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CURRENT INSIGHT ON THE BIOLOGICAL TRANSMISSION OF TRAUMAS TO SUBSEQUENT GENERATIONS

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Abstract

Traumatic experiences can have long-term consequences as it increases the risk to develop disorders such as PTSD and depression. Moreover, it has been observed that the effect of traumas does not only impact the individuals that were exposed to the event but can be transmitted to subsequent generations as well. Next to social transmission, epigenetics mechanisms are suggested to be (partly) responsible for the biological transmission. However, the underlying mechanism behind epigenetic inheritance remains to be established. A growing number of studies investigate the transmission of effects via either DNA methylation or altered non-coding RNA levels. The aim of this thesis is to review the emerging literature on evidence of biological inheritance of effects induced by exposure to traumas. There is still no biological explanation for epigenetic inheritance. However, it has been found that an accumulating amount of studies support the hypothesis that there is an association between altered phenotypes and epigenetic modifications which influence gene expression. Further research needs to be conducted to determine inter- and transgenerational transmission of the effects of traumas to subsequent generations in humans. A better insight in biological transmission of traumas is of great importance as it may lead to identification of biomarkers of traumatic stress and to advances in the treatment of even prevention of disorders such as PTSD and depression.

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INTRODUCTION:

Survivors of the Holocaust were confronted with traumatic experiences and memories, a burden they have to carry for the rest of their lives. This is one of the many traumatic events our history counts. Nowadays, our daily newspapers are still dominated with reports of traumatic or violent events such as wars, sexual violence, childhood treatment. People that have been exposed to traumatic stress events throughout their lives are likely to develop diseases and mental disorders including PTSD, depression and anxiety (Christopher, 2004). Additionally, traumatic experiences also have shown to affect physical health. Norman et al., (2012) showed that people who have experienced trauma are at higher risk to develop diseases such as cardiovascular disease, diabetes and even cancer. Interestingly, numerous studies suggest that traumatic experience does not only impact the individuals that were exposed to the events, but also seem to influence their offspring. For example, the famous Dutch Hunger Winter study showed that pregnant women that were exposed to famine in 1944 had children and grandchildren who were prone to obesity and diabetes (Painter et al., 2008). The discussion about intergenerational effects of trauma began with observations that the Holocaust not only had an impact on the survivors themselves but also on their children. Offspring of individuals that have experienced traumatic events, will probably react to the narrative and altered behavior of the trauma survivor. However, this does not seem to adequately capture the extent to which traumatic events permeates the children's life. Besides the influence of social transmission, it is believed that biological inheritance may play a role.

The last decades, numerous studies have found a connection between an individual's experience and altered phenotypes of the organism. It has been shown that sociocultural variables, like diet, stress and maternal care, can affect our cells and behavior (del Blanco & Barco, 2018). Such factors influence the phenotype without changing the DNA sequence. Instead, molecular mechanisms can lead to alterations in gene expression, which can persist throughout life (Weaver et al., 2004). These mechanisms are referred to as epigenetic modifications; molecular alterations induced by life experiences and environmental factors which can have long-lasting influence on an individual's behavior and physiology. Some of them result in beneficial adaptive responses, while other can have detrimental effects (del Blanco & Barco, 2018).

Introduction to epigenetics

Multicellular organisms develop through differentiation of disparate cell types. All cells have an identical genome, yet cells will develop into different cell types by exhibiting different profiles of gene expression (Margueron & Reinberg, 2010). The term ‘epigenetics’ refers to non-genomic inheritance. This means that the heritable changes are not the results of altered DNA sequences but rather due to epigenetic modifications. Epigenetic modifications alter the chromatin structure and DNA accessibility. This in turn regulates the patterns of gene expression (Handy et al., 2011). Which genes are expressed depends on a variety of factors including the cell type, the phase of the cell cycle and environmental factors. Protein production determines the functioning of the cell. Which genes are able to be transcribed is dependent on the accessibility of the DNA. Two of the most studied epigenetic modifications are chromatin modifications and DNA methylation. In addition, non-coding RNA’s are also important modulators of gene expression (Handy et al., 2011). These modifications can either interfere or enable transcription. Hereby, the processes of epigenetic modification constitute cell-type specific gene expression which is essential for the differentiation and development of different cell types into an adult organism by repressing gene-expression in certain cells. Epigenetic mechanisms allow a stable propagation of gene expression from one generations of cells to the next. Moreover, epigenetic modifications mediate the interaction between external factors and gene expression, enabling an individual to respond and adapt to its environment (L. Liu et al., 2008). Elaborated description of the mechanisms can be found in box 1.

Box 1: Epigenetic modification

Histone modification:
Chromatin is the complex structure of DNA associated with proteins called histones. Chromosomal DNA is spooled around 8 histone complexes, together, such units are referred to as nucleosomes. DNA is further folded for additional levels of compaction in order to pack the DNA into the nucleus, these structures are called chromatins (Simpson, 1978). The chromatin structure commonly presents a barrier for the machinery responsible for transcription, replication or repair of DNA (Margueron & Reinberg, 2010). The chromatin is a dynamically adjusted entity that regulates the cellular pathways. The dynamics of the structure can be adjusted by a variety of histone posttranslational modifications (PTM) including methylation, acetylation, phosphorylation and ubiquitinylation (Boyce & Kobor, 2015). Most modifications on the nucleosome occur on the residues in the N-terminal tail that protrudes from the histone core. Amino acids that are positively charged, such as lysine, are commonly targeted for acetylation. The acyl group that is added masks the positive charge of the amino acid, hereby decreasing the affinity to DNA. This results in a relaxation of the chromatin structure which is associated with greater levels of gene transcription (Barnes et al., 2019).

DNA methylation:
DNA methylation is the epigenetic mechanism by which a methyl-group is added to a cytosine base that lies sequentially adjacent to a guanine base, called CpG dinucleotide. Areas with lots of CpG nucleotides are referred to as CpG islands which are often associated with gene promoters (Illingworth & Bird, 2009). DNA methylation is a relatively stable epigenetic tag which can foster or impede gene expression. However, DNA methylation in gene promoter regions is most often associated with diminished gene expression (Bird, 1986). It can suppress transcription by several mechanisms but in general it blocks DNA recognition and transcription factors binding. It is an important component in a large variety of cellular processes including genomic imprinting and embryonic development like X-inactivation. Also, DNA methylation has become viewed as a reversible process (L. Liu et al., 2008).

Noncoding-RNA's:
A third epigenetic mechanism is the expression of non-coding RNAs (ncRNAs). Different than coding RNA, non-coding RNA is not translated into a protein. Instead, ncRNA's interfere with gene expression by multiple mechanism, most of them are repressive. The role of long non-coding RNA's (>30 nucleotides) in gene silencing is due to their recruitment of remodeling complexes that foster histone methylation and deacetylation (Nagano et al., 2008). There are also different classes of small noncoding RNA's, each of them have been shown to mediate epigenetic histone and DNA methylation (Handy et al., 2011).

Numerous studies have shown that the altered phenotypes that are induced by external factors, are not only expressed by the individual that is exposed to the factors but are passed down to further generations. The neo-Darwinian theory postulates that new evolving phenotypes result from natural selection via genetic alterations and mutations (Skinner et al., 2015). However, since this selection appears slow and inefficient, the concept of transgenerational epigenetic inheritance as underlying mechanism has been established. This proposes that altered phenotypes induced by environmental factors, can be transmitted to subsequent generations via epigenetic modifications (Skinner et al., 2015).

Trauma transmission

It has widely been proven that acquired traits can be transmitted to subsequent generations (Lacal & Ventura, 2018). Transmission can occur via multiple pathways. Firstly, parents can model a certain behavior from which children learn and can start to exhibit themselves. Furthermore, parental deficits, such as in child rearing, can lead to phenotypic alterations in the offspring (Vostanis et al., 2006).

In case of traumatic events, offspring can be affected by the impact of the trauma by the burden of parental damage or by experiencing parental trauma's vicariously. This type of transmission, in which the parental trauma exposure affects the offspring without transmission of biological alterations is referred to as social or cultural transmission (Bowers & Yehuda, 2016). An alternative pathway of transmission if through molecular alterations. Parental acquired alterations can be transmitted via gametes. A potential mechanism would be via epigenetic modifications mediating biological inheritance (Bowers & Yehuda, 2016).
If the impact of an external factor is transmitted from parent (F0) to offspring (F1). This is typically called intergenerational transmission (Klengel et al., 2016). In case the exposure to environmental factors occurs during the development of the fetus (in utero: F1), the passing down to their offspring (F2) is also referred to as intergenerational transmission. Transmission of acquired traits across multiple generations is called transgenerational transmission. Notably, if exposure occurs before conception, transgenerational inheritance is already present in F2, otherwise the alterations should be passed down to F3 (Dias & Ressler, 2014).

So, epigenetic modifications may provide a basis for biological transmission of acquired traits to next generations. However, the underlying mechanism of epigenetic inheritance remains unclear. In almost all organisms, most of the acquired epigenetic modifications are erased during epigenetic reprograming during gametogenesis and embryogenesis (Casas & Vavouri, 2020). Yet, a small set of genomic sites escapes the reprogramming of DNA methylation. There are regions in the DNA that are protected against this removal of epigenetic markers, namely genomic imprinting processes prevents the removal of methylation at specific parts of the genome. However, it seems not likely that the epigenetic modifications induced by trauma exposure occur on these regions (Casas & Vavouri, 2020).

A few years ago, a research on four generations of a family showed that patterns in DNA methylation are passed down to the offspring during spermatogenesis. This indicates that DNA methylation modification across generations could lead to changes in phenotype of the progeny (Tang et al., 2016), which in turn suggests transgenerational epigenetic inheritance in humans. Still, transgenerational biological inheritance of trauma's generates heated debates due to unknown mechanisms and the impact of social transmission. In this thesis the current state of research on transgenerational epigenetic inheritance of trauma's will be investigated. It has extensively been observed that parental trauma exposure causes offspring to be at greater risk

to develop behavioral, physical and cognitive problems such as psychopathology. What is the role of epigenetic alterations in the the biological transmission of trauma?

Consequences of traumatic events

Traumatic experiences involve the confrontation with events that are characterized by a disruption of beliefs and expectation. The individual is a victim of circumstances and has lost control. Example of traumatic events are exposure to violence, war, serious illness, abuse, disasters and disturbing and overwhelming events (Kleber, 2019). The term early-life stress is used to describe a wide range of stressful and adverse event, considered as traumatic such as childhood abuse or neglect (Pervanidou et al., 2020). Exposure to traumatic stressors can lead to fundamental and enduring changes of an individual's life. It can be associated with the development of post-traumatic stress disorder (PTSD) and other mood and anxiety disorders. Importantly, not all individuals that experienced trauma's will develop PTSD (Sharma. N et al., 2015). From a biological perspective, it has been observed that traumatic stressful events lead to biological alterations of the HPA-axis and other biological systems (Yehuda et al., 2018). Most studies have focused on the impact of trauma on HPA axis functioning as they play a key role in the regulation of stress response both centrally and peripherally.

The HPA-axis

If an individual is exposed to stress, the hypothalamus secretes CRH which stimulates secretion of ACTH from the pituitary. The release of ACTH in turn stimulates the secretion of glucocorticoids from the adrenal cortex. Glucocorticoids play an important role in modulating immune and brain function. Additionally, glucocorticoids generate a negative feedback to the hypothalamus and pituitary by binding to glucocorticoid receptors (GR). Altogether, the HPA-axis mediates a proper response to stress and regulates many bodily processes (Stephens & Wand, 2012). Two important genes involved in the HPA-axis are FKBP5 and NR3C1 (Sherin & Nemeroff, 2011).

Dysregulation of the HPA-axis is associated with a wide variety of mental and physical disorders. The long-term consequences and the impact of trauma exposure depend on a variety of factors including severity, duration, type and the moment of exposure of stressors (Katharina Gapp, von Ziegler, et al., 2014). The altered HPA-axis functioning seems to be the consequence of altered glucocorticoid programming through epigenetic modifications (Brand et al., 2006).

As traumatic events can lead to a wide variety of consequences which are depending on many variables, this thesis will not focus on one specific process. Instead, this review will focus on the epigenetic transmission of 'any' effect of parental trauma exposure to offspring.

Multiple descriptive studies on the effect of trauma's have been conducted in humans. For the investigation of epigenetic inheritance of trauma's, research on the effect of paternal trauma on the offspring is often conducted. Rodent models are often used to simulate traumatic events and investigate its consequences. Additionally, in most rodent studies, fathers do not directly interact with their offspring, thus they are only able to pass down 'information' via germ cells instead of via social transmission (Bale, 2014). Therefore, experimental rodent studies come into play to investigate causal links between phenotypes observed in parents and offspring. Traumatic events are often generated by the use of social and psychological stressor are often used to generate a traumatic event for the animal. Thereafter, behavioral tests are used to measure and analyze behavioral responses and consequences of trauma exposure (Verbitsky et al., 2020).

Findings from human and rodent case studies will be discussed and combined to generate an insight in current knowledge on the transmission of traumatic event to offspring via epigenetics. There are subtypes of studies which involve epigenetic alterations due to traumatic events reviewed below. They focus on traumatic exposure either before or after conception (so the latter one during pregnancy) and on epigenetic alterations mediated by either DNA methylation or non-coding RNA's. They examined methylation of genes involved in the HPA-axis and non-coding RNA's levels in sperm.

Literature review

There is an accumulating amount of studies that demonstrate that children of traumatized or extremely stressed parents are at higher risk to develop mental or physical adverse outcomes. For instance, genocide exposures in Rwanda and Cambodia are linked to increased anxiety and depression in descendants (Field et al., 2013; Rieder & Elbert, 2013). Additionally, daughters of Finnish women that were evacuated in 2nd world War show an increased risk of psychiatric hospitalization (Santavirta et al., 2018) and Klarić et al., (2008) showed that children of war veterans with PTSD were at higher risk of developing psychological problems. Parental PTSD seems to be a salient factor in the transmission in some of these effects (Yehuda et al., 2001).

It is likely that the parent suffering PTSD and the symptoms that derive from it, affect the upbringing of the offspring and caregiving behavior of the parents. However, numerous studies suggest that besides social transmission of the effects, biological alterations contribute to the inheritance of trauma's as well. Only in a few human case studies, molecular analyses are conducted to identify the involvement of epigenetic factors. For a summary of the reviewed studies, see appendix A.

DNA methylation studies

Multiple studies have been conducted on the investigation of methylation patterns of the NR3C1 gene, which encodes the Glucocorticoid Receptor. The studies of Oberlander et al., (2008) and Radtke et al., (2011) have shown that exposure to environmental stressors early in life in humans increase DNA methylation at the promoter region of exon NR3C1, which maintains measurable later in life. Similar results were found in the studies of Mulligan et al., (2012) and Rodney & Mulligan, (2014). They showed that maternal exposure to war or rape is associated with increased DNA methylation on the promoter of NR3C1 gene in their offspring. These studies did not assess the mental health status of the children. The Tutsi study investigated the effect of exposure of the Tutsi genocide during pregnancy (in utero) on the offspring (Perroud et al., 2014). PTSD, depression severity, GR and MR levels and NR3C1 methylation levels were compared between women exposed to the trauma and control subjects. They found that both mothers exposed to trauma and their offspring showed higher PTSD and depression severity as well as higher NR3C1 methylation compared to non-exposed mothers and offspring. So, multiple studies in humans have shown that maternal traumatic stress events during pregnancy are associated with increased methylation patterns in NR3C1 promoter regions in their children. These increased methylation patterns were also found in studies that investigated the effect of childhood adversity and experiences of stressful life events during adolescence on methylation on the NR3C1 gene (Cicchetti & Handley, 2017; Van Der Knaap et al., 2014). The hypermethylation was also associated with greater depressive symptoms and emotional dysregulation. Current studies have shown that exposure to traumatic stress events during different moment in life is associated with higher NR3C1 methylation. Increased methylation of the NR3C1 promoter region constrains the expression of the Glucocorticoid Receptor. Hereby, the feedback sensitivity of the HPA-axis is diminished (Weaver et al., 2004).

Limitations of these studies include small sample size and possible confounding of the results such as that factors like health of participants during pregnancy (Perroud et al., 2014) and parenting could have played a role. Furthermore, methylation levels of peripheral blood cells were examined. It is not guaranteed similar methylation patterns would be observed in the NR3C1 genes in the brain. However, a postmortem human study showed increased DNA methylation in promoter regions of the GR gene in human brains associated with childhood abuse, suggesting these alterations are also present in the brain (McGowan et al., 2009).
Moreover, as the trauma exposure occurred during pregnancy, these finding do not yet identify inter- and transgenerational transmission of the effects induced by traumas. The studies that investigated childhood adversity or traumatic events later in life did not examine the offspring either. If the epigenetic modifications on the NR3C1 promoter, found in these studies, also occur in the subsequent generation, then intergenerational inheritance is present. Therefore, follow-up studies should be conducted to investigate further generations to explore the inter- and transgenerational transmission of NR3C1 methylation. Hereby, the impact of 'in utero', as well as, postnatal trauma exposure on NR3C1 methylation in offspring can be further explored.

These findings on epigenetic alterations on NR3C1 gene promoter are supported by a famous mice study. Pioneering studies by Liu et al., (1997) investigated the impact of early life stress on the offspring. Early life stress was measured as reduced levels of maternal care (licking and grooming). They observed that offspring from low licking mothers showed increased HPA-axis reactivity, reduced cognitive capabilities and increased anxiety behavior in adulthood compared to offspring from high licking mothers. A seminal study done by Weaver et al. (2004), showed that these altered responses were associated with an increased methylation of the NR3C1 promoter region and histone acetylation in the hippocampus. Together, this results in a decreased GR expression. These epigenetic differences persisted in adulthood and seem to be responsible for the different behaviors and responses to stress (Weaver et al., 2004).

Besides NR3C1, is FKBP5 an important gene involved in the regulation of the HPA-axis. FKBP51, encoded by the gene FKBP5, is an important regulator of the stress hormone system (Hawn et al., 2019). If FKBP51 binds the GR receptor complex, it decreases the affinity of cortisol. Thereby, it generally has an inhibitory effect on GR signaling. GR activation also leads to rapid FKBP5 expression, creating a short negative feedback loop that regulates the activity of GR (A. S. Zannas & West, 2014). Alterations in FKBP5 expression have been associated with PTSD and intergenerational effects (Lehrner et al., 2014).

Yehuda et al., (2016) investigated the effect of traumatic experiences on methylation status of the FKBP5 gene. The impact of the Holocaust on the survivors and their offspring has widely been researched. They found that there was an increased risk to develop psychopathologies in the offspring and grand-offspring (Yehuda et al., (1998); Yehuda et al., 2008). In a more recent study, they identified changes in DNA methylation patterns as potential epigenetic signature of the symptoms (Yehuda et al., 2016). They investigated whether alterations in methylation status of FKBP5 gene, as a result of Holocaust exposure, was transmitted to the next generation. The results showed higher methylation levels in intron 7 of the FKBP5 gene in holocaust survivors compared to control subject. Whereas, significantly lower methylation was observed in Holocaust offspring. Despite the methylation alterations occurred in the opposite direction, they were found in the same site. These findings provide the first demonstration of epigenetic alterations in both exposed parents and their children due to preconception stress in humans. Therefore, this suggests intergenerational alteration in epigenetic modification in humans. Other studies have shown that the impact of the Holocaust trauma on psychosocial functioning is also transmitted to the third generation of the survivors (Scharf, 2007). This generation should also be examined on epigenetic marks to determine transgenerational epigenetic inheritance of trauma exposure. Plank et al., (2021) showed that prenatally traumatized mice is associated with an increased methylation status of the FKBP5 promoter in the hippocampus, these findings support the results of methylation analysis in the survivors. However, other studies showed that early life stress and trauma exposure induced FKPB5 demethylation in rodents in the amygdala (Anthony S. Zannas et al., 2016). So, distinct methylation patterns are found in different areas in the brain by studies investigating the effect of trauma exposure on FKPB5 methylation. This shows that further research is needed to get a better understanding on the effect of trauma on FKBP5 methylation.

Limitations of this study include again a small sample size, as well as that other factors that could have also contributed to observed effects are present. For instance, the extreme starvation condition for the survivors are impossible to control for, yet it is another environmental factor that can influence the epigenome. Furthermore, they only investigated blood samples which does not permit ascertainment of transmission depending on gametes-transmission.

Further studies on other genes have been conducted in mice. The study of Franklin et al., (2010) investigated the transmission of the impact of unpredictable postnatal stress (comparable to human childhood ‘trauma’) across generations. They ‘implemented’ early life stress by chronic and unpredictable maternal separation which led to detrimental effect on the upbringing. To

produce a second generation, males (F1) subjected to maternal separation, as well as a control group, were mated with naïve females. These mice were reared under normal conditions (so no unpredictable maternal separation or stress). For the third generation, again male offspring (F2) were mated with naïve females. Importantly, in both cases, the males were removed from the cage after mating. Therefore, the males exposed to maternal separation never had contact with the offspring. This serves as a control for behavioral/social transmission of paternal behavior.

The results show that the early life stress led to altered behavior such as depressive symptoms and different response to aversive and novel environments. Most of the behavior alterations were also observed in the offspring of males subjected to maternal separation as well as in the subsequent generation. However, not all behavioral changes were observed in across the generations consistently. For instance, males of F2 generation did not show depressive-like behavior, whereas this behavior was seen in F3. This suggests that mice can act as 'silent carriers' of altered behavior. Looking at epigenetic inheritance, early life stress appeared to alter methylation patterns which were also transmitted to offspring.

To investigate the effect of DNA methylation patterns, they examined methylation status in the promoters of multiple candidate genes such as MeCP2, CB1 and CRFR2. These candidate genes were associated with depressive or emotional behavior and those that were known to be involved in epigenetic regulation of gene expression. Either hypomethylation or hypermethylation was found in the results, depending on the gene. Some of the DNA methylation alterations were observed in F2 and F3 generation as well. Overall, these findings suggest that that early life stress can cause changes in DNA methylation in the male germline. Some of the alterations are maintained and transmitted to subsequent generations. The transmission of altered DNA methylation and behavioral responses across multiple generations indicates the presence of transgenerational epigenetic inheritance.

Non-coding RNA studies

Non-coding RNA's have also been implicated in the transmission of the effect of traumas.

The study of Dickson et al., (2018) investigated the relation between Adverse Childhood Experience (ACE) and the levels of a specific miRNA marker in sperm that promotes stress-associated behavior in the offspring in humans. The ratings of ACE are based on a questionnaire quantifying childhood abuse. High scores, indicating traumatic experiences, are associated with increasing risk to develop mental and physical maladies (Waite et al., 2010). Furthermore, ACE scores seems also to be correlated with the sensitivity to PTSD (Leardmann et al., 2010;

McGuinness & Waldrop, 2015). Additionally, they did a comparable study in mice. They investigated the effect of stressful/traumatic events on same miRNA across generations in mice. The mice were exposed to chronic social instability (CSI) stress, according to the researchers, a similar experience for the mice as childhood adversity in humans. The expression of those members has been implicated in the regulation of stress response in the adult brain (Andolina et al., 2017). In this study, they found an association between reduced levels of multiple members of 34/449 miRNA family and early life stress exposure. The results of the human study showed that high ACE score samples had significant lower levels of miR-449a and miR-34c compared to samples with low ACE scores. Previous studies found that neither obesity nor smoking influences the levels of these sperm members. This implies that early life stress in men, quantified by ACE score, is associated with lowered levels of miR-449a and miR-34c. The results of the mice study showed that mice exposed to chronic social instability (CSI) stress had decreased levels of miR-449a and miR-34c as well. In mice these impact of stress on the expression of miRNA's is transmitted to the next generation as their offspring also showed severely suppressed levels of miR-449a and miR-34c.
This study shows that childhood adversity leads to a reduced level of miR-449a and miR-34c in the both human and mice. Furthermore, the mice study showed that these same alterations in miRNA levels were transmitted to the next generation, suggesting intergenerational inheritance.

The findings in the mice study suggest that the altered miRNA's levels may also be transmitted to their offspring. For the determination of intergenerational/transgenerational epigenetic inheritance in humans, miRNA levels in subsequent generations should be investigated. Importantly, this study has several limitations: the rating/answering of the ACE is subjective measurement. Also, more recent traumatic experiences and current psychiatric state that can impact miRNA levels as well, are not surveyed by the ACE questionnaire. Therefore, a larger follow-up study in combination with a survey covering severe stressful/traumatic experiences should be done to explore the association between lowered levels of the miRNA members and early life adversity.

The behavioral alterations in the study of Franklin et al., (2010) described earlier, were supported by the study of Gapp et al., (2014). Similarly, they investigated the impact of traumatic stress early in life, induced by unpredictable maternal separation, on subsequent generations. The mice exposed to maternal separation showed altered behavioral response. These behavioral traits were passed down to the F2 and F3 offspring. Different than the study

of Franklin et al., (2010), Gapp et al., (2014) analyzed the role of miRNA, instead of DNA methylation, in the transmission across subsequent generations. The mice were exposed to a similar maternal separation procedure as described in the study of Franklin et al., (2010). The results revealed an effect of the traumatic early life stress events on several sncRNA's, different than the ones investigated by Dickson et al., (2018), populations in mice sperm. Either up- or downregulation was observed, depending on the RNA member. These alterations were also found in the serum and brain structures that are involved in the stress response. Surprisingly, in the F2 generation, these alterations in miRNA levels were observed in the brain but not in the sperm. Gapp et al., (2014) suggested that this could be explained by a transfer of changes in miRNA to other non-genomic or epigenetic marks like DNA methylation. However, there is no evidence for this phenomenon. The causal link between sperm RNA's and the effects of traumatic stress early in life across generations was tested by the injection of RNA from sperm from the traumatized males into naïve fertilized mouse oocytes. The mice and their offspring showed comparable behavioral and molecular effects as the mice and the offspring that were directly exposed to traumatic early life stress.The findings in this research show that sncRNA's are potential mediators of nongenomic inheritance of phenotypes or traits acquired across life. These findings are in line with previous studies showing altered sncRNA's contribute to the transmission of acquired traits (W. M. Liu et al., 2012; Rassoulzadegan et al., 2006). Rodgers et al., (2013) showed that stress exposure during puberty and adulthood also led to alterations in sncRNA's levels and behavioral responses in offspring. This indicates germ cell vulnerability to external factors during both puberty and adolescence. However, the underlying mechanisms remain unknown.

A few years later K. Gapp et al., (2020) investigated whether long RNA in sperm also plays a mediating role in the transmission of traumatic stress early in life to offspring. Again, they also tested the causal link between both sncRNA's and longRNA's and the effect of traumatic early life experience in the offspring by injection of the RNA's into fertilized oocytes of naïve females. The behavioral phenotypes of the offspring resulting from sperm RNA injections were analyzed. This showed that both sncRNA's and longRNA's were necessary to mimic behavioral changes that were known to be induced by maternal separation in natural offspring. The findings in these studies show that non-coding RNA's are very sensitive to traumatic stress experiences and that the dysregulation of non-coding RNA's is associated with inheritance of the effects of traumas.

Discussion

Multiple studies showed that in utero, as well as, postnatal exposure to traumatic events leads to hypermethylation of the NR3C1 gene promoter in humans. The changes in methylation lead to a decreased sensitivity of the HPA-axis feedback loop which seems to contribute to the altered phenotypes. The transmission of the alterations to subsequent generations is not yet well investigated. Therefore, further research in offspring is needed to determine the role of epigenetics in inter- and transgenerational inheritance. Similar results were found in the famous rat licking study, supporting the finding that traumatic stress events are associated with hypermethylation of the NR3C1 gene promoter (Weaver et al., 2004).

The study of Yehuda et al., (2016) suggests intergenerational inheritance of altered methylation patterns in humans. They showed that alterations in methylation of the FKBP5 gene occurred in opposite direction in parents and offspring. The authors suggest that this might be attributed to biological accommodation in their children. More research is needed to support this explanation. Research in mice have shown that altered methylation patterns in candidate genes are transmitted across multiple generations. This suggest epigenetic transgenerational inheritance. The study of Dickson et al., (2018) showed an association between childhood adversity and lowered levels of specific miRNA in humans. Similar results were found in the mice study. In addition, they found transmission of these altered levels in offspring as well. Importantly, the ACE questionnaire is subjective and experiences and exposure after childhood are not covered in the rating. Therefore, it is not possible to draw a strong conclusion about the association between traumatic events and the altered miRNA levels.

The association between non-coding RNA's and trauma transmission has also been demonstrated in several other rodent studies. Interestingly, Gapp et al., (2014) showed that certain traits that are not expressed by the parent, can be passed down to progeny and subsequently expressed. This indicates that individuals can act as an 'silent carrier' of certain alterations. This phenomenon is also seen in humans (Pembrey et al., 2006; Roseboom et al., 2006). However, the underlying mechanism is still unclear. Awareness and further understanding of the process of silent carrier is important as it suggests possible 'invisible/unnoticed' transmission of traits to subsequent generations.

Altogether, the limited amount of available studies in humans suggest that altered methylation patterns or non-coding RNA's levels are associated with the transmission of the effects of

trauma exposure from parents to offspring. The findings were supported by several experimental rodent studies. The alterations in epigenetic modifications may be the due to the combined influence of both social and biological transmission of traumas. There are some important limitations that should be taken into account during interpretation of the results. It has widely been proven that factors other than traumatic events can affect epigenetic modifications as well. In human studies, it is impossible to control for the influence of other environmental factors. Therefore, we are not able to identify the causal link between the traumatic event and the altered phenotype as it is possible, and very likely, that other experiences and exposures were (partly) responsible for the modifications. That is the reason why experimental animal studies are of great importance. Especially the studies investigating paternal trauma exposure serve as a control for social transmission as the father is often separated from the offspring. This implies that the observed phenotypic changes are the result of biological inheritance rather than behavior or social transmission of traumas. Additionally, human studies mainly investigate blood or saliva. Importantly, we can not say for sure that these alterations would also be measured in the brain. The study of Dickson et al., (2018) showed converging observations from mice and humans (same alterations in similar miRNA types). It is a promising finding that supports the translation of findings in animal studies to human cases. However, due to the genomic distance between rodents and humans, findings in experimental investigations in mice and rats are for limited use to transfer knowledge to understanding of the human body. Additionally, it is unclear whether trauma and PTSD models in animals are well characterized. I think it is doubtful whether traumatic events induced in animals are comparable to the experience of traumatic events in humans. Next to the problem that it is impossible to control for behavioral transmission in humans, our long lifespan makes it hard to conduct longitudinal research and investigating multiple generations. It is also important to keep in mind that methylation analyses were restricted to certain genes. Therefore, the impact of trauma's is probably not restricted to alterations in the genes discussed in this thesis. Many more genes are expected to be altered, as well as intragenic regions which were not analyzed in these studies. Furthermore, for instance prove of the contribution of a specific miRNA does not exclude other underlying mechanisms, like DNA methylation. Moreover, non-coding RNAs are transcribed form genes. So, it would be valid to suggest that the altered expression levels are due to an upstream epigenetic modification such as DNA methylation as driving force.

Follow-up studies need to be conducted to explore the underlying mechanisms of transmission of epigenetic modifications. Also, further research could be conducted on the effect of the

moment of trauma exposure on the transmission. Furthermore, promising studies show that these modifications are reversible and that enriched environments could prevent the transmission of parental trauma (Katharina Gapp et al., 2016).

Conclusion

The field is still in a premature phase to actually prove that traumas are transgenerationally inheritable via epigenetics. However, despite the limitations of current studies, there seems to be accumulating evidence of the transmission of parental trauma experiences to subsequent generations via epigenetics. The findings in rodent studies show the transmission of the effect of trauma are transgenerational. For determining the role of epigenetics in inter- and transgenerational inheritance of traumas in humans, more studies should be carried out on epigenetic marks in brains across subsequent generations. This will be facilitated by the development of brain banks like the 'National Posttraumatic Stress Disorder Brain Bank'. In addition, the ability to identify the persistence of epigenetic effects of exposures to trauma across generations will be enhanced by data from birth cohorts that across multiple generations such as the 'Drakenstein Child Health Study' (Stein et al., 2015). It is speculated that the altered phenotypes across generations is the result of a complex interplay between factors like DNA methylation and different RNA fractions.

In conclusion, the field has already made great steps in investigating the role of epigenetic mechanisms in the regulation of the HPA-axis to traumatic events. It is important to draw attention to the consequences of trauma experiences on the individual, as well as, on subsequent generations. Future research in this field may lead to the identification of markers of traumas. This may lead to greater advances into the treatment and prevention of disorders such as PTSD and depression that develop through (parental) traumatic experiences.

Literature

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Appendix

A: Epigenetic modifications or altered behavior induced by traumatic stress exposure

	Reference	Parental measure	Time of exposure for offspring	Measured mechanism	Observed effects
Human	Oberlander et al., (2008)	Depressed / anxiety mood	In utero	NR3C1 promoter methylation	Positive association
	Radtke et al., (2011)	Partner violence	In utero	NR3C1 promoter methylation	Positive association
	Mulligan et al., (2012)	Exposure to war and rape	In utero	NR3C1 promoter methylation	Positive association
	Rodney & Mulligan, (2014)	Exposure to war	In utero	NR3C1 promoter methylation	Positive association
	Perroud et al., (2014)	Tutsi genocide	In utero	NR3C1 promoter methylation	Positive association
	Cicchetti & Handley, (2017)		Childhood adversity	NR3C1 promoter methylation	Positive association
	Van Der Knaap et al., (2014)		Stressful life events during adolescence	NR3C1 promoter methylation	Positive association
	Yehuda et al., (2016)	Holocaust experience	Not direct/preconception	FKBP5 gene methylation	Positive association in survivors, negative association in offspring
	Dickson et al., (2018)		Adverse Childhood Experience	miR-449a and miR-34c levels in sperm	Negative association
Mice	Weaver et al. (2004)		Reduced maternal care	NR3C1 promoter methylation	Positive association
	Plank et al., (2021)	Exposure to traumatic shock	In utero	FKBP5 gene methylation	Positive association
	Anthony S. Zannas et al., (2016)		Early life stress	FKBP5 gene methylation	Negative association
	Franklin et al., (2010)	Early life stress	Not direct/preconception	Methylation status of candidate genes such as MeCP2, CB1 and CRFR2	Both positive and negative association depending on the

					gene in both F2 and F3
	Dickson et al., (2018)		Chronic social instability	miR-449a and miR-34c levels in sperm	Negative association
	Gapp et al., (2014)	Early life stress	Not direct/preconception	Levels of multiple sncRNA's in sperm	Either positive or negative association depending on the RNA member. However, this was not found in sperm in F2 although the behavioral effects were still observed as well as in F3
	Rodgers et al., (2013)		6 weeks of chronic stress	9 different types of miRNAs	Altered offspring stress-responsivity
	Gapp et al., (2020)		Short and long ncRNA from MSUS mice injection in fertilized oocytes	Behavioral changes	Altered behavioral such as increased behavioral despair and risk-taking behavior