The Psychological and Physiological Effects of Stress on the Human Epigenetic Profile and Brain

Jana de Kock

The University of Tampa

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The Brain

In the words of the American physicists, Michio Kaku, "*The human brain has 100 billion neurons, each neuron connected to 10 thousand other neurons. Sitting on your shoulders is the most complicated object in the known universe.*"

Before we can understand how the brain functions, and particularly how it responds to stress and trauma, we first have to consider its physical structure. We will consider how the brain's architecture has evolved over time, why human brains are so different from most other species, how the brain has evolved to become the complex organ we know it to be, and why scientists still know very little about it when compared to any other organ in the human body.

When I think of the brain, I picture a Christmas tree - one of those magnificent trees that you always longed for as a child, with every ornament you could imagine, multi-coloured string lights, and thick, fluffy branches. Just as those trees always seem to carry a twinkle of magic in their magnificence, the brain has that same mysterious charm about it if we dare to look closely. Weighing in at approximately 1232g[1], our brain, and really our whole nervous system, is littered with string lights we call neurons, that light up systematically, like a Christmas tree, in response to stimuli from the environment.

Ultimately, the lump of fat, protein and electricity that lives between our ears is mostly made from only two types of cells, glia and neurons, billions of them[2]. Our neurons make up the fundamental units of the brain, consisting of a cell body with projections known as axons and dendrites that facilitates the transmission of vital electrochemical signals that allows me to write this sentence, and allows you to read it. Glial cells were long thought of as the 'body guards' of neurons, since they come in many different forms and protect the neurons.[3] Together, this plethora of cells come together to offer us the things that make us who we are. Once coined "the three-pound universe", our brains do

[1] Herculano-Houzel, S. et al. pg. 7
[2] Johns Hopkins Medicine
[3] Johns Hopkins Medicine

not merely relay information from the outside world, but creates it. Every single experience you and I have had has been carefully curated in our brains, customized for us to remember from our personal perspective[4].

In the early 1700s the Swiss philosopher, Jean-Jacques Rousseau, suggested that similar to how our physical body requires exercise, our brain holds a clear degree of plasticity and thus, have to be stimulated continuously in order to support optimal connectivity between the over 100 billion connections[5] that exists in the brain. In the late 1800s, when the glial cell was identified, the concept of neural connectivity transformed, and these cells would later prove to play a key role in the comprehensive functioning of the brain.

Neurons are littered throughout our entire body, working in tandem to create our peripheral nervous system which allows the body and brain to send and receive chemical messages on a continuous basis. Since the axons and dendrites of neighbouring neurons do not have direct contact, these electrical messages are relayed through synapses, where charged ions flow from one neuron to the next with the help of protein pore receptors on the surface of these cells[6]. The electrochemical signal is initiated in the dendrites, flowing through the cell body and along the axon until it is transmitted to the next neuron. The speed and integrity of the signal transduction is enhanced by a fatty myelin sheath that covers the length of the axons, leaving small gaps in the sheath where the electrical signal 'jumps' from one point to the next instead of constantly moving within the axon. This increases the speed of the signal transmission by up to 100 times, compared to neurons without the myelin sheath, which can be very helpful in cases such as the spinal cord where one axon could be close to a meter in length[7].

[4] Chopra, D., pg. 9
[5] Chopra, D., pg. 11
[6] Parrington, J., pg. 63
[7] Parrington, J., pg. 64

Since neurons occur throughout the entire body, it would be sensible to note that there are various different types of neuron, all with their own unique structure and function. The main three neuron types are the sensory, motor, and interneurons. Together these three structures allow us to receive, process and respond to information from both our external and internal environment in an effective and timely manner. When considering burning your hand on a hot surface, the sensory neurons are responsible for relaying the information from the pain receptors in your hand to your central nervous system. Here the interneurons take over, to process and sort the incoming information. Once organised, the motor neurons can use this information from your central nervous system in order to stimulate the muscles in your arms to lift your hand from the hot surface to prevent further damage. All of this happens in a matter of milliseconds, and most of the time we are totally unaware of its complexity[8].

The discovery of mirror neurons comes as a surprise to anyone who is not too familiar with the science behind their behaviours. These specialised cells have proven to play a significant role in our ability to recognise and comprehend the actions, emotions and intentions of those we interact with[9]. They are active in almost every interaction we are involved in or observe – whether it is a baby who looks at their caregiver's smile and smiles back at them, or when we see someone we care about that is hurting and it feels as though our own heart is breaking as well. Mirror neurons have been studied extensively and will be discussed further when we look at how social interactions and human relationships affect how we cope with stress, learn, and store memories.

In the outer sphere of the brain, we find the largest part of the brain, the cerebrum – a remarkable structure that makes our human brains unique in their development, with deep grooves that allow for maximum surface area, and therefore maximum functioning. Divided into 2 hemispheres and 4 regions, this structure processes, relays and transmits most of the information we receive from

[8] Parrington, J., pg. 67
[9] Parrington, J., pg. 67

our outside world and determines how it influences our internal world. The frontal lobe is situated towards the front of the brain and is responsible for learning, talking, feeling and moving, only fully developing at the age of X. Behind this we find the parietal lobes that sense our pain, registers our touch and allows us to distinguish temperature differences, among many other sensory information. At the rear end of our brain lies the occipital lobe, responsible for sight. Lastly, the temporal lobes lies above the ears and aids in hearing and memory, and emotion[10].

The second largest structure in the human brain is the cerebellum which is responsible for more primitive functions such as mobility and stability and is located below the cerebrum. At the core of the human brain lies the diencephalon, which encompasses several structures like the thalamus, hypothalamus, limbic system etc. It controls our hormones, relays nerve impulses, maintains temperature, monitors hunger, and controls our emotions. The brain stem is the final structure to mention, and it is our most conserved structure in the brain of a mammal[11], coordinating our reflexes and involuntary functioning such as heart rate, breathing, the sleep cycle, and blood pressure.

Although the above-mentioned structures constitutes the majority of the brain structure, one element that is vital to brain functioning and has not been addressed is the blood brain barrier (BBB). Although discussed in more detail further in the review, the BBB is what allows for stability and protection for the brain and spinal cord from any toxic or harmful environmental factors. This semi-permeable gated layer covers most of the nervous system organs, allowing nutrients and oxygen to diffuse from the bloodstream while simultaneously providing a barrier against harmful substances that could damage nervous tissue[12]. This poses a significant obstacle in clinical trials and other research related to the effect of specific molecules on the brain, since crossing the BBB can be challenging.

As researchers refine their studies of the structure of the brain and technology improves, we are able to gain more insight on the structure of this complex and interconnected organ. In 2015 the

[10] Parrington, J., pg. 68
[11] Johns Hopkins Medicine
[12] Johns Hopkins Medicine

total number of neurons in the human brain was estimated to be 86 billion, of which 69 billion resides in the cerebellum. What is important to note is that the architecture of the brain, rather than the size, is what enables this vast amount of cells to exist and function in unison. This is seen in the African Bush Elephant's brain, which is more than double the weight of a human brain, 2848g, but only houses 5.59 billions neurons[13]. Another aspect of the brain that allows for its complexity and productivity is its high energy demand. Despite only making up roughly 2% of the human body weight, it requires upwards of 20% of the energy expenditure when the body is at rest[14].

The reason for this energy trade-off that occurs in humans, but not in other primates, has been traced to a regulatory sequence related to muscle and brain-related glucose-transporting genes. Messenger RNA levels of these genes were measured and found to 3.2 times more active in human brains than in chimpanzees, and 1.6 times more active in chimpanzee muscle than in human muscles[15]. In humans there are signs of accelerated evolution where these genes experienced more mutations than would naturally occur by chance, insinuating that there must have been a strong evolutionary pressure for redirecting the energy from the muscles to the brain. A further analysis of the glucose-transporting proteins was done by analysing the metabolites, small molecules including carbohydrates, nucleic acids and neurotransmitters within the brain and muscle of various primates, including humans. When comparing the metabolite profiles of humans and chimps brain metabolites of humans were four times higher than that of chimps[16], further confirming a difference in gene expression in this region which could have led to the evolution of the complex architecture within the human brain.

Although this complex, multi-faceted, magnificent structure that sits within our skull holds immense power, its connection to our body is what allows it to flourish. In conjunction with our brain size, bipedalism, manual dexterity and our vocal tract contributed greatly to the overall development

[13] Herculano-Houzel, S. et al., pg. 3
[14] Herculano-Houzel, S. et al., pg. 3
[15] Herculano-Houzel, S. et al., pg. 7
[16] Herculano-Houzel, S. et al., pg. 8

of human intelligence, and ultimately consciousness. These factors worked in tandem to give rise to the modern human, as complicated, messy, ingenuous, and lovely as we all are.

In a universe as complex as ours, stretching billions of light years and for all we know to infinity, the human brain has struggled most with understanding itself. The reasons for this are debatable: a lack of technological advancements to accurately map the connections, the likelihood that our brain is more fluid and adaptable in structure than we can comprehend, or possibly that we were never meant to understand the complexity that exists in the few inches between our temples. The neurophysiologist, Tony Zador, wanted answers, and so he chose to investigate technology that will be able to accurately map neuronal connections as his path to getting them. According to him, the problem is that "we don't understand enough about the circuitry of the neurons" to accurately comprehend the complexity of the human brain. His argument hinges on the fact that studying the human brain without understanding the circuitry is like "trying to understand how a computer works by looking at it from the outside, sticking an electrode in and probing what we can find... Without ever knowing the hard drive is connected to the processor and the USB pod provides input to the whole system, it's difficult to understand what's happening."[17]

His technique, MAPseq, involves the injection of genetically engineered viruses carrying an array of known RNA sequences that infiltrate neurons with a distinct combination of sequences. This allows researchers to track individual neurons when they dissect the brain. Zador recently published this work in a new *Nature* paper, when he mapped close to 600 neurons in the mouse visual system, three times more neurons than the technology used at Harvard[18]. Their results showed the axons in the visual cortex to be highly distributed, offering an incredible level of cross-connectivity, making one-to-one mapping techniques nearly impossible. Seeing that specific neurons projected to particular regions in the visual cortex also suggests that there could be specialised cells within this cortex that

[17] Han Y., et al pg. 54
[18] Han Y., et al. pg 51

have not yet been identified. Zador foresees MAPseq to be able to process 100,000 neurons within a week, offering an insightful tool to studying psychiatric disorders, as well as autism[19]. Although this technology cannot directly reveal the functioning of these neuronal connection, its ability to provide brain maps with an incomparable level of detail is vital to neurological research due to the power it has to enable and provide a solid foundation for transformative research in the future.

Although the physicality of the brain serves as an important foundation to understanding the brain, what is far more important is the relationship we as humans have between our mind and our brain. We know now that our brain is capable of growing new axons and dendrites up until the late years of a human life, but without regular stimulation and new experiences, the brain becomes complacent in its regeneration. This connection is what gives rise to human consciousness. Rather than the architecture, weight or physical matter of the brain, it is our patience, curiosity, hope, and dedication that ultimately making us who we are.

Considering a stroke patient can aid in understanding the importance of distinguishing between the physical brain and the mind-brain connection. When specific areas of the brain have been deprived of oxygen for long enough, the neurons of that region will lose all functionality, often leaving the patient semi- or completely paralyzed. Two decades ago, physicians would have sent the patient home with some rehabilitation instructions, but essentially no hope for recovery. Today, the possibilities for recovery are endless, although often coupled with long periods of intensive rehab, pain and frustration. Despite it might be thought to be impossible, it is clear that when a paralyzed patient visualizes lifting up their hand while actively trying to move their muscles, new connections are formed in the brain. Slowly but surely, as the action is repeated enough times, these connections become sufficient to the point where the patient can move their fingers, possibly even their wrist. Full recovery is possible, but remains highly patient-specific. Yet, this new research is evidence that our thought processes,

[19] Han Y., et al. pg 52

diligence, and willingness to force our brains to make new connections despite the severe discomfort, can result in real change.

When we only discuss the biology of the brain and disregard its full complexity, we limit our understanding and capability of what we believe the human brain is capable of. Of course, we all have our own unique set of genes that offers some blueprint to the structure and functioning of our brain, but this blueprint is much more fluid than one might suspect. With the discovery of epigenetics, the idea that humans and their environments can alter their gene expression became a revolutionary concept in understanding the plasticity of our brain in new ways[20]. From a purely biological perspective, many support the idea that humans are controlled by our brains, but what if it worked the other way around? What if we had the ability to control our brain? To extend our functionality to a new level? What if we dared to set higher expectations for our brains and in essence, for ourselves?

Deepak Chopra, both a doctor and prolific author writing about the intersection of the physical and the spiritual, , compares the human brain to a Steinway grand piano. As a piano player myself, this analogy is one that brings home the importance of the mind-brain connection as it relates to the physical structure to of the brain[21]. When a Steinway grand is placed on the stage, all of its keys are sitting there, waiting for the music to come alive. The individual sitting in front of the piano could be a complete beginner, or a pianist progeny, such as Mozart or Bach. Sound will come out regardless of whose fingers touch the keys, however the quality and potential of the sound is greatly amplified when a virtuoso lays his or her fingers on the keys than if a beginner does. The beginner can barely utilise 1% of the potential of the piano, whereas Mozart pushed the limits of what the piano can offer, creating master pieces with those same 88 piano keys. The physical structure of the piano never changes, but the one in control does. Just like the piano, our brains are waiting to come alive, but most of us roam around unable to tap into more than 1% of its potential. The good news is that incoming research has

[20] Chopra, D., pg. 32
[21] Chopra, D., pg. 62

provided glimpses of the brains that have been pushed to new limits, with brain scans form various individuals, from monks to chess geniuses[22].

Theories of the connection between the physical brain and the human mind have been a matter of debate for close to a century, if not longer. Some of the most influential theories include introspective psychology (established by psychologists such as William James), behaviourism which Ivan Pavlov introduced with his notorious experiments where dogs salivated at the ring of a bell due to association with food, and lastly, Sigmund Freud's development of psychoanalysis. Although these theories will be further developed when discussing the history of neuroscience and psychology, what is astounding about these theories is that they all seem to somehow contradict each other, and often themselves. As the philosopher Vygotsky noted, these theories of the mind seem to have evolved into such sweeping ideas that they exclude many other perspectives. Another critique he made was that these views of 'the mind [expanded] to the point that it became almost a parody to the original insight.'[23] In this way Freud's idea that our unconscious tendencies are influenced by the suppressed sexual impulses led to him aiming to explain all of human society through this lens. Sweeping across communism, conviction, cultural icons, occultism, and even Da Vinci's inventions, Freund attributed these pillars of human society to nothing more than 'a libido in disguise'[24].

With more than a hundred years between Vygotsky's words and modern neuropsychology, one might wonder what the relevance of these remarks could be. It turns out that in the 21st century our understanding of how the human mind functions are not necessarily any closer to unification than it was back then. The paradigm of modern neuropsychology rests on two main theories, namely cognitive behaviorism (supported largely by clinical psychologists) and the biomedical model of the brain (accepted more readily in the psychiatric field)[25]. These models both provide fascinating insights into

[22] Chopra, D. et al., pg. 45
[23] Parrington, J. pg.15
[24] Parrington, J. pg. 17
[25] Parrington, J. pg. 18

the matter that sits in our skull, yet they share very little in common. The question remains: What if a common ground in our comprehension of the brain is what might edge us closer to possibly understanding how we have developed human consciousness, and how we can alter it over the course of an individual's lifespan to ultimately be happier, healthier, and more content human beings?

Epigenetics

"Though this be madness, yet there is method in't."

- Hamlet, Shakespeare

Although these words ring from more than four centuries ago, this is many people's response when they learn more about the depth and breadth of the power of epigenetics. In many ways, it is madness to think that our thoughts, environment, and life choices have such a significant influence on our genetic expression. The good news is that there is a whole bunch of method behind it. Yet, there are so many of us who still believe that we are the victim of our genetic heritage, blaming health issues, learning disabilities, personality traits, etc. on nothing more than the fate we were given at birth. Realising that this theory is not only false, but a limiting belief that has been disproven by the science of neurophysiology and -psychology, might give us as individuals the opportunity to take responsibility for our mental, physical, and emotional health in a plethora of new ways.

The concept of epigenetics was first defined by an embryologist, Conrad Waddington, in 1942 when he related his work in drosophila to 'epigenesis' studies from the 17th century. His ideas of the human genome not being completely hardwired took decades to gain traction with the general public, but after the abundance of dissimilarities found in identical twins were further research, Waddington's concept became apparent. How can two people with the exact same genome grow into such different people, both physically and mentally? The answer lies in the power of epigenetics, the transformation of their gene activity as a result of how they individually experience the world around

them[26]. Since Waddington defined the term as the relationship between the genotype and phenotype, a plethora of research studies have addressed the concept of gene regulation where chromatin alterations such as histone differences or DNA modification occur without changing the base genome sequence[27].

It remains a common misconception in our society today that an individual can be born with 'good genes' or 'bad genes', but if we take closer look and disregard the rigidity of this paradigm, we will find that our genome expression is much fluid than we might imagine. Through actions such as exercise, meditation, diet, and regular sleep, we have the ability to transform our bodies and minds through regulating the expression of our genes. Just like our brains, it might surprise you that we as humans have an astonishing degree of control over our genes rather than the other way around. This revolutionary new science of transformation tells a story much different to the concept of 'good' and 'bad' genes. It has revealed that in the midst of DNA's incredible complexity, lies useful tools that we can harness in order to elevate our bodies, minds, and lives to a higher level. A large degree of this control has been attributed to simple lifestyle changes, and this is where the destructive impact of high stress on the human brain and body is alarmingly evident, but more on that later.

The base unit of any gene is a nucleotide, consisting of a phosphate group, a nitrogen base, and a sugar. In the more stable, double stranded structure known as DNA, this sugar is deoxyribose. However, in RNA, which is single stranded and less stable, but vital to all protein expression and cell functioning, the sugar is a ribose. Together, our DNA and RNA will be regulated by different enzymes and other proteins, making us who we are in response to what we expose ourselves to. As humans we each house a set of 46 chromosomes (23 pairs), which can be found in almost all human cells regardless of their differentiated function. In the same way the cells of our small intestine have

[26] Chopra, D., & Rudolph, T. pg. 23
[27] Deichmann, U. pg 252

certain genes that are activated within the human genome in order to allow it to digest nutrients, our neurons have alternative genes that have been activated to transmit billions of electrical impulses.

This activation and suppression of specific genes is largely due to differences in gene expression, transcription factors and the role of epigenetic mechanisms in cell-specific environments. Epigenetic modifications control which genes are transcribed into proteins through various mechanisms such as histone modifications, non-coding RNA expressions, DNA methylation and changes in chromatin structure[28] allowing cells to respond and adapt to their environments.[29] Simply put, genes are constantly being switched on and off, and regulated up and down. The total sum of these changes as a single human genome is altered over the course of an individual life is known as the *epigenome*.

Connecting the Dots - Glial Cells, microRNA and Enzymes

The complexity of the brain and its neurons has been studied for centuries, however its close interconnectedness with genetics, enzymes, ions and the immune system has revolutionised our understanding of its function. By gaining a deeper understanding of the various spheres that influence brain activity, we are able to more accurately consider why stress has such catastrophic and lasting effects on this complex, mysterious mass of fat that exists between our ears. One of the breakthroughs that transformed the concept of neural connectivity was that of glial cells in the late 1800s. Originally thought to not be related to neuronal connections, glial cells would later prove to play a key role in the comprehensive functioning of the brain.

Traditionally, neurons were regarded as the only effective participants in the formation of brain oscillations, neural communication and the interpretation of sensory and motor inputs[30]. Following the discovery of glial cells, they were initially hypothesised to be nothing but "glue"[31] in

[28] Stenz, L. et al.
[29] Marcho, C. et al.
[30] Pascual, M. et al., pg. 797
[31] Buskila, Y. et al.

the brain, offering structural support and protection to the neurons. Since then, it has been found that glial cells, particularly astrocytes and oligodendrocytes, are actively involved in facilitating the communication between neurons and separate brain regions[32].

It is a known phenomenon that extracellular neurotransmitters and ion concentrations, as well as several other factors, influence the formation of brain waves, yet astrocytes have been found to be directly involved in this process due to their "close association with synapses*"[33],* allowing for two-way communication with neurons through gap junctions[34]. Astrocytes regulate these neuronal oscillations across larger regions in the brain by means of calcium signalling and ion channels.

As neurons initiate brain waves, the electrical impulses are synchronised within the brain in order to produce "rhythmic voltage fluctuation*"[35]* which are subjective to specific cell membrane properties and highly dependent on the speed of the conduction at the axon. These neurons can exist in a "*down state*", at a resting potential, or in an "*up-state*", when action potentials are firing in a network of spatially organised units. This results in a coordinated communication grid that is essential for cognitive plasticity, as well as the processing of sensory and motor information[36]. These sensory and motors neurons cross-communicate with one another in convoluted and highly complex lattice, making it challenging to determine exactly how the conduction of brain waves truly operate.

Since the discovery of glial cells in recent history, one can no longer interpret neurons in isolation from glial cells, with the evidence for their association being overwhelming in modern science. There are a number of different types of glial cells, yet when considering astrocytes specifically it has been established that they do not only influence the physiological functioning of the brain and synaptogenesis[37], but also play a role in and cognitive plasticity[38].

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[32] Durkee, C. A. et al., pg. 73; Buskila, Y. et al.
[33] Buskila, Y. et al.
[34] Pascual, M. et al., pg. 797
[35] Buskila, Y. et al.
[36] Buskila, Y. et al.
[37] Durkee, C. A. et al., pg. 76.
[38] Pascual, M. et al., pg. 798

While these astrocytes are unable to fire electrical impulses, they communicate bidirectionally with neurons via Ca2+ ion signals which are organised in distinct anatomical and functional ensembles[39]. They also play an essential role in the regulation and modulation of neuronal oscillation since their Ca2+ signals can travel longer distances in the brain than neuronal impulses. Astrocytes' ability to express a large number of receptors and ion channels, allows them to interact with neurons in dynamic pathways across diverse regions of the brain. Through facilitating glutamate clearance as well as administering K+ homeostasis, astrocytes have a direct influence on the excitability of neurons and synaptic transmissions[40] across the brain. It is important to note that individuals who experience chronic stress and have been diagnosed with Major Depression Disorder have significantly low levels of glutamate, which directly influences and inhibits this process from functioning optimally.

It has been proposed that these transmissions are not solely affected by the strength of an active synapse but could also involve gliotransmitters, which are defined as chemicals released by the glial cells to the neurons aiding in the communication between these cells[41]. Although this concept is still highly debatable, since there has only been evidence of their existence in manipulated *in vitro* studies, the suggested gliotransmitters are said to be dependent on the physiological and functional properties of glial cells[42], and act as signalling mechanisms in larger brain areas. These interactions are precise and complex, allowing freedom in connectivity and a variety of mechanisms to take place simultaneously in order to contribute to the larger network of communication in the brain[43].

There has been substantial evidence throughout *in vivo* trials where astrocytes were detected to respond to a large number of neurotransmitters and can express most of the same neurotransmitter

[39] Buskila, Y. et al.
[40] Pascual, M. et al., pg. 797
[41] Torres, F. et al.
[42] Durkee, C. A. et al., pg. 77.
[43] Chopra (2013). Pg. 105

receptors as neurons, despite "not being stimulated by electrical impulses"[44]. Neurons are capable of integrating information and synaptic inputs from various sources. However, single hippocampus astrocytes[45] also seem to have this ability. Although astrocytes are able to respond to a multitude of neurotransmitters, whether or not they are capable of signal integration remains debatable. However, the conclusion has been made that the intracellular calcium signals affect the functionality and ability of astrocytes to regulate synaptic signals through gliotransmitter release within the brain. Thus, further research into the integration of signals by astrocytes could reveal beneficial information about how information is processed within the brain and thus, how the stress response inhibits this integration.

Another key discovery and mechanism used in order to comprehend the interconnectivity of the brain and nervous system has been the use of single-cell sequencing in order to identify unique morphological and genetic features of neuronal cell types. Originally these sequencing techniques were only used to identify cells in tissues such as the spleen or pancreas. However, using this single-cell technology in neurobiological systems has helped to shed some light on the highly complex nature of the brain[46].

Through considering the role of genetics and transcriptomes in the brain can offer a novel understanding of how the brain operates and the billions of cells that exists within the neuronal communication network. Sequencing conducted in the somatosensory cortex and the hippocampal region has revealed nine distinct clusters of cells, including interneurons, oligodendrocytes, astrocytes, and microglial cells[47]. The results were confirmed by the presence of specific functional markers and single-molecule RNA fluorescence in situ hybridization (RNA-FISH). When a transcriptomic profile within the human cortex was outlined using neural, glial and vascular cells[48]

[44] Pascual, M. et al., pg. 797
[45] Pascual, M. et al., pg. 798
[46] Guillaumet-Adkins, A. et al., pg. 497
[47] Guillaumet-Adkins, A. et al., pg. 398
[48] Guillaumet-Adkins, A. et al., pg. 402

based on marker genes, it revealed neurons can be separated into “excitatory” and “inhibitory” subclasses[49]. These transcriptomes and genetic markers were analyzed from pre-natal to the adult brain, presenting that the neurogenetic expression differs vastly between fetal and adult brain tissues, aiding in the understanding of the plasticity and interactions within the cortex. This substantial difference between fetal and adult brains reveals the power of epigenetics, but also emphasises the vulnerability of our brains as we grow up. This could explain why early life stressors and childhood trauma has such intense, long-lasting, and complicated effects on the brain structure and functioning.

In conjunction with glial cells, small membranous vesicles called exosomes facilitate communication in brain. These vesicles contain proteins, RNAs and lipids which are often taken up by surrounding cells whose internal structure is then altered accordingly[50]. Specifically, exosomes act as messengers between neurons and glial cells, facilitating communication throughout the central nervous system (CNS). This includes their function during pathological conditions in the brain where exosomes contain inflammatory molecules and interact with neurons in order to induce neuroinflammation, as well as neurodegeneration. Each exosome’s function is dependent on the neural cell from which they originated. Yet, a vast number of exosomes play critical roles in the development of CNS, regeneration after injury and the maintenance of an adult brain, specifically removing unwanted proteins and other molecules. Exosomes thereby influence the neuronal network communication through reorganising synapses and stimulating microglial phagocytosis[51].

A further vital function of the glial cells is their ability to identify and clear toxic compounds and induce neuroinflammation as an immune response, especially when an individual is experiencing acute or chronic stress[52]. They respond to endocrine signals sent to the brain and are able to readily cross the BBB enabling communication channels that regulates physiological processes in the CNS

[49] Guillaumet-Adkins, A. et al., pg. 400
[50] Guillaumet-Adkins, A. et al., pg. 395
[51] Pascual, M. et al., pg. 799
[52] Williamson et al., pg. 37

via systemic inflammation[53]. When neural damage occurs or glial cells are activated by specific signals, the microglia (the macrophages of the central nervous system) together with the astroglia induce an innate immune response resulting in neuroinflammation. This often occurs when misfolded pathogenic proteins or mutated microRNA are transported into the neurons via astrocytic exosomes and could result in neural death or degeneration[54].

Thus, when examining stress and neurodegenerative diseases such as Alzheimer's and Parkinson's disease, it is important to note that exosomes also contain miRNA which can deregulate the gene expression of neighbouring cells. Then, through astrocytes transferring proteins in exosomes, these proteins can induce neural cell death and neurodegeneration. In autoimmune diseases and Multiples Sclerosis there has been close to 100 mutations found in these microRNAs of tissues which reduces the immunity of cells through inhibiting the process of cell differentiation[55].

It is important to note that exosomes have the potential to act as therapeutic agents for various neurological disorders, to monitor neurodegenerative diseases, and to reverse possible changes caused by previous trauma or periods of enduring stress[56]. They are ideal agents for therapies since they are able to deliver antigens to the peripheral nervous system and other specific tissues by crossing the BBB. In a recent study done by Pascual et al. exosomes that were injected with curcumin, a naturally anti-inflammatory molecule, increased the stability and availability of the molecule in vitro, significantly improving its survival within the tissue. This curcumin treatment was effective in mice that exhibited brain inflammation and many other therapies have been developed since in order to relieve neurological disorders and traumatic brain injuries. Since exosomes a can cross the BBB they can also be used as biomarkers to diagnose brain disorders and early neural alterations linked to emotional or physical distress[57]. However, additional research is required in

[53] Williamson et al., pg. 41
[54] Pascual, M. et al., pg. 799
[55] Pascual, M. et al., pg. 799
[56] Pascual, M. et al., pg. 800
[57] Durkee, C. A. et al., pg. 78.

order to develop technology capable of accurately identifying and detecting these biomarkers to successfully use them in diagnoses.

Similarly, the exosomes derived from hypoxia-preconditioned mesenchymal stem cells alleviated cognitive decline in mouse models of Alzheimer's disease by rescuing synaptic dysfunction, regulating astrocytic and microglial activity, thereby decreasing pro-inflammation factors. These mesenchymal-stem-cell-derived-exosomes also revealed to promote neurogenesis and the recovery of cognitive function in Alzheimer's disease mouse models[58]. This is significant evidence in the hope for a cure of the Alzheimer's disease in the future.

A region of the brain that is highly affected by neurodegenerative diseases such as Parkinson's disease, stress, addiction, schizophrenia and depression is known as the mid-brain. In a study done using the single-cell RNA-seq mentioned earlier, there has been ground-breaking evidence regarding the neurogenesis and gene expression. Since the midbrain controls functions such as "vision, hearing, motor control, alertness, and temperature regulation", it was significant to note that when considering the dopaminergic system, neurogenesis and diversification occurred at different stages of development[59]. This neuronal diversity was also detected in the somatosensory nervous system, as well as in olfactory and retinal neurogenesis, which corresponded to astrocytes, microglia and endothelial cells.

Oligodendrocytes, another type of glial cells, are responsible for producing the myelin sheath which surrounds the axon and maintains the integrity of the action potential. Their function has been proposed to be related to exosomes as these vesicles travel from oligodendrocytes to neurons containing the myelin protein molecules. These exosomes provide protection and promote survival of neurons in stressful conditions pertaining to oxygen and glucose deprivation[60].

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[58] Pascual, M. et al., pg. 801.
[59] Guillaumet-Adkins, A. et al., pg. 401
[60] Pascual, M. et al., pg. 801

Although oligodendrocytes are considered to be functionally consistent, through the single-cell RNA-seq there has been thirteen dissimilar cell populations identified. Through analyzing these diverse cell clusters, a transcriptional range was discovered between distinct oligodendrocytes, each committed to a different expression of the genes involved in development, differentiation and myelin formation. These oligodendrocytes include oligodendrocyte precursor cells (OPCs), differentiation-committed oligodendrocyte (COPs), newly formed oligodendrocyte (NFOL1 & NFOL2), myelin-forming oligodendrocyte (MFOL1 & MFOL2), mature oligodendrocyte (MOL1 & MOL6), and vascular and leptomeningeal cells (VLMCs) of which each expressed different genes that contributed to the overall functioning of oligodendrocyte[61]. As a result, it has been recognised that oligodendroglia affect the physiology and gene expression of neurons by controlling specific signalling pathways and miRNA transfer[62].

In conclusion, glial cells do not only provide structure or protection to neurons, they integrate and organise the synaptic activity in the brain to enable an array of unique and flexible responses within the brain. This bidirectional communication between glial cells and neurons is highly specific in terms of cell structures, synapses and circuits, and therefor there are unique gliotransmitters released by glial cells in order to coordinate and integrate information with the surrounding neuronal environment[63]. All of these interactions allow for a complex and intricate network of communication in the brain with a liberating sense of plasticity and flexibility that allows for individually tailored responses, particularly to the stress we experience in our daily lives. Due to this network-like construct of communication in the brain and the limitless quantity of connections that are possible, it is challenging to make accurate conclusions as to how the networks in the brain function and how they are altered as a result of childhood trauma, acute stress, or prolonged periods of distress.

[61] Guillaumet-Adkins, A. et al., pg. 400
[62] Guillaumet-Adkins, A. et al., pg. 406
[63] Buskila, Y. et al.

However, with every study and new breakthrough neuroscientists discover more of this void of unknown territory that is the human brain and its consciousness.

Epigenetics and Stress

The role of stress in the brain has been an intense topic of debate in the 21st century due to the spike in societal pressure, depression and anxiety globally. However, before diving into how stress affects our daily lives and correlates with increase in various illnesses and a shorter lifespan, it is important to understand how stress affects the brain even before we are born.

In the brain, epigenetics plays a vital role in the development and maturation of synaptic circuitries and neuronal pathways[64], since these structures drastically adapt to environmental conditions during adverse experiences, early-life stress (ELS) and traumatic events during stages of developmental plasticity.[65] These alterations often result in neurological deficits later in life and are able to be transmitted to future generations. Hence, they are significant in understanding conditions such as anxiety, depression, Alzheimer's disease or post-traumatic stress disorders.[66]

An organism's environment as well as their genome influences the extent of epigenetic changes. However, depending on their interaction during ELS, individuals tend to either be at higher risk for stress-related psychiatric diseases or, in contrast, display advanced resilience towards traumatic circumstances later in life. Considering the effects of early life stress (ELS), as well as stress in adulthood, on the epigenome in both animal models and humans help us to gain a better understanding of the interactions between the environment, an organism's genes and their behaviour.

The most common form of epigenetic alterations is DNA methylation, which occurs at CpG dinucleotides or CpG islands (regions of high CpG density) and involves the addition of a methyl group to a specific locus on the gene. As a result of methylation, the gene is not actively transcribed,

[64] Groger, N. et al., pg. 1038
[65] Groger, N. et al., pg. 1038
[66] Bergink, V. et al.

but rather silenced[67]. This occurs due to histone acetylation and chromatin compaction which prevents the transcription of the gene.[68] Recently, it was established that the genetic and behavioural responses to the environment is dependent on the SAM (s-adenosyl methionine) compounds which is produced by the liver and required by the enzyme responsible for DNA methylation, since SAM is a methyl-group donor. During stressful events, levels of SAM increased, enhancing DNA methylation in specific genes which led to a subsequent change in the individual's behaviour[69]. Methylation can occur in both peripheral tissues, mainly blood cells, and in brain tissue, which more common in cases of ELS, depression and epigenetic inheritance.[70]

These epigenetic changes are mostly established during sensitive periods of brain development in mammals, which fluctuates depending on genetically programmed phases of elevated synaptic plasticity. The extent of plasticity is also dependent on the maturity of the brain, with fetal brains displaying different functional neuronal connectivity than adolescent or adult brains.[71] Thus, adverse environmental events at different stages of life will affect the development of regions such as the amygdala, hippocampus and frontal cortex of the brain in unique ways. One of the most significant long-term consequences of stress, whether periconceptional, prenatal or ELS, influences the hypothalamic-pituitary-adrenal (HPA) axis activity which induces structural and functional changes in the brain, ultimately influencing behaviour. This alteration has been found to either increase anxiety-related behaviour later in life or promote a degree of resilience to adult stressors, depending on when the ELS was experienced[72].

Early Life Stress

When studying the inheritance of the effects of stress on the epigenome through generations, a newfound emphasis has been placed on both maternal and paternal preconception stressors (PCS).

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[67] Marcho, C. et al. pg 2
[68] Stenz, L. et al.
[69] Saunderson, E. A. et al.
[70] Penner-Goeke, S. et al., pg. 400
[71] Groger, N. et al., pg. 1041
[72] Penner-Goeke, S. et al., pg. 401

PCS has been linked to a significant decline in social interaction[73], long-lasting effects on behavioural distress, and learning difficulties later in life. Paternal PCS has also been found to cause sex-specific changes in dendritic morphology in the medial prefrontal cortex of their offspring, linking to behavioural variations such as reduced avoidance and fear[74]. These changes are dependent on the time between PCS and conception.

When considering sperm epigenetics and paternal PCS in male mice models, stress-levels, nutrition, exercise and drugs were all found to influence fertility and fetal development. Male rat cells that were exposed to different dosages of testicular cancer therapies, showcased 143 loci that were altered in spermatids and spermatozoa compared to controls. The effect of recreational drugs also contributes to PCS since the negative effects of smoking tobacco, as well as marijuana, targets multidimensional pathways associated with epigenetic alterations in the somatic tissues of offspring[75]. When comparing sperm methylation in men who smoke to non-smokers, there has been 141 uniquely methylated CpG sites identified that influence spermatogenesis. These aberrations were found to cause behavioural impairments and learning deficits in both male and female offspring[76]. The transmission of these epigenetic changes is associated with the release of microRNAs in the epididymis, triggered by stressful events. Since the epididymis is the site where sperm cells mature, it is hypothesized that these stress-related microRNAs are incorporated into the genetic material of the sperm cell and thereby influences offspring DNA[77].

A stressor that has been a universal experience, although varying in its severity, to most people around the world has been the COVID-19 pandemic which has stretched over almost 2 years and has become a part of our everyday lives to some extent. However, this event and many other chronic stressors, does not only take a significant toll on a person's mental health, but affects future

[73] Shachar-Dadon, A. et al., pg. 11
[74] Stenz, L. et al.
[75] Marcho, C. et al.
[76] Marcho, C. et al.
[77] Rodgers, A. B. et al.

offspring, specifically due to stress experienced by men prior to reproduction. The father's stress levels prior to sperm production have been found to have significant impacts on fetal brain development[78].

As mentioned before, changes in a male's extracellular vesicles (microRNA specifically) can enter the nucleus of developing sperm and have a noteworthy impact on both sperm maturation and DNA integrity. When analyzing these effects in mice treated with the stress hormone corticosterone, several changes were observed regarding the size, protein and microRNA levels of extracellular vesicles. When these mice produced sperm and researchers used the "stressed" sperm to fertilize eggs[79], the offspring showed great variation in brain development patterns and as adults they responded to minor stressors much more intensely than the controls.

When looking at human sperm and related stress levels, researchers asked students at a university to donate sperm once a month over the course of 6 months and asked them to complete a "stress survey" each time, reporting on their own stress levels and behaviours. The same changes observed in mice were seen in the extracellular vesicles of the human sperm of those individuals that reported they had been experience an unusual amount of stress[80]. This confirms the fact that long periods of stress have a significant impact on the sperm production, and possibly on the development and mental health of future offspring.

In previous studies completed in generations who had children during times of substantial stress, such as Vietnam war survivors, holocaust survivors, and individuals living in severe poverty, it was found that children showed a significant increase in mental health struggles, bipolar disorder, depression, and schizophrenia[81]. As studies reveal more and more about the detrimental impact that our stress could have on the lives of our children, it is important to stay cognizant to the way we

[78] Chan, J. C. et al.
[79] Chan, J. C. et al.
[80] Chan, J. C. et al.
[81] Chan, J. C. et al.

manage stress, the choices we make to minimize stress, and whether there are new ways to prevent our stress from negatively impacting future generations.

Animal models reveal significant evidence that maternal stress during the gestational period has lasting effects on epigenetic changes in brain development and the onset of various diseases in offspring. Prenatal Stress (PS) results in synaptic and morphological changes in neuronal networks, influences the limbic system[82] and often dysregulates cortical circuits[83] which enhances depression and anxiety in offspring.[84] This behavioural dysfunction was studied in mice where offspring that experienced PS had low motivation in the force swim test when compared to controls.[85] Similarly, when PS rhesus monkeys were studied they exhibited amplified anxiety, strongly associated with the dysregulation of the HPA axis, and lower cognitive performance than unexposed monkeys.[86]

In another study that assessed the effects of long-term maternal stress on the growing fetus correlated with increased levels of corticotropin-releasing hormone (CRH) in the amniotic fluid. This CRH regulates the administration of cortisol, a hormone released during stressful periods. Due to the fact that the placenta can release CRH during pregnancy, it enters the amniotic fluid, and thus the fetal metabolism. Studies is tadpoles revealed that elevated levels of CRH and cortisol has been found to increase growth rates of the fetus, which could theoretically be true for humans, although not yet proven[87]. Thus, despite the fact that PS does not directly fall under ELS, it is observed to have significant influence on offspring and future generations.

A link between PS, ELS and future eating habits in mice populations further indicates these effects. Scientists genetically engineered a generation of mice in order to manipulate the brain's response to stress, focussing on the corticotropin-releasing factor (CRF) system which is linked to high levels of anxiety, suppressed appetite and inflammation. This system was activated by changing

[82] Groger, N. et al., pg. 1044
[83] Penner-Goeke, S. et al., pg. 403
[84] Dickerson, P. et al., pg. 589
[85] Frye, C. et al., pg. 321
[86] Clarke, A. S. et al., pg. 270
[87] La Marca-Ghaemmaghami et al.

the drinking water of the female engineered mice during the third trimester of pregnancy, since handling them would trigger additional uncontrolled stress responses which could compromise results. The offspring from these engineered female mice displayed epigenetic methylation in specific genes of their hypothalami[88]. However, this transmission of the epigenetic methyl groups was not sufficient to result in immediate behavioural changes. Only once pups were placed in a stressful environment with limited access to food, was the epigenetic trait activated with all of the offspring from the engineered mice exhibiting binge-eating-habits, whereas the unaffected mice did not[89]. This research highlights the powerful impact of gestational stress on the fetus's life.

There are several distinctive forms of ELS such as abandonment, weaning, handling and exposure to different smells or sensations. One of the most significant of these ELSs in mammalian childhood is the shift from the mother's milk to independent nutrition, known as weaning[90]. There has been evidence that early weaning can be related to long-lasting behavioural changes and alterations in the brain structure of offspring. Due to psychological, physical and nutritional stressors, early weaned mice exhibited an increase in anxious behaviour in adulthood linked to a dysfunction in the HPA axis.[91] As these early-weaned mice were exposed to stressors in adulthood, they appeared to be more aggressive compared to normally weaned mice.

Another form of ELS is the absence of maternal care in the initial stages of development. In one of the first studies on epigenetic changes in mice, variations in maternal care seemed to have an effect on endocrine and behavioural responses of the offspring. In mice pups that experienced an extensive level of grooming and licking from their mothers, "reduced HPA axis reactivity, decreased anxiety behaviours, and enhanced cognitive capabilities in adulthood" were observed[92]. However, when infant rats were exposed to neglectful mothers, they displayed enduring changes in what is

[88] Schroeder, M. et al., pg. 1271
[89] Schroeder, M. et al., pg. 1272
[90] Groger, N. et al., pg. 1045
[91] Kikusui, T. et al., pg. 90
[92] Liu, D. et al.

known as brain-derived neurotrophic factor (BDNF) gene expression, which was detrimental to neural growth and development[93].

Notably, these epigenetics alterations have been observed to be transmitted through generations, implying that traumatic events in childhood could have multi-generational effects. In a study where infant male mice were exposed to unpredictable maternal separation periods; they were found to "transmit depressive-like phenotypes over two generations when bred with stress-naive female"[94] suggesting that ELS could result in germline-dependent inheritance. The transference of these epigenetic adaptations can be spread across two generations, known as intergenerational inheritance, however when the epigenetic changes persist in the F3 generation, this is classified as transgenerational inheritance[95]. This transgenerational inheritance has been seen in multiple studies, specifically in the previously mentioned study where licking and grooming by the mother to the pups induced a genome wide change in the DNA methylation and histone acetylation at specific sites in the hippocampal GR promotor, causing declined levels of anxiety and stress-related activity. This change was transmitted to future generations, while the extent of methylation was influenced by maternal behaviour in those respective generations[96].

Interestingly, the effect of ELS has been found to either correlate with an increase in depressive-like, anxious behaviour with a lack of motivation, or it could induce a degree of resilience to enhance stress coping mechanisms in adulthood. This resilience is defined as the ability to maintain a healthy mental state despite adverse life events[97] and can be observed in animal and human studies where ELS enhanced emotional and cognitive development, allowing for resilience to traumatic or stressful events in later in adulthood[98]. When monkeys were studied to determine whether resilience is established through a stress inoculation concept during the weaning period, it

[93] Roth et al., pg 761
[94] Groger, N. et al., pg. 1049
[95] Groger, N. et al., pg. 1049
[96] Groger, N. et al., pg. 1050
[97] Penner-Goeke, S. et al., pg. 404
[98] Fuentes, S. et al.

was found that the "stress inoculated monkeys" had a lower level of HPA-activity, were less apprehensive to later-life stressors and were able to better regulate their emotional responses to triggering events[99].

Although ELS could have beneficial outcomes later in life for cognitive and emotional competence, it is highly dependent on the intensity and the timing at which the trauma occurs during infancy. The stress coping mechanism relates to ELS in a "match/mismatch" manner. In a study done by the scientist Champagne, he detected those individual mice who experienced ELS were capable of higher functioning cognitive behaviour during a stressful event later in life that "matched" their experience of ELS. However, during low-stress periods they tended to express lower brain activity than healthy individuals[100]. This information is vital in order to understand the complex mechanisms of behaviour, the impact of ELS and how it influences genetics and brain structure.

A major source of trauma in early human childhood is historical events such as war, genocide, famine etc. The epigenetic alterations resulting from these events and their transmission to future generations has been studied in several publications. One such study focused on the Tutsi genocide in Rwanda, which resulted in the transmission of abnormal HPA-axis activity from mothers to offspring. Mothers that were pregnant during the time of the genocide, as well as their offspring, were more likely to be affected by post-traumatic stress disorder (PTSD) and major depression disorder. This is due to the methylation of the NR3C1 promotor in the blood, which resulted in lower levels of cortisol and NR3C1 plasma levels[101] compared to healthy mothers and children. In a similar study of Holocaust survivors, HPA-axis activity that induced epigenetic changes through DNA methylation in the FKBP5 gene was shown to be transmitted intergenerationally. The FKBP5 gene is has been associated with PTSD and major depression disorder, since it caused children of Holocaust

[99] Parker, K. J. et al.
[100] Weaver, I. C. et al.
[101] Perroud, N. et al.

survivors to be more susceptible to these disorders later in life[102]. Although both of these above-mentioned studies were poorly regulated and failed to provide concrete evidence due to the lack of controls, they shed some light on the effect that trauma has on the epigenome and brain activity in humans.

In a similar study that looked at the epigenetic profiles of individuals who we born from women who were expecting during Quebec's Ice Storm in 1998, significant changes were found in their genetic expression compared to other individuals in their age group[103]. In particular, it was the T-cells of the immune system that showed the most significant epigenetic alterations, suggesting that maternal stress could influence the extend of DNA methylation in immune cells. This study also suggests that the degree of stress is to always a determining factor in epigenetic changes for the foetus, since these women experienced the ice storm in various degrees of severity. However, they all had the underlying stress of the natural disaster and all 36 of their children who were born during that time show similar patterns in DNA methylation of the T cells[104].

These children, who are now in their early twenties have been found to have a greater risk for asthma, diabetes and obesity than the average child[105]. This is yet another example of how important it is for humans, but especially expectant mothers, to manage and respond healthily to various stressful events. We are not always able to control things like an ice storm, or a global pandemic. However, acknowledging that our stressed states have lasting impacts on our own lives and possibly the lives of our children, allows us to take action and work towards managing and minimizing those stressors that we are able to control.

[102] Yehuda, R. et al.
[103] Cao-Lei, L. et al.
[104] Cao-Lei, L. et al.
[105] Cao-Lei, L. et al.

Telomeres

Although DNA methylation and transcription factors are key features of epigenetic changes, telomeres are also altered due to high-stress environments which relates to aging and risk of disease. Although these changes may largely be due to ELS, epigenetic variations can also result from short term stress. Telomeres are found at the end of chromosomes and serve as protective DNA-protein complexes to prevent chromosomes from deteriorating and helps maintain the integrity of genetic material. When telomeres shorten or shrink, the risk of cancer, cardiovascular disease, dementia and pre-mature mortality has bene found to increase significantly.

High levels of anxiety have been strongly associated with shortened telomeres and in a recent study conducted on middle-aged women, it was found that telomere length of women who were highly anxious corresponded to that of healthy women who were six years older, showing how stress-related psychiatry can induce premature aging. It is important to mention that this study was not able to identify whether shortened telomeres caused anxiety or vice versa[106]. Related to this study, there has been novel interest in the effect of prenatal stress on the premature shortening of telomeres of offspring. Researchers measured telomere lengths in 319 babies and 318 mothers, by collecting saliva samples from the mothers and umbilical cord blood from babies postpartum. Interestingly, infants of mothers who experienced increased levels of stress during pregnancy had shortened telomeres. However, those new-borns whose mothers suffered from long-term psychological disorder showed no abnormalities in telomere length[107]. This study also found that females were born with longer telomeres than males, and that smoking during pregnancy did not affect the telomere length of babies, however shortened the mother's telomeres.

When measuring the telomere length of new doctors at the start of their first residency year compared to the end of that year, their telomeres had shortened six times faster than usual. Doctors

[106] Brigham and Women's Hospital
[107] Send, M. et al.

who were quick-tempered and highly anxious were found to have shorter telomeres compared to the others and those who worked longer hours during the week experienced an increased rate of telomere shortening[108]. This shortening of telomeres exposes individuals to many risks, especially in terms of disease and cancer. These results are significant when considering the stress that is placed on entry-level doctors and residents, calling for a re-evaluation of how the work environment effects the wellbeing necessary for physicians to provide the optimal healthcare.

Why Stress Persists for Some but Not Others

In studying our response to stress, looking at a specific incidence at an exact time is both insufficient and uninformed. We need a broader picture in order to make accurate observations and knowledgeable assumptions. As a result, researchers studied stress by looking at it at the three basic levels: neural, cellular, and behavioural. After analyzing brain images, epigenetic profiles and behavioural reports from 49 young health males they found that our susceptibility to stress is largely related to differences in microRNAs[109]. The study looked specifically at fMRI images and miRNA exerts (via a blood test) during and 3 hours after participating in a socially stressful activity. About 20 minutes after the activity, it was clear that the group was separated into **sustainers** (still stressed) and **recovered** (no longer stressed) individuals. A change in the expression of miRNA-miR-29c was what influenced this discretion between groups. An increased level of miR-29c predisposed individuals to be more stressed and maintain that level of stress for a longer period of time after the stressful occurrence passed[110].

Imaging the brain whilst experiencing stress is one thing but measuring its resilience to stress is an emerging concept that has been made possible with ground-breaking technology and has provided invaluable insight into the human stress response. This has made it possible to facilitate early intervention programs in individuals that find themselves in high stress environments such as

[108] Ridout, K. et al.
[109] Vaisvaser, S. et al.
[110] Vaisvaser, S. et al.

soldiers and those in emergency medicine[111]. Through the future possibility of blood tests, scientists are looking for ways to determine a person's resilience to stress and as a result, provide the needed treatment or prevention therapy.

Each of us experiences stress in our daily lives, and a healthy amount of it is beneficial for our overall physical and mental health. However, the challenge comes in when these daily occurrences become paralyzing, leaving you stressed for days, weeks, months, or even years. When the body exists in a constant state of stress, we are unable to process and regulate our bodily functions at an optimum level. Through this study it is clear that there is a traceable cause for why some are more prone to stress than others, and through blood tests we can treat these individuals with personalized plans to help prevent or combat future and current stress[112]. As the work of interdisciplinary studies of stress continues, researchers will focus on the role of our brain oscillations and how their dynamics influence our stress perceptions and responses. Although very insightful, it is important to note that this study was only conducted in a cohort of young men[113]. In order to make accurate and helpful assessments for treatment in the future, one would have to extend the study to other demographics, especially women, since their stress response differs greatly from that of men.

The Clock is Ticking

Every single one of us, whether we care to admit it or not, has a biological clock ticking every second of the day, and whether we like it or not, many of the decisions we make about our fitness, health, careers, and the places we live will impact how fast or slow this clock ticks. As it turns out, we all age at different speeds, according several studies that have analysed the chemical transformations that occur within our DNA as we age and found that the time at which these changes

[111] Vaisvaser, S. et al.
[112] Vaisvaser, S. et al.
[113] Vaisvaser, S. et al.

occur are different for various people[114]. It has also been determined that this mechanism of looking at age, coined the "GrimAge" by Yale researchers[115], is much more accurate than looking at our chronological age.

The main thing that makes this clock tick faster? Stress. If you live in our society today this is not great news, because the more time passes, the more it seems as though chronic stress is unavoidable. However, the good news is that although stress accelerates our epigenetic aging process, we can combat these changes in our DNA through various internal and external changes to our environment[116]. Mainly, developing emotional resilience and a strong sense of self-control were the two key markers for people who experience the same level of stress but had a lower GrimAge compared to those who had little self-control or emotional maturity and resilience.

These findings come from a study done on 450 individuals aged 19-50 who reported on their stress levels and emotional resilience, and then provided a blood sample to analyse DNA markers. There was a clear correlation between individuals who perceived high stress levels and those who showed early aging in their DNA profiles, also exhibiting different physiological symptoms such as early onset heart disease, metabolic issues, or mood disorders[117]. The interesting part of the data was that these chronic stress levels affected people differently, and those who had a higher emotional intelligence score were less affected by their stressful experiences[118]. These findings provide valuable insight into the power of strengthening our emotional resilience and ability to process, control, and accept our emotional experiences. If anything, it might just add a few years to your life.

Everyday Stress

Despite the clear correlation between stress and epigenetic changes in organisms which can result in various behavioural and developmental alterations and deficits, emerging research has

[114] Harvanek, Z. et al.
[115] Harvanek, Z. et al.
[116] Harvanek, Z. et al.
[117] Harvanek, Z. et al.
[118] Harvanek, Z. et al.

correlated practices such as meditation and exercise with beneficial epigenetic changes[119, 120]. These customs could potentially be used to counter-act the effects of stress, depression and trauma. As research in this field continues, it will provide us with a more comprehensive understanding of epigenetics, the brain, the process of inheritance and how the interactions between these concepts lead to certain behavioural changes, pathological risk and brain development.

When we stress, our body responds in ways that we might not always be consciously aware of or see the effects of in our daily lives. However, as the field of epigenetics grows and the Human Genome Project reveals more and more data with regards to genetic markers transferred throughout generations, it is evident that stress, more than most other things, effects the genetic make-up and epigenetic profiles of our offspring.

Most of the epigenetic effects of stress have been linked to cancer and chronic diseases in future generations through complex and rudimentary cellular mechanisms that are only being discovered as technology improves. The changes in our epigenetic profiles due to environmental changes and stress is often undetectable. However, as we learn more about the human genome, it is evident that these changes could have significant, possibly detrimental impacts on the future human genome. The positive thing is that we can influence this hereditary through lifestyle choices and changes, most noticeably, through living slower paced, less stressed and more active lives.

Neuroscience and Stress

When studying the brain, it important to note how the brain's architecture plays a role in its functioning, particularly, how important communication between different brain regions are in order to comprehend, integrate and respond to the world around us. Due to its complexity, researchers have formulated several different theories for how our brains function and interpret information[121]. As we become increasingly aware of the brain's sheer complexity, it could be helpful to consider a

[119] Puterman, E. et al.
[120] Kaliman, P. et al.
[121] Hahn, G. et al., pg. 120

combination of various theories in order to provide a more holistic and accurate view of the neurological mechanisms.

Looking at the brain as a dynamic system of various networks helps us understand why activity in one region could have profound effects on other, seemingly unconnected, responses. By combining the synfire communication, communication through coherence, and communication through resonance theories, it is possible to provide a more comprehensive model of how the brain processes information. Particularly, it provides a more complete explanation for how neurons interact with each other depending on their stimulus level, and whether or how a signal in one region of the brain reaches another region[122]. Having a better understanding of these two fundamental concepts is foundational to understanding how the brain functions as a whole, and how it responds to different types of stress.

A particularly important part of our brain's capability to process information and respond to stress is the role of oscillations and brain rhythms with regards to neuronal communication[123]. These effect not only specific neural circuits, but could influence entire brain regions, altering the brain's ability to effectively communicate between different areas in different degrees of severity depending on the level of the stress or trauma experienced. This could explain why we sometimes feel as though we "shut down" in moments of immense stress, emotional difficulty, or physical trauma[124].

Throughout the evolutionary history of human beings, our "survival mode" has shifted a great deal from our early existence to now. Our fundamental survival concerns up until as recent as 100 years ago were mostly concerned with food security, wild animals, a safe shelter, procreation, birth, and being protected from the natural elements. Although much of this still constitutes our underlying concerns today, we have become much more complicated as our world has progressed to a more technological, globalized, and competitive environment. Some of the things that could elicit

[122] Hahn, G. et al., pg. 121
[123] Hahn, G. et al., pg. 121
[124] Dispenza, J., pg. 266

this “survival mode” stress respond in an individual today are filing your taxes, having health insurance, paying college fees, managing your debt, unemployment, social media comparisons, modern-day terrorism, and contrasting political views between you and someone you love. However, even though the external stressors are not directly threatening our lives, we still respond in a similar neurologically mechanism than we did when we were concerned with a predator killing us in.

The most important part of this stress response is whether or not we feel prepared for the expected events. While our neocortex analyses our external environment and tries to predict what might happen next, we become aware of all sorts of possible dangers that exist beyond our control. In these moments, when our brains recognize a potential danger or stressor or threat to our survival (physically or mentally), our neocortical functioning changes and the stress response is initiated[125]. Our minds take us back to a similar experience we might have had before, moments we remember feeling the same type of threat, or when we saw similar event transcribing. In doing so, we re-activate previously existing neural connection that we have used before via the chemical stress response. As a result, we respond in the exact same way we have done before when a stressor was perceived in our environment. The response becomes automatic, uncontrolled, and often comforting because we do not have to think about it. The question arises is whether this automatic, subconscious response is healthy for us in relation to acute stress, and whether we could intentionally change the circuits we activate in these critical moments[126].

The key to understanding the stress response is acknowledging that when we experience stress our body shifts out of its state of homeostatic equilibrium. In order to correct this imbalance in our internal environment, the body launches the stress response, which includes a rush of hormones, inflammatory cytokines, and other physiological transformations in order to bring the body back to its usual state of homeostasis. This response could be due to a stressor that is currently experienced,

[125] Dispenza, J., pg. 266
[126] Dispenza, J., pg. 268

or because of the way we think about the past, or fear the future. For each of us, these responses look different, and while some might seem incredibly stressed both inside and out, others do a really good job at constructing a calm facade whilst slowly giving way to the internal stress that they are experiencing. Hiding our stress from other people or denying it for ourselves only leads to chronic pain, lasting medical issues, and deteriorating mental health.

Putting yourself in a constant state of high stress, looking for any stressors that might cross your path in the near or far future, activates your body's survival mode, making you hyper-vigilant. In this state our bodies do not have the time to rest, repair, regenerate, and reset the necessary systems that help us function in a healthy and balanced way. Our metabolism slows down, our blood vessels are constantly constricted, we produce an excess of adrenaline, and our intelligence is silenced because we are unable to calmly think about the situations at hand. When we experience these stressors (past, present, or future) our mind responds in both a neurological and chemical way.

Neurologically, we respond almost automatically when our nervous system registers a threat in our environment, or in the future. The autonomic nervous system is activated and immediately communicates with the adrenal glands via the peripheral nerves. Once the adrenal glands have received the signal of the stress response, it produces a cascade of adrenaline directly into the bloodstream[127]. This process initiates the overall stress response in the human body and brain. All non-essential systems shut down, most of our blood is redirected to the muscles, our pupils dilate, breathing increases, and we prepare to fight or flee.

The chemical response is slightly different and takes longer to transpire. This process is initiated by our thoughts and the neural networks involved in registering and responding to mental or physical stressors. The hypothalamus is largely responsible for the initiation of this stress response, registering the activation of various nerves that signal a potential stressor, and then using this

[127] Dispenza, J. (2017). – pg. 270

information to respond through chemical messenger proteins known as peptides. These peptides, mainly *corticotrophin releasing hormone* (CRH), alerts the pituitary gland of the stressor. *Adrenocorticotropic hormone* (ACTH) is released by the pituitary in response, and it incites the adrenal glands to release *glucocorticoids* which sends the stress signal throughout the body and plays a large role in the shift out of homeostatic equilibrium[128]. Although this takes a couple of hours to occur, it produces the same changes in the body as the neurological response does. However, these changes last much longer. For someone who experiences chronic levels of stress, this is an ongoing and never-ending process, which leads to the health issues related to stress – such as heart disease, strokes, diabetes, depression, persistent joint pain, etc.

When our brains register that we are experiencing a stressful situation, this stress response is designed for a quick onset and a quick resolution. However, as we have evolved and our world has become increasingly complex, humans live in a persisting state of stress. We experience stress in every sphere of our existence – physical, emotional, and chemical. The constant worrying, planning, anticipating, and preparing for any and all potential change that might offset our routine results in our bodies experiencing a chronic state of stress. We experience physical stress due to our environment – we get in a car crash, or break a limb, or fall down the stairs, or burn ourselves on the stove, or most commonly, we sleep too little to rest and recuperate fully. Emotionally we stress about our relationships, whether we will get the job, or impress the girl or guy, or be able to pay our rent, or lose a loved one. And lastly, often the one that is best hidden from plain sight, is the chemical stress we experience when we are exposed to environmental pollutants, toxins in our foods, an excess of fat, sugar or salt, or any other allergens[129]. Due to stress having invaded our lives to an extent it has never done in the history of the human existence, it makes sense that close to 90% of Americans who are admitted into hospital have a health concern that relates to chronic stress[130].

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[128] Dispenza, J., pg. 270
[129] Dispenza, J., pg. 272
[130] Dispenza, J., pg. 276

Neurologically, the most concerning fact about the stress response is that the *glucocorticoids* that are secreted throughout the process has severely damaging effects on the hippocampus. Being the center for learning, information encoding, memory retrieval and a host of other critical functions, damage in this brain region leads to detrimental influences on our ability to think clearly, adapt, and learn new information. In a study done on mice brains, scientists mimicked this effect by targeting the hippocampus of the mouse and inducing damage via radiation[131]. Prior to radiation the mice were eager to explore their environments when placed in a new location. However, once the hippocampus was targeted the affected mice remained in the place they were positioned by the researchers. They showed no interest in exploring or learning more about their surroundings. We know that the hippocampus is involved in making sense of new experiences, but it turns out that without a functioning one there is very little motivation to seek out any novel understandings[132]. This sheds light on why we resort to our most comfortable routines when we are chronically stressed. However, what happens when our daily routines include being stressed? When we make "being stressed" a part of our daily routine, when it becomes a personality trait rather than a fleeting and temporary experience, we are trapped in a never-ending, incurable cycle of slowly deteriorating. The hardest part, possibly the most stressful part, is to break this cycle for good.

Contrary to general beliefs, our brains are capable of regenerating neurons well into our late adulthood, and so, it is never too late to break this cycle. The good news is neurogenesis is especially active in the hippocampus. Changing our routines, letting go of the comfort that "being stressed" brings us, and seeking out new and exciting experiences will not only improve our mental health and critical thinking abilities, but it will make our brains healthier, and possibly save our lives. Further effects on the brain and memory will be discussed in later chapters.

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[131] Dispenza, J., pg. 282
[132] Dispenza, J., pg. 283

Brain and Body Connection

As mentioned previously, what makes the human brain as powerful and complex as it is, is not necessarily the structure itself, but its connection to the rest of the body and its ability to adapt to changes in both our internal and external environments. The human stress response is dependent on a plethora of factors; however, our experience of stress is predominantly dictated by this connection between our brain and body. Our mind's ability to effectively process these emotions, experiences, and traumas determines whether and how we react, what memories we hold onto, which we block out, and how we move forward from the stress and trauma we have experienced. When we are stressed, we experience something that is often referred to as an "internal storm"[133]. The severity of this storm depends on the degree of stress or trauma, as well as our resilience to it. However, whether mild or catastrophic this internal storm almost always involves the following: intense emotions, hyperactivation of the limbic system, the release of inflammatory cytokines, the inability to effectively integrate information, changes in metabolism and digestion, extreme fluctuations in heart rate and breathing, and by the end – tremendous fatigue. These things occur in a unique way for each of us, whether we recognize it or not, but if we are unable to actively process through these fluctuations in a systematic and meaningful way, issues such as PTSD or chronic somatic disfunction (constant pain, discomfort, and exhaustion) sets in. Observing the changes in our minds and bodies at both a cellular and behavioral level allows for the development of educated, effective intervention methods.

Decisions, Decisions…

One of the processes in our brain that is most affected by our bodily functions is our decision-making process. When in a high-stress, heightened state of arousal the internal dynamics of the body has a fascinating effect on the brain's ability to effectively make decisions in time-pressured

[133] van der Kolk, B., pg. 243

scenarios. A heightened state of arousal is categorised by a racing heartbeat, high blood pressure, shortness of breath, and often poor decision-making. When studying this in non-human primates, two centres within the brain's decision making system housed neurons that are specifically designed to monitor the internal state of the body[134]. These neurons seem to be rewired when the body is in a high-stress condition, transforming some of the decision-making neurons into internal monitors.

These findings provide valuable insight into the functioning of the decision-making process and the integral role that the body plays in it. It also allows researchers to gain a better understanding of the cellular functioning that underlies our stress response, why we often make irrational decisions during periods of prolonged stress, or intense trauma, and how we can approach this behavior with therapy in the future.

Muscles Matter

Given the significance of the motor cortex in the brain-body connection, its functioning also plays a large role in providing insight into our stress response and its influence on our bodies. When studying these neurons in rats it was found that they could be separated into the externally focussed and internally focussed groups[135]. Those that were labelled 'externally focussed' predominantly functioned as the neurons that relayed information to the rest of the body, controlling movement and sensation, whereas the 'internally focussed' neurons did not communicate with the rest of the body, but rather processed and sorted through the information already present in the cortex[136]. What was fascinating was that when researchers suppressed some of the internally focussed neurons in the cortex, those that were originally externally focussed, switched to being internally focussed in order to counteract the neuronal inhibition.

This manipulation mimicked the effects of some brain disorders and severe cases PTSD. It revealed that once the externally focussed neurons orient themselves inward due to the inhibition, it

[134] Atsushi et al. pg. 118
[135] Patrick A. et al, pg. 3
[136] Patrick A. et al. pg. 5

can lead to compromised signals reaching the muscles, due to the newly reprogrammed internal signals that are usually not present[137]. This inhibition is seen in soldiers returning from deployment in war torn territories, children who experienced abuse in the home, and individuals who went through an unexpected, catastrophic change in their lives during a concentrated period of time. Through studying these neuron conversions and finding a way to prevent or reduce inhibition in key parts of the brain, novel therapies could be possible in the future.

One of the many reasons why exercise has such significant impacts on our ability to handle stress is its stimulation of our skeletal muscles. The changes and growth that occurs to our skeletal muscles during periods of exercise allows our body to discharge many of the inflammatory and damaging chemicals that are produced in the blood stream due to the body's stress response[138]. Most importantly, this mechanism prevents any lasting damage to the brain specifically, getting rid of destructive chemicals before it reaches the BBB.

One of the main proteins involved in muscle growth and conditioning is PGC-1a1, increasing fitness and muscle mass[139]. When researchers over expressed this protein in mice they also displayed all the signs of a mice that frequently exercised (even without any physical activity). After exposing these mice and a control group to mild stressors for 5 weeks the normal mice became depressed and unresponsive, whereas the PgC-1a1 mice showed no signs of depression. The secret to their resilience? An enzyme known as KAT. Mice who had increased PGC-1a1 proteins had notably higher KAT levels than the control group[140]. This enzyme breaks down a substance known as kynurenine, which is produced in high amounts during the body's stress response, into kynurenic acid which cannot pass the BBB like kynurenine can. Kynurenine levels are also high in patients with depression, anxiety and bipolar disorders.

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[137] Patrick A. et al. pg. 8
[138] Agudelo, L., pg. 351
[139] Agudelo, L., pg. 351
[140] Agudelo, L., pg. 351

As a result, it is clear than an increase in skeletal muscle stimulation and PCG-1a1 protein allows individuals to convert harmful chemicals such as kynurenine, produced during stress, into indigestible derivatives. Thereby, mitigating their effect on our brains and ultimately our overall mental health[141]. This study provides promising information in future treatments of chronic stress, depression and other conditions that impact our daily lives.

The Guts and The Glory

The brain-gut connection has been well established over the past 20 years, acknowledging that our neural pathways, thoughts, and emotions has a significant impact on our gut health, and vice versa, with the gut microbe influencing our susceptibility to anxiety, depression and other neurological complications. Unsurprisingly, stress has a major influence on a person's gut health, often leading to stomach ulcers, detrimental constipation, or an imbalance in microbiota in the gut.

When looking at stomach ulcers caused by chronic stress specifically, scientists have identified the neural pathways that directly influence the stomach. Uncovering the process by which stress results in stomach ulcers is vital in understanding the way stress influences our body, and how measures can be put in place to prevent these outcomes. This research not only reveals the importance of gut health, but sheds light a very important principle regarding how our brains control organ functioning and why our brain-body connection is so complex and imperative for our overall health.

More than a century ago Pavlov proved that the environment and previous experiences can greatly influence our central nervous system in order to stimulate and improve our gut health and digestion. Through studying the human stress response with regard to unemployment, it is known that as unemployment rates increase, the death rates attributed to stomach ulcers associated with stress follows the same trend. In order to locate the neural networks connected to the gut, researchers

[141] Agudelo, 2014

used a strain of rabies virus, injected it into the stomach of rats, and tracked its movement to the brain as it travelled through the central nervous system. This technique is the same one used by the rabies virus when it enters the nervous system through a bite or scratch, but in this case, it revealed the areas of the brain involved in controlling the stomach.

It was found that the rostral insula, a brain region involved in the regulation of emotion and gut sensation, was linked to the parasympathetic control of the stomach. This infers that the stomach and cortex are in communication with each other, sending signals back and forth that are somewhat subjective to our emotions, previous experiences and circumstantial awareness which is stored in the rostra insula. One could then argue that our "gut feelings" might actually be rooted in some degree of truth. The sympathetic nervous system (which is active during stress), on the other hand, linked to the primary motor cortex when tracked from the stomach by the rabies virus. The implication is that our voluntary control of our skeletal muscles is influenced.

Tracing these connections between the gut and the brain is vital in understanding why stomach ulcers form from stress, or from bacteria such as Helicobacter pylori. It is clear that our brain chemistry and signaling have a direct connection with the gut, possibly influencing the gastric composition, altering bacterial growth concentrations, and causing the stomach to exist in an unbalanced state, which could lead to ulcers.

Knowing that there is such a clear connection between the brain and gut could help future treatments of other gut disorders such as irritable bowel syndrome, Chrone's disease, or dyspepsia. In the future, neurological treatments in conjunction with gut therapies could provide a novel and more effective way of treating these complications.

The Heart of The Matter

The detrimental effects of stress on our heart health have been established, however what mechanisms causes a stress induced heart attack or stroke has been something that researchers have been increasingly more attentive to in the last decade. These occurrences usually result from a surge

of atherosclerosis (damage to blood vessels) in areas around the heart and brain. The link between atherosclerosis and prolonged stress comes from the negative emotions we feel when we are stressed for extended periods of time[142]. When our body experiences negative emotions our pro-inflammatory cytokines increase, and these chemicals raise blood pressure and increase our risk for cardiac disease.

The link between increased inflammation and chronic stress is confirmed by our neural activity, since the same regions of the brain that regulate our emotions are responsible for the body's inflammatory response. In a study of 157 healthy adults, researchers analysed brain activity while participants viewed an upsetting image and attempted to regulate any negative emotional response. They also looked for any indication of blood vessel damage and inflammation within the bloodstream. Participants who had greater neural activity in areas of emotional regulation within the brain also had elevated levels of interleukin-6 (pro-inflammatory cytokine, expanding the thickness of carotid artery wall) in their bloodstream[143]. This is a major sign of atherosclerosis, as well as premature death.

This is a clear sign that our emotions are inextricably bound to our heart health, even if we are not always aware of the increased inflammation. These findings not only emphasise the importance of stress management, but also propose a novel way of treating heart disease, possibly targeting these regions in the brain which cause the initial inflammation[144]. Knowing that our emotions and heart health are directly connected also presents the opportunity for interfering with the detrimental physical health implications of disorders such as depression and anxiety.

In conclusion, our body and our brains are inextricably bound together in every way possible. Denying this connection not only puts us at risk for various life-threatening health issues, but also

[142] Gianaros, P. et al., pg. 740
[143] Gianaros, P. et al., pg. 741
[144] Gianaros, P. et al., pg. 741

allows us to acknowledge the responsibility we hold to ensure that both our brains and bodies are healthy. Managing our stress levels could not only benefit our every-day lives, it could save it.

Memory and Learning

Now that we are aware of the effects that stress had on both the brain and the body, we can look more in depth into how we remember stressful situations, how our brains change our memory of what happened and why it often feels like our brain "blocked out" some of the hardest, most traumatic events of our lives. When we think back to a time when we lost a loved one, got fired from a dream job, got our hearts broken, was caught up in a detrimental car crash, or became so burnt out that every single task felt like an incomprehensible challenge – we usually remember very little, and tend to focus on the glimmers of hope we experienced in that time. This is a tactical survival mechanism that our brain uses to protect us from what happened to us in the past and to help us move forward to new experiences. Stress, whether emotional, professional, or physical is the thing that alters our memories the most. Understanding why and how this process works allows us to untangle many of the mysteries of the human experience, especially PTSD recovery.

The process of how information is stored in the brain has long been studied and is known to be complex and often challenging to understand. The "father of neuropsychology", Donald Hebb, stated that "increments in synaptic efficacy occur during learning when the firing of one neuron repeatedly produces firing in another neuron to which it is connected". Synapses are the junctions between neurons, and they are responsible for passing nerve signals through the brain and nervous system[145]. Neurons facilitate the storage of information, which occurs through repeated stimuli and firing neuronal signals. A crucial aspect of memory formation and cognitive function is the dynamic strengthening and weakening of synaptic junctions known as long-term potential (LTP) and long-term depression (LTD). This synaptic plasticity determines how memories are formed in the brain

[145] Joshi, V. et al.

and is defined as “long-lasting activity-dependent alterations of synaptic structures” and the efficacy of their connections, ultimately referring to the ability of synapses to adapt as individuals form new memories and no longer need older ones. Through integrating the neuronal function and improving the strength of synaptic connections the plasticity is improved and memories are consolidated[146].

As proposed by Hebb, this is today known as Hebbian plasticity and has long been thought of as a critical element to memory formation and behavioural change in the mammalian CNS. However, recently there has been a novel concept that opposes LTP and LTD, highlighting the crucial roles of “transmembrane signal transduction, N-methyl, and neural membrane properties in behaviour-modifying circuits”[147]. Another consideration in terms of synaptic changes is that of gene transcription, since DNA methylation and alterations in chromatin structures greatly impact learning capabilities. The discovery of *the protein synthesis dependence theory of memory and learning* has been linked to intellectual disabilities and memory disorders[148]. These are just a number of different theories that highlight the complexity in understanding how memory is truly formed.

In the context of exploring these theories of memory formation, the storage of information is defined as the process where “activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation” which then regulates information and stores it as engrams in the brain area where the plasticity is observed[149]. It is an ever-changing process initiated by new information from the environment which is then encoded and processed into unstable intermediate memories, before an engram is stored as a long-term memory. This long-term storage of the engram could be established for a lifetime or be destabilized and re-stabilised depending on memory retrieval[150]. This is where stress and trauma play a large role in our ability to accurately retrieve memories, or in the alteration of some of our memories due to conflicting new experiences that

[146] Joshi, V. et al.
[147] Joshi, V. et al.
[148] Joshi, V. et al.
[149] Korte, M. et al., pg. 677
[150] Korte, M. et al., pg. 678

destabilise our previously formed engrams. The process often involves the hippocampus which is responsible for imprinting spatial, contextual and episodic memories through processing information from the entorhinal cortex to form these "context-specific representations"[151].

As humans, we are exposed to a plethora of stimuli and experiences every single day. However, only some of these experiences will trigger the strengthening of synaptic connections between neurons to form memories, of which some will last a lifetime. Initially, the memory traces are fragile. However, through the process of consolidation they are transformed into persisting long-term memories[152]. The brain consolidates a memory through producing new proteins which strengthen the initial memories, but when other stimuli flood the brain whilst consolidation is happening, particularly stimuli that trigger the stress response, it could disrupt or even modify the process, resulting in fractured engrams being stored. In order to minimize this effect, the majority of the consolidation tends to occur during sleep when the exposure to new stimuli is low[153].

Although most of us go about life unaware of the miracles in our brains, we all have a remarkable ability to integrate information and connect different memories and ideas in record time. In the past decade, neuroscientists have immensely improved in identified rhythmic brain oscillations, known as theta oscillations, that activate and strengthen different brain regions in order to integrate memories.

The importance of brain waves during memory formation is vital, allowing for communication between the temporal and frontal brain regions. The activity of these theta oscillations fluctuates up and down several times per second and is crucial in memory formation, as well as the facilitation of the interaction between several brain regions[154]. Since brain waves are challenging to measure from outside the brain, computational techniques are used to restructure the

[151] Korte, M. et al., pg. 657
[152] Levy, R. et al.
[153] Levy, R. et al.
[154] Backus, A. R. et al., pg 453

oscillations from the hippocampus to make them more comprehensible. In doing so, an increase in these theta oscillations were observed in hippocampal signals whenever someone linked two separate memories and that these oscillations were evident in the medial prefrontal cortex, which is a brain region responsible for information-storing-networks[155].

Based on these findings it has been established that distant brain regions are able to interact and communicate with one another through synchronizing their theta oscillations, enabling the processing and integration of previously stored memories. It is this ability to link memories from the past to new experience which enables us as humans to make cognitively informed and complex decisions[156]. However, this connection is the part of our memory most effected by stress sand past trauma. The theta oscillations in the brain drop dramatically in individuals who have experienced recent or chronic stress. In future research, finding ways to optimize these brain waves could provide relief for neurological damage and disorders, specifically with regards to in PTSD, since PTSD involves the incorrect linkage between a previously traumatic memory and an everyday situation. Understanding the integration of memories in the brain also allows us to understand the complexity of memory formation and the linkage between memories, which highlights the fact that memories are not solely stored in isolated regions of the brain, but rather operates through a network of communication and brain waves.

The Fluidity of Memory

When studying memory, there tends to be a strong focus on the individual brain regions and molecular processes involved in learning and memory formation. However, there has been significant evidence suggesting a more network-like facilitation of memory formation in the brain, with different brain regions interacting in order to establish short- and long-term memories[157].

[155] Backus, A. R. et al., pg 455
[156] Backus, A. R. et al., pg 456
[157] Staresina, B. P. et al.

This is evident in the formation of short-term memories where the memory traces are first stored in the hippocampus, but then relocated to the outer regions of the brain. Brain wave activity plays a pivotal role in this process of memory consolidation. During non-rapid eye movement (NREM) sleep, there are very slow oscillations (SOs) present in the brain with other types of oscillations hidden within them[158]. When studying human epilepsy patients during their natural sleep processes it has been discovered that three different oscillations are all hidden inside of the hippocampus yet have a joint function.

Within the SOs, clusters of oscillations known as spindles were found at an intermediate speed. When they looked deeper, they found rapid oscillations within these spindles that are called ripples, which are thought to be responsible for transporting memory traces to the cortex[159]. This study suggests that there is a degree of connectivity between the cortex and the hippocampus which is essential for the formation and consolidation of short-term memory.

Richard Semon introduced the concept of an engram into the world of memory and neuroscience more than a century ago, however it is only recently that technological methods have become available to provide direct evidence for its existence. Today, an "engram complex" is known as an ensemble of excited neurons in a region of the brain, such as the hippocampus or amygdala, that connects with other regions of the brain, such as the cortex, in order to form a retrievable memory in the brain. Through synaptic plasticity engrams are connected and maintained as neurons are able to forge new connecting pathways throughout the brain[160]. Evidence in rodent models has revealed that memories stored in engrams can be retrieved through reactivation the complex, or could persist silently even when " memories cannot be naturally recalled" such as in Alzheimer's.

In recent times, researchers analysed specific mechanisms of memories and found intriguing data proposing that memory formation is much more dynamic than previously thought. It is known

[158] Staresina, B. P. et al.
[159] Staresina, B. P. et al.
[160] Miller, E. K. et al., pg. 468

that neurons communicate in the brain through synaptic connections, neurotransmitters and brain oscillations, in order to facilitate and establish memory formation. It is the strength of these connections that determines how well the memories are formed and the cellular mechanism involved is known as long term potentiation (LTP)[161].

The persistence of the memory depends on the LTP connections and the stronger the connections, the more vivid the memories are likely to be. The brain is often described as a "plastic" organ due to its ability to "reconfigure and restructure itself", strengthening synapses which then form the foundation for memories[162]. However, when studying the consolidation and formation of memories it is evident that memory involves various brain regions and could be responsible for behavioural changes such as addiction and anxiety. According to recent studies there are multiple different regions that are all connected in the brain in order to set up a complex memory network[163]. This contradicts the traditional viewpoint of memory formation and opens up the argument of whether memory could be more interconnected and complex than previously thought.

The Genetics of Memory

When studying the large-scale facilitation of memory, merely decoding neurons and brain regions might not be sufficient to truly understand memory mechanisms. The role of genetics in all aspects of the body is vitally important, and in a recent study there has been a clear link between microRNA molecules in the brain and their contribution to memory formation and interaction between brain regions.

MicroRNAs are non-coding genes and control the quantities and functions of target proteins that facilitate cellular mechanisms in the brain. Although these microRNAs lay various different roles in the brain, there has been one that particularly interested researchers which is expressed in neurons responsible for memory formation. These microRNAs that are involved in memory are seen

[161] Texas A&M University
[162] Texas A&M University
[163] Texas A&M University

as a novel regulatory mechanism within the brain and relies on the interaction between different RNA molecules[164]. Since both sides of the double helix of DNA can each produce a microRNA, the two microRNAs are mirror images of each other. However, they do differ slightly in sequence and thus regulate distinct protein producing RNAs, which therefor affects processes in the brain such as memory formation[165].

When studied, it was clear that acute changes to the microRNA genes could result in major changes in brain functioning and could influence memory consolidation and lead to neurological diseases. This reveals a fascinating aspect of how memory is formed and consolidated within the brain, because it suggests that memory depends on more than brain regions and neurons but is much more dynamic and facilitated by various different mechanisms[166]. In future research, the role of microRNAs will have to be considered when hypothesising how distinctive memories form in the brain and in the development of treatments for neurological deficits.

Memory-formation also requires the gene transcription for neural modification to occur, which is where epigenetics comes to play and results in changes in neuron excitability, assembly and synaptic activation. New connections are formed, selective strengthening of synapses occurs, and the existing synapses are eliminated. For this synaptic plasticity to occur, several proteins and steroids are required and expressed in regulated DNA methylation histone tails post-translational changes[167]. When synapses are strengthened to reinforce information, proteins build these synapses to aid in memory consolidation. This production of new proteins occurs when RNA that encodes for these proteins is activated. RNA sequences are silenced by microRNAs until they are activated by synapses when stimuli enter the brain[168]. Since the RNA and microRNA together form a protein complex, the activation of a synapse deconstructs the complex, freeing the RNA to synthesize a new

[164] Scott, H. et al.
[165] Scott, H. et al.
[166] Scott, H. et al.
[167] Joshi, V. et al.
[168] University of California - Santa Barbara

protein. The degradation and synthesis of proteins occurs simultaneously, since the deconstruction allows for the synthesis of novel proteins that strengthens synapses[169]. Every single one of these steps of building our memories will be influenced by our stress levels, our ability to manage them, the lifestyle choices we make, and our mental health.

Proteins, Enzymes and Hormones with Memory

One of the key proteins involved in the regulation of LTP and LTD as well as memory formation is brain-derived neurotrophic factor (BDNF). Its pro-peptide is secreted in neuronal cells and multiply the posttranslational mechanism enhancing the action of BDNF and connects with its highly sensitive receptor, tropomyosin receptor kinase B (TrkB), in order to regulate memories. The regulation of memories and BDNF is highly dependent on input specificity, "provided by spine-restricted Cdc42 activity", and this established biochemical computation in dendrites, contributing to synaptic plasticity[170].

The role of BDNF in synaptic plasticity influences the initial development of the CNS as well as the adult nervous system. It is said to play a vital role in transforming functional adaptations into structural changes. In a genetic study of mice, it was found that BDNF knockout mice had significantly lower synaptic plasticity, especially in the maintenance of LTP. However, this could be reversed through a virally mediated local expression of BDNF or through a recombinant form of BDNF inserted into hippocampal slices of the knockout mice[171]. When the same methods were applied to wild-type mice, there was an increase in LTP induction and synaptic strength. Since BDNF is essential in the process of learning and memory, the knockout mice showed clear deficits in specific learning tasks and information storage. The genetic evidence is obtained from the common human single-nucleotide polymorphism in the BDNF gene where a methionine (Met) is substituted for valine (Val) at the 66th codon which then results in the impairment of BDNF secretion. This

[169] University of California - Santa Barbara
[170] Joshi, V. et al.
[171] Korte, M. et al., pg. 662

compromised Val-Met genotype results in the misfolding of the BDNF protein and eventually leads to learning and memory difficulties due to the damaged performance of the hippocampus. Although BDNF has been identified to have serious implications for memory and learning abilities, it must be noted that this molecule functions in unison with other molecules in the brain and there is no "exclusive memory molecule"[172]. In future research on this concept in synaptic plasticity and cognitive functioning could lead to potential therapeutic measures for neurogenetic pathologies and give a better understanding on how BDNF is influenced by trauma, ELS, and chronic stress[173].

Another vital protein known as Npas4, which was traditionally thought to be heavily involved in the epigenetic expressions influenced by neural activity, has also been linked to our ability to efficiently build long term memories. It was found that this protein impacts the strengths of the connections between neurons in the CA3 region and dentate gyrus regions of the hippocampus, implying that a lack of Npas4 results in the inability to create long-term memories. This study reveals the cellular pathway that selectively controls "an experience-dependent synaptic mechanism" when encoding a memory in CA3[174]. It is important to note that experience dependent plasticity is not the same as synaptic plasticity and greatly impacts learning. This form of plasticity involves homeostatic regulation of neuronal firing within a dynamic network and has been directly linked to many neuropsychiatric disorders such as anxiety, phobias and post-traumatic-stress-disorders[175].

Contextual memories are formed in CA3 and are memories which include information such as where and when a specific experience took place. The gene for Npas4 has been identified, which is activated instantly in a new experience, controlling the gene expression for long-term memory encoding. This protein was most active in CA3 when an individual was learning something new and its functioning is essential to fast contextual learning, specifically fear and trauma conditioning[176].

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[172] Korte, M. et al., pg. 664
[173] Joshi, V. et al.
[174] Weng, F. J. et al., pg. 97
[175] Joshi, V. et al.
[176] Weng, F. J. et al., pg. 97

When analysing memory in mice, researchers allowed them to navigate a maze, but each time they entered a specific room they would receive a mild shock (stressor). Within minutes the mice feared this chamber of the maze and froze when approaching it, fearing the shock. When the gene for Npas4 protein was knocked out in CA3 of these mice, they could not remember the shock, however when it was knocked out in other areas of the brain there was no change in behaviour. Researchers also observed that synaptic strengthening in CA3 required Npas4 and some selective strengthening originated from the dentate gyrus of the hippocampus. In the absence of Npas4, the synapses from the dentate gyrus projecting to CA3 could not be strengthened, thus weakening memory encoding[177].

The study identified one gene (plk2), which influences Npas4 when strengthening synapses. This gene is responsible for shrinking postsynaptic structures and Npas4 activates this gene to weaken specific synapses[178]. It suggests that Npas4 does not explicitly strengthen synapses, but rather only allows them to strengthen, when necessary, thereby preventing them from becoming too strong that they cannot be activated when new memories have to be encoded. In the absence of Npas4, the strengthening is saturated causing little strengthening to occur during learning and thus memories are not accurately encoded.

In a related study, a research group studied the CaMKIIalpha gene in mice and when silenced, it resulted in complete and exclusive lack in kinase activity, which is strongly linked to the stress response. This was significant as it impacted the hippocampal synaptic plasticity, which is involved in learning and memory, resulting in major impairments. This gene codes for an enzyme which adds phosphates to protein substrates in order to influence their functions, but since CaMKIIalpha is saturated in the hippocampus, it is essential in mediating memory functions[179]. When the behavioural and functional memory in the brains of mice who lacked the CaMKIIalpha gene was examined, LTP and cellular mechanisms of memory in postsynaptic spine enlargement

[177] Weng et al.
[178] Weng et al.
[179] National Institute for Physiological Sciences

were negatively affected[180]. These mice were also observed to exhibit impairments regarding "inhibitory avoidance learning" in the hippocampus and they could not form any new memories, having no recollection of events that had just happened. This study reveals the vital importance of CaMKIIalpha in the formation of new memories and this could be used for the development of new therapies for individuals with severe memory deficits caused by chronic stress, "blocked out" trauma, or PTSD.

When our brains process new information, the points of contacts between neurons and regulation of signal strength adapts by continuously modifying the quantity of receptors in the neuronal membranes. When we use information on a daily basis, it is much easier to remember than that which we learnt years ago, since the receptors are constantly renewed[181]. The nerve cells in the hippocampus are able to control which receptors are "switched-on" or off, and there have been three main molecules identified (GRIP1, ephrinB2, and ApoER2) which play a vital role in this regulation. Through inhibiting these proteins, mimicking the effects of chronic stress in many individuals, it was seen that adult individuals displayed deficits in long-term plasticity, suggesting that together they are involved in enhancing neuroplasticity, which is the cellular basis of all learning and memory[182]. The ApoER2 and ephrinB2 molecules have both been related to the progression of Alzheimer's and through studying the interactions of these molecules with the rest of the brain, especially in terms of regulating learning and memory, it could lead to new treatment strategies for this disease.

Optogenentics

Optogenetics is a technique that uses light to precisely control the activity of neurons in living cells, allowing researchers to study various neurons to understand the higher function of the brain. This is done by inserting a gene that codes for a light-sensitive protein into the neurons of an individual. Once these proteins are produced on the outside of the cell membranes, an electrical

[180] National Institute for Physiological Sciences
[181] Pfennig, S. et al., pg. 87
[182] Pfennig, S. et al., pg. 87

channel is formed which is activated when light is shone on the specific neuron, causing electrical ions to flow into the cell[183]. The voltage in the neuron is altered and initiates an electrical impulse.

When analysing the synaptic signals and the role of interneurons, researchers identified different types of neurons through two-photon microscopy imaging, which also revealed that the transmissions of signals were highly dependent on the type of interneuron that received the impulse[184]. Through the use of optogenetics researchers have been able to identify key structures within the memory regions of the brain, yet more research must be done in order to create the full picture of how neurons interact.

A question that remains is whether it is possible to stimulate specific learning behaviours through directly activating clusters of neurons that have previously been activated during the learning period. When studied in mice, it has been shown that through opto-genetically stimulating cells in the hippocampus which are involved in active learning, including fear conditioning, it was enough to induce freezing behaviour. These results indicate that the recall of light-induced fear memory can be activated through the optogenetic protein channelrhodopsin-2 (ChR2)[185]. However, in control groups which were not exposed to the fear conditioning, no freezing behaviour was induced when upon ChR2 optogenetic stimulation. All of these subjects suggested that the fear-induced recollection of memories and behavioural changes are highly context specific.

Working Memory

Another large category in memory formation is known as working memory and forms the platform where individuals store and alter thoughts, forming the foundation for purposeful and goal-driven behaviour. It is known that neurons involved in the higher-order cortex, including the prefrontal cortex, display “delayed-activity” with higher levels of spiking during the stress response. However, when an optogenetic stimulus is given to these neurons over a brief period of time, it results in

[183] Pala, A. et al., pg. 71
[184] Pala, A. et al., pg. 71
[185] Korte, M. et al., pg. 687

increased working memory activity[186] Even when the stimulus fades, the elevated level of activity continues in the neurons at a lower rate, but above normal levels. This delayed neuron spiking activity is what helps maintain working memory of stimuli and activates higher functioning in the frontal cortex to encode specific content in memories[187]. The downside to these memories that are only maintained by persistent spiking is that when the activity is interrupted, these memories could be altered or destroyed, which is often the case in individuals who experience trauma or extreme stress during the period of memory consolidation.

Current research has revealed that brain signals involved in working memory, tend to be longer and store more information when you are learning about something new compared to information you are familiar with. Researchers artificially lengthened these brain signals involved in working memory in mice and observed that these subjects were 10-15% better at recalling the best way to go through a maze than the control group[188]. It was also found that neurons fire quick signals in rhythmic cycles in order to encode complex stimuli in the hippocampus, which was later termed "short wave ripples". These ripples are said to combine the information being learned in order to create the individual's memories.

The ripples were extended through optogenetics and found to include slow-firing neurons when recruiting memories in the brain. Slow-firing neurons had a high degree of plasticity when something new is being learnt, whereas the fast-firing neurons were more rigid throughout experiences since they tend to encode familiar, rather than novel, signalling pathways when stimulated by new information[189]. The future of this research entails finding a way to prolong the short-wave ripples in a non-invasive way in order to treat memory disorders.

[186] Miller, E. K. et al., pg. 472
[187] Miller, E. K. et al., pg. 472
[188] Fernández-Ruiz, A. et al., pg. 1084
[189] Fernández-Ruiz, A. et al., pg. 1085

Spatial Memory

Neurological diseases such as Alzheimer's often target the neurons responsible for memories and spatial orientation, which has also been linked to neuronal activity in the retro-splenial cortex. It is known that when an individual enters a new location, place cells fire in the hippocampus in order to start the process of encoding an engram. There is only a small number of neurons involved in this processing to minimize energy input, while maximizing the integrity of information that is stored[190]. Although the hippocampus is essential for memory, lesions in the retro-splenial cortex has been shown to correlate with disorientation and memory deficits, which could explain why childhood trauma and severe adulthood stressors, such as war combat and abuse causes severe memory impairments.

In this study, the brain circuitry in mice that were placed on treadmills with tactile stimuli was analysed. The activity of neurons in the hippocampus and the retro-splenial cortex was compared using highly sensitive microscopy techniques and the genetic labelling of cortical neurons[191]. Before this, it was not possible to analyse all of these brain cells simultaneously, therefore this study provided a rich analysis on the processing of spatial memory in the brain. The neuron cells in the retro-splenial cortex were found to fire in a similar manner to the place cells of the hippocampus but seemed to carry different aspects of rich spatial activity[192]. This information allowed for a more comprehensive understanding of where memories are processed and stored in the brain cells, but future research will have to include how the development of the retro-splenial cortex relates to various neurological diseases.

In another recent study the medial temporal lobes, as well as the entorhinal cortex (EC) were shown to be involved in declarative memory, which includes recalling things like your home address and your sibling's name. After the discovery of "grid or place cells", which are the neurons that are

[190] Mao, D. et al.
[191] Mao, D. et al.
[192] Mao, D. et al.

activated during navigation mentioned earlier, in these regions it is evident that the EC is also involved in spatial cognition. Neuro-engineers isolated neurons that are involved in targeting specific memories in the human brain for the very first time[193]. This was done by analysing human patients who had electrodes implanted in their brain which recorded their brain signal responses when completing "virtual reality object-location memory tasks"[194]. All 19 subjects played a virtual reality game on a laptop where they had to navigate through specific locations and find 4 objects.

Thereafter researchers removed the 4 objects and asked the patients to mark the location of one specific object as they moved through the same locations. After analysing the brain signals, "memory trace cells" were found which were directly associated with the location of the objects that individuals remembered seeing[195]. It was interesting to note that although there were specific neurons that were only activated based on the location and spatial awareness of the person, other neurons were dependent on the exact memory the individual was trying to retrieve whilst navigating through the location. Activity in those neurons changed when subjects were asked to remember the location of one of the objects compared to another[196]. Thus, the findings suggest that the brain tracks experiences we are consciously recalling and is able to activate separate neurons in order to differentiate between memories.

The majority of these memory-trace cells were found in the EC, which is highly affected by diseases such as Alzheimer's. Since these neurons are responsible for retrieving memories, it is highly likely that memory deficits in Alzheimer's is due to the malfunctioning and destruction of these neurons and their synapses[197]. This study revealed that neurons are concerned with where a memory occurred, and that the location plays a role in the individual's ability to recall that specific experience.

[193] Qasim, S. et al., pg. 2080
[194] Qasim, S. et al., pg. 2080
[195] Qasim, S. et al., pg. 2084
[196] Qasim, S. et al., pg. 2085
[197] Qasim, S. et al., pg. 2085

In future research the focus lies on whether other factors such as time or specific events influences neuronal activity.

The Frontal Cortex

The fact that certain areas of the brain are responsible for different functions has long been studied. However, how these regions interact and whether they are able to function beyond the expected purpose has only recently been explored. In a study investigating the cerebellum, which was once considered to be important for the control of movement, it was revealed that this brain region played a vital role in higher cognitive functions such as short-term memory and decision making[198].

The frontal cortex has always been considered to be the control centre of most of the higher cognitive functioning in the brain. Although the connection between the frontal cortex and the cerebellum is widely accepted, new evidence suggests that the interaction between these regions and the cerebellum itself is involved in memory and cognition. Through examining the cerebral activity in mice during periods where they are "thinking" rather than moving, researchers trained mice in activities that required them to make decisions based on short term memory[199]. They were exposed to a single object in a specific location and after some time the mice had to remember where the object was and indicate its location by licking in a left or direction.

The study revealed neural activity in the frontal cortex during the delay, which is likely to be involved in predicting the future motion of the mice, but there was also memory activity detected in the cerebellum. When the researchers silenced parts of the cerebellum during the delay, the mice were unable to correctly identify the location of the object and the activity in the frontal cortex depleted[200]. This suggests that the memory activity observed in the frontal cortex is dependent on the cerebellum.

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[198] Gao, Z. et al., pg. 115
[199] Gao, Z. et al., pg. 115
[200] Gao, Z. et al., pg. 116

When the connection between these two brain regions was disrupted, there was clear memory deficits observed. Therefore, this study confirms the fact that neural activity related to memory and behaviour is orchestrated by multiple regions of the brain and involves a communication network that is interdependent[201]. Future research in this field includes testing whether the cerebellum is able to regulate thoughts in the same way it does motion.

The Hippocampus

Since none of our memories are exactly alike, they are each stored in their own unique combination of cells that carry everything associated with that memory. Novel studies have recently shown that different parts of the hippocampus are associated with separate types of memories, making it a possibility to locate exactly where traumatic memories are stored in the brain so that personalized treatment could be developed in the future. Focussing on PTSD in particular, the data suggested that by activating the lower part of the hippocampus, traumatic memories that cause such disorders could become even more influential[202].

However, when the upper region of the hippocampus is activated, where positive memories are stored, the effects of past trauma and stress could be less vividly remembered. Using optogenetics, researchers tracked which cells were activated when mice were making positive, neutral and negative memories. This was done by using a glowing green protein that would light up whenever a cell was stimulated and with the use of laser light, these memories could be reactivated later. When activating the top part of the hippocampus, the traumatic memories seemed to be less debilitating and vivid, whereas when stimulating the lower half of the hippocampus, these past distressing experiences became more enervating[203]. These findings suggest that the lower region is responsible for storing memories which are so emotionally dense and draining that they negatively affect the individual's behaviours and mental state. The overall goal is to treat people with disorders

[201] Gao et al.
[202] Chen, B. K. et al.
[203] Chen, B. K. et al.

such as PTSD, but memory manipulation is a concept that could be taken much further to enhance brain functioning and memory for other advantages.

The Amygdala

Similarly, neuroscientists have investigated regions of the brain involved in memory formation, specifically analysing the amygdala, which is considered to be involved in memory formation with emotional content[204]. Many believe that the loss of the amygdala would result in the inability to form new memories linked with emotions. However, it was found that when the amygdala was removed or damaged, other brain regions seemed to take over its functions.

The area of the brain that compensated for the amygdala's function was the bed nuclei, which are found in the forebrain grey matter surrounding the stria terminalis. Since the amygdala is also responsible for activating several biological systems that protects the body in times of danger, in the absence of the amygdala information from the prefrontal cortex and hippocampus is processed by these forebrain neurons and then relayed to lower brain regions that control stress responses and defensive behaviours. Usually, the bed nuclei are much slower at learning than the amygdala and are only involved in memory formation when the amygdala is not processing new information. However, in the absence of the amygdala, these bed nuclei, together with neuroplasticity, are able to process emotional experiences and form memories[205].

These findings suggest that when a specific brain region is damaged or lost, other brain regions could potentially compensate for its functions. It also highlights the fact that the formation of new memories is not selectively dependent on specific structures, again implying that there is a dynamic network-like system involved in the processing and formation of memory in the brain[206]. This is vital in understanding the vulnerability of the memory network, how easily it can be changed and influenced by stress, and how adaptable it is to our past and present experiences. In future

[204] Poulos, A. M. et al.
[205] Poulos, A. M. et al.
[206] Poulos, A. M. et al.

research there will have to be more studies done on how this compensation could be promoted to help patients with memory loss and other neurological injuries or deficits.

Addiction and Drugs

Another fascinating aspect of the cellular basis of memory is the role addiction and drugs play in these mechanisms. In a study conducted on the neurological pathways involved in addictive behaviours, the brain was analysed to identify how cellular memory and brain functioning plays a role in addiction. When rats learned to self-administer cocaine, researchers observed an increase in communication in dopamine neurons, the source of which is the ventral tegmental area (VTA), also known as the reward and motivation circuit in the brain[207].

This increase in communication via LTP was seen in rats that self-administered cocaine, but also with food and sugar. The vital difference was that the LTP due to cocaine-use continued for 3 months after abstinence, whereas the naturally LTP only lasted about 3 weeks after the food or sugar intake. Another notable observation was that rats that were given the cocaine in a passive manner, administered by researchers, did not show signs of LTP in VTA dopamine neurons[208]. This study indicates that the cellular memory of cocaine-use remains intact long after the absence from the substance, regardless of the new behaviour of individuals and thus relapses could be easily triggered. The fact that 3 months for rats could be equivalent to several years in humans, it is the cellular basis of the "memory" of self-administered drugs that is likely to lead to relapse[209]. This research is vital in understanding how the use of drugs affects the brain and how memory regions of the brain interplays with the tendency of addicts to relapse.

When considering the molecular basis of memory there are a plethora of avenues to explore in this topic. It is clear that the formation of memories is highly dependent on the synaptic plasticity in the neurons of those regions of the brain involved in engram formations. The concept worth

[207] University of California - San Francisco
[208] University of California - San Francisco
[209] University of California - San Francisco

considering is whether the cellular mechanisms are solely responsible for our memories, or whether the interconnectivity of neurons and synaptic communication between brain regions plays an equally significant role in the formation and consolidation of memories.

Memory is a Network

One of the leading neuroscientists that share the view that memory and learning in the brain involves more of a network interaction between different regions of the brain, is Earl Miller. His hypothesis includes the idea that neurons involved in working memory interact with different regions of the brain in a network of communication through firing simultaneously at distinct frequencies, leaving a temporary "impression" of the information in various networks of neurons[210].

Although there has been no experimental evidence with how this relates to learning and behaviour, the hypothesis is plausible in the sense that there is increasing evidence that suggests various areas of the brain are involved in the processing of specific stimuli and that their interaction is essential for memory formation and retrieval. The problem with Miller's concept is that it seems to oppose doctor's findings in patient who have experienced brain injuries and seem to display no existing link between working and long-term memory. However, there are neuroscientists who have embraced Miller's idea of the "network of memory" and as research commences this might reveal a completely new perspective for neuroscience[211].

The research included here shed light on the studies and ideas that have opened up the concept that memories might be stored in a much more dynamic process than previously thought. It also provides incredible insight on how influential our stress response and experience are in the way we form memories, keep them, and retrieve them. Although there is much more to be done in order to grasp the mechanisms involved in the functioning of brain oscillations as well as microRNAs, researchers are closer than ever to understanding how we tore memories and make cognitive

[210] Miller, E. K. et al., pg. 7015
[211] Miller, E. K. et al., pg. 7015

decisions. It is clear that sleep plays a vital role in this process and that the interaction of different brain regions is pivotal to the formations and consolidation of memories. Having a better understanding on how our memories work, we can now consider how trauma and stress affects us in a more in-depth way.

Childhood Trauma and Our Response

When we think of trauma, the events and experiences that come to mind will be different for all of us. Some of the most common traumatic experiences include physical violence, emotional abuse, domestic and verbal maltreatment, sexual assault, generational secrets, cultural traditions (such as mutilation, child marriage, extreme violence as initiation), etc. What we have come to learn about trauma is that it shapes our lives, and the lives of people around us, in ways that we are often unaware of. Impacting our relationships, brain chemistry, emotional resilience, biological development, immune response, and our ability to fully experience the joys, heartaches, loves, and adventures that life has to offer[212]. When we carry trauma with us in our everyday lives it not only impacts our ability to live happy, fulfilling lives, but it also has profound impacts on the people that are closest to us. This concept is known as interpersonal neurobiology and looks at how our emotional state influences the behaviours and mental health of those around us.

It has been established that trauma not only limits us in terms of our emotional experiences, but also leads to physical biological changes, increasing the body's stress response, restructuring our abilities to detect danger, and reducing our ability to filter information from our external and internal environments in an effective and rational way. Most importantly, trauma effects the human ability to experience that natural, innate feeling of being brilliantly alive[213]. Not being able to feel these concrete, physical experiences within our minds and bodies is an essential human need. Without it we are unable to recognised and respond to our environments and how they make us feel, leading to

[212] van der Kolk, B., pg. 3
[213] van der Kolk, B., pg.3

a faded existence, regardless of our intelligence, experiences, or material achievements. The incapacity to fully experience the rollercoaster of love, or the disappointment that comes with failure, or the pure joy that we experience when we are laughing with the people we care about most, leaves people lukewarm, and often numb to the ups and downs of life.

Childhood

One of the most susceptible periods to stress for humans is our childhood years up until puberty. With much of the brain still developing, neural pruning occurring, and general maturing of neural circuits in full force, experiencing severe stress has lasting negative impacts on brain chemistry and development. The most common stressors include childhood abuse, parental divorce, extreme poverty, uncertainty in the home, and in some cases, bullying. When a child experiences these things for extensive periods of time, it alters their brain chemistry and silences their response to stress hormones such as corticoids and adrenaline. These effects become cumulative and worsen as the person continues throughout their childhood whilst experiencing emotional and physical stress.

This results in a network of adverse effects regarding concentration, self-control, organisational abilities, empathy, and a variety of other health and behavioural issues[214]. Researchers specifically looked at the presence of diurnal cortisol, a hormone that "helps us rise to the challenge", in 306 children 3-5 years old from various socioeconomic, racial and ethnic backgrounds, with economic status being the main determinant of adversity. Children were asked to perform a variety of functional and critical thinking challenges and provided a saliva sample to test the hormone levels.

Diurnal cortisol is a hormone that is highest in the morning and decreases throughout the day, aiding us to get up and start our day with as much energy as possible. However, in children from stressful backgrounds this was not the case. Those that experienced chronic forms of stress in the

[214] Lengua, L.J., pg. 550

home or at school showed an extremely low level of this stress hormone in the morning, with no changes throughout the day[215]. This not only means that these children do not have the alertness needed to face the emotional and cognitive challenges of each new day, it also suggests that they have become immune to the stress response.

Children with more normative levels of diurnal cortisol were also found to be more attentive to instructions, better at interacting in social settings and could regulate their own emotions at a higher level. This deficiency in regular diurnal cortisol not only impacts the child's development, but also adds up over time through their different experiences and abilities compared to other children[216]. This researchers gives vital insights into the importance of stress management, even at a young age, and how it can affect an array of different areas in an individual's life. This also provides some scientific background to why childhood trauma and experiences still have such a vivid and sometimes debilitating effect on our adult lives, and why it is so important for us to work through those experiences in order to move forward in a healthy way.

In a similar study, researchers found another vital mechanism of the stress response that is impaired by psychological childhood trauma, revealing a biological link between childhood stress and future depression and anxiety. It is known that most experiences of adversity leads to changes in our epigenetic profiles, but in a recent study it was found that childhood trauma specifically alters the human glucocorticoid receptor gene[217]. This is a vital part of our normal response, and could lead to increased susceptibility for psychiatric disorders.

Changes in the methylation of this specific gene has been found to be particularly affected by parenting styles. Rats with little to no maternal care displayed an increase in methylation with regards to this gene and had a significantly greater susceptibility to adult stress and showed increased fear and avoidance to any possible stressful situation. When 100 human adults, aged 22-34, who had

[215] Lengua, L.J., pg. 551
[216] Lengua, L.J., pg. 552
[217] Tyrka, A. R.

experienced childhood trauma related to parental care or loss, were studied by extracting DNA via blood samples, this same increase in methylation on the GR gene was observed. The increased methylation caused immunity against the cortisol response, leading to less stress resilience and a variety of other poor health outcomes, specifically psychiatric disorders[218].

This study has since been expanded to a much larger scale, studying 30,000 individuals over the course of 12 months to determine whether lifestyle changes could alter these epigenetic markers that occurred in childhood[219]. Whether we can reverse childhood trauma is not what is at question here. Through psychological and neurobiological research, scientists have found that one of the keys to overcoming these trauma responses is through language, speaking or writing about the trauma, and thereby accepting it[220]. Once we accept our past, no matter how difficult it might be, it sets us free to live differently in the future, to let go of the hurt, anger, and pain that we experienced. Overcoming trauma could take years, but through every conscious decision to move forward, we come closer to the light.

Our Response

How we respond to trauma is heavily dependent on the society we grew up in, when the trauma was experienced, and how our emotional resilience has developed. Some of us live in denial of the experience, others shut down completely and struggle to function in social environments, some experience PTSD or subconsciously recreate the events of their trauma in their everyday lives, while others develop neuropsychological disorders in response to an event that our brains cannot comprehend.

Freud suggested that some humans are prone to experience “the compulsion to repeat”, which is when an individual puts themselves in the same traumatic experience over and over again in different ways. This is often seen in people who experienced violent or sexual abuse as children, who

[218] Tyrka, A. R.
[219] Tyrka, A. R.
[220] Tyrka, A. R.

then grow up to be adults who subconsciously lean towards being in abusive intimate relationships, involved in violent prostitution, or "addicted" to the feeling of neglect or rejection. People who experience childhood trauma are 76% more likely to be involved in various forms of abuse in their adulthood, often because the re-enactment of trauma produces a sort of adrenaline- and endorphins-like rush[221]. Individuals repeat a pattern that they had gotten used to in order to suppress the original trauma, numbing and sedating themselves through a saturated stress response where the brain releases excess endorphins each time the trauma is re-hashed in a new environment. This repeated exposure to trauma offers some sort of temporary relief in the brain and body, which partially explains Freud's theory of "the compulsion to repeat".

Another one of the most noticeable effects of chronic stress is the loss of the desire for social interaction and inability to process and critically think through complex situations. In an attempt to study the biological mechanisms behind this "shut down" response, researchers looked for epigenetic changes and possible alterations in areas such as the hippocampus, amygdala, and surprisingly found a change in activity in the Broca's region (the area of the brain responsible for the formation of speech). When imaging the brains of individuals who have experienced childhood trauma to varying degrees, re-exposure to images, sounds and senses that were related to their specific experiences resulted in an extreme decrease in activity in this language region of the brain. When the Broca's region is essentially quiet this inhibits our brains to process thoughts, integrate experiences, and put our thoughts and feelings into words[222]. It is in these moments that trauma survivors "lose their tongues" and are pushed to the edge of the humanity, experiencing emotions and physical sensations that cannot be put into words. Knowing how our brain activity changes in response to trauma helps us understand why some people have great difficulty in recalling or vocalising their experiences.

[221] van der Kolk, B., pg. 29
[222] van der Kolk, B., pg. 43

When researchers looked at the chemical mechanism behind this, they also isolated a *nectin-3* cell adhesion protein in the hippocampus which was found to be responsible for neuron-to-neuron connections and communication[223]. These molecules are directly involved in maintaining synaptic integrity and ensuring efficient coherence of neurons in the hippocampus.

When scientists analysed this protein in rats who experienced chronic levels of high stress, it was seen that their *nectin-3* proteins were substantially lower compared to the control. This was caused by the MMP-9 enzyme, a degradation enzyme activated by the high amount of glutamate which is released as part of the brain's response to chronic stress[224]. After glutamate is released, it activates the NMDA receptors involved in neural plasticity and memory formation. When the NMDA receptors were activated it stimulated the MMP-9 enzyme to degrade *nectin-3* molecules, disabling their ability to maintain synaptic integrity and neural elasticity[225].

This cascade of neurological events lead the individual to become less likely to interact with others or form new memories due to the significantly low levels of *nectin-3*. When researchers manipulated the stress response to inhibit the MMP-9 enzyme, rats were found to reverse their antisocial behaviour[226], becoming more susceptible to peers and engaging in group activities.

The third, and possibly most dangerous, response to chronic stress and trauma is denial. It is when our body experiences the stress response, flooding our internal landscape with hormones, but our minds are incapable of paying attention to what is happening. Our bodies are fully aware of the stressor, however our conscious mind proceeds in a normal fashion, giving now notice to the stressor or trauma[227]. However, behind the scenes the amygdala continues to send out alarm signals, triggering an increase in the stress response, altering organ functioning, blood pressure, and muscle tensions. Eventually, if the denial persists for long enough, our trauma or chronic stress shows up as

[223] van der Kooij, M., pg. 67
[224] van der Kooij, M., pg. 67
[225] van der Kooij, M., pg. 70
[226] van der Kooij, M., pg. 71
[227] van der Kooij, M., pg. 73

a sudden collapse, a serious illness, or other physical effects that demand our immediate attention. In many cases, when we still chose denial, people numb these symptoms of stress with medication, drugs or alcohol[228]. The fact of the matter is that sooner or later the body will shut down, and the sooner we recognise our physical responses to stress, the healthier we can be.

Recalling Trauma

Memory, as we have established earlier, is a mechanism that can be easily interrupted by events in our lives, preventing or changing the encoding of information in our environments. Over time, as we retrieve memories less and less, our stories also seem to change, fogged with a sweet sense of selective amnesia that often tends to block out some of our less pleasurable memories. This is especially true for our autobiographical memories, which tend to represent our own perception of the occurrence, rather than the factual reality. Holding a memory of a traumatic or stressful event has several ways of manifesting, depending on the severity of the trauma, as well as how we perceived the event.

This was seen in a longitudinal study that interviewed 346 men that fought in WW2 in 1945/1946, and then again more than 40 years later in 1989/1990. The results were remarkable and showed that most of their memories of the worst atrocities and horrific violence that they accounted in the first interview seemed to have been released from their memories. Most of the men only gave a vague report of events that focused on resilience, war stories, and making it out alive. However, those men who had developed PTSD after the war showed very different recall abilities. Almost half a millennium later they could still recall the intricate details of some of the most traumatic experiences they had during their time in WW2. It was concluded that our ability to encode long term memory of events highly depends on how personally impactful the trauma or stress was to our lives, with a key factor influencing this being our degree of arousal during the original events.

[228] van der Kooij, M., pg. 74

It is the adrenaline rush that we endure when we perceive a threat or have a powerful stress response to trauma that aids in etching the experience into our long-term memories, leaving a lasting negative perception of the person, place, sound, or feeling, regardless of whether the detailed content fades. It has been found that the level of adrenaline that is produced during the stressful event is highly correlated with the accuracy of our memories[229]. However, this system has a certain threshold, after which our prefrontal cortex is overwhelmed with stimulus and shuts down, leaving our limbic system and brain stems (the more primitive side to emotional control) in charge. When this happens, we are unable to put words to our experience, we remember snippets of the event: specific sounds, smells, flash images, physical reactions, but nothing coherent. In this state the level arousal is so high that it disables the connection between the brain regions (hippocampus and thalamus) necessary for integration, encoding and sorting of incoming information[230]. This describes the "trauma induced amnesia" that we often hear of in victims that experienced horrible acts of violence and are unable to recall any relevant information from the events, despite having been fully conscious.

Although our daily experience of stress might not lead to a stress response that results in such a high level of arousal that our memory shuts down, it is important to note how trauma and high stress levels can alter our ability to form new memories. If you live in a state of constant high arousal, it could inhibit your ability to remember things like your wife's birthday, an intimate conversation over a cup of tea, a vulnerable moment with a friend, or the overwhelming joy of hugging someone you have not seen in a long time. These might sound like small, seemingly insignificant details, but these are the moments that make for a fulfilling and meaningful life, fosters human connection, and ultimately reduces stress and makes us feel safe.

[229] van der Kolk, B., pg. 178
[230] van der Kolk, B., pg. 179

Lifestyle choices

Knowing that stress has such catastrophic and lasting effects on our health, wellness, and survival can be an overwhelming reality to come to terms with – especially because in the 21st century stress is as normative as brushing your teeth. It has become a part of who we are. When we ask someone how they are doing the overwhelming majority of responses are some iterations of "Oh, I am busy" or "I'm so stressed but I'm fine" or "Hanging in there you know". What if that does not have to be our reality? What if we can defy this hustle-driven, high-strung, chronically stressed society, and just be perfectly calm and content? Seems like a high demand, but it is possible. There are a few scientifically proven ways to do so, and the sooner you implement them, the sooner you will find yourself drifting away from competing with the faceless mob of people who stress you out, and essentially find enjoyment in the small moments. Things like exercise, diet, socializing, and meditation are just some of the ways that we can trick our brains into becoming less stressed, more present, and ultimately, more alive.

Exercise

When considering how our lifestyles effect the way we handle stress, how fast we age, and whether or not we develop neurological diseases, exercise and diet are the two top contributors. It has been determined that even just one exercise session can alter and improve brain activity, specifically in the hippocampus, which is the region of the brain that ages the fastest and is strongly connected to Alzheimer's Disease.

When healthy individuals ages 55-85 were asked to remember the names of famous people, which activates the semantic memory within their brains, completing 30 minutes of medium intensity (70% effort) exercise on a stationary bike before retrieving these names significantly increased their performance. The task was performed once where the participants exercised and were

questioned 30 minutes afterwards, and again on a different day where there was no exercise prior to performing the memory task[231].

Semantic memory is known to deteriorate over time and is the most common form of initial memory loss. In this study, researchers proved that even the slightest form of exercise can initiate increased activity in four major brain areas – the middle frontal gyrus, inferior temporal gyrus, middle temporal gyrus, and the fusiform gyrus[232]. When an individual exercises, this causes increased activity in several brain regions that are not only related to memory, but also to how we experience stress. With repetition, these neural networks that are activated while exercising is likely to improve their integrity and increase memory efficiency in the long run.

Exercising is known to have positive effects for both the brain and body, however in a new study looking at the differences in high and low intensity exercises, it was established that these different forms of activity could affect the brain in distinctive ways. When looking at individuals' fMRI scans, researchers found that low-intensity exercise had a greater influence on cognitive functioning and attention, whereas high-intensity training stimulated the brain regions involved in emotional processing[233].

The use of functional neuroimaging has become a revolutionary tool in understanding the brain's structural and dynamic responses to several environmental changes, specifically transitioning out of a deskbound into a more active lifestyle. Analyzing fMRIs of 25 male athletes over the course of several days, researchers instructed the participants to run on a treadmill for 30 minutes, alternating between low and high intensities every session[234]. They also filled out a survey before and after every session, reporting on their overall mood.

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[231] Won, J., pg. 562
[232] Won, 2019
[233] Schmitt, A., pg. 43
[234] Schmitt, A., pg. 44

A significant elevation in mood was found across the board after both low and high intensity exercise. The fMRIs revealed that low intensity activity stimulated the functional connectivity in the prefrontal cortex regions, involved in cognition and attention. In contrast, high intensity sessions increased activity in the amygdala, hippocampus and other regions involved in emotional processing, but decreased brain activity in the motor cortex.

This is one of the first studies to distinctly differentiate between the benefits of both high and low intensity training, showing that both these activities help improve brain functioning. This study also indirectly implies that different forms of exercise could have separate benefits to combatting stress[235]. Since stress is known to affect cognition, memory, attention, and our ability to cope with emotions, exercising in both high and low intensity states could be a helpful tool to offset the stress response and advance our neural plasticity and functionality in those regions most affected by stress.

Our ability to deal with stress and stimulate brain health occurs through various processes, but two of the most important neurotransmitters involved in these changes are glutamate and GABA[236]. These are also the two chemicals that are most commonly involved in depression and other neuropsychiatric disorders, since they are heavily involved in the coordination of neural communication and health. In a study looking at the effect of high intensity training (such as kickboxing, running, tennis, etc.) on human stress response it was found that both of these hormones were significantly increased after periods of physical activity.

This is promising information for future treatment plans for major depressive disorder (MDD) specifically (which is often linked to chronic stress or childhood trauma), since it is identified by low levels of glutamate and GABA, which usually returns to normal after the individual no longer exhibits signs of MDD[237]. Through studying brain metabolism, researchers concluded that vigorous physical exercise is more demanding than any other activity for our brains (including

[235] Schmitt, A., pg. 54
[236] Maddock, R.J., pg. 36
[237] Maddock, R.J., pg. 36

mathematics and chess). During these sessions of exercise the brain producers an enormous amount of new neurotransmitters, which contrasts its regular metabolic activity.

A study of 38 healthy adults asked half of them to exercise to 85% of their maximum heart rate and the other half remained sedentary. Glutamate and GABA levels were measured before and after the respective exercise or stationary sessions. Both these neurotransmitters showed significant increase in the exercising group compared to those who did not exercise[238]. The visual cortex and anterior cingulate cortex (responsible for cognition and emotional processing) showed especially large increases. After a week of exercising the resting levels of glutamate and GABA were measured once again, and it was confirmed that the exercising group still showed an increase in these neurotransmitters compared to those who did not exercise.

This study provides imperative insight into the power of lifestyle changes in combatting depression and anxiety in our daily lives. It is possible that as research continues, exercise forms a more integral part of depression and anxiety treatment plans. However, as individuals, we can all make these changes now, whether we are stressed or not. Knowing that vigorous exercise has such immense health benefits[239] is reason enough to start living more active lifestyles and striving to live in a way that helps our brains function at an optimum level.

Meditation and Mindfulness

Another lifestyle change that has caught the eyes of many neuroscientists and psychiatrists in the last decade has been the practise of meditation and its health benefits regarding stress resilience, overall neurological health, and decision making. Becoming mindful of our experiences, calming the nervous system, breathing deeply and grounding our thoughts are all vital emotional resilience tools that have proven to be essential in working through and overcoming stress and trauma.

[238] Maddock, R.J., pg. 36
[239] Maddock, R.J., pg. 36

In one study, researchers focussed on the Kirtan Kriya meditation technique which involves a simple 12-minute practise of mantras and breathing exercises. In conjunction with the meditative practises over the course of a week, they also assessed participants on their degree of "purpose in life" (PIL)[240]. PIL alone proved to significantly impact their mental and physical health, showing that people with a higher PIL were 2.4 times less likely to develop AD, displayed better cognitive functioning, less pathological conditions, and an increased sense of peace and contentment, regardless of their daily stressors[241].

After the Kirtan Kriya 12-minute meditations were practised for a week, researchers used fMRI scans to compare brain activity before and after the study. These images revealed an increase in the blood flow to critical regions such as the hippocampus, prefrontal cortex and amygdala, specifically increasing neurological activity in grey matter[242] throughout the brain. These findings show that practising meditation for merely a week already positively influenced brain functioning, and could result in slower ageing of the brain.

When our minds get a chance to be quiet, such as in meditation, it is clear that something in our brains change. Whether that is because we get a chance to reset, to let go of stressful thoughts or emotions, or calm our breathing, is not specifically known. However, it is clear that meditation has some degree of positive influence in brain functioning. As the field of "spiritual and mental fitness" expands[243], similar studies are expected to reveal more about the mechanism behind these changes in brain activity and possibly provide helpful insights in how we can all implement these practises into our daily lives to become healthier and happier individuals.

It is when we live in a constant state of stress where these techniques are the most effective, quieting the voice of "what if" or worst-case scenarios. Being mindful, self-aware and taking the

[240] Khalsa, D.S., pg. 507
[241] Khalsa, D.S., pg. 508
[242] Khalsa, D.S., pg. 511
[243] Khalsa, D.S., pg. 513

time to center ourselves brings us back to the present moment, releasing tension in our shoulders, untying the knot in our stomachs, or easing the lump in our throats. When we fail to stay in the present moment and recognise our feelings as being temporary, we hold the tension and stress in our body, which makes us increasingly vulnerable to being engulfed by everyday stressors that others can navigate with ease. The first step to controlling our emotions and changing our mindsets is by coming aware of our inner world, our emotions, and embracing the ever-changing nature in which they come and go. The transience of emotions is what makes them so beautiful to experience. However, when we are chronically stressed we tend to fixate on the negative feelings, forgetting that they too will pass if we allow them to run their course without too much resistance.

Sleep

One of the most important influences on our resilience when it comes to dealing with stress is how much we sleep. The average human being, from young adults to about your late 60s, require 8 hours of sleep a night. When we are in a deficit, our whole body is thrown off balance, making us more vulnerable to stress, intense emotions, diseases, and allergens[244]. This fact has been proven time and again, despite many other sleep fads that have tried to disprove it. Once we reach the age of 70, our sleep needs change slightly, but only by an hour or two, requiring on average about 6.5 hours of sleep a night[245]. We sleep for a multitude of specific reasons, but in general it is to rest and regenerate. While we sleep our bodies are able to reset our homeostatic equilibrium, regenerate cells that might have been damaged or destroyed, encode memories of the day before, organise information in our brains to help with decision making in the future, and focus on digestion in order to provide us with the physical and mental energy we need to face a new day. When these vital processes are interrupted, cut short, or simply ignored, we become vulnerable to increased emotional

[244] Chopra, D., pg. 186
[245] Chopra, D., pg. 187

stress, our immune system weakens, and in extreme cases we could experience hallucinations and the failure of various bodily systems[246].

When we are experiencing a chronic state of stress, there is a constant level of elevated adrenaline circulating our bloodstream[247]. This counteracts the ability to experience the full restorative function of sleep, and often leads to inability to fall asleep, or waking up in the middle of the night. The elevated levels of adrenaline that still circles our bloodstream after a stressful day, or during a stressful period, results in a greater state of arousal than usual. In this case the brain is unable to relax and slow down, it is constantly racing to find the next potential threat, or to resolve the future problem that might arise. These thoughts stimulate our internal stress response even further and we wind up in a perpetuating cycle of internal arousal that becomes incredibly difficult to break. Days, weeks, or months ago by where we fail to recognise this and the longer, we remain trapped in the cycle, the more sleep debt we accumulate. Our minds and bodies cannot function optimally in this cycle, and breaking it is some people's biggest challenge in overcoming the effects that stress have on their mental and physical health[248]. One week (or sometimes even one night) spent sleeping 8 hours a night can significantly improve our moods, resilience to emotional and professional stress, and reverse a large extent of the chronic stress response that is causing inflammation, pain and panicked alertness within us[249].

The 21st century term known as *sleep hygiene* refers to how well we sleep, our routines before and after sleep, and how effectively we rest during we spend in bed. A few of the primary elements that constitutes good sleep hygiene is making your bedroom as dark as possible, limiting screen time an hour before bed, meditating 10 minutes before bed, consuming no caffeine 5 hours before sleep, and most importantly, getting the 8-9 hours of sleep a night that your body needs to

[246] Chopra, D., pg. 190
[247] Dispenza, J., pg 279
[248] Chopra, D., & Rudolph, T., pg. 199
[249] Chopra, D., & Rudolph, T., pg. 198

recuperate. Having a regular and enjoyable sleep routine is essential to prime our minds to wind down after a long, and often stressful day[250]. Our ability to do this, to relax and reset, allows the body to reverse whatever stress response might have been initiated during the day, to resolve inflammation in our cells, and to prepare us for new and unknown challenges that await the next day.

You Are What You Eat

As we have established, our gut microbe has a significant influence on our stress response, mental health, and brain functioning. Our stress response also involves inducing inflammation in the body which leads to serious physical and emotional pain. Through the foods we consume and the diet we follow we can make effective, scientifically proven changes to the how vulnerable we are to these physiological effects of chronic stress and how healthy our gut microbe is.

When we are chronically stressed, most individuals are experiencing a constant level of internal inflammation without even realising it. This increases the risk for obesity, heart disease, cancers, and leaky gut syndrome. Although dietary fads seem to change like the seasons, there are several dietary changes that we can slowly start implementing in our lives to reduce this inflammation and improve our overall physical, as well as emotional health in order to be less affected by the stress response. If we are physically healthier and balanced, it often becomes a lot easier to balance other tasks that caused the stress in the first place.

The key to these changes are to reset the gut microbiome, increase its diversity, and stimulate bacterial growth at an optimum level in order to extract as much vitamins and mineral out of the food we consume to nourish our brains and bodies. Although there are many toxins we consume on a daily basis without knowing it, the 5 white poisons are the most dangerous[251], and ironically also the best hidden in plain sight. They are pasteurized cow milk, refined rice, refined sugar, white flour, and processed salt. These 5 foods are hidden in almost any and every product we consume, and in excess

[250] Chopra, D., & Rudolph, T., pg. 189
[251] Articles, H.

they come together to contribute to the leading health issues in the United States and globally today[252]. The problem is, when we are stressed, pressured for time, or particularly exhausted, we tend to gravitate to exactly these five things – just think about a McDonalds drive through. Fighting the urge for junk food and the idea that unhealthy food is some sort of 'reward' for a difficult day is the first step to building healthier habits and committing to transforming your internal environment to eliminate inflammation.

Changes to Implement in Our Diets:

Beginner	Intermediate	Challenge
Prebiotics – Bananas, bran cereal, orange juice with pulp	Probiotic supplements and multivitamin every day	Become vegan or vegetarian.
Add a side salad to each meal	Cut out red meat	Adopt a Mediterranean diet.
Probiotics – Yoghurt, kimchi, sauerkraut	Limit alcohol	Cut out all alcohol.
Whole-grain bread and grains	Cut out any refined sugar, salt and flour	Reduce any packaged food
Limit snacking by portioning	Stop eating processed food	Eat mostly gluten free
Share desert	Eat more organic chicken, eggs and dairy products	Intermittent fasting
Eat fish once a week	Stop eating when you are not hungry	

Although these changes seem small, they make some of the biggest changes within our bodies. It has been proven that switching from a meat-eating diet to a Mediterranean diet (mostly fish, nuts, fruits, and vegetables) caused a shift in the gut microbiome within 3 days, with most individuals feeling more energized and less bloated and lethargic[253]. This not only influences our brain functioning, but also has positive influences on our epigenetic profiles, especially in pregnant women and how their internal environment affects the degree of methylation on the child's genome.

[252] Chopra, D., & Rudolph, T., pg. 124
[253] Chopra, D., & Rudolph, T., pg. 126

Some of the foods that aid in fighting stress-related inflammation in the gut: berries, nuts and seeds, fish high in Omega-3 fatty acids, turmeric, dark chocolate, soy and tempeh, ginger, garlic, and olive oil, etc. All of these foods are easily incorporated into modern dishes and can result in changes within our bodies that could add several years to our lives and reduce our risks for developing type 2 diabetes, as well as dementia, with one third of dementia cases linked to a poor diet[254].

Language, Relationships and Community

When we think of stress and trauma, we automatically think of the "fight or flight" response that we are all too familiar with. The classic reference to our stress response that is filled with adrenaline and high heart rates and heavy breathing and quick movements. However, since we are all very complex humans, it is fair to say that our response to stress is much more complicated than the evolutionary reaction of running away from a wild animal. The way we respond to our stress and how we overcome it heavily depends on the people around us, our ability to express ourselves, and how safe we feel in the moments after experiencing the stressor or traumatic event.

The first person to recognize this complexity in how we perceive and respond to danger in the environment was Stephen Porges, a researcher from the University of North Carolina. In 1994, Porges suggested that the human response to stress (including danger, trauma, etc.) involves the recognitions of instinctive changes within the body, as well as the facial expressions, behaviors and voices of those around us. He essentially explained why hearing a calm voice, seeing a friendly face, or experiencing a kind gesture makes us feel safe and seen. His researched also explained why we are so enraged when the people closest to us seem indifferent or dismissive towards us. This all involves our perception of the people around us. Whether conscious of it or not, our mirror neurons are constantly monitoring the people around us, picking up their internal emotional states and adjusting ours accordingly. Something as small as a creased brow, pressed lips, a tense jawline, or a

[254] Chopra, D., & Rudolph, T., pg. 128

finger tap by someone close to us, could signal something to our own emotional state and results in us changing our behavior accordingly. Most of the time, we are completely unaware of this. All of these signals allow us to judge whether we are safe in the presence of that person or not, fundamentally protecting us from any potential danger[255]. When we recognize a soft tone, a gentle smile, an open posture, we feel safe and our nervous system relaxes, regardless of the external stress we might experience. However, when the opposite is true, when the person is crass, frowning, and have their arms crossed, we immediately put our guards up and our internal alarm is triggered.

In society today, we like to believe that to be "unaffected" by the moods of others is the goal of a successful emotional state, yet it has been proven time and again that we are hardwired for connection, even at the simplest level of mirror neurons. Whether we care to accept it or not, our emotional states and stress response is greatly dependent on the people we surround ourselves with.

Community, Connection, and Courage

When we think of trauma and stress, many of the experiences that come to mind will inevitably involve other people, often those that were meant to be our biggest support systems who turned on us in our most vulnerable moments. Whether it was heartbreak, disappointment, abuse, chronic conflict, etc. Our human relationships can cause tremendous stress in our lives. However – they are also the single most effective treatment to work through and relieve stress and trauma.

Throughout history research and human experience has shown the power in solid support networks during times of intense stress or discomfort. The calming feeling of a familiar voice, of a long hug, and of being in the presence of someone we can be ourselves with allows us to release the tension of everyday life. It turns out that adults, just as children, find comfort in the same primitive practices: being held, swaddled, and swaying gently. This means that, just like the stress response of fight or flight stems from evolutionary behaviors, so does the anecdote for it. It is a globally agreed

[255] van der Kolk, B., pg. 80

upon fact that human beings recover from stress and trauma in the presence of others, in the connections that form in community, and the roots we can return to in our most vulnerable moments.

This is due to a multitude of reasons, but as we have established, many of the neurons in our brains are wired to be in sync with, interpret, and respond to those around us (namely mirror neurons). However, due to our pasts and the discomfort that is initially felt in fostering new relationships, we can fear relationships with others due to the lack of control we have in whether or not we get hurt. Our past traumas can have detrimental and lasting impacts in the relationships we have with those that love us most. This is why it is so incredibly important that we process trauma, that we try our best to not become chronically stressed to the point that we completely disengage from our realities and push away those that care about us most.

The key with a healing and healthy relationship that can alleviate stress and allow us to work through our deepest, darkest traumas has been found to be one where the is mutual trust, where you can speak your heaviest feelings and the other person can hold them without judgements, where someone can protect the wholeness of your heart while you slowly glue back the pieces that have been broken for so long, where you can release the things that stress you out the most without feeling as though they are irrelevant or insignificant[256]. It is in these relationships, no matter how few and far between they might be, that we can reconnect with the most human parts of ourselves and move forward without the heaviness we have carried for so long.

It is important to note that this type of relationship might not exist in a social setting or in your family. In these cases, finding a professional therapist could be a wonderful, life-changing step to recovery for the individual. There are also certain instances, especially in severe childhood trauma or domestic abuse, where you might not remember a time where you have ever felt safe with another

[256] van der Kolk, B., pg. 213

human being. It is here where therapy involving animals such as horses, dolphins, or dogs can be extraordinarily influential in coming back to who we are and feeling safe within ourselves.

Another aspect of community that has been particularly evident in recovery with regards to rape survivors and individuals that have experience extreme violence in their childhood or adulthood is the power of human rhythm. Growing up in South Africa, which has a vibrant and diverse tribal community network, I have seen the power of music and dance in healing trauma, firsthand. This has also been recorded by a multitude of researchers across the world. One quiet "hum" and a slow tap of the foot by an older woman becomes contagious in a circle of rape survivors, and before you know they are all singing and dancing to the same tune[257]. This group of women who came there with slouched shoulders, hollow eyes, and very limited words, were now dancing, smiling, and had a new glimmer of joy in their eyes. Whether it is the hum of a stranger, or a song on the radio, or dancing with a friend, these small moments are what allows for the most healing. Here, in our childlike joy that returns with music and dancing, we are able to break down some of our walls, experience the human experience, and release both physical and emotional tension that we might not be prepared to talk about yet.

So, what we have learned over the last 100 years of research regarding human behavior is essentially something the very first humans already understood somewhat 6 million years ago: we are made for community[258]. The sooner we accept this, the sooner we move towards healing the darkest, heaviest, and loneliest parts of our souls. When we realize that every single one of us in this moment, as you are reading this, is carrying something heavy in our hearts, it is much easier to connect, to foster relationships, and to have the courage to be vulnerable. As soon as we accept that trauma and chronic stress has become a collective human experience to varying degrees, we will be able to form stronger communities, deeper connections and live more present and meaningful lives.

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[257] van der Kolk, B., pg. 216
[258] van der Kolk, B., pg. 216

At the end of the day, as powerful as we are in hurting each other, we are infinitely more powerful in healing each other.

You Are Safe Here

Throughout all of the studies done on human mental health, stress, trauma, and emotional resilience, one outstanding feature that emerges from every realm of research is the idea of having a physical and emotional space in our lives where we feel safe, seen, and accepted for all that we are[259]. Over and over again we have seen that having safe connections with other human beings is the outstanding element in a rewarding and content life. The degree of social support that we experience during times of trauma and extreme stress strongly influences our recovery time and our ability to move forward. Feeling safe is not something that can be quantitively measured or created, but a key part of it is how reciprocal our relationships feel, whether we feel heard and seen, and whether the people in our lives respond to our needs (and vice versa). Individuals who are going through or recovering from chronic stress and trauma often feel detached, disconnected and removed from the ebb and flow of society, and thereby avoiding human connection. However, although it is far from being a prescription drug we can pick up at the nearest CVS and much harder to cultivate from scratch, safety and human connection through community, friendship and family has proven to be vital in our ability to work through and recover from some of our most painful trauma and stress.

When we are in this hyper-vigilant state of the human stress response, one of the main goals of therapy is to reprogram the nervous system, decreasing the emergency response and allowing ourselves to calm down so that we are able to engage in meaningful connection and reciprocal, safe relationships[260]. Experiencing or cultivating this sense of "safety" occurs in three different ways, the first is social interaction, but the latter two are strategies our brains use to imitate the idea that we are safe, whilst the stress response still runs wild within our bodily systems. When we are stressed and

[259] van der Kolk, B., pg. 81
[260] van der Kolk, B., pg. 82

we reach out to the people around us for help or guidance, we feel safe, and our nervous system can relax. This process is deeply connected to the ventral vagal system (VVC), largely involving the vagus nerve which controls our facial expressions, vocal expression and middle ear. The VVC recognizes that we are safe through picking up on the facial expressions, tone of voice, etc. of those that have come to help us in our time of stress, and then communicates to the heart to slow down, and our lungs to breathe deeper. Ultimately, we then become calmer, more centered, and able to relieve the stress response. Therefore, being in sync with the VVC systems of the people in your surrounding community is vitally important to mental health and our ability to deal with stressful or traumatic experiences.

However, when the people that we call on for help in our most vulnerable moments fail to show up, our minds and bodies go into the well-known *fight or flight* response. Our stress response increases, and the limbic system is activated, which initiates the sympathetic nervous system to increase heart rate, constrict breathing, decrease digestion, and mobilize muscles. Our emergency department kicks in and our metabolism is drastically reduced, our pre-frontal cortex's ability for rational and logical thought decreases, and we either run, or try to fight.

When this system fails us as well and we are still unable to escape or reduce the perceived threat, whether this is stress from work or emotional abuse in the home, we shut down. We simply disengage and freeze, because out bodies and minds reach a limit to which they are able to fight the stress response which disturbs the homeostasis in our internal environment. At this point, it feels as though your heart drops to your stomach, your metabolism is almost completely shut down, and you are at a loss for words. Getting to this edge of the human ability to tolerate stress (in any form: chemical, physical, or emotional) is dangerous territory, yet we find that so many people in society today are completely unaware that they are walking on this exact tight rope, with the chance of crashing down at any moment. It is here where physical symptoms, often disease or chronic pain, sets in and we find ourselves in the hospital with seemingly 'no idea' how we got there.

It is our connections with others, as research has proved time and again, that holds us in true safety, that prevents us from destroying ourselves, and that can ultimately save our lives. In order to foster and build these connections that make us feel safe we need to be willing to take some of our walls down, to be vulnerable, and to relax in the presence of novelty. As Porges would put it, "we need to turn off our natural vigilance"[261]. However, individuals who are under chronic stress or who have experienced trauma in their lives are often unable to let down their guards and make these connections. They are unable to enjoy the stable, simple joys in life and rather seek out adrenaline rushing, sometimes dangerous experiences to feel something, anything, other than the numbness that has resulted from their trauma. This is partially also due to the fact that these individuals have a harder time in distinguishing between when they are truly safe, or just feel that way because the events seem familiar to their past. It has been proven that women who experienced childhood sexual abuse were 7 times more likely to be raped in adulthood[262]. Many stressed and traumatized individuals feel safer if they can control their relationships, keeping a certain distance from everyone in their lives, limiting conversations to small-talk, and never letting anyone in to see their insecurities or stressors. However, this is a false and frail sense of safety because true connection requires trust, intimacy, and allowing yourself to be seen for who you truly are, despite the fear of rejection. It is through these moments of being seen, of sometimes uncomfortable vulnerability, that we acknowledge our stress. This enables us to let others help us to carry the heaviest parts of our hearts and minds, and eventually work through these things to let them go, and move forward to a lighter, safer, and more relaxed future.

Contagious Stress

When we think of stress, it is natural to wonder how it affects our brains and bodies, but have you ever wondered how your stress could be affecting those closest to you? In a ground-breaking

[261] van der Kolk, B., pg. 86
[262] van der Kolk, B., pg. 87

study in mice researchers found that the effects of stress on our brains can be transferred (or rather mimicked) by those around us. They also found that social interaction in a group plays a large role in guarding against these effects in the brain. However this was more effective in females, with male mice retaining some of the neurochemical responses even after social exposure.

Saying that our stress is "contagious" might be taking it a bit too far. However, when we think about our emotional response when we see those that we care about in stress, it makes sense that our brains and bodies are also experiencing some degree of stress or emotional discomfort[263]. When studying these effects, researchers looked at mice in pairs, exposing one of the mice to a mildly stressful encounter and then placing them with their partner in a secluded area. They specifically looked at the CHR neurons which are involved in the brain's stress response and found that these neurons were altered in the same way in both the stressed and control mice. The changes in the mice that were never exposed to the stressor, but only had contact with their stressed partner, were identical to those of the mice that experienced the stress directly[264].

When the researchers used optogenetics to activate the CHR neurons in one of the partners, even without inducing stress directly, these effects were still mirrored in the neurons of the partners who received no optogenetic interference. It was concluded that the CHR changes in the brain cause the stressed mouse to release pheromones that signalled their partner of the stress they had experienced[265]. However, when female mice were stressed and then exposed to their partners, their stress response in the CHR neurons were halved, suggesting that social interaction resulted in some degree of induced stress resilience. Yet, these effects were not seen in males. This is not only a way that mice protect and warn each other of critical information in their surroundings, but provides an insight into the biological benefits for species to exist within a social network.

[263] Sterley, pg. 34
[264] Sterley, pg. 34
[265] Sterley, pg. 37

As humans, we do not need pheromones to signal to others that we are stressed, we already do so through our body language, words and responses to certain triggers. When we sense each other's stress it provides an opportunity for social connection and to help each other through stressful times. However we must be cognisant of the lasting impact untreated chronic stress can have on those around us. They might be long-lasting, irreversible and damaging to future generations and those that we love most.

Men and Women

An interesting, and noteworthy issue to address regarding these studies about stress and the human response to it, is that many of these experiments only involved male participants. This is problematic for various reasons, but particularly because it has been proven that women and men differ majorly in their stress responses and coping mechanisms. Different parts of the brain are activated during psychological stress in men and women, causing the well-known "fight or flight" response in men, but a newly termed "tend-and-befriend" response in women. Understanding the differences in the various ways that men and women experience stress is crucial to determining how the stress response can be managed and how the detrimental effects of stress could be identified and treated.

This response stems from an evolutionary element of human nature. Men used to either confront the stress factor head-on, or run away, whereas women were much more likely to take on a motherly role, nurturing and caring for others in a social setting. In a study conducted with 320 individuals (half male, half female), researchers analysed the cerebral blood flow (CBF) patterns to determine which areas of the brain were most active during a performance-based stress test. The right prefrontal cortex in male participants experienced an increase in CBF, whereas the left orbital cortex had decreased CBF. On the contrary, women's limbic systems (the center for regulating emotions) were the main hub of CBF during these stress tests. The CBF levels also remained elevated in the females' brain for much longer than in males. Another interesting response was that

cortisol levels in men underwent a much larger change than in women, showing very little variation between stressed and neutral levels.

The stark differences in the stress response between men and women are not only fascinating, but vitally important to recognise and to explore further. Women are twice as likely to develop major depression disorder, anxiety and other related psychological disorders. This could closely be tied to the fact that women experience the majority of their stress in the area of the brain that controls our emotions, and that the stress response lasts much longer than in men. These alterations could greatly influence a woman's ability to cope with everyday stressors, emotions, and daily tasks.

Gene Editing & How It Can Help

The study of the interplay between human health, genetics, and behaviour is an ever-growing, interconnected field that aims to comprehend the different ways in which our brains and bodies respond to the environment and are influenced by our genetic code. Neurological analyses, brain imaging, and behavioural studies have all shed a light on the intricate dynamics of the connection between brain activity and epigenetics. However, with the advancement of gene editing and animal models a plethora of new opportunities have arose to study the specifics regarding the way the brain and human behaviour are influenced by the human genome, and vice versa.

Since the discovery of clustered regularly-interspaced short palindromic repeats (CRISPR), the possibility of detailed genetic editing has played a vital role in expanding our understanding on the significant impact of epigenetics on the huma genome. CRISPR revealed a world full of new opportunities in terms of gene therapy, enabling the study of neurological diseases in both animal and cellular models. Traditionally, CRISPR is initiated by double-strand DNA breaks (DSB)[266] at a targeted location, after which the break could be repaired either by a non-homologous end joining (NHEJ) mechanism, which is highly prone to errors, or by homology-directed repair (HDR)

[266] Rahman et al., pg 26

pathways in order to introduce new mutations or transgenes. CRISPR-Cas9 is guided by an sgRNA sequence and forms base pairs that are complementary to the targeted sequence.[267] The base pairing occurs with CRISPR RNAs (crRNAs) and is monitored by *trans*-activating RNA (tracrRNAs), forming a complex tracrRNA: crRNA. This is essential for facilitating the binding of Cas9 and thus, for gene expression. CRISPR's ability to be readily engineered and modified, makes it an ideal mechanism for gene editing and chromosomal manipulation to study neurological disorders and other diseases in animal models.[268]

CRISPR has enabled researchers to gain a more in-depth understanding of the functioning human genome, particularly in terms of the nervous system. Through homologous recombination in embryonic stem cells, human neurological diseases have been modelled in animals such as rats, rodents and primates.[269] By means of genetic editing involving zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR-associated (Cas) effector proteins, there has been development towards site-specific gene editing that could control transcription and modify genes associated with neurodegeneration specifically.[270] One of the greatest advantages of the CRISPR-Cas9 protein is that, together with single-guide RNAs, this complex is able to edit a variety of genes at once, saving time when considering the effect of multiple genetic modifications and their interactions in genomic disorders. [271]

Gene altering often involves the knockout and knocking-in of genes using the CRISPR technique. This has been studied in *ex vivo* brain slices, but also in *in vitro* analysis where research has been conducted with cultured neural cells of embryonic and adult mouse brains, investigating whether certain genes influence and promote given neurological disorders. Vectors of Cas9 and sgRNA were distributed into a hippocampal culture, specifically directed at two synaptic proteins

[267] Heidenreich et al., pg 38
[268] Rahman et al., pg 23
[269] Heidenreich et al., pg 40
[270] Heidenreich et al., pg 41
[271] Rahman et al., pg 25

(NMDA and AMPA) involved in a variety of neurological diseases. When this 'knock-out' was completed, "the electrophysiological function of the NMDA and AMPA receptors was eliminated, demonstrating efficient delivery of Cas9 ex vivo."[272] This is just one illustration of the power of CRISPR as a gene editing technique and provides promising evidence for therapies in future neurological research. In the interest of stress, this could provide a novel and direct mechanism to reverse or relieve life-long, debilitating changes in gene expression that has been caused by childhood trauma, chronic stress, or PTSD.

The techniques of "knock-in" genes are still relatively inefficient, yet with recent studies there have been encouraging results in genome editing where neurodegenerative diseases have been studied in animal models. With the use of chemically synthesized RNA in combination with the Cas9 protein, the gene insertion effectiveness increased by 50%[273]. Through genome editing in single-cell embryos of various animals, one can study the functions of specific proteins within the nervous system and compare that to the complex phenotypes that are observed during the human stress response and several neurological disorders.[274]

Due to the efficiency and simplicity of CRISPR knock-out techniques and its ability to eliminate multiple genes at once, there have been various studies in mice where several mutations in embryonic stem cells are observed simultaneously, enabling a much more comprehensive and accelerated model where genes can be manipulated within one generation, instead of repeated cloning in extended generations. This technique has also been investigated in both rodents and other non-human primates[275] where a successful study with primates used retroviral and lentiviral mechanisms in early embryos to insert the human gene Huntington (HTT) in order to analyse the

[272] Feng et al., pg 461
[273] Rahman et al., pg 24
[274] Heidenreich et al., pg 37
[275] Feng et al., pg 461

clinical signs of Huntington's disease (HD).[276] This provided a novel understanding of the complexity of HD and what future therapies could entail.

Although primates are more closely related to humans, rodents are currently the most desired subjects of study due to their conservation of cellular and molecular mechanisms in the brain and neuronal functioning. This allows for the introduction of human-induced-pluripotent stem cells into the rodents' genetics in order to study human neurological diseases such as Alzheimer's, Parkinson's and ALS, which is highly valuable for progressing to a better understanding of these complex disorders. There also seem to be fewer ethical concerns regarding the editing of rodent genomes for neurological disorders than there are with primates, due to the fact that primates are highly social animals and in many ways, extremely similar to humans.[277]

CRISPR is a promising tool for the future of genetic modelling, yet there are some limitations in terms of the geniting editing which have to addressed. One of these complications is the off-target effects that are often caused by shorter sgRNAs where there is a mismatch between the targeted locus and the actual locus that is affected by the CRISPR/Cas9 complex. Despite the fact that these differences are often minute, they do pose a threat since they able to cause oncogenetic mutations. A way to combat this is to produce highly sensitive sgRNAs which are able to withstand some mismatched bases to the sequence, but this may not always be effective.[278]

Another challenge faced by gene editing is the delivery inside of the host. This is either done by "direct administration (involving microinjections, electroporation, hydrodyamin injections, etc.), chemically mediated transfection (cationic polymers, dendrimers, etc.), or virus mediation (lentivirus, adenovirus, adeno-associated virus, etc.)"[279]. Direct administration is often not deemed as

[276] Heidenreich et al., pg 40
[277] Jennings et al.
[278] Rahman et al., pg 28
[279] Rahman et al., pg 29

effective as chemical transferring, yet it is still utilised in both in vivo and ex vivo experiments since it is relatively simple when there are larger units that could be influenced by cell toxins. [280]

The viral approach is non-pathogenic and allows for long-term expression of without gene integration which is advantageous, but is limited to transgene capacity. There are also concerns regarding immunogenicity, carcinogenesis and off-target effects. However, it remains successful in carrying sgRNAs and Cas9 when conducting *in vitro* and *in vivo* studies.

There are various ways in which to avoid these limitations, namely nanoparticle-based compositions, cell-penetrating peptide, etc. Notably, the use of exosomes is particularly valuable in neurological research due to their ability to cross the blood brain barrier and that they have been successful in *in vivo* delivery of the Cas9 complex into mesenchymal stem cells. [281]

When delivering the edited genome to the brain in particular, there are four pathways allowing for accurate expression of sgRNA and Cas9. These methods consist of a plasmid expression vector, viral transfusion, as well as a ribonucleoprotein (RNP) complex with recombinant Cas enzymes and sgRNA. This third method occurs quickly and is advantageous in avoiding an error in the genomic incorporation. Lastly, there is *in vitro* transcribed Cas mRNA and sgRNA which is preferred for pronuclear microinjections in an embryo. With viral transfusion, the size of the vector could hinder the accuracy, however to counteract this limitation a smaller strand of Cas9 is used in the brain, allowing for the transduction of genes into specific cell types throughout various developmental phases. [282]

Neurological diseases are often caused by the stagnation of brain development and a weakening in the uphold of neural pathway. These neurological deficits have been studied in animal models where mutations were experimentally introduced into the genome, as well as human-derived clinical cell samples, revealing convincing evidence of significant genetic influence on the

[280] Heidenreich et al., pg 41
[281] Rahman et al., pg 29
[282] Feng et al., pg 465

functioning and development of the brain[283]. Disorders such as autism, epilepsy, dementia, Alzheimer's Disease, Parkinson's Disease and brain tumours are all neurological, yet many stem from genetic mutations and signals. For example, in Autism there are 845 genes associated with the disease and the CRISPR technology provides the potential for genes to be edited or "knocked out", given that a safe mechanism is determined in the future[284].

Another aspect of CRISPR, gene expressions and human behaviour is the role that emotions and stress plays in the epigenesis of the human genome. When considering the effect of stress on a person's gene expression, health and behaviour it is evident that there are long lasting consequences, which has also been studied in mouse models. Since stress affects the neurotransmitters within the brain and influences the plasticity in the cortex, hippocampus and amygdala, it can permanently impact the functioning and structure of the brain[285]. Mostly, these changes that were detected in mice that were exposed to external stressors, led to neurodegeneration, cognitive deficits and changes in behaviour, memory and learning[286]. As the brain responds to stress, there is also an epigenetic response in the form of DNA methylation and dynamic gene expression which is expressed in the "synaptic plasticity of the brain, memory and cognitive processes."

These genetic alterations that are cause by chronic stress can result in gene silencing, inactivating X-chromosomes or exposing sections of DNA for varying amounts of time, which inevitably turns the gene on or off, changing the protein production[287]. This change can affect behaviour and can be passed on to the next cell within the same organism, or even be transmitted from one generation of mice to the next.[288] This is an important distinction to make when comparing natural changes in genes via epigenetics to man-made genetic alterations in animal models, since the role of epigenetics is highly influential and cannot be accurately predicted.

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[283] Feng et al., pg 465
[284] Feng et al., pg 464
[285] Stankiewicz, A. M., pg 80
[286] Lee at al.
[287] Roth et al., pg 761
[288] Roth et al., pg 761

As CRISPR technology improves and more research is done on the risks involved with genetic manipulation, there is a high possibility that most diseases that are deemed "incurable"[289] could have a cure in the near future. Seeing that the genetic mutations that are linked to neurological disorders are becoming more accurately identifiable, research into new and effective therapies can be conducted.

Mechanisms and strategies in gene editing are continuously upgraded with the altering of various genes simultaneously, enabling a multi-faceted efficiency that has not been revealed before. Integrating these methods into personalised treatment strategies will transform the perspective of neurological disease therapy in the future. Currently, the biggest challenge is the "site specific delivery" of the altered gene[290]. This must be further researched in order to observe viable mechanisms that could be used in humans in the future, but despite multiple animal models being studied, it is challenging to make accurate conclusions from these results since the human brain and genome is dynamic and differs greatly from other animals.

The Final Word

Understanding how stress affects our brains, bodies, and every other aspect of our existence both physically and psychologically is what gives us the power to act against it. As seen in the numerous different studies and scientific knowledge shared in this thesis, the negative effects of stress is undeniable, yet there is so much we can do to prevent, minimise, or reverse them. Whether we increase our daily exercise, become more cognizant about when and how we have children, the social exposure we receive, or even just by acknowledging the past trauma and stress in order to move forward – all of these practices shift us into the right direction. They bring us closer to a world where we are not all chronically stressed, suffering silently as we hustle to get the next promotion, or

[289] Rahman et al., pg 29
[290] Feng et al., pg 463

raise, or social status upgrade. Through educating ourselves in this field we gain the power to change our own lives and those around us.

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