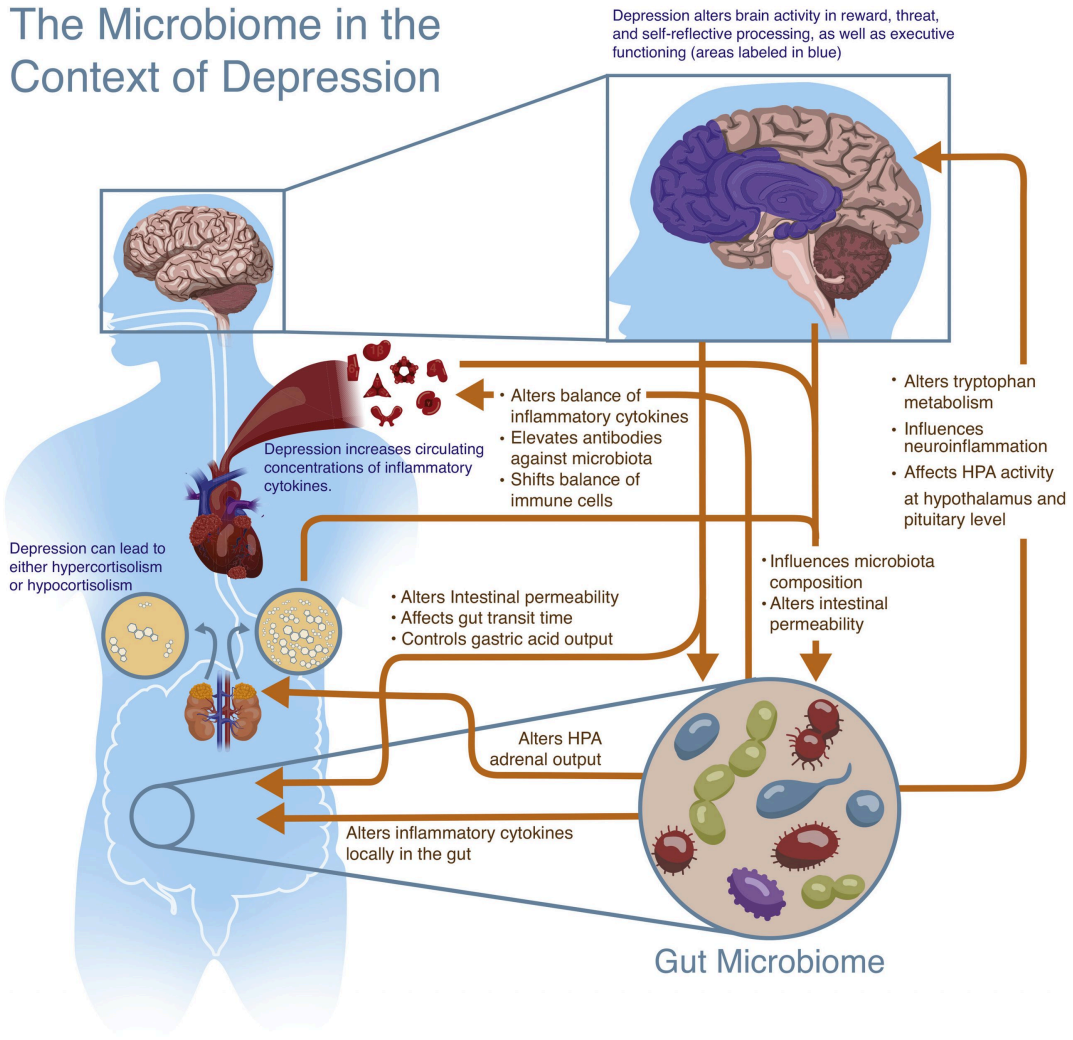

Figure 3. The effects of depression on multiple physiological factors and their collective influence on gut health as well as mental health. Adapted from "Finding intestinal fortitude: Integrating the microbiome into a holistic view of depression mechanisms", treatment, and resilience. Flux, M. C., & Lowry, C. A 2020, Neurobiology of Disease, 135(104578).

Challenging Questions

1. Design an experiment to investigate the directionality of the microbiome-gut-brain axis and its implication in depression.

2. How can the gut microbiome be used as a potential treatment for depression and what are some caveats to the gut-related treatments proposed above?

3. What is an effective way to address the imbalances in the gut induced by depression in the already existing treatment techniques?

Keywords

Gut-brain-axis; MDD; Probiotics; Prebiotics; Psychobiotics; FMT; Microbiome-gut-brain-axis

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5.4 EXERCISE AND THE BRAIN

Overview

For centuries, researchers have sought to elucidate the mechanisms behind the axiom that a healthy body leads to a healthy mind. It has now been established that exercise, even among minimal commitment exercise routines, has an array of robust effects on the brain, such as enhanced memory, mood, cognitive functioning, plasticity, and learning capabilities (Erickson et al., 2011; Spalding et al., 2013; Phillips et al., 2014). Most notably, exercise has been implicated in having anti-depressant effects and counteracting disease or age-related mental impairment and atrophy, such as Alzheimer's disease or dementia (Laurin et al., 2001). Yet, until recently, the intermediaries between exercise and its health benefits have not been well-understood.

However, it has been shown that—contrary to the age-old notion that the number of neurons in the brain remains static after prenatal and neonatal development—new neurons can be generated in the adult brain via a process known as neurogenesis, which can attenuate the deleterious effects of neurodegeneration (van Praag et al., 1999). This phenomenon has been linked to exercise, with a significant portion of subsequent neural growth occurring in the dentate gyrus of the hippocampus (Cotman and Berchtold, 2002). Since the hippocampus is critical for memory consolidation and learning, the generation of new neurons and increased plasticity in this brain region may explain the improved cognition and emotional state that accompanies exercise (Gandy et al., 2017; Trinchero et al., 2017). Furthermore, preliminary research has suggested that neurogenesis may also occur in numerous other areas of the brain, including the amygdala and hypothalamus, which may explain the diversity of exercise-derived benefits (Fowler et al., 2008). However, this research is not as extensive or conclusive as hippocampal neurogenesis research, nor is the extent to which neurogenesis occurs in other brain regions as robust as it is in the hippocampus, with the exception of the olfactory bulb (Cotman et al., 2007). One key molecule, brain-derived neurotrophic factor (BDNF), has been shown to modulate neurogenesis and exercise likely influences BDNF levels to alter areas of the brain.

Exercise and Hippocampal Neurogenesis

In rodents, hippocampal neurogenesis as a function of exercise has been extensively demonstrated and replicated. To test this, rodents are injected with bromodeoxyuridine (BrdU), which signifies actively mitotic cells and is incorporated by daughter cells, thereby allowing the tracking of cell division (del Rio and Soriano, 1989). In some of the earliest work in this field, it was shown that mice allowed to voluntarily exercise on a running wheel exhibited enhanced neurogenesis in the dentate gyrus. By utilizing BrdU as a tracing molecule,

it was observed that exercise not only increased proliferation of the progenitor cells in the subgranular zone but also increased their survival rate as they differentiated and matured (van Praag et al., 1999; Seri et al., 2001; for reviews of neural progenitors and lineage progression see Weissman et al., 2001; Seri et al., 2004; Göritz and Frisén, 2012).

Although it is much more difficult to study exercise-mediated neurogenesis in humans, there is significant evidence that neurogenesis occurs in the adult human brain, especially in the dentate gyrus. Indeed, exercise has been shown to increase the size of the hippocampus in human adults (Erickson et al., 2011). Through postmortem tissue analysis of cancer patients administered BrdU, it has been shown that mature granule neurons are continually generated from the subgranular zone, even in the later stages of life (Eriksson et al., 1998). Interestingly, the participants in this study were not assigned to exercise conditions, and since they were cancer patients near death, it is unlikely they participated in any exercise regimen. This suggests that the hippocampus has the capability to generate new neurons in adulthood independent of exercise. Later sections in this review, however, provide evidence that exercise accentuates neurogenesis in humans and addresses how the amount of exercise modulates the degree of neurogenesis.

BDNF Mediation of Hippocampal Neurogenesis

Given these early findings establishing a connection between exercise and hippocampal neurogenesis, researchers next turned to examine the biological underpinnings. One of the strongest candidates for bridging the gap between exercise and neurogenesis is BDNF, a growth factor categorized under the neurotrophin family widely expressed in the brain and throughout the rest of the central nervous system (Salehi et al., 2003). Early research on this molecule found that during development in mice, BDNF expression is low during prenatal development, but then increases during the first few weeks after being born and peaks during the shift from embryonic to adult neurogenesis (Bath et al., 2012). This provides key insight into its potential for facilitating neurogenesis, which then spurred much more research interest in its connection to neurogenesis.

BDNF

As a whole, the neurotrophin family polypeptides are vital to the regulation of the neural processes in neurogenesis, such as proliferation, differentiation, maturation, and plasticity. Within this family, BDNF exhibits the highest degree of expression in the brain and is primarily synthesized there during exercise (Reichardt, 2006). BDNF can also enter the brain via freely diffusing across the blood-brain barrier (Pan et al., 1998; Mousavi and Jasmin, 2006). Furthermore, during exercise, proteins and their metabolic derivatives secreted from peripheral muscles, such as cathepsin B and FNDC5/irisin, also cross the blood-brain barrier to mediate BDNF expression in the hippocampus and subsequent neurogenesis and memory improvement

(Wrann et al., 2013; Moon et al., 2016). Indeed, mice injected with skeletal muscle endurance factors had elevated levels of hippocampal neurogenesis and increased spatial memory (Kobilo et al., 2010).

BDNF functions by binding to tropomyosin receptor kinase B (TrkB), which is largely expressed in hippocampal neurons. Upon binding, the BDNF-TrkB complex serves as a docking site for numerous signalling cascades, protein phosphorylation cascades, and secondary signalling systems (Huang and Reichardt, 2003; Nykjaer et al., 2005; Yoshii and Constantine-Paton, 2010). Through these pathways (shown in Figure 1), BDNF can exert significant regulatory control over many facets of a neuron's function and thereby influence how these neurons function as a whole within the hippocampus.

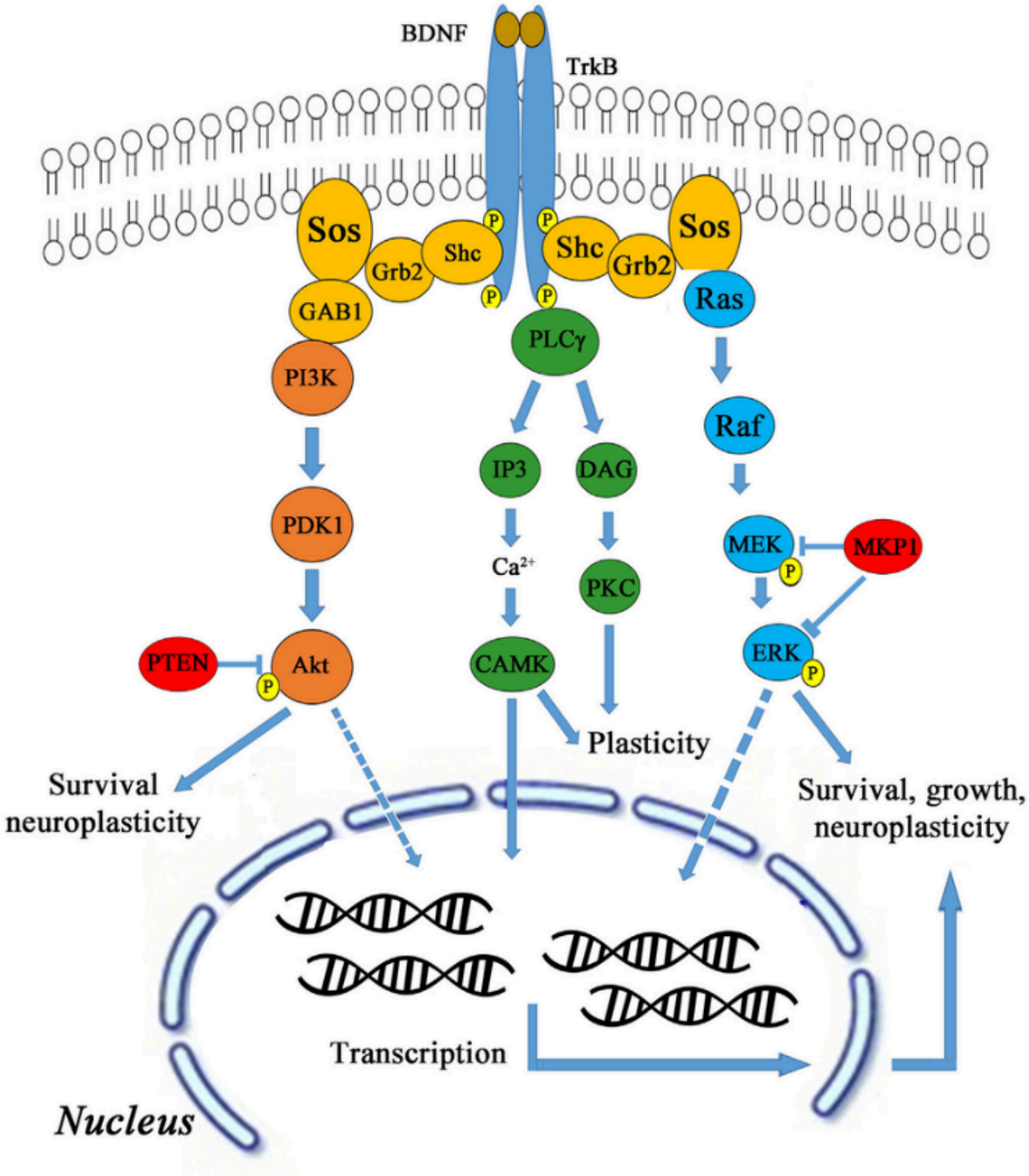

Figure 1. *Pathways that BDNF activates. Brain-derived neurotrophic factor activates TrkB through several downstream signalling pathways, such as AKT, CaMK, Ras/Raf/MEK/ERK leading to cell survival, growth, and neuroplasticity. BDNF activates TrkB stimulation via phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and also activates proteins like Shc, Grb-2, and Gab-1. The PI3K is also activated by binding to Ras homolog enriched by brain glutamine triphosphate (Ras-GTP). Image from: https://doi.org/10.3389/*

Exercise and BDNF Expression

Thus far, both exercise and BDNF have been shown to be associated with increased neurogenesis. Further research has extended this to show that treadmill exercise in mice and aerobic exercise in humans increases BDNF expression by regulating BDNF gene expression in the hippocampus (Kim et al., 2015). This process is largely mediated by neurotransmitter and neuroendocrine systems, with extensive literature supporting acetylcholine (ACh) as a key regulator (Knipper et al., 1994).

In mice allowed to voluntarily engage in wheel running, an increase in BDNF mRNA levels in the dentate gyrus was observed after only a few days of exercise (Neeper et al., 1995). Surprisingly, these levels were maintained throughout several weeks of exercise and corresponded to proportional increases in BDNF protein expression (Russo-Neustadt et al., 1999). When the exercise conditions were supplemented with antibodies blocking TrkB, however, the mice had attenuated learning capabilities involving the hippocampus. Furthermore, these mice also lacked synaptic-specific proteins in the hippocampus, thereby demonstrating that BDNF signalling is necessary to allow the benefits of exercise to manifest (Vaynman et al., 2004, 2006).

Research has also shown that metrics of overall health quality in humans follow a dose-dependent relationship with the duration and intensity of exercise, with the best outcomes linked to moderate exercise (Larson et al., 2006). Further work illustrates that mice show greater improvements in acquisition and retention based learning in hippocampus-dependent tasks following long-term exercise rather than shorter regimes of exercise (Handschin and Spiegelman, 2008; Parachikova et al., 2008; Ploeger et al., 2009). It was found that in mice, even just one session of exercise increased BDNF levels. This effect, however, became amplified following a period of exercise in mice that regularly exercised, with increased response in BDNF levels relative to mice after just a single session of exercise (Johnson et al., 2003; van Praag et al., 2005; Rasmussen et al., 2009). Consistent with these findings is a meta-analysis of 29 studies spanning 1,111 human participants that analyzed BDNF expression levels across various exercise paradigms. However, many of the studies only examined moderate exercise, and several studies did not report the intensity level. Interestingly, considerable evidence from this meta-analysis suggests that humans also experience a dose-response relationship in which each session of exercise corresponds to a dose of increased BDNF expression. Furthermore, regular exercise in moderate amounts has been shown to increase the magnitude of BDNF expression following individual sessions of exercise (Szuhany et al., 2015).

There is not a perfect positive correlation, however, between the amount and intensity of exercise and BDNF expression levels and subsequent health benefits. Extreme exercise has been shown to disrupt a number of metabolic and physiological processes and lead to impaired cognitive performance in humans (Aguiló et al., 2005). Since oxygen is rapidly metabolized during physical exertion, reactive oxygen species (ROS) are naturally produced as a metabolic byproduct. When produced at high levels, such as during bouts of intense exercise, ROS can lead to oxidative damage and increased cellular mortality in both rodents and humans (Radak et al.,

2016). Moderate levels of exercise enforce the human body's antioxidant defence system, but extreme levels of exercise lead to the generation of more ROS than the antioxidant system can defend against, thereby allowing their accumulation as oxidative stress (Mastaloudis et al., 2001). In fact, when treated with hydrogen peroxide, a potent ROS, hippocampal cell cultures taken from rodents showed an inverse relationship between BDNF expression levels and hydrogen peroxide concentration (Kwon et al., 2013). The *in vivo* production of BDNF as a function of ROS production, however, is less clear and warrants further study.

Not all BDNF is created equal

In humans there are frequent polymorphisms in the BDNF gene which are typically found on position 66 that converts valine to a methionine. Previous work in the early 2000s suggested that somehow this gene was not working well in people with schizophrenia and that this Met66 polymorphism would be linked to disorders of the brain including schizophrenia.

When researchers examined the polymorphisms in the gene and schizophrenia and effect on hippocampal dysfunction in a study of 641 individuals reported in Cell 2003 by Egan et al. cohorts of schizophrenia, siblings and normal individuals were examined to see if there was a relationship. However, it turned out that this polymorphism had no difference in cognitive recall skills in individuals with schizophrenia but normal individuals (i.e. their siblings) who did not have symptoms showed dramatically reduced memory recall with the Met66 mutation. Later research showed that this polymorphism makes it harder to release BDNF, therefore affecting neuronal health. So even if an individual exercised, there is no guarantee that more BDNF will be released! The effectiveness of exercise is literally in your BDNF genes!

License

5.5 NEUROSCIENCE AND ART

Amna Noor and Rena Patricia Seeger

Music

Music is one of the most abstract forms of art and yet can evoke real and measurable physiological mechanisms in the brain, allowing one to feel a part of a different dynamic — a purely musical dynamic (Walton, 1988; Trimble & Hesdorffer, 2017). The music-neuroscience interface has been studied by various researchers recently and in this section, some of the important findings, questions, and controversies will be discussed.

How is Music Processed?

It is important to learn how our brains process music to be able to implement music therapies and make informed choices for various brain traumas and neurological disorders. There is an inherent difficulty in the understanding of music processing due to the sheer plethora of music genres and the different neurological cascades they can kickstart by evoking different emotions such as stress, joy, happiness, melancholy, et cetera (Hernandez-Ruiz, 2019; Tai & Kuo, 2019). Each musical melody is made up of different elements which can be randomly categorized under pitch, tempo and rhythm, volume, timbre, texture, duration, and form. Out of these, only pitch and tempo and rhythm have been studied in detail (Hernandez-Ruiz, 2019).

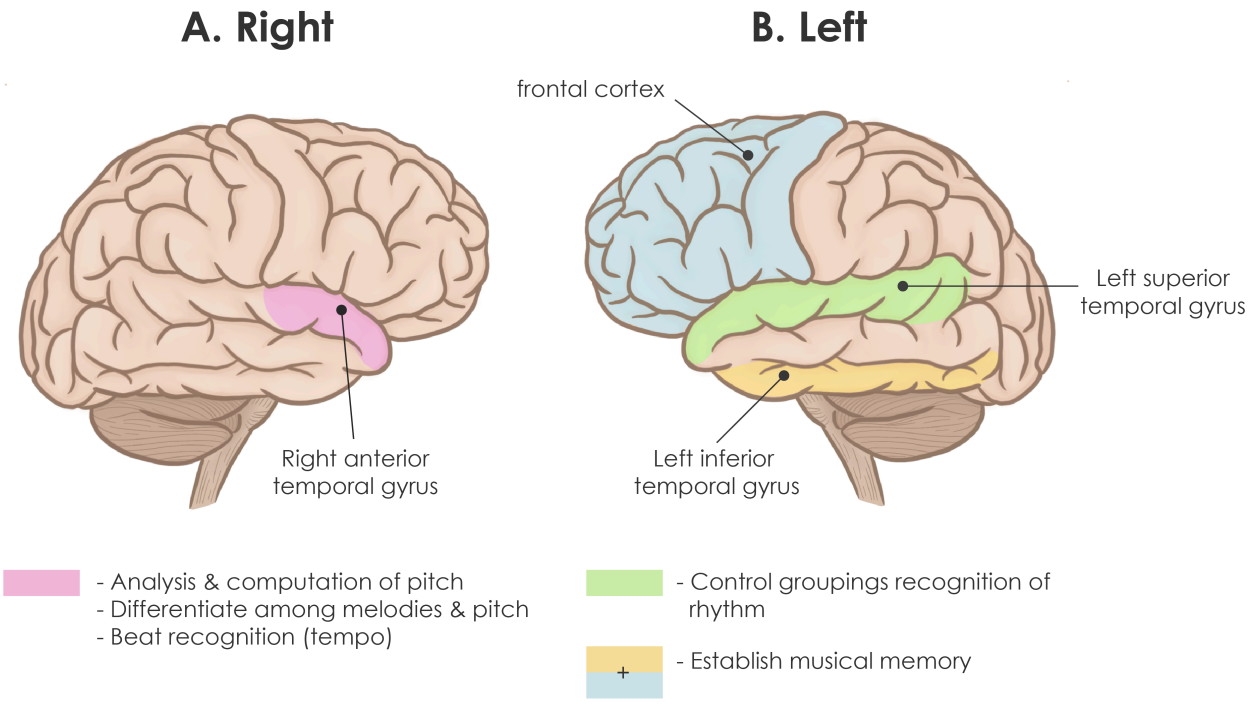

Figure 1. *Different functions of the left and right hemispheres in processing music.* ***A.*** *Right hemisphere* ***B.*** *Left hemisphere.*

Pitch is analyzed and computed in the right anterior **superior temporal gyrus (STG)** (Figure 1A). Changes in pitch can activate the posterior secondary auditory cortex. The right temporal cortex can also differentiate among melodies in the presence of contour information or pitch direction. The right anterior STG is also responsible for beat recognition (tempo) while the left STG controls groupings recognition (rhythm). This motor and perceptual timing also involve the functions of the cerebellum, basal ganglia, supplemental motor cortex, premotor cortex, and the parietal cortex (Peretz & Zatorre, 2005; Hernandez-Ruiz, 2019). As shown in Figure 1B, higher-order processing that involves the inferior temporal and frontal cortex is required for the establishment of musical memory (Peretz & Zatorre, 2005).

Overall, it seems that different components of a melody are processed by different areas of the brain. A study compared a musician with Alzheimer's disease to a musician with semantic dementia to evaluate their abilities to recognize compositions. Musical knowledge was preserved in the musician with semantic dementia even though he experienced a loss of both normal expressive and normal verbal functions. This alludes to the double dissociation theory that suggests musical elements are processed independently and not sequentially (Omar et al., 2010; Hernandez-Ruiz, 2019).

Music Therapy in Neuroscience

Music therapy, defined as "clinical, evidence-based use of music interventions to accomplish individualized goals within a therapeutic relationship by a credentialed professional who has completed an approved music therapy program" is being increasingly used to enhance neural executive functions and neuroplasticity in recent years (American Music Therapy Association, 2018). Taking part in a musical performance, or listening to complex music makes use of multiple parts of the brain which is beneficial for the brain as it gives the brain a chance to enhance dendritic sprouting and promote synaptic plasticity (Vik et al., 2019). This chapter will discuss some of the neuroscience fields benefitting from the therapy.

Neonatal Brain Development

Many epigenetic factors contribute to neonatal brain development such as auditory, visual, or somatosensory factors (Galvan, 2018). Fetus's auditory system develops around the 16th week of gestation. Following the development, the fetus can detect and process sound stimuli between the 26th and 30th weeks of gestation; this period is considered critical for neurodevelopment. Research has shown that a cacophony of loud people's voices or lack thereof in the NICU can have detrimental effects on neonatal neurodevelopment which can manifest themselves as delayed language capacity in later years (Loewy & Jaschke, 2020).

A study by Loewy et al. in 2013 demonstrated improvement in vital signs, sleep patterns, and heart rate patterns of infants born prematurely when exposed to either parental or music therapist singing conditions (Loewy & Jaschke, 2020). Studies such as the one mentioned are common but the longterm transfer of these improvements as well as longitudinal studies that record the impact of early music therapy on different cognitive activities are infrequent. However, a study by Hennessy et al. in 2019 suggested that music-based

training can play a role in expediting the development of inhibitory controls and associated neural pathways well over childhood (Hennessy et al., 2019).

It is also important to consider different musical parameters when discussing neonatal development, as we have discussed that different musical elements are processed individually before. Recently, a study highlighted the importance of timing of music therapy. Listening to ~8 minutes of music 5 times per week at times of feeding, alertness, or sleepiness before 32 weeks of gestation is associated with a functional brain that is similar in architecture to that of a full-term newborn (Loewy & Jaschke, 2020). Sleep regulation is tightly linked with a strong neurological function. A study explored the relationship of timbre and function of brain regions in the frontal lobe, the thalamus, the hippocampus, the amygdala, planum temporal, and the temporal plane and discovered a positive neurobehavioral development correlation between the two. The study made use of a breathing bear contraception that mimicked breathing babies in the vicinity by making an "ah" sound. Infants that received the device had better respiratory patterns than those who did not and eventually had a quieter and calmer sleep at ~45 weeks (Loewy & Jaschke, 2020; Ingersoll & Thoman, 1994). Other factors that are involved in better development of neural structures and associations in neonates include music that is predictable and familiar (Loewy & Jaschke, 2020).

Traumatic Brain Injury and Neurological Disorders

Traumatic brain injury (TBI) is associated with lifelong impairment of cognitive functions, especially executive functions. Music-supported therapy can improve cognitive functions, particularly in the case of a mild injury by enhancing neuroplasticity — the ability of the brain to form new connections (Siponkoski et al., 2020). TBI usually involves damage to either the orbitofrontal cortex alone or combined with temporal pole damage (Vik et al., 2019). Both of these scenarios have great implications for behaviour because the orbitofrontal cortex is an integration centre for emotional processing (Vik et al., 2019).

A study by Vik et al. trained victims of a TBI to play the piano. They found an enhancement of synaptic connections in regions of the brain that process episodic and semantic memory networks. Elements of repetition can also strengthen the newly established connections, as is observed in learning. Furthermore, during the training the 'goal' for the patients was to learn to play the piano when this was achieved, dopamine was released (sense of reward is involved in the release of dopamine). Dopamine release causes the limbic system, the amygdala, and association cortices to be activated and form new connections. Their last finding indicated that post-music intervention, patients were more likely to engage in a social situation rather than withdraw, which indicated an improvement in the orbitofrontal cortex and rostral anterior cingulate gyrus and solidified the use of music therapy as a successful therapy (Vik et al., 2019). Similar results were found in a study that investigated the impact of music therapy on patients with mild TBI (Vik et al., 2018).

Autism Spectrum Disorder (ASD) is characterized by avoidance of social interaction and "repetitive patterns of behaviour, interests or activities" and is linked to deficits and impairments in frontotemporal and frontoparietal regions, the amygdala-hippocampal complex, cerebellum, basal ganglia, and anterior and

posterior cingulate regions (Brancatisano et al., 2020). It was found that children suffering from ASD are more likely to respond to musical stimuli rather than normal, verbal stimuli and can focus on musical stimuli longer than controls. The finding has favourable effects on attention, memory, and social interactions for children with ASD (Brancatisano et al., 2020). Music therapy inventions have also had valuable results for patients suffering from dementia, stroke, and Parkinson's disease (Brancatisano et al., 2020).

Limitations and Conclusions

Although many studies have tried to establish a causative link between music-supported therapy and enhancement of neural connections, the number of long-term studies is minimal, and some studies are not replicable. These limitations can potentially overestimate the effect of music therapy, especially over the years. Furthermore, the studies that use behavioural testing and assume a connection to a particular region of the brain grossly underestimate the complexity of neural pathways which can be activated by a multitude of confounding factors such as mood, environment, and expertise (Hernandez-Ruiz, 2019).

In conclusion, music-supported therapy is an emerging topic in neuroscience that still has a long way to go. The interdisciplinary nature of the music and its effect makes it a difficult topic to study. However, a mechanistic understanding of how all the different elements of music are processed and analyzed by the brain can lead us to the ultimate therapeutic uses of music as a neurological therapy.

Dance

Dance, defined as a choreographed routine of movements usually performed to music (Hui et al., 2009) is one of the more synchronized activities that human body can perform – by involving perceiving and performing rhythm, it is similar to music, however, dance also can convey ideas in ways that are analogous to language. Unlike most of our daily activities, dance integrates brain functions involved in kinesthesia, musicality, and emotion (Teixeira-Machado, Arida, de Jesus Mari, 2019). In this section, we will be exploring how dance affects neuroplasticity, and how dance therapy can be used for neurodegenerative disease.

Neuroplasticity and Dance

Due to its integrative nature, dance is believed to provide a unique model for studying the way the human brain integrates both movement and sound, and how motor experience can be influenced by creativity and performance. A systematic review by Teixeria-Machado, Arida, and de Jesus Mari from 2019 looked at eight studies that explored the influence of dance practice on neuroplasticity in mature brains. All studies demonstrated positive structural and/or functional changes, including increased hippocampal volume, increased gray matter volume in areas like the left precentral and parahippocampal gyrus, as well as significant improvements in memory, attention, and psychosocial parameters. Other studies have shown the impact of

dance on the human brain even in the short-term. A study by Kulinna et al. from 2018, looking at the effects of dance on elementary school students, demonstrated that an acute bout of aerobic dance was able to significantly improve students' processing speed and concentration performance in a selective attention test.

Dance As Treatment

In recent years, there has been an increasing interest in using dance as a form of therapeutic intervention to treat various developmental disorders such as Down syndrome, neurological disorders such as schizophrenia, mood disorders like depression, neuromotor disorders like Parkinson's disease, and even dementia (Burzynska, Finc, Taylor, Knecht, Kramer, 2017). As dance typically involves motor, cognitive, visuospatial, social, and emotional engagement, compared to other forms of treatment that may only target physical fitness or cognitive abilities, dance is seen as a more holistic form of treatment that is easily accessible and enjoyable.

Age-related changes, including volume reductions in brain areas such as the prefrontal and temporal cortices are primarily due to the shrinking of cells and reduced synaptic density (Rehfeld et al., 2018). With increasing research suggesting that the adult brain is capable of neuroplasticity, there has been an increased interest in discovering new treatments that can counteract these detrimental effects of ageing on the brain.

Animal research has demonstrated that only a combination of physical activity and sensory enrichment can have lasting impacts on adult neuroplasticity. Dance is thus seen as an appealing intervention as it demands both physical and cognitive functions. When comparing dance therapy to traditional forms of exercise interventions, although both are associated with increases in physical fitness levels, individuals who had undergone dance therapy saw larger volume increases in brain areas such as the cingulate cortex, insula, corpus callosum, and sensorimotor cortex (Rehfeld et al., 2018). A study by Rehfeld et al. from 2018 found that participants who had undergone a dance program, and not the conventional fitness program, showed increases in plasma BDNF levels. BDNF is an important growth factor supporting functions such as synaptic plasticity in the CNS.

Parkinson's Disorder and Dance

Parkinson's disease is a neurodegenerative disorder characterized by motor impairments such as bradykinesia, rigidity, resting tremor and gait problems, as well as non-motor symptoms including sleep disorders, sensory alteration, cognitive impairment and depression (Nemes et al., 2019). Although often classified as a 'motor disorder', its effects on psychological, emotional, social and financial functions of life are often what make PD so debilitating (Sharp and Hewitt, 2014). As a result, there is a strong need for interventions that cater towards treating not just the motor symptoms, but other aspects of quality of life.

There is accumulating research suggesting that regular physical activity is associated with a lower risk of developing PD, as well as can slow down the progression of its physical manifestations (Romenets et al., 2015). Regular exercise has been shown to improve gait speed, strength, functional capacity, and has been shown to

reduce falls in PD patients (Romenets et al., 2015). However, many exercise interventions are unappealing for patients with PD and have low compliance and participation (Heiberger et al., 2011a). Since less than half of PD patients meet recommended daily levels of physical activity, there is a need to find interventions involving physical activity that can help patients overcome barriers to participating (Ellis et al., 2013).

Dance in particular, is a promising intervention for PD patients as it offers auditory, visual and sensory stimulation, social interaction, motor learning and memory (Kattenstroth et al., 2010). Compared to other forms of exercise therapy, patients with PD have higher compliance rates when it comes to dancing therapy and is more motivated to attend the classes even after the study period (Hackney and Earhart, 2009). This is particularly important since research shows that exercise interventions are only beneficial if performed regularly over a longer period (Goodwin et al., 2008).

One study by Westheimer et al. from 2015 employed both quantitative measures pre and post-intervention to examine the dance intervention's effects on motor function and quality of life in Parkinson's patients. Over eight weeks they found improvements not only in gait and tremor, but the qualitative interviews that took place post-intervention revealed benefits related to the general quality of life and well-being, including improvements in social interactions and increased happiness. However, these results are not consistent across studies. Studies by Hackney and Earhart (2009a), Duncan and Earhart (2012), and Foster et al. (2013) found no differences in gait between participants who had undergone dance intervention and those had no intervention. However, a study by Brichetto et al. (2006) found that scores of Freezing of Gait Questionnaire, but not Unified Parkinson's Disease Rating Scale (UPDRS) scores significantly improved after treatment, suggesting that the type of quantitative measure being employed in these studies also affects which motor symptoms show improvement.

Limitations

Although there are studies that demonstrate the potential of dance therapy for treating Parkinson's disorder, there are many discrepancies across studies that make it difficult to know what aspect of the treatment was responsible for improving certain symptoms. For example, many of the studies administered interventions across different timelines. The study by Westheimer et al. ran 16 dance sessions over 8 weeks, while another study by Duncan and Earhart from 2012 ran their study over a course of a year. The frequency of having dance sessions, as well as the duration of each one and the length of the entire study all would influence the impact of the intervention. Looking at the literature, it appears that studies that had longer interventions showed more significant improvements in symptoms compared to the current study. However, there is a lack of understanding of whether longer intervention periods or longer sessions lead to more superior results (de Dreu et al., 2012).

Another important factor that likely influenced the results across studies is whether participants were tested while on medication. Although testing participants while on their best ON medication state can help provide insight into how they perform daily activities, some of the deficits of PD are not fully seen. As a

result, these studies lack a full picture of the effects of dance intervention on symptoms, and whether the intervention is disease-modifying (Duncan and Earhart, 2012). Although testing participants while they are on medication appears to be common, as is shown in the meta-analysis by Kathryn Sharp and Jonathan Hewitt (2014), for future studies, testing off medication is warranted. A study by Duncan and Earhart (2012) found improvements in UPDRS scores at 3, 6, and 12 months in patients who were off medication. This allowed them to identify that participation in the dance program had a disease-modifying effect.

Although most dance training employs visual focus, rhythm, and reproducing distinct parts of dance sequences, it is possible that different types and regimes of dance would have different effects on motor symptoms. One study by Westheimer et al. from 2015 employed ballet steps such as tendus, port de bras, and plie in addition to modern, jazz, and tap moves. This is different from many other dance studies that employ tango dancing as an intervention for Parkinson's patients (Romenets et al, 2015, Hackney et al., 2007a, Hackney et al, 2007b, Hackney and Earhart 2009, Duncan and Earhart, 2012). It would be interesting for future studies to compare different forms of dance on Parkinson's patients to determine which forms of dance are the most effective and if some forms improve motor symptoms more than others. It is likely that some forms of dance are more effective at treating specific motor symptoms, which has important applications when using dance therapy as treatment. Depending on what symptoms a patient is experiencing, and which are the most severe, a different kind of dance may be prescribed.

Since the study by Westheimer et al. (2015) found improvements in motor symptoms and quality of life aspects reflected in the qualitative interviews but not the quantitative interview, likely, the type of tests used also influence whether improvements are seen. Further research in the field could determine standardized ways to test for the effects of dance interventions on Parkinson's patients that include both quantitative and qualitative measures.

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5.6 INTEGRATIVE AND CONTEMPLATIVE NEUROSCIENCE

Sunny Sanghee Baek and Bushra Ahmed

Introduction to Contemplative Science

Neuroplasticity is the innate property of the brain to alter its structure and function depending on our experiences. The theoretical framework of neuroplasticity is often associated with learning a new skill, such as how to juggle or speak a new language. A new field of scientific study, contemplative science, is concerned with contemplative practices and their effect on neuroplasticity. Contemplative practices involve training a complex array of cognitive processing, including attentional and emotional regulation. In this chapter, we explore how a contemplative practice like mindfulness meditation, influences neural activity and the architecture of the brain. We will also take a closer look at the methodology used to understand the effects of contemplative therapy on the brain and behaviour.

Meditation

Mediation is defined as a technique to train the mind or induces an altered state of consciousness, to increase mindfulness. The practice of meditation has rich roots that extend back to the century-old traditions of Eastern religions. In Western society, the practice of meditation has been adopted and utilized as a self-help tool for stress reduction (Cutshall et al., 2011). Both secular and non-secular forms of meditation involve the regulation of attention and emotions, utilizing techniques such as **focused attention (FA)**, **open monitoring (OM)**, and controlled breathing. FA requires that individuals actively direct their attention to an object, sensation, or idea (i.e. a mantra). OM involves attending to the transiency of thoughts and bodily sensations as they change from moment-to-moment (Lutz et al., 2009).

The most widely practiced form of meditation is **Mindfulness meditation (MM)**, which is based on the concept that mindfulness is the ability to; pay attention, on purpose, to thoughts and sensations in the present moment, in a non-judgemental manner. (Kabat-Zinn,1994). Mindfulness meditation has been integrated into therapies to treat psychiatric disorders such as major depression and anxiety, as well as neurodegenerative diseases like Parkinson's disease and Alzheimer's disease (Shonin & Van Gordon, 2016).

Making the Connection Between Meditation and

Neuroscience

Identifying Effects on Neural Activity

Initial research on meditation focused on identifying how long-term practice influenced cortical activity using **electroencephalography (EEG)**. Seminal work by Lutz et al. (2004), uncovered that long-term Buddhist practitioners exhibited EEG patterns in the lateral frontoparietal area that were indicative of phase synchrony of gamma-band oscillatory activity (25-70 Hz). This pattern of cortical activity has been associated with the integration of information across neural networks through short and long-term synaptic changes that underlie processes of cognitive and affective regulation. These findings suggest that the practice of meditation may promote functional changes of activity in the brain, consistent with the experience-dependent nature of neuroplasticity (Pascual-Leone et al. 2005; Travis & Arenander 2004).

Although these studies provided evidence for the meditation-brain connection, it is important to consider the limitations of utilizing EEG to characterize neural changes associated with meditation practice. EEG is a method that measures cortical activity by using electrodes placed on the scalp to detect extracellular currents produced by postsynaptic apical dendrites of pyramidal neurons. These electrical signals however are distorted as they pass through the skull. As a result, using EEG makes it difficult to infer the source of observed cortical activity. To this point, it is also difficult to detect changes in neural activity in deeper cortical structures due to their distance from surface electrodes. These limitations are referred to as the inverse problem of EEG. The advantage of EEG is that the technique offers a high degree of temporal resolution, as it can be used to detect changes in cortical on the order of milliseconds (Grech et al. 2008).

To address the limitations of EEG, subsequent studies utilized techniques such as **functional magnetic resonance imaging (fMRI)** to measure local changes in blood oxygenation as a level of neuronal activity, referred to as the BOLD response. It was found that individuals that practiced meditation exhibited reduced activity in the **default mode network (DMN)** during the practice of meditation compared to rest. The DMN is a collection of cortical structures including; the posterior cingulate cortex, medial prefrontal cortex, and the angular gyrus. The DMN has also been referred to as a task-negative network as activity in these regions is associated with self-referential processing and mind-wandering that divert attention while completing a task. Considering this, the practice of attentional regulation through meditation practice has been hypothesized to suppress the activity of this network to improve focus on tasks outside of meditation practice (Garrison et al. 2015; Pagnoni 2012).

Knowledge Checkpoint

1. What changes in neural activity can be observed in meditation practitioners and what are these patterns of activity associated with?
2. What are the limitations of using EEG to infer the localization of changes in cortical activity?
3. Which imaging technique can be used to improve spatial resolution to determine changes in the activity of brain structures that occur during the practice of meditation?

Identifying Effects on Cortical Grey Matter Structure

Using **magnetic resonance imaging (MRI)**, researchers found that long term meditators exhibit an increased cortical thickness in the prefrontal cortex and right anterior insular cortex which correlated with their level of experience (Lazar et al., 2005). The prefrontal cortex has been shown to integrate emotion and cognition, a skill that is hypothesized to develop through meditation practice (Gray et al. 2002). As well, an increased thickness of the right anterior insular cortex is related to visceral awareness that is developed by attending to bodily sensations through OM. These findings support the theory that long-term meditation practices induce changes in cortical structure. The cross-sectional design of this study did not allow for the observation of how meditation practice changes the structure of the brain over some time, especially in naive meditators.

A longitudinal study conducted by Holtzel et al., (2011) was conducted to observe changes in grey matter volume throughout the brain after individuals completed an 8-week long mindfulness-based stress reduction program (MBSR). It was found after program completion, individuals exhibited increased grey matter volume in regions of the brain including the posterior cingulate cortex, part of the DMN. Other studies have found significant changes in grey matter volume in emotion processing and regulatory regions including the **insula**, **anterior cingulate cortex (ACC)** and the **amygdalae** of meditators (Marchand, 2014).

Identifying Effects on White Matter Structures

In addition to examining changes in grey matter volume in the brain after meditation practice, white matter tracts that integrate information across brain regions have also been a focus of study. Using **diffusion tensor imaging (DTI)**, it was found that after a short period of meditation practice, individuals exhibited an increase in white matter connectivity from the anterior cingulate cortex (ACC) through the corona radiata to other regions of the brain. This supports that meditation increases connectivity in a region of the brain that is involved in self-regulation, a skill that is central to the meditation practice. This finding is extremely interesting because the observed alterations in white matter structure were viewed after a short training period (11 hours),

whereas in previous studies examining the white matter effects of skill training, months to years were required to induce changes in white matter structure (Tang et al., 2010).

Knowledge Checkpoint

1. Explain which type of study design can be used to measure changes in brain structure that occur over some time and how this differs from other study designs.
2. What neuroimaging techniques can use to alterations in grey matter volume and white matter structure?

Applications of Meditation

Psychotherapy

Given that meditation has been found to induce neuroplastic changes in brain structure and neural activity, extensive research has been conducted focusing on the psychological effects of these changes. For example, mindfulness interventions have been integrated into traditional cognitive behavioural therapies to treat individuals that suffer from affective disorders This form of mindfulness-informed psychotherapy is referred to as **mindfulness-based cognitive therapy (MBCT**). A study by Ives-Deliperi et al. (2013), examined how an MBCT intervention influenced the brain activity of bipolar disorder patients, using fMRI, and how these changes were associated with behavioural factors such as anxiety and emotional regulation. After treatment, patients were found to have improved anxiety and emotional regulation. As well, patients exhibited a decrease in mPFC activation during a mindfulness task compared to controls. This indicates that mindfulness practice suppresses activity within the default mode network to improve attentional regulation during the practice of mindfulness.

A recent systematic review on the efficacy of mindfulness-based therapies found that this therapy has similar efficacy to traditional cognitive behavioural (CBT), behavioural therapies, and pharmacological treatments. This indicates that mindfulness is a valid therapeutic intervention for the treatment of anxiety-related disorders and affective disorders (Khoury et al., 2013). Although these findings are promising, little is known about the long-term efficacy of mindfulness-based therapies in relapse prevention. Recent studies have shown that MBCT is important for the prevention of major depression relapse patients (Matthew et al., 2010), however more studies of this nature must be completed.

However, not all forms of mindfulness psychotherapy rely heavily on the principles of mindfulness

meditation. A psychotherapist can offer therapy as a mindfulness clinician (where they practice meditation themselves), mindfulness informed (where the clinician is informed of mindfulness theory and asks directed mindfulness questions) and lastly mindfulness-based, where the clinician offers formal mindfulness practice to the client. (Katz, 2020). The reason mindfulness interventions are offered in diverse forms is to cater to various client's needs. For example, a client suffering from post-traumatic stress will not benefit from MBCT as much as they would from mindfulness informed psychotherapy, where the latter provides a safer environment for the client to explore their past trauma. Moreover, for those with more than two episodes of depression may not benefit from MBCT, since mindfulness meditation is better suited to treat mild to moderate depression symptoms (Katz, 2020). Therefore, to address various mental health issues, mindfulness meditation is not a one size fits all model, rather it is modified to cater to the varied needs of the client.

Athletic Training

There has been an increased demand for mindfulness interventions for athletes to promote better cognitive, emotional, and physical experiences of performance as mindfulness reduces performance-impairing traits and improves overall athletic performance (Noetel et al., 2017; Zadeh et al., 2019). For instance, the **Mindfulness-Acceptance-Commitment program (MAC)**, introduced in 2004 by Gardner and Moore, is a mindfulness intervention that is specifically designed for athletes (Josefsson et al., 2019). MAC incorporated well-established therapy called **psychological skills training (PST)** principles and acceptance-and-commitment therapy while implementing mindfulness practices. When athletes completed the MAC, they showed an increased emotion regulation abilities, increased **working memory**, and decreased competition anxiety and pain rumination (Dehghani et al., 2018; Josefsson et al., 2019; Scott-Hamilton et al., 2016). Athletes also exhibited an enhanced perception of pain and stress, resulting in faster recovery on sports-related injuries (Exsci, 2019; Zadeh et al., 2019). In summary, athletes can train the ability to accept negative emotions and regulate meta-cognitive functions, therefore improving performance through mindfulness exercises. (Dehghani et al., 2018; Schiphof-Godart et al., 2018).

Anxiety, Stress Management in Post-secondary Students

Perhaps one of the most significant impacts mindfulness meditation has made is on stress reduction. Being exposed to mindfulness meditation training can lead to lower cortisol secretion, reduced heart rate variability and anxiety symptoms, which are often expressed as a result of highly stressful stimuli (Tang, Hölzel and Posner, 2015).

Post-secondary students are highly susceptible to chronic stress due to the significant life transition students go through such as living away from home, living in isolation, difficulty finding a sense of belonging, taking up work, a decline in academic performance due to high expectations set by the institution, coupled with an expression of suicidal thoughts and ideations (Auerbach et. al, 2016; Bruffaerts et. al, 2018; Wilczyńska et. al, 2019 and Mortier et.al, 2018). Moreover, they are at a higher risk of experiencing burnout.

To mitigate this stress and burnout experience, mindfulness meditation is considered an effective tool. **Mindfulness-Based Stress Reduction or MBSR**, have been shown to reduce anxiety symptoms (Vøllestad et. al, 2011). Regular practice of meditation lowers anxiety, stress and burnout levels amongst healthcare professionals, who lead highly stressful lives (Taylor et al, 2016; van der Riet et. al 2018; Ratanasiripong et. al, 2015). Practicing mindfulness meditation led to lower expression of symptoms associated with social anxiety disorder, enhancing emotional regulation and amygdala inhibition (Khusid and Vythilingam, 2016). Therefore, there is increasing evidence that mindfulness meditation is making an impact on anxiety symptoms.

The same efficacy of mindfulness meditation applies to young adults as well, particularly post-secondary students. There is a significant reduction in state anxiety and decreased stress levels due to meditation practice (Edwards et. al, 2018; Burgstahler and Stenson, 2018; Lemay et al, 2019 and Basso et al, 2019). Due to these promising results, mindfulness meditation has evolved as a cost-effective and accessible alternative for treating anxiety and is being implemented in various post-secondary settings.

Mobile Mindfulness

Although in-person guided meditation is a popular form of accessing meditation, it might not be practical for those with a hectic schedule, as it may be a challenge to attend in-person meditation sessions regularly. In this regard, mindfulness meditation apps present as a time-saving alternative to classroom-based guided meditation. Users of meditation apps such as Calm, Headspace and Smiling Mind reported reduced depression, stress and anxiety levels (Huberty et.al, 2018; Flett et. al, 2019). There is increasing popularity of mindfulness-based apps, which are providing a promising alternative for those with packed schedules.

However, these promising results must be evaluated carefully. One major issue of the mindfulness meditation study is the use of self-report questionnaires, which can lead to self-report bias and skew the overall results of the study. Furthermore, a high attrition rate and an unregulated intervention period and risk for security and privacy (Price et al, 2014) are some of the major concerns raised for meditation apps. There need to be more controlled and rigorous study designs to replicate these results and render increased legibility to the claims that the meditation apps used to market to the public.

Conclusion

As outlined in this chapter, meditation practice induces neuroplastic changes in the brain much like practicing

other skills. Meditation induced changes occur at the level of grey matter and white matter, as well as neural activity. These changes are associated with regions of the brain that are involved in the regulation of attention and emotion, skills that are developed through a regular meditation practice. These findings have important clinical implications for supplementing traditional cognitive behavioural therapies to treat psychiatric disorders such as major depression, anxiety, and bipolar disorder. The field of contemplative science, however, still requires improvement in the methodology utilized to study changes in the brain and behaviour that occur as a result of meditation practice.

Knowledge Checkpoint

1. What are the behavioural and functional changes observed after mindfulness-based interventions?
2. Who can benefit from mindfulness-based interventions?
3. What is a limitation of studies conducted on the efficacy of mindfulness-based interventions?

Case Study: PODCAST

The PODCAST can be found at https://youtu.be/v0J8JenhYdE

The transcribed version can be found at https://issuu.com/sunnyshbaek/docs/podcast_transcribed_version

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I graduated from the University of Toronto where I specialized in Cells & Systems Biology and Health & Disease. As a graduate student at the department of Cells & Systems Biology, it has been an absolute joy working with the team to make this textbook a reality. *Neuroscience* has been a collaborative effort of many hours of researching, illustrating, editing and discussing. I hope that this will be an insightful and engaging window into neuroscience for current students and a reliable reference for all in the future.

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I'm a fourth-year student studying Human Biology, Physiology, and Immunology at the University of

Toronto. I am very passionate about the clinical applications of Science. Music is the primary medium of expression for me, so to be able to research and combine that with neuroscience for this textbook has been a delight. I hope that as you advance through this textbook, you will have as much appreciation for neuroscience as I do.

Hannan Algitami

I am a fourth-year student studying Neuroscience, Psychology and Physiology at the University of Toronto. I have a deep passion for scientific research and a secret love for writing. Contributing to this textbook has allowed me to work in both of these avenues. I have thoroughly enjoyed the experience and sincerely hope that this will be a useful resource for students.

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I am a first-year nursing student at the University of Toronto, and I have a bachelor's degree in Human Biology, Cell Systems Biology, and Buddhism Psychology & Mental Health. My passion is to create a platform where mindfulness and neuroscience can produce synergy. I am glad to introduce how meditation and neuroscience can coexist, and I look forward to seeing how this textbook can enrich growing brains!

Rena Seeger

I'm a fourth-year student studying neuroscience, art history, and physiology at UofT. As someone who has strong interests in both medicine and the arts, I love learning about the possibilities of combining the two to help treat patients. After having worked with organizations like Dancing with Parkinson's, it was particularly rewarding for me to learn more about recent neuroscience research in the field of dance therapy and potential future directions.

Bushra Ahmed

I am a recent graduate of the University of Toronto. I completed my Honors B.Sc degree with a major in Neuroscience, and a double minor in Physiology and Buddhism, Psychology and Mental Health. I am excited to contribute to this textbook and help create an opportunity for readers to learn about exciting new ways neuroscience impacts us in our day-to-day lives.

Andrea Pinto

I'm a third-year undergraduate studying Neuroscience and Physiology at the University of Toronto. Collaborating on this textbook seemed to be the perfect opportunity to combine my passions for neuroscience and literature. Our team has worked diligently to produce an informative and engaging

interface. I hope this textbook provides a broader perspective on neuroscience by exploring unconventional topics and its applications to numerous disciplines.

Omar Hassan

Buna! I'm a second-year studying Neuroscience from Romania/Canada/Iraq pursuing a specialist degree. I'm thankful for my role in question creation because it gave me a greater perspective on teaching and learning management. It has also been a wonderful excuse to catch up on the many papers that I've been meaning to read. I hope I highlighted the more practical aspects of research in neuroscience in a fun and engaging manner to give insight into lab life and current problems students may face soon.

Di (Andy) Wu

I'm a recent graduate from the University of Toronto with an Honors Bachelor of Science. I completed my degree with a major in Neuroscience and a double minor in Immunology and Physiology. I enjoy writing as well as teaching, so I was excited to take on this project. I hope this textbook serves as useful reference material for anyone with an interest in learning more about neuroscience!

Stefani Mihilli

I am a fourth-year undergraduate student pursuing a double major in biochemistry and immunology. I have developed an interest in emerging research in neuroscience and the future applications of these findings. Working on this textbook has allowed me to investigate these fields and techniques while highlighting the importance of a multidisciplinary perspective.

Beatrix Yu

I am a fourth-year student pursuing a degree in Neuroscience, Immunology and Psychology at the University of Toronto. Having deep interests in both science and visual art, I hope to bring science into vibrant images to evoke curiosity as well as inspire others to engage in and learn more about emerging topics in Neuroscience. Being able to take part in this textbook was a great opportunity to work with like-minded peers and it was such a delightful experience!

Mira Chow

I am graduating from the University of Toronto with a Bachelor's degree in anthropology and physiology. I will be entering the Master of Teaching program with Biology and Social Sciences as my teachable subjects. I am particularly interested in making education more accessible and equitable. It is my hope that this textbook can be a useful resource for future students of neuroscience and physiology.

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