The Effect of Early Life Stress on Epigenetic Programming of the Brain

Bachelor Thesis

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Abstract

Early life stress causes epigenetic changes in the brain and those changes often result in altered behavior. A stressful experience can activate the HPA axis. The HPA axis releases glucocorticoids to prepare the organism for the stressor. Most of the epigenetic changes are regulated by DNA methylation and histone modification. Early life is the most vulnerable period for epigenetic changes to take place. Early life stress can alter gene expression in many brain regions and this effect is most of the time gene silencing. Most importantly, *Bdnf* expression in the hippocampus is reduced as a result of early life stress.

Furthermore, there are gender differences in the epigenetic response to early life stress. Studies discussed in this review show conflicting results. Some studies suggest that early life stress affects males stronger, but others demonstrate that females experience a larger effect of early life stress on their behavior. Moreover, both escitalopram and new potential antidepressants are discussed in this review. Therapeutic interventions with these compounds can reverse the negative effect of early life stress on the epigenome and behavior.

Future studies should take into consideration that the protocol that is used for creating stressful experiences can change the outcome of the experiment. Different stressors can induce different physiological responses. On top of that, the timing of the stressor can alter the effect of the stress. Different stages in early life are more or less sensitive for epigenetic changes.

1. Introduction

Hundreds of millions of people suffer from mental disorders, including depression and anxiety. This causes not only to a lot of discomfort and disability for many people, but it also leads to economic loss (World Health Organization, 2008). In the last years, a lot of research has been done on the causes of mental illnesses and their treatment. It has been shown that environmental conditions, like stress exposure, can establish epigenetic changes in genes that are involved in depression and anxiety. On top of that, it has been suggested that therapeutic interventions could reverse these changes. (Weaver et al., 2004; Park et al., 2018). These results emphasize the important role of stress in epigenetic programming of the brain and suggest that stress exposure during early life can cause mental disorders by inducing epigenetic changes. Therefore, this review will focus on the latest developments of the research into the effect of early life stress on epigenetic programming of the brain.

1.1 Physiological response to stress

The experience of a stressful situation activates the hypothalamic-pituitary-adrenal (HPA) axis, as shown in Figure 1 (Lanoix et al., 2014). An environmental stressor causes hypothalamic neurons to release corticotropin-releasing factor (CRF). CRF stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH). In response to ACTH, corticosteroids, like cortisol, are released from the adrenal glands into the bloodstream. These corticosteroids have a negative feedback on their own release by inhibiting the secretion of CRH and ACTH by respectively the hypothalamus and the pituitary gland (Lupien et al., 2001). Furthermore, they have many effects on the body to prepare it for a stressful event (Gjerstad et al., 2018). First of all, the division of energy changes, so that more energy goes to the muscles and brain. Secondly, heart rate, blood pressure and immune function increase. Finally, reproductive behavior and appetite are inhibited (Sapolsky et al., 2000).

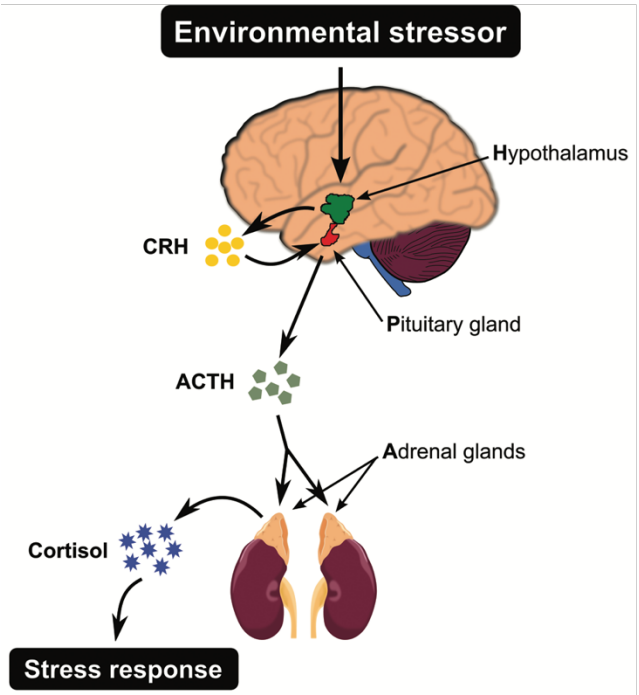

Figure 1: The HPA Axis. *An environmental stressor causes the hypothalamus to secrete CRH and this stimulates the pituitary gland to release ACTH. The adrenal glands produce cortisol in response to ACTH (Lanoix et al, 2014).*

Corticosteroids exert their effects by binding to the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). These types of receptors are expressed in different tissues and they also differ in their affinity for cortisol. In the brain, the level of GR is high in the hippocampus, amygdala, prefrontal cortex and the paraventricular nucleus of the hypothalamus. MR levels are also high in the hippocampus, but lower in the amygdala and prefrontal cortex. Furthermore, MR's are already activated when cortisol concentrations are low, while GR are only occupied when cortisol levels are higher, such as in response to stress (Gjerstad et al., 2018).

During pregnancy, cortisol is being released by the maternal adrenal gland. This cortisol can cross the placenta and enter the general circulation of the fetus. Cortisol has a lipophilic structure, so normally it would cross the placenta readily, but the placental enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD2) converts the maternal cortisol into its inactive form: cortisone. When maternal cortisol concentrations are high, it may overwhelm 11β-HSD2 and if this happens, not all the maternal cortisol is converted. Moreover, it has been shown that even minor increases of maternal cortisol can double the fetal cortisol level. Maternal stress thus influences the fetal glucocorticoid concentrations and therefore maternal stress can induce psychological changes in the fetus (Cao-Lei

et al, 2016). Furthermore, glucocorticoids are thought to induce epigenetic changes during both the prenatal and postnatal phase (Zannas et al., 2015; Bernoit et al., 2015). In the next paragraph, the basic principles of epigenetics will be outlined.

1.2 Epigenetics

The term 'epigenetics' comes from the Greek language, in which 'epi' means 'on top of'. Epigenetics can be defined as: chemical modifications to chromatin that alter the genomic transcription without altering the DNA sequence. Epigenetic modifications can be changed by environmental conditions but can also be stable and inheritable (Cao-Lei et al, 2016). The two major molecular mechanisms of epigenetic changes are DNA methylation and histone modification. DNA methylation is most often associated with gene silencing, while histone modifications can result in both active transcription and silencing of genes (Gräff et al., 2008).

DNA methylation most commonly occurs at cytosine residues on CpG islands. CpG islands are DNA sequences that have a high CpG density. The majority of gene promotors are located here, implying that methylations in these regions are functionally important. The methylation of DNA is catalyzed by DNA methyltransferases (DNMTs). DNMT3a and DNMT3b are involved in *de novo* DNA methylation, establishing new methylations in unmethylated DNA. DNMT1 is involved in copying existing DNA methylation patterns during cell replication. The gene silencing effect of DNA methylation can be mediated by methyl-CpG-binding domain (MBD) proteins, such as MeCP2. MeCP2 is highly expressed in the brain and can bind to methylated DNA and repressor complexes to silence gene expression. Furthermore, MeCP2 fulfills a critical role in maintaining DNA methylation by binding to DNMT1 and recruiting this protein to maintain methylation patterns (Moore et al, 2013).

Most histone posttranslational modifications take place at the N-terminal tail of the sequences of the histone proteins H3 and H4. This part is exposed and accessible for histone-related proteins due to the structure of the nucleosome (Janssen et al, 2017). Histone acetylation is associated with the activation of transcription, since it neutralizes the positive charge on lysine residues in the histone tail. This decreases its affinity with DNA, so the DNA is less tightly bound to the histones and can be transcribed more easily. The methylation of histones can occur in different forms and therefore it is associated with both actively transcribing and silencing of genes (Gräff et al., 2008). The processes of DNA methylation and histone modification are summarized in Figure 2 (Mukherjee et al., 2015).

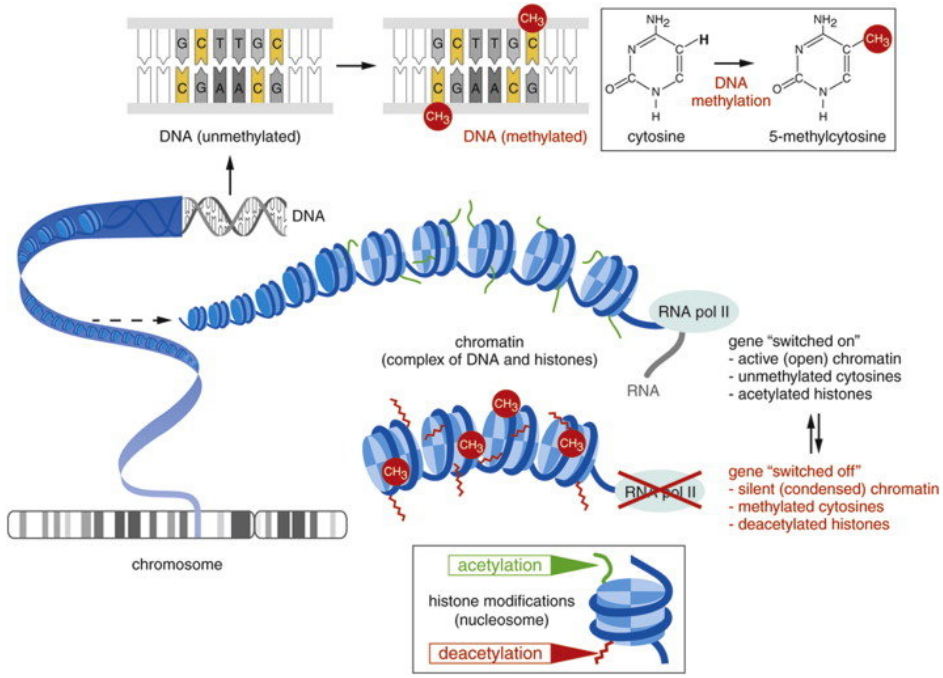

Figure 2: Process of Chromatin Modifications. *Gene expression can be regulated by chemical modifications of DNA or histone proteins. The addition of a methyl group (CH_3) to a cytosine residue alters the way in which DNA interacts with transcription proteins. The addition of acetyl groups produces a loose chromatin structure that favors transcription (Mukherjee et al., 2015).*

Although it has been shown that chronic stress later in life can affect epigenetic modifications and gene expression patterns (Abassi et al., 2017; Mychasiuk et al., 2016), it is assumed that early life environments have a relatively high impact on epigenetic properties. During early life, neurons are still developing and undergoing changes, and this may be the reason that epigenetic programming is most sensitive to change early in life (Nagy et al., 2012).

Weaver and colleagues (Weaver et al., 2004) were the first to show that early life circumstances alter the methylation pattern of the GR promoter in the hippocampus. Their study showed that a lack of maternal care in the first week of life altered the cytosine methylation of the GR promoter. This was associated with histone acetylation and alliterations in transcription factor (NGFI-A) binding to the GR promotor. Furthermore, those changes in epigenetic programming of the GR promotor persisted into adulthood and were reversed by cross-fostering. Following the research of Weaver et al., many more studies were conducted to gain more knowledge about the effect of early life stress on epigenetic regulation of other stress associated genes and brain regions. These studies will be discussed in the next chapters.

2. Brain Regions Affected by Early Life Stress

Although epigenetic changes can occur in many genes and tissues throughout the body (Vaiserman, 2015), this review will focus on the epigenetic modifications that occur in the brain and can affect behavior. Franklin et al., induced stress in mice during the first two weeks of their life. They determined the level of the serotonin receptor 5HT1AR in different brain regions of the offspring of these mice. Early life stress reduced 5HT1AR levels in the lateral periaqueductal grey, dorsal raphe, thalamus, dentate gyrus and CA1 region of the hippocampus. On top of that, second and third generation of mice in the experimental group showed abnormal behavior that was associated with lower 5HT1AR levels in these brain regions (Franklin et al., 2011). These results suggest that the epigenetic changes take place in different regions of the brain and that they can persist across several generations.

2.1 Hippocampus

A lot of research focused on the epigenetic programming of the hippocampus; a brain region that is mainly involved in learning and memory (Xiao et al., 2016). Furthermore, the hippocampus acts synergistically with the amygdala to form long-term memories of emotional events. There are many projections from the amygdala to the hippocampus and these brain regions are located very close to each other (Richter-Levin et al., 2001). This is shown in Figure 2 (Dumas et al, 2013).

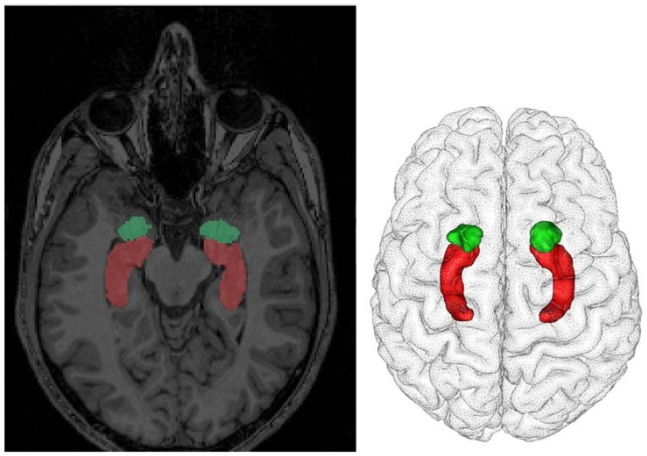

Figure 3: The Hippocampus and Amygdala. *The hippocampus (in red) and the amygdala (in green) are located next to each other. An MRI image is shown on the left and the right image is a computer image produced from the same MRI scan (Dumas et al., 2013).*

A study by Benoit et al. looked at the effects of prenatal stress (PNS) on memory formation and the epigenome of hippocampi in mice. This was done by administering chronic unpredictable stress to their mothers before and during gestation. Mice that experienced PNS showed increased DNMT1 levels and lower acetylation levels of histone protein H3 in the hippocampus compared to control. Furthermore, PNS mice had an impaired memory that may be caused by the alliterations of DNMT1 and histone acetylation in the hippocampus (Bernoit et al., 2015).

Other studies focused on the gene coding for Brain-Derived Neurotropic Factor (BDNF) in the hippocampus. BDNF is a growth factor that is important for the maintenance and development of the nervous system (Sandhya, 2013). Moreover, serum levels of BDNF are abnormally low in people suffering from depression and can be elevated using antidepressants (Sem et al., 2008). Therefore, BDNF is seen as an important molecular factor in the pathophysiology of mental disorders (Monteiro et al., 2017).

A study by Seo et al., used maternal separation (MS) as a model for early life stress. They found that the level of *Bdnf* exon IV expression in the hippocampus was lower in the mice that experienced MS than in the control group. In accordance with these results, they found a lower level of acetylation of histone proteins H3 and H4 at the *Bdnf* promotor IV and a higher level of histone deacetylase (HDAC5) mRNA in MS mice. On top of that, MeCP2 concentrations at the *Bdnf* promotor IV were higher in MS mice (Seo et al., 2016). Similar results were found during a study that focused on the *Bdnf* exon I promotor in the hippocampus. Lower levels of BDNF and *Bdnf* exon I mRNA were found in the hippocampi of MS mice compared to control. MS also reduced the levels of H3 acetylation and increased DNMT1 and DNMT3a levels. Surprisingly, no reduction in MeCP2 binding at the *Bdnf* exon I promotor was found (Park et al., 2018).

2.2 Amygdala

Another brain region that has been studied frequently is the amygdala, a brain region that is most important in emotion regulation (Phelps et al., 2005). It has been shown that MS results in higher *Dnmt3a* expression levels and MeCP2 concentrations in the amygdala of adolescent rats. The changes in *Dnmt3a* expression and MeCP2 concentrations persisted into adulthood. The levels of other proteins that are involved in DNA methylation (TET3, REST) and histone modification (H3K14ac, H3K14me2, H3K14me3) were also altered in the amygdala by the experience of early life stress. Together, these changes resulted in lower *Bdnf* expression in the amygdala during adolescence and adulthood. This altered the behavior of the rats during behavioral tests. The MS rats showed impaired social behavior: they showed less social interaction with stranger rats than control did. This was the case in both adolescence and adulthood (Karen et al., 2019).

A study by St-cyr et al., confirmed that the transcriptions of *Nr3c1* and *Fkbp5* in the amygdala are also altered by early life stress. *Nr3c1* is the gene coding for the GR and *Fkbp5* codes for FKBP5, a co-chaperone of several steroid receptors. Stress was induced in mothers by exposing them to predator odor. The transcription levels of *Nr3c1* and *Fkbp5* were measured in the amygdala of their offspring. The transcription level of *Nr3c1* in the amygdala was increased by prenatal stress in female offspring directly after birth but not in adulthood. On the other hand, *Fkbp5* transcription was increased in females in adulthood but not directly after birth. Furthermore, a negative correlation between methylation of CpG sites in the *Fkbp5* intron V and *Fkbp5* expression was found in this study (St-cyr et al., 2017).

2.3 PVN

The paraventricular nucleus (PVN) of the hypothalamus is another brain area at which early life stress can have a functional effect. The PVN plays an important role in the stress response, since CRH+ neurons in the PVN secrete CRH that stimulate the pituitary gland to secrete ACTH. Those CRH+ neurons can also release oxytocin and vasopressin, both of which can activate the secreting of ACTH and can have a negative effect on the PVN, independent of CRH (Qin et al., 2018).

It has been shown that early life stress affects the epigenome in the PVN. Bockmühl et al. induced early life stress in male mice using MS. This resulted in site specific hypermethylation at CpG islands of the *Nr3c1* promotor of CRH+ neurons in the PVN. This hypermethylation was maintained with age. Mice that experienced early life stress did not show normal CRH upregulation when they were exposed to chronic stress during adulthood (Bockmühl et al., 2015). These results suggest that early life stress diminishes the effect of subsequent lifetime stressors and that this can be mediated by hypermethylation of the *Nr3c1* promotor in the PVN.

All the researches discussed in this section highlight the important role of early life stress on epigenetic programming of the brain. Early life stress causes changes in gene expression in different brain areas like the hippocampus, amygdala and PVN. Although the study of St-cyr et al. suggested that stress experience resulted in lower methylation levels, most studies show that stress causes DNA methylation and histone acetylation. Since DNA methylation and histone acetylation both repress gene transcription, it suggests that early life stress has a gene silencing effect on several genes in different brain areas. In the hippocampus and amygdala, BDNF expression is reduced by stressful experiences during early life. This caused impaired social behavior. In the PVN, the *Nr3c1* promotor was hypermethylated after early life stress experience, resulting in lower a stress response in stressful environments later in life.

3. Sex Differences in Response to Early Life Stress

Even though male and female brains are very much alike, existing literature suggest that sex differences in brain structure and organization are present across the lifespan. There is much evidence that these differences develop and appear during childhood and adolescence (Kaczkurkin et al., 2019). For example, Figure 4 shows that serotonin signaling also differs between male and female brains (Karolinska Institutet, 2008). This might be the reason that the prevalence of mental illnesses differs between males and females. Women experience depression and other mental problems more often than men (Centraal Bureau voor de Statistiek, 2019). Since there is a clear difference between sexes in brain structure and mental health, the effect on epigenetic programming and behavior of early life stress might also differ between males and females.

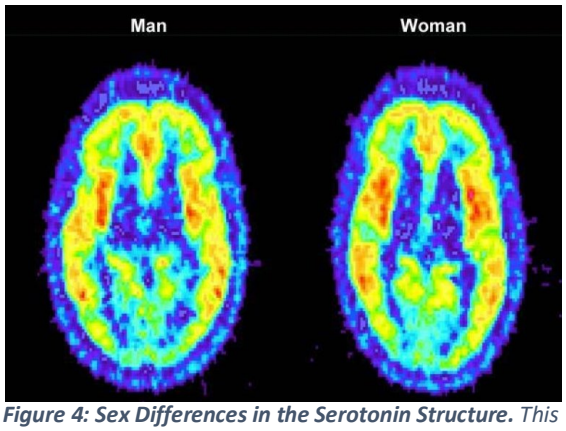

Figure 4: Sex Differences in the Serotonin Structure. *This pet scan shows central serotonergic neurotransmission. It differs between males and females (Karolinska Institutet, 2008).*

The study by Franklin et al. that induced stress during the first weeks of life in mice also observed differences in social behavior between males and females. As discussed in the previous chapter, Franklin et al. found reduced 5HT1AR levels in both male and female mice. Social behavior was assessed in the offspring of mice that experienced early life stress. The time they investigated same sex conspecifics was measured. Only the male offspring of stressed mice showed a decrease in investigation time. Impairment of memory due to stress experience did not differ between males and females (Franklin et al., 2011). These results suggest that early life stress influences behavior more negatively in males than in females.

On the contrary, Bernoit et al. found that chronic unpredictable stress during the first weeks of live had a less negative influence on male mice than on female mice. Chronic plasma corticosterone concentrations were significantly higher in females that experienced chronic unpredictable stress than in males that experienced this stress. Plasma corticosterone concentrations in males and females did not differ in the control group. In line with this result, Bernoit et al. also found that the epigenome of female mice was more affected by chronic unpredictable stress. Acetylation levels of histone H3 decreased more in females than in males that experienced early life stress. DNMT1 levels were only increased in females and not in males (Bernoit et al., 2015).

As a result of a research performed in male Wistar Kyoto rats, McCoy et al. even stated that early life stress could have a positive influence on behavior. Wistar Kyoto rats, that are known for their anxiety-like phenotype, were exposed to MS. Global DNA methylation levels were much higher in rats that experienced MS. Enhancing DNA methylations by a diet with methyl donors, improved the results in behavioral tests and augmented stress resilience. This indicates that stress resilience in male rats may be mediated through DNA methylation (McCoy et al., 2016).

St-cry et al. also studied these sex differences and got confronted with contradictory results. They exposed pregnant rats to predator odor and did not find a sex difference in cortisol response to predator odor in their adult offspring. They did find a sex difference with respect to the ACTH response. Only in female rats that experienced prenatal stress, ACTH levels stayed did not drop and stayed elevated for more than 120 min. Furthermore, transcriptions of *N3rc1* and *Fkbp* in the amygdala were increased as a response to prenatal stress in female rats only. Surprisingly, behavioral test suggested a bigger effect of early life stress in males instead of females. The research showed that anxiety-like behaviors like inactivity, laying boli, risk aversion and center visiting in open field were increased by early life stress in male rats only. The only behavior that was increased in females but not in males was avoiding of the dark zone in a light-dark box (St-cry et al., 2017).

As suggested by the studies mentioned above, gender effects have indeed a large influence on developmental programming of the brain. This was confirmed by a recent study of Mattern et al. that studied the expression of nine different genes in six brain areas. Mice were exposed to a standard or enriched environment during pregnancy. Their offspring was exposed to an adverse (MS) or an enriched environment in their first weeks of life. Methylation levels of several genes were measured in control and experimental groups. DNA methylation differed already a lot between male and female in control groups. For examples, *Maoa* methylation levels were more than 10 times higher in female mice than in male mice in all brain regions. Moreover, all observed epigenetic effects of early life experience were sex-specific (Mattern et al., 2019).

In summary, researches looking at gender differences on early life programming of the brain find conflicting results. Some show that early life stress has a less negative influence or even a positive influence on male behavior. Until some point, early life stress could prepare organisms for future stress experiences via enhancing epigenetic changes. Other studies state that behavior of males is more impaired than behavior of females by early life stress.

4. Therapeutic Agents

Previous chapters emphasized that early life stress affects the epigenome of the brain and that the experience of early life stress can alter behavior. In most cases, these behavior changes are negatively. If therapeutic interventions could prevent or reverse the effect of early life stress on the epigenome of the brain, the behavioral alliterations can also be diminished. As stated in the introduction, research into therapeutic interventions that reduce the effect of early life stress, can be used for preventing or curing mental illnesses.

4.1 Escitalopram: a classical antidepressant

Escitalopram is a selective serotonin reuptake inhibitor (SSRI) that is commonly used as an antidepressant (Sakka et al., 2017). SSRI's block the reuptake of serotonin, resulting in higher serotonin levels in the synapse. This process is outlined in Figure 3 (Lattimore et al., 2005).

The study of Park et al., that showed that MS resulted in lower *Bdnf* expression in the hippocampus, also looked at the effect of antidepressants on *Bdnf* expression. As adults, the rats that experienced MS received chronic escitalopram. The administration of chronic escitalopram fully reversed the effect of MS on *Bdnf* expression and histone protein H3 acetylation. Furthermore, the effect of MS on *Dnmt1* and *Dnmt3* mRNA levels were reversed by chronic escitalopram administration (Park et al., 2018). Seo et al. found similar results while studying the *Bdnf* expression after MS. Escitalopram reversed the effect of MS on histone H3 and H4 acetylation, MeCP2 levels and *Bdnf* expression. In addition to this, they showed that behavioral changes associated with lower *Bdnf* expression were reduced after escitalopram intake (Seo et al., 2016).

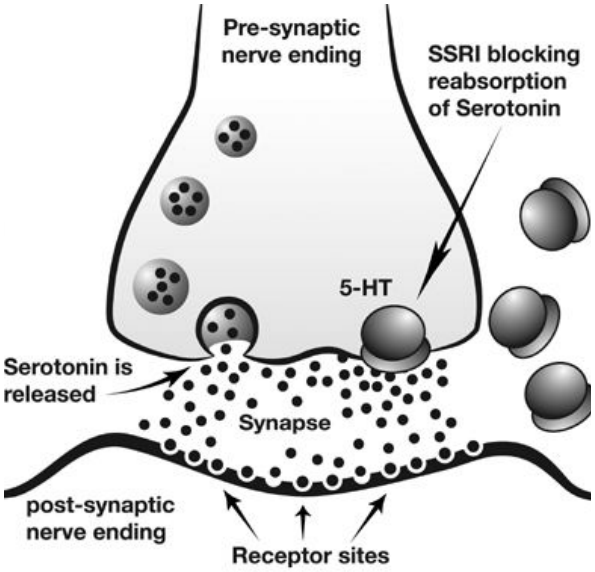

Figure 5: Mechanism of action of SSRI's. *Serotonin is released in the synapse and exerts its effect by binding to receptors on the post-synaptic nerve ending. SSRI's block the reabsorption of serotonin, so serotonin concentrations in the synapse stay elevated (Lattimore et al., 2005).*

The effects of escitalopram on the GR promotor I_7, have also been studied by Park et al. This research demonstrated that GR expression in the hippocampus was reduced by MS and persisted into adulthood. This was in accordance with the results of the study of Weaver et al. that was discussed in the introduction. The effect of MS on GR concentrations and GR exon I_7 expression levels in the hippocampus was reduced by escitalopram and became almost equal to control. On top of that, levels of histone H3 acetylation were also increased by escitalopram (Park et al., 2017).

4.2 Potential antidepressants

In rats, it has been demonstrated that the selective serotonin 5HT1AR agonist, 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT), exerts antidepressant effects. 8-OH-DPAT is a selective serotonin 5HT1AR agonist, so it mimics the effect of serotonin (Miyake et al., 2014). This potential antidepressant can reduce the effects of MS. As discussed in chapter 2, unpredictable MS can deteriorates serotonergic functions and as a result of that it decreases social exploration. Acute administration of 8-OH-DPAT in mice that experienced MS reverses the effect of unpredictable MS on social exploration. After 8-OH-DPAT treatment, mice that experienced MS spend equal time investigating strangers as control mice (Franklin et al., 2011).

Trichostatin A (TSA) is another compound that is thought to be able to prevent the negative effects of MS. TSA is a histone deacetylase (HDACs) inhibitor, so TSA will inhibit the gene silencing effects of HDACs (Tóth et al., 2004). Weaver et al. showed that TSA injections in rats during adulthood prevented the effects of MS on histone H3 acetylation, NGFI-A binding and cytosine methylation. Their research also showed that TSA eliminated the effect of early life stress on the HPA response to stress. Plasma

cortisol response to restrained stress of the TSA treatment group was equal to the group that did not experience early life stress. The cortisol response of the TSA rats was significantly lower than that of the vehicle treated group (Weaver et al., 2004).

As highlighted in this chapter, pharmaceutical interventions can diminish effects of early life stress on the epigenome and behavior. Escitalopram that is nowadays used as an antidepressant, can increase BDNF and GR concentrations in the hippocampus. 8-OH-DPAT can mimic the effect of serotonin and can improve social exploration in mice that experienced MS. TSA prevents effects of MS on the epigenome of rats and lowered cortisol response in those animals.

5. Discussion

Concluding, early life stress causes epigenetic changes in the brain and this results in altered behavior. Stressful experiences activate the HPA axis that release glucocorticoid to prepare the organism to react to the stressor. Most epigenetic changes are regulated by DNA methylation and histone modification and occur the easiest during early life. Many different models are used to induce early life stress in animals, like exposing them to MS, predator odor or restrained stress during pregnancy. Early life stress alters gene expression in several brain regions and this effect is meanly gene silencing. For example, several studies demonstrated that *Bdnf* expression in the hippocampus was reduced by early life stress.

Furthermore, there exist clear gender differences in the modulation of the epigenome and behavior by early life stress. Studies done so far show conflicting results, so nothing can be concluded about the nature of those differences and the mechanism by which they take place. Further research will be needed to establish the way in which male and female responses differ. Since the entire brain is affected by sex hormones (Marrocco et al., 2016), the interaction between epigenetic mechanisms and sex hormones could at least partly explain the gender difference of the epigenetic response to early life stress. Understanding gender differences is highly important since it can enhance personalized medicine.

Finally, both escitalopram, a commonly prescribed anti-depressant, and new potential antidepressants are able to reverse the negative effects of early life stress on the epigenome and behavior. 8-OH-DPAT can reverse the effect of MS on serotonin signaling and TSA is able to prevent the effect of MS on the epigenome by inhibiting HDACs. These results are very promising, since these studies could eventually lead to the production new and more effective antidepressants.

It is hard to compare the results of different studies done in the field of early life stress. This is mainly due to the fact that different models are used to create early life stress and those different types of stress can induce different stress responses. A research done by Skoluda et al. compared the psychological and physiological response of several laboratory stressors. Their results emphasize that stress protocols induce different responses and that the magnitudes of the responses vary enormously (Skoluda et al., 2015).

Not only the stress protocol that is used, but also the moment that stress is induced is also a variable that is unneglectable. The timing of the stress experience is very important for the outcome of epigenetic changes. Exposing an organism to the same environment early and late in gestation can result in different epigenetic outcomes (Tobi et al., 2009).

So far, all studies researching the effects of early life stress changed the environment to induce an epigenetic response. Until now, no studies have established the direct effect of glucocorticoids on epigenetic programming of the brain. A stressful experience does not only alter glucocorticoid concentration, but it also affects other hormones like oxytocin and vasopressin (Oyola et al., 2017). In my opinion, no conclusions can be drawn about the mechanism by which stress brings about epigenetic changes if stress is induced by environmental changes. The hormones and neurotransmitters that are involved in epigenetic changes due to stress exposure cannot be identified in this way. By injecting glucocorticoid directly in the bloodstream of pregnant or newborn animals, the effect of glucocorticoid concentrations on epigenetic programming during early life can be established. The effects of other stress related hormones on epigenetic changes can be assessed in the same way.

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