Eco-Neurobiology, and How the Environment Shapes Our Brains

Eco-Neurobiology, and How the Environment Shapes Our Brains

By

Andreas M. Grabrucker

Cambridge
Scholars
Publishing

Eco-Neurobiology, and How the Environment Shapes Our Brains

By Andreas M. Grabrucker

This book first published 2019

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-4035-9
ISBN (13): 978-1-5275-4035-4

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Many things have to come together symbiotically (and otherwise) to enable someone to produce something as significant as a book. I am very grateful that they did and I am aware that besides hard work, lots of luck and the help of others are inexorably involved. I was and am very lucky that various funding sources have supported my research, including Deutsche Forschungsgemeinschaft (DFG), Alzheimer's Association - and currently the Autism Research Institute and Else Kröner Fresenius Foundation - that fund my projects on environmental factors in Autism. I am also delighted to have a great research team at the University of Limerick, with Ph.D. students that never fail to generate lots of exciting data while I am busy with other things, and final year project students like Amy Donoghue, who stumbled across Neuroscience in my lab and became so intrigued that she agreed to proofread this book.

The Bernal Institute of the University of Limerick gave my research group a home and provided a network of other PIs that are, in no uncertain terms, incredibly supportive. The Health Research Institute at the University of Limerick helped me connect with many like-minded researchers. However, I did not have to look far for one of the most supportive researchers, as my wife is one of the best behavioural neuroscientists I know. Being a scientist is not a 9-5 job; instead, it is an attitude towards life. It is impossible to stop working on a set of new and exciting data just because it is time to go home or eat, or sleep. Having someone on your side that shares this love and passion for research makes the journey a lot easier.

Thanks.

PROLOGUE:
THE POST-GENETIC AGE OF NEUROSCIENCE

As is usual for Ireland, it was a cold and rainy day at the University of Limerick. My colleague Nicholas Fleming and I enjoyed a break between our Anatomy and Physiology labs that we were teaching for the previous two hours, with two more hours to go. Sitting in the University Café over a Latte, we started discussing our research and exploring the possibility of collaborating someday. This is not an uncommon conversation to occur between colleagues of a department, except that Nick happens to be a marine ecologist, and I am a neuroscientist focusing on human brain disorders.

As distant as these two scientific fields of research may sound, it turns out that it is not hard to find areas of convergence, because although not a very aquatic species, humans do have a strong connection with the marine ecosystem. On the one hand, humans influence the marine ecosystem – usually negatively – by dumping all kinds of material into the oceans (often waste). On the other hand, - also generally in a bad way – we influence the marine ecosystem by excoriating things from our oceans (salt, fish, algae and anything edible). However, what we often seem to miss is the fact that this system is susceptible to backfire on us because although the things we put into oceans do seem to disappear from our minds, that is not really the case. The reason for this overlook may be that some waste like chemicals in liquid waste are hard to see and also more massive waste, over time, is getting too small for us to be been seen by the naked eye. Plastic being a prime example: Quite ironically, uncountable tons of plastic can be found in our oceans – the very same oceans we get our food from. Over time, the plastics disintegrate and get increasingly smaller until they become so-called micro and nanoplastic.

So, as it turned out, Nick and I were sitting and he was telling me about how much these nanoplastics have become enriched in the oceans over the last number of years, with the possibility that they will be consumed by small organisms, which will be eaten by larger ones, eventually ending up in fish and on that account, on our dinner table entering the human food chain. I was talking about brain disorders, in

particular, my favourite research subject autism, and how much the incidence rate of autism has increased in the last few years. So, we were asking ourselves, do we actually know all the human-made things in our environment that influence our brain and its functioning, how our brains develop, and how environmental factors affect our mental health?

The answer is that sometimes we can. As a matter of fact, we are doing this more and more. We know how a nutritional component or the lack thereof changes brain functioning. We know about some pesticides that have nasty effects on the developing brain, and we can increasingly talk about gut-brain interactions, which implies that all the bacteria in our guts, the gut microbiota, are affecting our brain. And we manipulate them by using, for example, probiotics. We all know that stress affects us and that staying mentally active in old age may help maintain cognitive function. However, oftentimes, we fail to decipher the mechanisms of how these environmental factors act on our brain and, it is likely that the list of factors that we know of is far from complete.

The question is: why do we know so little? One answer is that Science as we know it is influenced by the methods currently available to study the subject at hand. In Neuroscience parlance, especially biomedical neuroscience research, the ability to sequence whole genomes at an ever-increasing pace and decreasing costs was a major driving force that led to genome-wide association studies and the identification of many candidate genes for brain disorders. The availability of tools to specifically delete genes in mice, creating so-called “knockout mice” has significantly facilitated the process of generating mouse models for brain disorders based on previously identified mutations in candidate genes. This, in turn, led to a scientific community that, for several years, has focused on genetics and the question of how specific gene mutations cause brain disorders. For example, approximately 800 candidate genes have been found (according to the SFARI gene database) in the case of autism. Based on this number, one might assume that autism is a genetic disorder, and that mutations in genes are the primary causative factor. However, this is not the case. Even after identifying all these candidate genes, a good 40-50% of cases cannot be explained by genetics. This is unlikely to change dramatically in the future as the latest studies identifying candidate genes with only weak associations with autism were only able to do so by using large numbers of participants. For example, researchers can identify whether a specific DNA sequence is statistically more likely to appear in the group with autism by comparing the genome of 1000 individuals with autism to an equal amount of individuals without autism. A gene that is

amongst the most frequently mutated, such as SHANK3 (SHANK3 mutations may be found in 1.5% of all patients with autism) might turn up showing a specific sequence roughly ten times in the group with autism, but this sequence of the gene is rarely found in the control group. Therefore, it can be concluded that SHANK3 is an autism candidate gene, and that the specific difference in the sequence may be a mutation of the gene that eventually leads to autism. This, in turn, means that in order to detect candidate genes that only contribute little to the pathology or only in combination with other gene variants, it is important to sequence many more genomes. This is because the variant may turn up in the control group from time to time and only after the analysis of thousands of genomes does it become evident that the variant is more often found in the patient group. Therefore, the latest studies were already comparing the genomes of more than 130,000 controls with about 16,000 individuals with autism and that should have identified autism candidate genes even if they are only weakly associated with the disorder. Thus, it is highly unlikely that we have missed major candidate genes for autism so far that can explain the remaining 40-50% of cases we cannot assign to a known mutation in a gene.

Besides, the number of individuals with autism is ever-increasing, a phenomenon that is too steep to be explained by genetics. Even if having autism would impart a significant benefit for an individual in our current world, meaning that someone with autism has more offspring than someone without, thus spreading the gene and associated disorder, the time course of the increase rules out this model. Moreover, individuals with autism are often riddled with difficulties in social situations and thus, are less likely to have many children. Changes in diagnostic criteria and heightened awareness of the disorder in the population and amongst doctors may explain some of the increase in diagnosed individuals. However, the fact remains that it cannot explain all of it. Therefore, if it is not genes and the increase is real, a non-genetic factor or several factors must contribute to the development of autism, and one wonders what is it in our environment that contributes to the rise of this disorder?

It is not autism alone. Most of the major brain disorders that certainly a lot of us have heard of already, for example, Parkinson's disease, Amyotrophic lateral sclerosis (ALS) (the disorder Stephen Hawkins was diagnosed with), Alzheimer's disease, Depression, and Schizophrenia, not only have a genetic component, but also an environmental one. Sometimes, it is even surprising how small the genetic component really is. Take for example Alzheimer's disease. In some individuals, this disorder may have an early-onset (about 10% of Alzheimer's disease

cases), whereas for the majority, the disorder is diagnosed after 60 years of age (there is an ongoing debate whether the early-onset and the late-onset form of the disease are the same disorder). Among 10% of patients with early-onset Alzheimer's disease, mutations, in some cases, in one of three known candidate genes can be found. For 90% of patients with late-onset, researchers have been unable to find a specific gene that directly causes the disease so far, and interaction between environmental factors and gene variants is likely. Unfortunately, in the recent past, the determination of neuroscientists to explain the brain, its function and its dysfunction in diseases by genetic factors sometimes created a climate, whereby research on environmental factors was pushed aside, under-funded, and looked upon as esoteric. However, environmental factors seem to be a significant contributor to the development of brain disorders.

Another answer to the question of why we know little about the mechanisms with which environmental factors act on our brain might lie in the complexity of environmental factors. Humans in general, including the scientists among them, are drawn to stories that they can easily understand — stories, where the rules are simple and clearly defined in binaries. Good and evil, heaven and hell, gene "on" or "off". Focusing on genetics restricts the number of possible parameters to be considered in an experiment. For example, in knockout mice that like us humans have two copies of every gene, either both copies are kaput (homozygous knockout), which implies that there is no functional protein at all; one copy is mutated and the other one intact (heterozygous knockout), or both copies are intact (homozygous wild type). In the context of environmental factors, it becomes apparent that they come in many shades of grey. For example, let's think about the exposure to a toxic metal such as lead. High levels of lead are known to damage our nervous system and result in a decline in cognitive abilities. However, the amount of lead we are exposed to may vary considerably, depending on how much water we consume from lead pipes and how long or whether our bedroom is covered in lead-containing paint.

Also, knocking out one gene that may only be expressed in specific cells that only occur within a particular part of the brain immediately narrows down the focus of observation to these cells. Contrastingly, in the case of environmental factors, it is usually our entire body that gets exposed to them. Therefore, it is difficult to discern where to begin looking for effects, as there might be a problem in the liver or kidneys and the cells there might secrete factors that ultimately affect brain function. Or, there could be a problem in the gastrointestinal system, maybe an

altered composition of the microbiota that secrete hundreds of factors and one of them may be responsible for what happens in the brain. It could also be the case that the environmental factor is indeed directly affecting a protein in the brain. Most likely, however, these things are happening in parallel.

This difference almost certainly ruins any chance to publish research in Nature or other esteemed scientific journals, given that the impact of a single study is limited. It will always be incomplete as it may only look at one aspect of action that the environmental factor triggers in our body. Equally, it will also never be a perfect *black and white story* where all the graphs illustrate the significant difference at the right place, since the dosage effect is too big and researchers are required to choose one concentration of the factor as they cannot replicate their experiments for each and every possible dose that the environmental factor may come in. Thus, studying an environmental factor requires more extensive research, more tests, is more expensive, and takes longer until the results can be published, and also probably because the complete story will exceed any word limit of a scientific journal; it will be published in pieces and not in an appealing, all-explaining closed story.

Thus, on this day in the Café, Nick and I chatted about “Eco-Neurobiology” or “Environmental Neuroscience". It is a branch of Neuroscience that focuses on the interaction of environmental, non-genetic factors with our brain, and we were surprised to learn that a few structured activities in this area do indeed exist. We could find hardly any Research Institute, study programme or even Department focusing on “Environmental Neuroscience” at Universities worldwide.

I never cease to be amazed by how little we know about the things we expose ourselves to in our everyday lives. We continue to egregiously and negligently ignore the interaction of the environment with our brain, although the past has given us ample evidence that this is a dangerous move. In 2003, Andy Meharg of the University of Aberdeen in Scotland had reported the presence of arsenic in the green pigment of a patterned wallpaper produced sometime between 1864 and 1875. This poisonous emerald green wallpaper that was quite in fashion at the time, if damp, might have produced toxic fumes containing arsenic. Napoleon had such a green-painted wallpaper in his bedroom during his exile on the island of St Helena, and according to one particular theory, he was poisoned by it. Arsenic influences the brain often by producing hallucinations. Thus, if even a past emperor was not impervious to environmental factors affecting his brain, how can we believe that we will be insulated from such harms?

Indeed, testing of drugs and other materials has come a long way. Every new drug that is brought to the market is nowadays undergoing rigorous testing for safety and toxicity – for the right reasons. The same is true for many new materials we get in contact with, and that have to undergo tests for carcinogenicity, mutagenicity, and teratogenicity, meaning that manufacturers are required to investigate whether something may increase the risk of getting cancer or may lead to congenital disabilities in babies. Nowadays, these parameters are relatively easy to measure. In my research lab, we often test new nanoparticles that are developed for drug delivery into the brain for their toxicity. Many model systems are used to do this. Cells can be grown in a petri dish and exposed to a compound, and it is possible to measure how many show signs of cell death and at what concentration and over what lengths of exposure.

Similarly, the rate of mutations can be assessed using cells growing on plastic. However, cells cannot tell you whether they have hallucinations or are depressed. Therefore, it is much harder to know whether a compound has neuropsychiatric effects, because this is a much more complicated phenomenon to measure compared to malformations, mutations or cell proliferation (unwanted in case of cancer). Although it is possible to know that a new substance such as 3,4-methylenedioxy-methamphetamine (ecstasy) produces enhanced sense of well-being, increased extroversion, empathy toward others, and enhanced sensory perception (according to the NIH National Institute on Drug Abuse) due to the immediate and undeniable effects, it is much more difficult to know whether a new substance produces neuropsychiatric effects after years of chronic exposure. More so, it is even harder to understand what happens, if a child is diagnosed with a neurodevelopmental disorder such as autism, which often occurs when the child is already three years or older, but the causing environmental factor was something that the developing brain was exposed to within the mother's womb. Due to the lag between exposure and measured effect, and the difference between the exposed person (mother) and the individual with autism (child), it is difficult to identify the responsible factor. To date, there is no real test for any materials or drugs that would predict their neuropsychiatric effects after chronic exposure over long periods with varying doses.

It is, therefore, time to better understand what and how environmental factors influence our brain. For this purpose, neuroscientific research on environmental factors needs to progress from epidemiological studies resulting in possible associations to real testable model-based mechanistic studies. Hopefully, Environmental Neuroscience will be a new field of

research that, together with genetics, can lead to a holistic explanation of brain function in health and disease. This book should serve as an introduction to this exciting topic.

CHAPTER 1

NATURE VS. NURTURE

In the history of science, the discussion of whether nature (our genes) determines what we do and who we are, or whether nurture (our environment, defined as "everything except that which is genetic") is the driving force behind our behavioural characteristics has been fiercely debated. In reality, many discussions could have been avoided by merely acknowledging the fact that these two concepts are not mutually exclusive. Indeed, we have a genetic setup that sets boundaries to what we can do, who we are, how we usually react, whether we get sick often or not, whether we are tall, energetic, intelligent, and much more. Sometimes, these boundaries are set very narrowly, which means that the environment can push a trait only a little; at other times, the environment is a major determining force and decides whether or not the potential for something that lies in our genes is awakened.

In my lectures, I usually like to explain this with an example that often surprisingly leads to astonishing Eureka moments, despite its simplicity: Some of us want to look up from our smartphones from time to time, and on some occasions, we even leave the house. When we are outside, we sooner or later encounter a plant. Let's say we spot an apple tree (*Malus Domestica*). Now, this plant is one individual, which means that every cell it is made of contains the same genetic information (I like the 'Granny Smith' cultivar; in this case, it would be pairs of 17 chromosomes (34 in total), but if you prefer "Golden Delicious", it is the same). However, the very same apple tree will look very different depending on what time of the year you step outside. In spring, for example, blossoms begin as pink buds and will later bloom as beautiful white flowers. The key is that the information to produce flowers is present in the genome of the apple tree *at all times*. Theoretically, it possesses the capacity to bloom at any time of the year. The environment alone determines whether it starts flowering. In the case of blooming, it is the ambient temperature (for the production of fruit, it is the amount of sunlight (6+ hours per day in the growing season)) that determines what transpires. Before blooming, apple trees have chill requirements. This means that they need to be exposed to

temperatures below 7°C for some time. ‘Granny Smith' only requires 400 hours of cold temperature, while other cultivars may need more. After the chill period, the temperature rises (and stays optimally around 22-25 degrees Celsius because bees tend to work best at these temperatures), which is the signal to produce flowers.

Thus, there is an individual with a genetic setup that allows it to radically change over a period of time. This change is only triggered by the environment. But what about us humans? Isn't it likely that in our genome, we have information and capabilities that allow us to do the same, wanted or not? Capabilities we are not even aware of because we did not receive the correct environmental trigger so far? What if, for example, we all have the unfortunate capacity to develop a brain disorder, but only some of us do because an external stimulus triggered it?

The answer is yes; we all have genes that may make us prone to developing brain disorders, some more and some less. We know that this is the mechanism that may cause several of the major brain disorders humans can suffer from. Therefore, many cases of brain disorders are both genetic and environmentally caused simultaneously; in most cases, this is a complex interaction. We have genes and variants (mutations) that may cause a disorder alone in some cases, but there are also substantial environmental triggers of diseases that may be causative on their own. In the majority of cases, there will be genes that, in combination, shift our brain either more towards the red line beyond which a brain disorder lurks or farther away, and there will be environmental factors that do the same. However, they may also combine and push you over the line in some disastrous event.

For scientific research on brain disorders, this entails significant consequences. Researchers that work on genetics (and this is by far the majority) have very limited options in changing genes in a patient with a brain disorder. To be able to repair a genetic mutation, we must alter the DNA of uncountable cells. Although there are different ways to do this, for example, with the help of viruses, the technology is not yet available in the clinics. Therefore, researchers are looking for drugs that can compensate for the defects caused by mutated genes. Small proteins, molecules, etc. are screened to find a treatment strategy. If researchers are lucky enough to find a compound, a pharmaceutical company will invest the time and money to develop a drug from it, and then earn money in return. This concept and type of research keeps a whole domain of industry alive. The key here is ‘treatment’ because the preventive altering of genes is not possible, at least as of now.

This is different for environmental factors. While it is difficult to alter the genes of a person, sometimes it can be quite easy to modify the environment. Once we know the environmental factors that facilitate the development of brain disorders, all we have to do is to avoid them. Thus, the key here is 'prevention', and not treatment, although some environmental factors can be considered as a treatment. As a case in point, a change in diet may alleviate symptoms of some brain disorders. But then, any drug you take is an environmental factor as well.

Although many of us would agree that prevention is better than treatment, the fact of the matter is that research on environmental factors is in its infancy and not well financially supported. At this stage, one might speculate that the interest of the pharma-industry and that of governments in keeping the lucrative sector of drug development and its job-market running has something to do with the slightly biased distribution of money between research projects focusing on genetic and those dealing with non-genetic factors of brain disorders. Before having said too much in this regard, let's go back to our apple tree.

In the case of our apple tree, we encountered an interaction between genes and the environment. These genes were activated once the correct environmental stimulus was present. Extending that analogy to a brain disorder, it would mean that we have a gene and that it will be activated or deactivated by changing the signalling pathway and physiological process it is involved in so as to alter brain function in a way that we diagnose a person with a disorder. Older models of gene x environment (GxE) interactions viewed genetic and environmental factors as easily separable components. This is a rather static interaction, where the genetic risk (having a gene contributing to the development of a brain disorder) and the environmental risk (exposure to a non-genetic factor contributing to the development of a brain disorder) were simply added together. If the addition reaches a certain threshold, the line is crossed, subsequent to which the disease manifests.

This is still the most common and a purely statistical model based on the analysis of variance. For example, if you have several dogs of two different breeds, let's say Greyhounds and Golden Retrievers (both are classified as domestic dogs (*Canis lupus familiaris*), albeit with different gene variants), you can note down their weight gain after feeding them two dissimilar diets (environmental factor). If one breed gains more weight than the other irrespective of the diet, you can safely infer that this is purely based on their genes. On the other hand, if one diet in comparison to the other leads to weight gain irrespective of the breed, the increased

weight is attributed to the environmental factor. In many cases, there are effects of genes on the phenotype (weight of the dog), effects of the environment on the phenotype, and no interaction, which means that the impact of genes and environment will be "just" added together. However, we now know that the relationship between genetic and environmental factors is more dynamic, and also includes a temporal component.

In our example of the apple tree, we have a more complicated situation. Although the apple tree (as we do) inherits its genes from its parents, their activation is highly dynamic and variable over time. This so-called ‘gene expression’ can be dependent on environmental factors. This was demonstrated in a famous experiment performed by Jacques Monod (1910-1976) and François Jacob (1920–2013). These researchers studied *E. coli* bacteria and demonstrated that the bacteria turn on their genes to digest lactose only in the presence of lactose as a source of sugar in their environment. More importantly, they were able to decipher the underlying molecular mechanisms behind this feature (today known as the Jacob-Monod model). With regard to the apple tree, this means that genes required to produce flowers are only activated after a rise in temperature following the chill period. Thus, in contrast to the older model, it is not that little flower production takes place all the time and the pro-flowering effect of the environmental factor is added on top to produce the full performance. Instead, the trait is directly controlled by the environmental factor controlling gene expression.

Also, in humans, the control of gene expression can be under the control of or influenced by environmental factors. In fact, as for apple trees, this occurs not only in relation to pathological events (except you consider the flowering of a plant as its disorder), but is also part of our normal physiology. To illustrate, it has been shown that in humans, menses and menopause in women is heavily influenced by environmental stimuli.

Nevertheless, a GxE interaction can be linked to disorders of the central nervous system (CNS). As the tree only starts flowering after the chill period, an individual at genetic risk may only become ill after being exposed to a specific environmental stressor. Under this model, from a low-risk environment to a high-risk environment, the severity of the disorder–associated phenotype increases regardless of the genetic level pre-determining the trait. Thus, in this case, genes and the environment act independently, but the environment influences the phenotype.

One example is the link between famine (as an environmental factor) and schizophrenia. A recent study conducted by scientists from Aberdeen

University and colleagues at Shanghai Jiao Tong University in China investigated babies born during a period of famine from 1959-1961 in the Chinese province of Anhui. The researchers looked at the incidence rate of schizophrenia in subjects born before, during, and after the period of extreme famine. The results (based on thousands of schizophrenia case records) reveal that the starvation experienced during critical stages of early pregnancy leads to a two-fold risk for children to show the signs of schizophrenia later in adulthood.

Sometimes, a more complex GxE interaction exists, where the impact of genes depends on the environment, whereas the effect of environmental exposure hinges on the impact of genes. As this is an actual dependence, these complex interactions reflecting the rule rather than the exception for many disorders of the CNS, do not favour the one cause, genetic or environmental, over the other as primary pathomechanism.

Probably the most common model occurring in nature for disorders of the CNS postulates that the genetic predisposition has few influences on the phenotype in a benign environment. However, with increasingly severe environmental conditions, some genes might be protective, and therefore, have a much more potent impact on the phenotype in a stressful environment. In a way, the genes influence the sensitivity of an individual to its environment.

With many transitions in between, this model may result in a more extreme situation, where an individual with a particular genetic setup is doing well in one environment but will be at a high risk of developing a brain disorder in another environment, while others are less prone to environmental alterations. Further, the exposure to an environmental factor may not follow a black and white principle, and gene-environment interactions may have to consider dose-response type interactions.

In general, especially for psychiatric disorders, two general modes of gene-environment interaction seem plausible today. Under the simpler model, disease susceptibility results from a combination of genetic factors and the environment, with the environment influencing the individual. However, a more complicated situation might exist in which genetic factors determine certain behaviours that alter the environment, which then feeds back to the individual. Here, it is not as if only the environment is influencing the individual; instead, the individual creates or seeks a specific environment. While the principles of GxE interaction are relatively easy to understand, investigating them experimentally and decoding GxE interactions on a molecular level is a challenging task. Moreover, our theories of how the environment mechanistically interacts with our body were not always correct in the past.

When I walk through the Louvre in Paris, I might look at the paintings in an entirely different manner than most connoisseurs. I try to determine whether the subjects in the pictures can be considered beautiful persons, possibly being the ‘top-models’ of their time. Probably some of them are. The reason for my intrinsic curiosity is that in the early 1800s, it was a common practice among noble ladies in Paris to get wheeled around in the Louvre in easy-chairs during pregnancy to gaze at the paintings and in particular, the pictures depicting beautiful people. They did this because they wished their future children to resemble these beauties.

This behaviour was based on the theory that experiences of the pregnant mother affect the unborn child, a concept known as ‘maternal impressions,' which has been around since medieval times. The belief that "maternal impressions" can have a lasting and severe effect on the developing embryo probably arose because people wanted to explain certain birth defects. Although in industrialised nations, we rarely come across morphological anomalies such as cleft plates (a non-closure of the upper lip as we see in rodents, for example) and extra fingers or remains of a tail, because they are surgically corrected very early in life, these defects are not rare. Indeed, defects would have occurred sometimes, and explanations for a baby with facial malformation or other anomalies were likely to have been sought. One explanation, for example, was that an animal might have startled the woman during pregnancy. A rabbit could have surprised the mother of a child born with a cleft palate. While in shock, the image of the rabbit was impressed onto the embryo. Until the 17th century, the notion that being frightened by a cat, fish, dog or rabbit, etc. during pregnancy could result in a malformed child resembling the frightening object was widely acknowledged. As a matter of fact, Claude Quillet (1602-1661) even dedicated a book “Callipaedie” (an art on how to have handsome children) to this topic advising pregnant women to be discerning about what they look at.

Surprisingly, there are documented cases where women indeed seem to have given birth to babies, so transformed by a startling event, that they completely resembled the animal that caught the woman by surprise. Mary Toft (born around 1701 in England) was one such case. In 1726, Mary became pregnant but had a miscarriage and claimed to have given birth to animal parts, which was blamed on the sighting of a rabbit. The local surgeon who investigated Mary indeed delivered several pieces of animal flesh (supposedly from a cat and a rabbit) (The cat's parts were claimed to be caused by a cat she was fond of, and slept on her bed at night). The case piqued the interest of several prominent physicians, and to everyone's amazement, Mary gave birth to a complete rabbit, and after few days, to

four more rabbits, followed by further deliveries on the next days. Every time she delivered a dead rabbit, she and the rabbit were carefully examined. Eventually, even the king (King George I) was notified. Mary's story was published in newspapers, and her story became a national sensation. Mary was brought to London. However, under ever-closer observation, Mary was unable to deliver more rabbits, and on 4th December 1726, it was discovered that the entire story was a hoax. Her husband, Joshua, had been spotted buying young rabbits and a Sir Thomas Clarges confessed that Mary's sister-in-law, Margaret, had bribed him to sneak a rabbit into Mary's bedroom. Mary finally confessed on 7th December after being threatened that a painful operation would be performed on her in order to investigate the case. Mary was imprisoned for a short time but eventually released without charge and returned home. Although some attending doctors did remain sceptical throughout the whole sequence of events, the career of several doctors was ruined, and the reputation of the entire medical profession suffered a major damage at the time.

In their defence, it was practice at that time that the mother would deliver sitting on a birthing stool wearing a dress, and not on a bed; moreover, male doctors usually would, if at all (because childbirth was the job of midwives), only have their hands underneath the dress. Therefore, it was easier to stage the birth of rabbits.

Several theories had been put forward on how maternal impressions work on a mechanistic level. One of them was based on the then-current theory of how our vision works (which was also wrong). According to that theory, everything we see is reproduced as a tiny version (maybe a molecule) in our eye. This substance traverses into our brain through our optic nerves where we process the substance and actually "see" the object. During pregnancy, these substances would not only go to the brain, but also to the embryo where they can modify its development. There is even a published scientific study that claimed to have identified a connection from the brain of the mother to the baby, running as a nerve through the umbilical vein, thus, delivering maternal impressions.

However, it was not always an object; emotions too, were believed to be "impressed" on the baby. Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim ~1493-1541) advised that pregnant women should live morally and be careful with their thoughts since their imagination will shape the developing baby. It was believed that if a woman was sorrowful during pregnancy, the child will later develop into a person with what we would diagnose today as depression (indeed, we now

know that there is a relationship between perinatal stress and mental disorders in offspring).

While the past idea of mechanisms behind “maternal impressions” certainly was indeed wrong, the underlying theory that the environment can influence the development of a developing baby does bear some truth. Today, we still often are not entirely sure how this works on a molecular and cellular level.

In many brain disorders such as autism, the effect of an individual gene variant can be small, and researchers are confronted with the predicament that it is extremely hard to decipher not only whether a particular genetic variant impacts the trait of interest, but also whether those gene variants interact with environmental factors. The primary task is to measure environmental exposure quantitatively in biologically meaningful ways as well as to look at processes such as alterations in gene expression, the proteome, neurochemistry, and behaviour. In the future, for research to identify the mechanisms by which causal environmental factors exert their effects, the crucial step will be to identify biological mechanisms that underlie vulnerability to a disorder along with the molecular targets of environmental factors.

CHAPTER 2

HOW THE ENVIRONMENT SHAPES OUR BRAIN

I'm a massive fan of the 1999 movie “The Matrix”. In the plot, people are submerged into a gel-like substance, and their sensory input is connected to a computer generating and controlling a programme that they perceive to be the environment that surrounds them. Because all input to the brain is simulated, it is conceivable that a person connected in this way may not be aware that the environment he or she lives in is not reality.

What if everything around you
Isn't quite as it seems?
What if all the world you think you know
Is an elaborate dream?

(From the Lyrics to "Right Where It Belongs", a song by Nine Inch Nails)

I like this idea because if such a technology did indeed exist, it would provide the basis for the ultimate experiment for the investigation of environmental vs. genetic influences on our brain. What we need for this experiment are several clones (100% genetically identical). These, let’s say, 10 males or 10 females, and, let’s say mice, need to be born from a mother without any sensory or environmental input and raised in such a machine that simulates the exact same environment for all of them up to the point where the mice enter our experiment (the software may not allow the mice in the simulated reality to make their own decisions and simulate alternative environments based on those). In the experiment, they could face a simple situation that requires them to make a decision: left or right, for example, or maybe a more interesting proposition: fight or flight.

We would expect that regardless of what decision they take, all ten individuals should make the same decisions. Now, we could mutate candidate genes and ascertain whether we get a different outcome. Maybe a mutation makes the mice more aggressive and now, they prefer to fight. However, and this is where it gets really important, we could change the programme. We could control every single event in their lives up to this

experiment. We could also include stressful early life events (encounters with a cat), or rewarding events such as becoming the dominant male. We could even simulate life in a permanent winter or everlasting summer, life alone or life in groups. Would those events change the decision of the animals in the experiments and if yes, which ones?

The idea that our environment can influence our brain function is not novel; it has been around for centuries. For example, we know very well that being hungry can turn the sweetest little child into an aggressive raging monster. Thus, the presence, or in this case absence, of an environmental factor (food) must change the activity of neurons, the way they communicate with each other and the way brain regions are exchanging signals with each other that ultimately leads to a shift in our emotional stability – in this case for the worst. Most of us are perfectly aware that during this transient mood swing, our genetic code does not change. How should it? After all, we are still the same individual. We are not magically transforming into a monster with a different genetic code. However, what we are doing is showing signs of a psychiatric disorder. We have trouble controlling our emotions and become obsessed with thoughts of sizzling steaks and vanilla ice cream that inexorably affect our ability to focus and make rational decisions.

In 2015, researchers from the University of Gothenburg found that the hormone ghrelin (released when we are hungry) has a negative effect both on decision-making and impulse control. If this situation were to continue chronically, at some point, we probably would be diagnosed with a neuropsychiatric disorder. This is one instance where hunger is not as innocuous as it may seem.

Interestingly, although the knowledge that hunger changes our mood has been around for a very long time, as being hungry was possibly a state that people in the stone-age were very used to, to this day, we still do not exactly know the mechanisms of being hungry makes us grumpy, and why some tend to be more affected than others. How is the presence or absence of an environmental factor shaping the physical world of our brain?

Although we do not know the exact answer for every case and every factor, scientists have identified several general principles on how the environment can interact with our bodies. One defined mechanism has founded a whole new scientific field of study called epigenetics.

As it turns out, our DNA is made up of four basic building blocks called bases. These molecules are abbreviated with A, G, T, and C, based on the first letter in their names Adenine, Guanine, Thymine, and Cytosine. Since James Watson and Francis Crick discovered their orientation

towards each other in the 1950s with the help of Rosalind Franklin, we know that they form a long string wound up in the shape of a double helix. Connected by a phosphate bound to a five-sided sugar, these bases are aligned in a specific sequence forming the genetic code. The genetic code can also be used as a template to translate this sequence into a chain of amino acids that ultimately create a protein.

Sometimes, by accident, bases may be exchanged for one another, inserted or cut out, creating what we call a mutation. Mutations are not always harmful, although, in our daily life, we frequently associate them with genetic disorders. In fact, they form the basis of evolution as from time to time, a mutation will create a protein that works better and improves the chances of its carrier to produce offspring. It is pertinent to mention here that actually, the environment decides whether a mutation is good or bad, as mutations without the context of an environment cannot be classified in that manner. For example, we can place an animal in two different environments in a very simplified thought experiment: An icy arctic one with freezing temperatures, and a hellish hot desert-like environment. Let's assume we have a protein that is part of the process which allows our bodies to cool down. However, a mutation might impair this function. If this mutation occurs in an animal living in the cold environment, the mutation might be neutral as no cooling is necessary, or maybe even beneficial, given that the animal is losing less temperature if the cooling process is active for some reason.

If the animal, however, lives in the hot environment, an impaired cooling mechanism may be deadly; thus, the mutation is undoubtedly a bad one. This example already illustrates that the environment always influences us because it determines whether or not our body finds itself in a place suited to our physiology or a place where we can barely survive. However, this form of a selection of animals based on mutations that make it more or less adaptable to the environment occurs on an evolutionary timescale and is a mechanism that we should be less concerned about.

Therefore, shaped by millions of years of evolution, we are born with our set of DNA, and our specific sequence of bases. From that moment on, every cell of our body contains this one particular set of DNA. Although sometimes altered by nasty viruses that integrate their genes into our genomic DNA, this information is fixed, and we know that it will not change our entire life. It is the genetic setup we are born with, unique to us, except if we have an identical twin, and the environment is unable to change this genetic code.

Interestingly, the sequence of our genome is only one part of the story. Our long string of DNA is not floating freely through cells, but it is neatly organised. Sometimes, it is wound around proteins called histones and more curled up or more extended. Moreover, there are occasionally other molecules attached to our bases within the DNA modifying individual bases at specific points within the string. Although the sequence of GTACTCTCGCGCGAs does not change, these secondary features called epigenetic modifications respond to the environment.

Epigenetics, as we understand the term today, means that although our DNA is essentially the same in all of our cells, patterns of gene expression (which means that the protein encoded by the gene is produced in the cell) differ greatly among different cell types; these patterns can be controlled and inherited by epigenetic modifications. An important epigenetic modification, for example, is DNA methylation. A methyl group (CH_3) is a molecule that can be attached to the base C (Cytosine). This is controlled by several different enzymes in our body, which means that the process can be turned on or off. These so-called DNA methyltransferases can be activated in different cells and tissues. Therefore, not every DNA in every cell of our body follows the same pattern of methylated Cs. The importance of this process is that the methylation does not occur in a random manner. Not every C gets methylated, and the ‘Cs’ that do get methylated are at specific locations in our genome. We often find them in groups in so-called promoter regions within the DNA. Here, a C is often followed by a G in the DNA, which then acts as a signal for the enzymes to methylate the C in such a pairing. Thus, these CpG islands (p stands for the phosphate in between the two bases) are preferred sites of methylation. This is because the promoter region is a stretch of DNA with a particular purpose. It controls whether a gene gets expressed or not.

Although each gene codes for a protein built from the information encoded by the gene's sequence, a gene is more than a blueprint for the alignment of specific amino acids in a chain that together forms the protein. A gene also contains elements that allow control over whether and how much protein is generated from the gene. This assumes great importance. It is the basis of multicellular organisms because it allows cells to become specialised. Not every cell needs the same set of proteins. For example, a cell in the pancreas has different requirements as compared to a nerve cell. The cell in the pancreas might produce insulin, which means that the gene encoding insulin is turned on. The nerve cell, in contrast, is busy conducting electric impulses in our brain and is happy to leave the duty to regulate sugar levels in our blood to the cells in the pancreas. To be able to function correctly, the nerve cell will need other

proteins. Therefore, although the insulin gene is turned off, other genes that the pancreas cell doesn't care much about are turned on. The system is even more flexible than this. Even if a gene is turned off, this may only be a temporary phenomenon. For example, if the pancreas cell senses that its store of insulin is full, production of the protein might be stopped for a while until we eat our triple chocolate fudge brownie and the protein is needed again to lower our sugar levels in our system.

The on/off switch for a gene lies in the promoter region. Here, specific sequences are recognised by proteins (transcription factors) that allow a gene to be expressed (turned on) in case the DNA sequence allows the transcription factors to bind. Whether they can attach is contingent on many factors, but one of them is whether or not the 'Cs' in this region are methylated. In most cases, the presence of methylation turns the gene off and disallows its expression. Thus, the production of proteins can be regulated by generating a pattern of methylated or non-methylated promoter regions within the DNA of a cell. And, it turns out that the methylation pattern is something that reacts to environmental stimuli.

A gargantuan number of environmental factors may influence DNA methylation patterns. For example, a study published in 2017 showed that DNA methylation in the brain changes following exposure to noise. When rats were exposed to 70-75 dB during the night (their active phase) and 45 dB during the day (when they usually sleep) for three days, the DNA methylation of a specific gene in the medulla oblongata (located in the brain stem) was altered. It is difficult to translate what the required noise level for humans is, and it is indeed possible for rats to have more sensitive ears and stronger reactions to noise than humans. However, 70-75 dB is only the loudness of a passing Mercedes S class (2017 model S450 4Matic: 71 dB) and a 3-day visit to a rock festival exposes you to sound pressure levels that can easily reach 100 to 120 dB (the Swedish rockers Sleazy Joe recorded in 2008 at record 143.2 dB in Hasselholm, Sweden).

An important feature of DNA methylation patterns is that they can also be inherited. Thus, if an environmental factor we are exposed to can change the methylation pattern of a germ cell (the oocyte from the mother or the sperm cells from the father) the pattern is transferred to the next generation. This epigenetic transgenerational inheritance sounds a bit like Lamarckism (also known as the inheritance of acquired characteristics). According to Lamarckian inheritance (named after Jean Baptiste Lamarck (1744-1829)), an individual may pass on the characteristics it has obtained through use or disuse during its lifetime to the next generation. Using an

example provided by him, this means that a blacksmith who strengthens the muscles in his arms will have children that would have similar strong muscles as adults (although they may not be blacksmiths themselves). It therefore contradicts the idea that changes occur through genetic mutations and selection, and as we know today, this is not correct. However, the transgenerational inheritance of methylation patterns has been shown, for example, after exposure to vinclozolin, a fungicide used on fruits and vegetable crops. Thus, it is possible that an environmental factor that hits us today can affect our children in the future, although they have never been exposed to the factor, through epigenetic mechanisms.

There are more epigenetic modifications than DNA methylation. Another illustration of this is the histone modification. If the DNA inside a human cell were stretched out, it would be a string that is 2 meters long. If we include all cells in a human body that contain DNA (10^{13} cells), it means that we have DNA stretching from the earth to the sun and back 70 times. Therefore, we evidently have to tightly pack our DNA and wind it up inside a cell, almost like a ball of wool. However, this process of condensing DNA is controlled and regulated. This DNA is wound around small proteins, so-called histones. Eight histone proteins each cluster together and DNA wraps around them 1.65 times, before leaving for the next group of histones, again wrapping around 1.65 times, before again leaving, and so on. Ultimately, through this process, DNA is packed densely into a structure that we call chromosome (humans have 23 pairs of them =46 in total). The trick is that it is possible to modify how tightly DNA is packed by altering the structure of histones slightly. Histone proteins can be methylated (as we have seen for the Cs in the DNA), phosphorylated, acetylated, ubiquitylated, and sumoylated. Let's just say a lot of things can be attached to them. Moreover, the attachment of one of these molecules, which is again done by enzymes, forces the DNA into a different type of wrapping. Palpably, the way DNA is packed also influences gene expression. Usually, genes situated at very tightly packed regions are less likely to produce their encoded protein. Interestingly, the pattern of histone modification and the enzymes doing this are again responsive to environmental stimuli.

Although we are nowhere close to a stage where we know everything about environmental factors that may influence histone modification, we do know that stress is a potent one (more about stress in a later chapter). Acute stress, as a case in point, has been shown to alter histone methylation within 45 minutes in the brains of rats (for obvious reasons it is difficult to design experiments where the brain is extracted from human