university of groningen / faculty of science and engineering

Prenatal Stress and Psychopathology

Bachelor Thesis

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Abstract: Prenatal stress is associated with a wide variety of psychopathologies in later life such as ADHD, anxiety disorders, schizphrenia and autimsm. During pregnancy offspring is exposed to maternal signals that give information about the extra-uterine environment. When in a stressfull environment maternal glucorticoids reach the developing fetus and potentially alter its developmental track. Prenatal stress and glucorticoid exposure during pregnancy alter offspring HPA-functioning and brain morphology. These effects are mediated by epigenetic mechanisms. Many effects of PS can be put in an evolutionary perspective and give evidence for a predictive adaptive response to stress.

Keywords: Fetal Programming, Prenatal Stress, Psychopathology, Neurodevelopment, HPA-axis, Predictive Adaptive Response, ADHD, Anxiety, Conduct Disorder

1 Introduction

Early life adversity such as stress or undernutrition during development can have a profound impact on behavior, physiology and metabolism. This adverse influence early in life can lead to increased susceptibility to disease in adult life. The developmental origins of adult disease hypothesis (also known as Barker hypothesis) was first posited by Barker and his colleagues who made the observation that low birth weight serves as an indication for adult health. Low birth weight increases the risk of coronary heart disease, hypertension, type 2 diabetes mellitus and hyperlipidaemia[1] The Barker hypothesis is supported by findings from European birth registries during famines. These large registries recorded birth details and made it possible to identify the outcome in subjects later in life.

The Dutch famine of 1944-1945, caused by an embargo on food transport and an unusually harsh winter, lead to severe malnutrition, including pregnant woman, during this period. It was found that undernutrition during gestation affected health later in life. Differences in effects of undernutrition on adult health was dependent on the timing during gestation. Adults whose mothers were subjected to undernutrition during early gestation displayed an atherogenic lipid profile and a higher BMI[2],[3]. Those who were subjected to undernutrition during mid or late gestation showed a reduced glucose tolerance[4]. Furthermore it was found that undernutrition may affect adult health without affecting the size at birth, indicating that adaptations that enable the fetus survival may have adverse consequences later in life[5].

The siege of Leningrad in 1944 lead to a famine spanning 800 days. In contrast to the Dutch famine the subjects of the Leningrad famine did not display insulin resistance, dyslipidemia, hypertension, or coronary artery disease6. The Russian subjects showed a thrifty phenotype[6] that was well adapted to extra uterine life. The Dutch however displayed catch-up growth; what guaranteed survival during pregnancy, but lead to problems in adult life when the conditions did not match the intrauterine environment.

It is also becoming increasingly clear that stress experienced by the mother during gestation can have many long term effects on the neurodevelopment of the offspring both in animals and humans[7, 8, 9, 10]. Using cross fostering in animal models have shown that there is a permanent prenatal component to the effects on neurodevelopment of offspring. These effects are not solely due to postnatal effects[11].

The hypothalamic-pituary-adrenal-axis (HPA-

axis) is essential for regulating the stress response. Research done on mammals and birds has shown that prenatal stress (PS) can modulate HPA-axis responsivity[12]. The modulation of HPA-axis reactivity is believed to be an adaptive response necessary to maximize fitness[13]. It is therefore plausible that PS can cause a mismatch between the intrauterine environment and the amount of stress later in life which could lead to psychopathology[14, 15].

Furthermore PS has been observed to affect several brain regions that include the hippocampus, amygdala, corpus callosum, neocortex, cerebellum, and hypothalamus[16].

The relationship between intrauterine conditions and the effects later in life have been termed fetal programming, a concept that falls within the broader concept of developmental plasticity. Developmental plasticity is the process that generates alternative phenotypes in a single genotype exposed to different environmental cues[17]. In this manner fetal programming allows an organism to prepare for the expected environment. If the expected environment does not match the actual environment a mismatch may occur such as in the case of the Dutch famine. How this concept of fetal programming and a mismatch relate to prenatal stress and the development of psychopathologies later in life is largely unknown. In principle fetal programming is regarded as an adaptive response [10, 13]. Stress signals in the form of maternal hormones provide useful information about the external world. So in this context it is a predictive and adaptive response. In this paper I will discuss the mechanisms of developmental plasticity, including fetal programming, epigenetics and the modulation of the HPA-axis. Furthermore I will attempt to elucidate the evolutionary advantages of prenatal stress, mismatch in current society, sex differences and the role of transgenerational epigenetic effects.

2 Mechanisms of Fetal Programming

2.1 Fetal programming

Most organisms can express a variety of structure, physiology and behavior within individuals of the same species regardless of genotype. Phenotypic plasticity is the ability of an organism to react to an environmental input with a change in form, state, movement, or rate of activity[17]. Developmental plasticity however is not just the responsiveness of the phenotype of a single genotype to the environment. Individual development starts with an inherited bridging phenotype, a responsive and organized cell provided by the parents that is adapted for survival and interaction in the embryonic environment. The genotype of the offspring and subsequent embryonic/fetal environment then act on the bridging phenotype[17]. Thus developmental plasticity starts before birth and is subject to maternal signals.

Signals from the mother during pregnancy carry useful information about the outside world to the developing fetus. This information can be used to express a phenotype that matches the outside world, a process called fetal programming, and a form of adaptive developmental plasticity[17, 18]. This form of developmental plasticity can be considered a predictive adaptive response (PARs) as the advantage of the induced phenotype is manifested in a later phase of life[19]. The information received from the mother thus acts as a predictor of the environment after birth. How advantageous the adaptation is depends on how well the prediction matches reality (see Figure 2.1). If there is a mismatch later-life pathology may occur. There are many mechanism underlying phenotypic plasticity but often epigenetic changes in DNA expression[20] are involved.

2.2 Epigenetic Mechanisms

Literally meaning on top of genetics, epigenetics are the causal interactions between genes and their products that lead to the phenotype[22]. Over the years the definition has narrowed a bit. Today epigenetics has been defined and is most commonly used as: the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence[23]. This section will encompass the molecular epigenetic mechanisms involved in fetal programming. DNA methylation, histone modification, chromatin structure modification, Non-coding RNA and RNA interference (RNAi) all possibly play a role in fetal programming, however, I will limit myself to DNA methylation and histone modification.

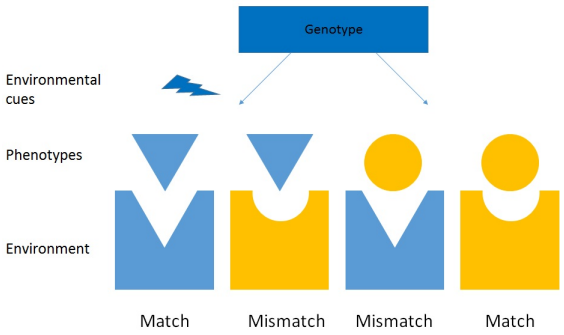

Figure 2.1: Environmental cues act as predictors, determining the path of developmental plasticity to match. If the subsequent environment doesn't change then the organism is well adapted. If the environment does change a mismatch of phenotype and environment occurs. Represented in the diagram by matching shapes and colors (redrawn from [21])

2.2.1 DNA Methylation

DNA methylation is the process of a covalent addition of a methyl group to either the carbon-5 of the cytosine ring (5mC) or the nitrogen-6 position of adenine (m6A)[24]. Methylation of cytosine in mammals occurs on cytosine that is 5 adjacent to guanine. These so called GpC islands can contain up to hundreds of base pairs. GpC islands are present in roughly 60 percent of promotor regions in humans. This covalent addition is catalyzed by DNA methyltransferases (DNMT). Cytosine methylation is generally associated with a decreased expression of relevant genes[25].

After fertilization the paternal and maternal epigenome is contained in the zygote. The paternal epigenome then undergoes rapid demethylation whereas the maternal epigenome undergoes gradual demethylation. After the methylation pattern is erased, de novo methylation occurs. New methylation patterns are established in the growing embryo via DNMT3a and DNMT3b, DNMT1 is responsible for maintaining methylation patterns. During development its then possible for changes in methylation pattern to occur due to environmental influence passed on through the mother[26].

In controlled animal experiments effects of prenatal stress (PS) on DNA methylation have been shown. Pregnant rats exposed to 1h/day of randomly timed restraint stress from gestational day 14-20 showed an increase in DNMT1 expression in the fetal cortex and hypothalamus. In the placenta an increase in DNMT3a expression was found. suggesting that PS has an influence on DNA methylation patterns[27]. In the placenta a decreased HSD11B2 transcription was found following PS exposure. HSD11B2 is responsible for the conversion of cortisol to the inactive cortisone, in this manner more cortisol is able to pass through the placenta to the developing fetus[27]. Another study done on rats used indirect prenatal stress. The indirect stress was administered by exposing cage mates of pregnant dams to bright light for 30 minutes twice a day. The researchers found an increase in global DNA methylation in the frontal cortex and the hippocampus, further indicating that the epigenome is adjusted by PS. Furthermore global DNA methylation in the frontal cortex was higher in females exposed to PS compared to males, suggesting a sex modulating effect of PS on the epigenome. When looking at the gene expression profile 558 genes in the frontal cortex and hippocampus were found to be expressed differently in the indirectly stressed grouped from the control group. Of those 558 genes there was an overlap of only 10 genes between male and female rats, indicating a sex effect on the effect of prenatal stress in brain gene regulation. Interestingly NR3C1 in the hippocampus and SLC6A1 in the frontal cortex were found to be differentially expressed in both sexes. NRC1 codes for the glucocorticoid receptor and plays an important role in the hippocampus by modulating HPA-reactivity. SLC6A1 codes for GABA transferase and in the prefrontal cortex a down regulation of SLC6A1 could lead to alterations of attention in offspring[28].

In humans the effect of PS on DNA methylation has also been demonstrated. Devlin and colleagues found that increased maternal depressed mood was associated with a lower SLC6A4 promotor methylation status in maternal peripheral leukocytes and in umbilical cord leukocytes[29]. The SLC6A4 region codes for the serotonin (5-ht) transporter (5-htt, SERT) which is responsible for transporting serotonin from the synaptic cleft back in to the presynaptic neuron. In this manner this may lead to lower intrasynaptic 5-ht levels. 5-HT has an important role during fetal development[30] and alterations in 5-HT levels during development alters

affective behavior in adult life in mice[31]. Furthermore prenatal stress in the form of maternal depression, war stress and partner violence had an effect on the methylation status of the NR3C1 promotor region[32, 33, 34].

2.2.2 Histone modification

DNA is wrapped around nucleosomes, protein structures consisting of histones. This nucleosome consist of two copies of four different histone proteins (H2A, H2B, H3 and H4), in total eight histones. Histones contribute to higher-order chromatin structure and is essential for fitting the large genomes of eukaryotes inside of cells. Furthermore histones play an important role in gene regulation. Chemical modifications such as methylation, acetylation and phosphorylation of amino acid residues on histone tails contribute to formation of either active or inactive chromatin structures[25]. Acetylation of histone tails reduces compactness of the chromatin structure and thus activation of the gene[25]. Methylation can have either a repressive or activating influence on transcription.

The role of histone modification during development and the effect of PS on the fetus has not been described in much detail yet with the majority of studies being done with regard to DNA methylation[35]. However, the enzymes that maintain and initiate histone modification may be altered by a developmental insult and in that manner play a role in fetal programming[36].

2.2.3 HPA-axis Modulation

The HPA-axis regulates corticosteroid synthesis and secretion during basal and stress conditions and plays an important role in regulating the stress response (see Figure 2.2). In the paraventricular nucleus (PVN) neurons secrete corticotrophin releasing hormone (CRH) and vasopressin (AVP). The releasing hormones then act on the pituitary to promote POMC which is the precursor of adrenocorticotropic hormone (ACTH) which is then in turn secreted. ACTH acts on the adrenal cortex where glucocorticoids are synthesized and released. Glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) regulate HPA activity via negative feedback. A decrease of MR and GR in the HPA-axis attenuates the negative feedback and results in increased HPA-activity. Conversely an increase of these receptors leads to a reduction of HPA activity and higher feedback sensitivity[37].

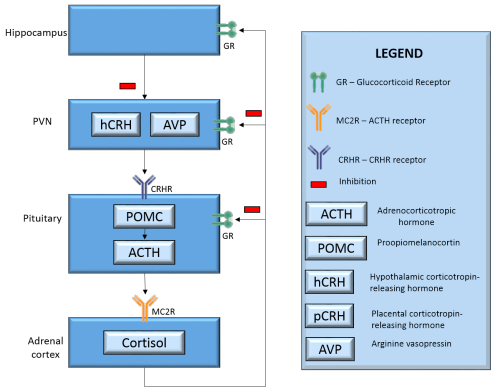

Figure 2.2: Schematic simplified overview of HPA-axis functioning. In the paraventricular nucleus (PVN) neurons secrete corticotrophin releasing hormone (CRH) and vasopressin (AVP). The releasing hormones then act on the pituitary to promote POMC which is the precursor of adrenocorticotropic hormone (ACTH) which is then in turn secreted. ACTH acts on the adrenal cortex where glucocorticoids are synthesized and released[38]. Glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) regulate HPA activity via negative feedback. A decrease of MR and GR in the HPA-axis attenuates the negative feedback and results in increased HPA-activity.

The ability of the HPA-axis responsiveness to be modulated by the environment was first demonstrated in the 1950s. Neonatal exposure to stress was found to be able to modify adult HPA responsiveness in rats[39]. Since then substantial evidence supporting long term effects on HPA responsiveness following early life stress and prenatal stress[10, 40, 41] have been established.

Exposure to PS during fetal development has long-term effects on HPA functioning and responsiveness. In rats PS generally leads to an extended peak or duration of the HPA response. However there appear to be many variations possible to this pattern[7]. Differences in findings could arise from the nature and timing of PS. Furthermore there are very significant sex differences in outcome of HPA responsivity following PS[10]. Rhesus macaques that underwent PS showed considerably higher cortisol values and higher ACTH values in both basal and stressed conditions than controls[42]. In humans prenatal stress has been associated with an increase in basal cortisol levels. This effect is greatest if the stress was experienced

during late gestation[43, 44, 45, 46]. Furthermore using the still-face procedure, an interactive social stressor, Grant and colleagues found an increase in the cortisol response 40 minutes post procedure[47]. Entringer and colleagues found that in prenatally stressed young adults that were subjects to trier social stress test showed an increase in ACTH and cortisol response in comparison with controls[48]. Taken together these results suggest a more active HPA-axis in humans who experienced PS[12].

The mechanisms in which maternal signals program the HPA-axis remain largely unknown. However epigenetic mechanism and glucocorticoids are likely to be involved. There are several key regulatory genes of the HPA-axis that are subject to DNA methylation and histone acetylation, namely: NRC1, CRH, POMC and HSD11B2. NR3C1 that codes for the GR as discussed previously sees an increase in methylation following prenatal stress in humans that experienced PS which leads to an increased stress response due to reduced negative feedback on the GR[34]. The promotor region of CRH undergoes hypomethylation in male offspring of rats exposed to chronic random stressors during early gestation[49]. HSD11B2 expression is correlated with absence of methylation at its promotor region, evidence for epigenetic regulation of this gene[50]. Elevated maternal stress leads to reduced placental HSD11B2 expression and an increase of methylation at its promotor[27]. HSD11B2 modulates glucocorticoid levels in the fetus. This leads to an increase of fetal glucocorticoid levels and can have an influence on the developing HPA-system and other brain regions[27]. The promotor region of proopiomelanocortin (POMC) also shows GcP islands and its expression has been linked to methylation status of this region[51], suggesting that PS could potentially affect POMC expression. Furthermore fetal baboons exposed to betamethasone (synthetic glucocorticoid) showed reduced POMC mRNA in the anterior pituitary and reduced ACTH receptor (MC2R) expression in the Adrenal cortex[52], suggesting that ACTH from the fetal pituitary regulates MC2R expression in the adrenal cortex through epigenetic mechanisms. Prenatally stressed rhesus macaques showed increased pituitary-adrenal activity at 2 years of age, as shown by an increase in cortisol after a dexamethasone suppression test. This indicates a long term effect of PS on HPA functioning[53]. In fetal sheep an increase of NR3C1 which codes for GR was found after exposure to maternal stress[54]. Taken together these results indicate that prenatal stress and glucocorticoid exposure play an important role in modulating the HPA-axis. These effects of antenatal exposure to glucocorticoids is highly dependent on species, sex, age and timing of exposure[55].

3 Effects of prenatal stress on the developing brain

Prenatal stress (PS) can have effects on cognition, behavior and susceptibility to psychosocial problems. For example, children of a mother who experiences stress during pregnancy, have a higher risk of anxiety disorders[56, 57], attention-deficit disorder (ADHD)[58, 59], schizophrenia[60, 61], and autism spectrum disorders (ASD)[62]. The effects of PS are likely mediated by the effect it has on the structure and function of the developing fetal brain.

Cortisol has an important role in the bodys stress response and during development. During late gestation maternal cortisol levels are naturally elevated. Indeed, cortisol is a critical developmental trigger and essential for healthy development of many organ systems such as thyroid, kidney, brain, pituitary and lungs[63]. If fetal cortisol levels are elevated at the wrong time normal developmental trajectories could be altered. The fetus is protected from the influences of cortisol through the placenta. In the placenta the enzyme HSDB11 facilitates the conversion of cortisol to the inactive cortisone. This gatekeeping role of the placenta with regards to cortisol is an imperfect one however, as maternal cortisol is able to pass through the placenta and maternal and fetal cortisol levels are correlated[64]. Furthermore placental CRH (pCRH) secretion is increased by cortisol in contrast to hippocampal CRH (hCRH) production which is suppressed by cortisol (see figure 3.2). In this manner an increase in cortisol also leads to higher CRH concentrations in the fetal brain which can then lead to a positive endocrine feedback loop. pCRH stimulates ACTH production in the fetal pituitary which in turn stimulates the secretion of cortisol by the fetal adrenal cortex. Fetal cortisol then further leads to an increase of pCRH[65].

pCRH can potentially alter brain regions rich in CRH receptors such as the fetal hippocampus, amygdala or other limbic areas[66, 16]. Furthermore cortisol itself has a potentially detrimental effect on the developing brain. In animal studies it has been readily demonstrated that fetal exposure to high doses of glucocorticoids can lead to decreased brain weight, alterations in HPA functionality[67] and hippocampal neuronal degeneration of the CA regions[68]. PS can lead to a decrease of placental HSDB11 activity thereby increasing cortisol level in the developing fetus even further. Altogether, the maternal and fetal HPA-axis and the placenta mediate the effect of glucocorticoids on the developing brain. Because stress may have differential effects in the brain, I will discuss three specific brain structures (hippocampus, amygdala and hypothalamus) and their development under PS.

3.1 Hippocampus

Development of the hippocampus, consisting of the hippocampus proper (CA1-4) and the dentate gyrus (DG) (see figure 3.1), is primarily formed during gestation in rodents as well as in primates[69]. When extrapolating data from rats to humans/primates, however, it is important to keep in mind that the brain of rats and primates do not maturate at the same rate. Rats can be considered post-natal brain developers, the postnatal period of rats is comparable to the third trimester in primates[70]. There are several differences in timing of formation of hippocampal neurons between primates and rodents. The neurons of the hippocampus proper form during the first half of gestation whereas in rodents this forms in late gestation. The granular cells of the DG in primates form largely prenatally and in rodents they form postnatal[69]. In the DG neuronal proliferation continues in adult life[71, 72].

Rhesus monkeys prenatally stressed in either early or late gestation showed reduced neurogenesis in the DG and a reduced overall hippocampal volume, regardless of the timing of the stressor[53]. Other studies using rhesus monkeys, but dexamethasone (synthetic glucocorticoid) as a stressor, also found reduced hippocampal volume and in addition found degeneration of pyramidal and dentate granular neurons[68, 74]. Rats that had been

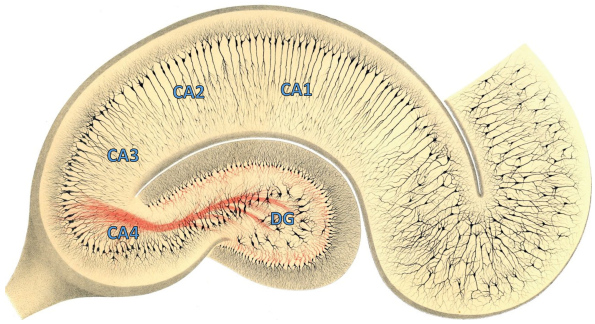

Figure 3.1: hippocampus proper (CA1-4) and the dentate gyrus (DG). Drawing of the hippocampus by Camillo Golgi [73]

subjected to PS in the form of random restraint from gestational day 15-19 showed a reduction in hippocampal wet weight in males and females[75]. Whereas a different study done by Schmitz and colleagues that looked at hippocampal volume following restraint stress only found an effect in females. However in the study done by Schmitz the stressor consisted of 20 minutes of restraint on gestational day 18[76]. Both studies used restraint as a stressor, however the difference in intensity of the stressor used suggests that the hippocampus responds different to prenatal stress in males and females. Neurogenesis in the DG region of the hippocampus is also subjected to effects of PS. Using restrain + bright light during gestation led to a decrease in DG neurogenesis in adult rats, however neonatal handling reversed these effects. An indication of the regenerative properties of the hippocampus[77, 78]. This effect appears to be sex specific as the two studies mentioned previously only used males whereas another study that employed both male and female rats only found an decrease in DG neurogenesis in males[79]. Interestingly limited stress seems beneficial for DG neurogenesis. When rats were subjected to 240 min/d of PS, neurogenesis was decreased. This contrasts to when rats only received 30 min/d of PS, where neurogenesis was increased[80]. In the CA1 region synaptic loss and a decrease in dendritic arborization has been reported in rats following prenatal stress in the form of daily restraint from gestational day 14-21[81]. Furthermore hippocampal glucocorticoid receptors in the hippocampus are downregulated in female rats that experienced prenatal

stress using flashing lights and noise throughout the pregnancy[82].

In humans little data are available on how PS affects the structure and function of the hippocampus. One study was performed in a large cohort following children who were subjected to PS by an ice storm in Canada in 1998. MRI was performed on children aged 11, the study is not yet published but preliminary findings show reduced right hippocampal volume in male but not in female adolescents[83]. Another study linking maternal cortisol levels with hippocampal volume found no effect of higher maternal cortisol levels on hippocampal volume in offspring[84]. This is in contrast to animal studies but could be explained by the fact that these women had not been exposed to stressful events such as disaster during pregnancy. Furthermore a common finding in the brain of people suffering from post-traumatic stress disorder (PTSD) is a decrease in hippocampal volume compared to subjects exposed to trauma but without PTSD and subjects not exposed to trauma[85]. In combat veterans of the Vietnam war it was found that PTSD was associated with a reduction in hippocampal volume, however the same reduction was found in their monozygotic twins who did not go to war[86], indicating that a reduced hippocampal volume may also be an indication for susceptibility to PTSD.

3.2 Amygdala

The amygdala is part of the limbic system and plays a central role in emotional responses and memory. It consists of several nuclei including the central, lateral, basolateral, and cortical amygdalar nuclei. The amygdala develops early in development[87] and is sensitive to glucocorticoids during this period[88]. The amygdala has a facilitator effect on the HPA-axis by causing CRH release following amygdala stimulation[89]. Furthermore its role is implicated in a number of psychopathological and neurodevelopmental disorders[90].

In rats PS has been shown to decrease the volume of the basolateral, lateral and central nuclei; the number of neurons in these nuclei and the number of glia cells. Interestingly after post-natal day 45 this reduction resolves itself[91]. A study done earlier by the same group and using the exact same stressors found that after postnatal day 80 an increase in volume of the lateral nucleus was found as well as an increase in neuronal density in that region. For the other amygdalar nuclei no difference was found compared with controls[92]. In the central nucleus a higher expression of CRH and a reduced CRHR2 expression was found in offspring of rats that underwent stress from gestational day 13-21[93]. These findings combined suggest that there is an early divergence of amygdala development following PS that could ultimately function quite differently in adulthood compared with controls.

In humans higher maternal cortisol has been linked to an increase in the volume of the right but not left amygdala. This effect was only seen when mothers had elevated cortisol levels during early gestation and not late gestation. Furthermore Buss and colleagues found more affective problems in girls that were exposed to elevated cortisol during early gestation, an increase in affective problems was also associated with an increase in volume of the amygdala[84]. Furthermore using MRI functional connectivity of the amygdala was found to be altered in early post-natal life due to prenatal maternal depressive symptoms[94]. Excessive activity of the amygdala has been linked to higher risk to develop PTSD and major depression[88].

3.3 Hypothalamus

The hypothalamus primary function is the link between the central nervous system and the endocrine system via the HPA-axis. The hypothalamus forms during the early fetal period[16]. The PVN is an especially interesting area of the hypothalamus as the neurons that secrete CRH reside here to control the HPA-axis.

In rats long-lasting PS leads to neurotoxicity of neurons in the PVN whereas short PS facilitates development of these very same neurons indicating that a little stress is healthy for PVN development[95]. In the PVN, PS induces a higher expression of CRH mRNA in females[93]. In both male and female rats it was reported that there was a reduction in CRH-binding protein (CRH-BP) mRNA in the PVN. This protein modulates CRH activity by inhibiting CRH-induced ACTH secretion[93]. Piglets that were exposed to PS exhibited a reduction of glucocorticoid binding sites. This could indicate a reduction in negative feedback at the PVN site[96]. Furthermore, the size

of the SDN-POA, a sexual dimorphic nuclei of the hypothalamus, is shown to be affected by PS in male rats. Normally the SDN-POA is almost twice the size in males compared to females. However when males are exposed to PS the size is almost 50% smaller which brings it closer to the size in females[97]. Combining these findings suggests that PS can have morphological effects on the hypothalamus and that these effects are modulated by sex[16].

Taken together these findings show that there are many different brain regions that are susceptible to alterations via prenatal stress. Furthermore in the different brain regions there are several sites in which alterations take place. The effects we see show a clear sex component and also the type and intensity of stressor could potentially play a role. The alterations in brain morphology and HPA-axis shed light on the developmental origins of psychopathology

4 Prenatal Stress and Psychopathology

It becomes apparent that the fetus can be programmed through maternal signals. Prenatal stress can through various mechanisms influence offsprings physiology and behavior. Changes in brain morphology and HPA axis responsiveness have been observed following PS. Indeed PS has been linked to a variety of psychopathologies in humans. Most notably anxiety, attention deficit/hyperactivity disorder, conduct disorders, cognitive defects, schizophrenia and autism spectrum disorder[15, 61, 84, 98]. Therefore it makes sense to look at psychopathologies from a proximate angle, what are the mechanisms involved and how does the trait develop over time? However, to fully understand these phenomena we must also look at the ultimate questions. In what way does the trait impact fitness and how has it developed over the history of the species? There are contending views on how this developmental plasticity came to be. One important realization then to make is that negative feelings and emotions, such as anxiety and fear, are useful and the systems governing them are delicate98. What we now call disorder or disease could once have been a functional adaptation. Heightened anxiety could lead to an increase in vigilance and alertness to danger. People with ADHD might have a greater sensitivity to potentially dangerous signals. Depression especially during winter as with seasonal affective disorder could help conserve energy. Increased aggression makes sense in a dangerous environment where the need to defend could arise at any moment. The capacity for these kinds of disorders exist only because there are situations in which they are useful[99]. The idea that fetal development is altered in such a way that it is prepared for the world in which it will find itself has been termed the predictive adaptive response (PAR)[21]. Maternal signals act as a forecast for the world that the offspring will find himself in. The majority of the focus on the PAR hypothesis has been on fetal growth and nutrition. The variation in availability of food being a basic parameter that systems in the body can respond to. In the same way variation in stress in the environment is another parameter that can be transferred to the developing fetus in the form of glucocorticoids where systems such as the HPA axis can respond to. The effects observed after prenatal stress may well have given an adaptive edge in more primitive conditions. The non-adaptive phenomena we observe now could be due to a mismatch in modern conditions or non-adaptive side effects. Others could be explained by being on an extreme of an adaptive spectrum. For example a fear of spiders could be beneficial however when it results in a debilitating phobia of spiders it can be maladaptive[15]. In the following sections I will outline specific psychopathologies associated with prenatal stress and discuss their advantages and disadvantages and briefly examine sex differences.

4.1 ADHD

ADHD has been linked with prenatal stress in a wide variety of studies[15, 56, 98, 100, 101]. With the prevalence of ADHD occurring in humans it stands to reason that the behaviors we see may not just be symptoms of a disorder, but are functional adaptations. ADHD is characterized by inattention, hyperactivity and impulsivity. Traits which are all potentially adaptive. Inattention could be effective in an environment containing a high amount of threats. Vigilance in such surroundings is paramount for survival. Over-focused

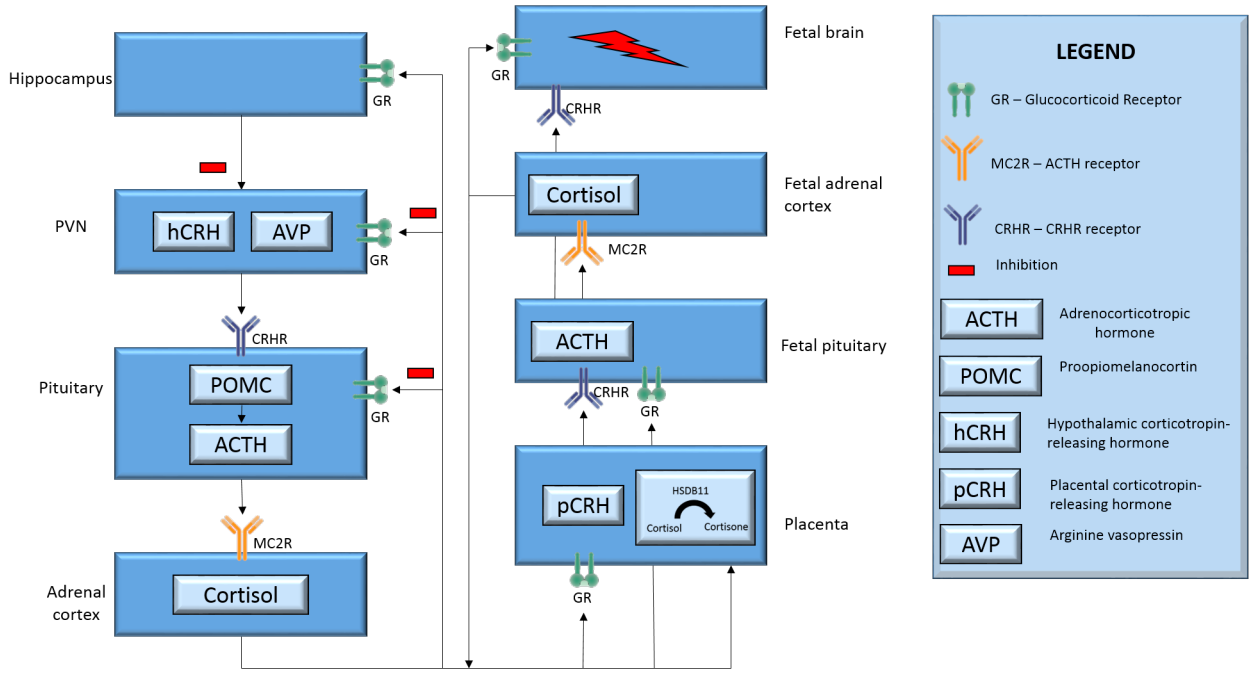

Figure 3.2: Fetal HPA-axis. **Neurons from the PVN secrete hCRH and AVP that then act on the pituitary to promote POMC which is the precursor of ACTH. ACTH then acts on the adrenal cortex to stimulate cortisol synthesis. Cortisol attenuates the HPA-response via negative feedback at the pituitary and PVN. In the placenta the enzyme HSDB11 facilitates the conversion of maternal cortisol to the inactive cortisone. This barrier is not perfect as cortisol is able to pass through the placenta in to the fetal bloodstream. Furthermore GR on the placenta stimulate pCRH production which then through the fetal HPA-axis further stimulates cortisol production. pCRH is increased by cortisol causing a positive endocrine feedback loop.**

attention could be potentially dangerous in a high threat environment whereas it could be beneficial in a low-threat setting.

Exploration helps in finding new food sources and identifying threats and in such a way is essential for any organism in order to adapt successfully to a given environment. From this perspective increased hyperactivity and motor activity is potentially adaptive. Impulsivity is a quick response to environmental cues without considering alternative options. If you dont quickly pounce on potential prey or dodging out of the way you may not get another chance. Therefore impulsivity is a potentially beneficial trait[102]. Furthermore it has been argued that for an individual clamoring for maternal attention can be adaptive. Among the Masai of eastern Africa during droughts more difficult or fussy children have been suggested to have a greater survival[103]. In groups having individuals who are impulsive and who do things that can be detrimental to the individual but could be beneficial for the group, such as eating unknown berries, might be poisonous but the knowledge learned could be beneficial[104].

A mother experiencing stress could be signaling to the fetus via glucocorticoids that the outside environment is one full of dangers and unpredictability and as such prepare them for it. One study using self-reported anxiety measures found that maternal anxiety during pregnancy lead to a decrease in attention regulation at 3 and 8 months[105]. Another study found that maternal anxiety during late gestation lead to an increase in ADHD symptoms in four-year old boys but not girls. When looking at postnatal effects on anxiety and ADHD symptoms no correlation was found suggesting that it is a fetal programming effect at work here[100]. Interestingly in contrast another study found that ADHD symptoms increase when the mother was experiencing anxiety 12-22 weeks post-menstrual age[106].

One possible mechanism responsible for PS causing ADHD are epigenetic changes to the SLC6A4

and NR3C1 promotor regions. Both these regions methylation status is implicated in ADHD symptoms[107, 108]. Furthermore the methylation status of these regions are affected by PS[32, 109]32,109. What the exact relation is between these factors and developing ADHD however remains largely unknown and many more gene regions factor in to the equation.

4.2 Anxiety

Prenatal stress has been associated with an increase in anxiety in the offspring in several studies[57, 100, 110]. Perceived threats lead to a general increase in anxiety that prepares the body for incoming danger. Anxiety is a normal emotion necessary for defending against a wide variety of threats and dangers. Threats such as predation or heights but also social danger. Anxiety is only beneficial when carefully calibrated. Too little anxiety leads to dangerous behaviors and too much disables us. The regulation of anxiety has been described as a benefit-cost tradeoff[111]. Anxiety disorders in a stressful and dangerous environment could be beneficial. Overreactivity to stressors might be an inconvenience or even debilitating in modern society, however in more primitive times failing to respond to a threat could very well mean death. Thus, having a fetal programming mechanism that heightens anxiety-like traits in the offspring could be adaptive in high threat environments. External threats arouse general but also specific responses. Heights induces freezing behavior, social threats submission, predators provoke flight and so on[111]. Thus, exposure to different types of threats could lead to different effects on outcome of fetal programming.

Exposure to elevated maternal cortisol levels was in one study associated with an increase in child anxiety at 6-9 years of age[57]. Furthermore, children with clinical levels of anxiety where twice as likely to have been exposed to elevated maternal cortisol levels[57]. Maternal psychological stress was only found to influence child anxiety when it was concerning pregnancy specific anxiety. This could be an indication of different types of anxiety/stress having different outcomes, or perhaps one of timing as the self-reported pregnancy specific anxiety decreased as pregnancy progressed[57]. Another study found that prenatal maternal anxiety 18-22 weeks post menstrual age increased anxiety in 8 and 9 years old, this result was found for anxiety and ADHD[106].

The HPA-axis is crucial in the regulation of the stress response and alterations in HPA-axis functioning have been associated with anxiety disorders[112]. A number of HPA regulatory genes (NR3C1 NR3C2, CRH, POMC and HSD11B2) are regulated by epigenetic mechanisms and are thus a possible target for fetal programming of anxiety[35]. As mentioned before PS has been shown to alter methylation status of the promotor regions for NR3C1, HSD11B2 and CRH[49, 50, 32, 34]. In such a manner PS can through epigenetic mechanisms alter HPA reactivity and cause anxiety disorders. When looking at brain morphology of the anxious brain, a hyperactivity of the amygdala is most commonly observed[113]. In one study general anxiety disorder (GAD) was associated with an increase in right amygdala volume and a total increase in amygdala volume[114]. In contrast a different study showed a decrease in left amygdala volume[115].

PTSD can be considered an anxiety disorder and was indeed officially so before the new DSM-V classification[116], this new classification is not without controversy however[117]. For this section I will consider PTSD as an anxiety related disorder. The proximal cause of PTSD is a distinct event or events in which there is a threat to an individuals life. The symptoms of PTSD are characterized by hypervigilance, avoiding similar situations (triggers) and reliving the event in flashbacks. Veterans returning from war often suffer from PTSD and this makes integration back in to civilian life difficult. From an evolutionary perspective the prevalence of PTSD after traumatic events makes sense, Few failures are as unforgiving as the failure to avoid a predator: being killed greatly decreases future fitness[118]. In the case of a threat to your life there is one chance to survive and one chance to learn from it. It is therefore adaptive to be wrong a lot of the time if you get it right the one time when survival is on the line. A reduction of hippocampal volume is observed in veterans suffering PTSD but also in their monozygotic twins that did not go to war and dont suffer from PTSD[86]. This implies a vulnerability to PTSD with a reduction in hippocampal size. One effect seen from PS is a reduction of hippocampal size[61, 63, 64, 83]. There is a significant link between mothers having PTSD

and their offspring subsequently being at risk for developing PTSD later in life[119] indicating that a fetal programming mechanism might be at play here. Further research in to specific stress during pregnancy and the risk of developing PTSD later in life is necessary to elucidate this relationship.

4.3 Conduct Disorder

Conduct disorder refers to behavioral and emotional problems in childhood and adolescents. Its is characterized by repetitive and persistent pattern of behavior in which basic rights of others are violated or violation of societal norms[116]. Prenatal stress shows a significant association with antisocial behavior or conduct problems in children, especially when exposed at late gestation. Interestingly conduct disorders are more prevalent in boys than girls but in relation to prenatal stress no significant differences between the sexes was found[100, 120, 121].

An increase in aggressive behavior may be adaptive in a stressful environment where resources are scarce or threats from outsiders are significant. Aggression helps defending from predators and hostile humans. The rule braking aspect of conduct disorder can be beneficial in discovery of new environments or strategies[15].

In the brain of boys with conduct disorder aging 12-17 years a reduction of left amygdala and left hippocampus was found[122]. As discussed previously PS is known to potentially lead to a reduction in these brain areas. Further mechanisms on the cause of conduct disorders can be found in HPA-axis functioning. Interestingly conduct disorders are associated with lower HPA activity[123]. This is in contrast with findings of PS leading to more reactivity of the HPA-axis. Furthermore PS has been shown as discussed previously to disrupt glucocorticoid receptor expression which is associated with aggression.

These findings taken together suggest that PS exposure may lead to conduct problems later in life. The increase in aggression and rule breaking behavior can potentially be explained by alterations in HPA-axis functioning and morphological changes in the amygdala and hippocampus

4.4 Diversity in Outcome

A large variety of outcomes as a result of prenatal stress is observed. In some cases, but not all, it leads to psychopathology, and the psychopathologies that occur can be very different in effect. What causes this breadth of plasticity as a result of PS? From an evolutionary perspective having genetic diversity in a population is generally seen as beneficial when faced with a changing environment. In the same manner having diversity in strategies in responses to PS can give an evolutionary edge. A basis for this could be in genetic factors underlying fetal programming responses. For example there exist a polymorphism of the SLC6A4 gene. This polymorphism come in two different variants, namely a short (s) and a long (l) variant and is associated with anxiety related traits[124]. Interactions in genotype and epigenetic regulation could help explain the diversity in outcome.

5 Conclusion

In this thesis I explored the relation between prenatal stress, neurodevelopment and subsequent developing of psychopathology. I looked at fetal programming from an evolutionary perspective and discussed some of the molecular mechanisms involved. Research on the effects of prenatal stress on offspring show a persistent effect on epigenetic regulation, HPA-axis activity, brain morphology and behavior in infancy as well as in adult life

DNA methylation is the most commonly researched epigenetic mechanism with regard to fetal programming via PS. Promoter regions of DNA that are rich in GcP islands that undergo methylation are generally silenced. Genes of interest among others that undergo hyper- or hypo- methylation following PS are SLC6A1/4, HSD11B2, NR3C1, CRH and POMC[35]. The HPA-axis which controls the stress response is, among others, regulated by HSD11B2, NR3C1, CRH and POMC[35]. HSD11B2 converts cortisol to the inactive cortisone in the placenta. This mechanism protects the fetus from excessive amount of cortisol. Research shows that placental HSD11B2 is lowered following PS. This could indicate an increased sensitivity to maternal cortisol of the offspring. NR3C1 codes for the glucocorticoid receptor and is expressed in a

great variety of regions and tissues. It was found that PS leads to an increase in methylation of the N3C1 promotor regions and a lowered expression of GR receptors has been found in the hippocampus where the GR modulates the HPA-axis via a negative feedback-loop (see figure 2.2). POMC and CRH both play a role in cortisol secretion. The CRH promotor region is found to be hypomethylated following PS, this is in accordance with the finding that CRH levels are increased in the amygdala and hypothalamus. Brain regions that are high in CRH receptors ,such as the limbic system, could be altered during development by excess CRH levels. No research was found on epigenetic alterations by PS on POMC, however its promotor region is rich in GcP islands and has been shown to be differentially expressed due to methylation. Furthermore synthetic glucocorticoid exposure during development has been shown to reduce POMC mRNA in the pituitary indicating that PS could have an effect. SLC6A1/4 code for proteins that transport respectively GABA and serotonin from the synaptic cleft to the presynaptic neuron. SLC6A1 is found to be downregulated in the prefrontal cortex following prenatal bystander stress. SLC6A4 is hypomethylated following prenatal stress.

In the brain of offspring exposed to PS we see morphological changes at several sites. In this thesis I limited myself to the hippocampal, amygdalar and hypothalamic regions. In the hippocampus a reduction of neurogenesis in the DG, degeneration of pyramidal and dentate granular neurons and a reduction in hippocampal volume is reported following PS. In the right half of the amygdala an increase is found in volume. Furthermore reductions have been found in several sites of the amygdala in rats. In the hypothalamus the SDN-POA nuclei shows reduction in size due in males due to PS. Increased CRH expression is observed in the PVN and a reduction of glucocorticoid binding sites is reported. Concluding that these areas are subject to alterations by PS and can subsequently alter behavior and lead to development of psychopathology in adulthood.

There is abundant evidence linking PS to development of psychopathology in adult life. Chances of anxiety, ADHD and conductive disorders increase after prenatal exposure. There may be evolutionary benefit to what we consider disorders in contemporary society. People with ADHD are quick to react to stimuli and are more prone to impulsivity, beneficial traits in high threat environments. Increased anxiety and susceptibility to PTSD can also be beneficial in such environments. Rabbits without an innate fear of foxes have an often fatal anxiety disorder98. The overreaction to fear stimuli can be beneficial considering that in nature a threat is often a life or death situation, and death greatly decreases fitness. Conduct disorders are accompanied by more aggression and anti-social behavior. Aggression and anti-social behaviors can be beneficial when resources are scarce or there is threat of hostile humans or predators. Not only do these apparent psychopathologies have evolutionary benefits, the mechanisms for PS to implement them are present in the brain. This can be considered evidence for the PAR hypothesis, maternal signals regarding the state of the environment influence the development in such a way that offspring is better prepared for it in adult life. There are however some criticisms regarding the validity of this hypothesis. This model assumes that the maternal signals always serve the fetuses best interest but overlooks that maternal manipulation of fetal development could be used to promote her own biological fitness at the expense of the offspring[18]. Indeed maternal stress can be extremely detrimental to the developing fetus often leading to miscarriage in the first trimester of pregnancy[125]. Glucocorticoid exposure and PS exposure in late gestation is associated with an increase in preterm birth[126]126 and quicker maturation of the brain and other organs[35]. Avoiding the final weeks of pregnancy when brain development is most rapid may help in preserving resources that are scarce. Preterm birth is not without risk for the offspring as it leads to a higher chance of morbidity, mortality, and altered developmental trajectories, this may be a risk worth taking from a maternal perspective in order to balance current and future offspring and thereby improving overall reproductive succes[127]. The psychopathologies that occur later in life could simply be a side effect of an altered developmental track.

Regardless of the evolutionary origin of the effects of PS and the subsequent development of psychopathology later in life it is essential to further understand the mechanisms involved. Being able to identify risk factors for developing psychopathologies may enable us to prevent or rem-

edy said pathologies. The molecular mechanisms identified could potentially prove to be targets for pharmaceuticals. However it might also be important to realize that not-neurotypical brains do not always necessarily have to be fixed and that neurodiversity may have intrinsic value in and of itself.

Further understanding of these phenomena is necessary. One interesting avenue of exploration could be research that implements genotyping together with epigenetic alterations in order to elucidate the high diversity of outcome. Furthermore, epigenetic programming has been shown to be able to persist over generations it would be interesting to see if psychopathology can be passed down via epigenetic mechanisms.

References

[1] D.J.P. Barker. The Developmental Origins of Adult Disease. *Journal of the American College of Nutrition*, 23(sup6):588S–595S, 2004.

[2] T. J. Roseboom, J. H.P. Van der Meulen, C. Osmond, D. J.P. Barker, A. C.J. Ravelli, and O. P. Bleker. Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. *American Journal of Clinical Nutrition*, 72(5):1101–1106, 2000.

[3] Gian-Paolo Ravelli, Zena A. Stein, and Mervyn W. Susser. Obesity in Young Men after Famine Exposure in Utero and Early Infancy. *New England Journal of Medicine*, 295(7):349–353, 1976.

[4] Tessa J. Roseboom, Jan H. P. van der Meulen, Anita C. J. Ravelli, Clive Osmond, David J. P. Barker, and Otto P. Bleker. Effects of prenatal expossure to the Ductch famine on adult disease in later life: an overview. *Molecular and Cellular Endocrinology*, 185(5):93–98, 2001.

[5] T. J. Roseboom, J. H P Van Der Meulen, C. Osmond, D. J P Barker, A. C J Ravelli, and O. P. Bleker. Adult survival after prenatal exposure to the Dutch famine 1944-45. *Paediatric and Perinatal Epidemiology*, 15(3):220–225, 2001.

[6] C Nicholas Hales and David J P Barker. The thrifty phenotype hypothesis: Type 2 diabetes. *British Medical Bulletin*, 60(1):5–20, 2001.

[7] Marta Weinstock. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain, Behavior, and Immunity*, 19(4):296–308, 2005.

[8] Marie Paule Austin, Leo R. Leader, and Nicole Reilly. Prenatal stress, the hypothalamic-pituitary-adrenal axis, and fetal and infant neurobehaviour. *Early Human Development*, 81(11):917–926, 2005.

[9] Bea R.H. Van Den Bergh, Eduard J.H. Mulder, Maarten Mennes, and Vivette Glover. Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: Links and possible mechanisms. A review. *Neuroscience and Biobehavioral Reviews*, 29(2):237–258, 2005.

[10] Amita Kapoor, Elizabeth Dunn, Alice Kostaki, Marcus H Andrews, and Stephen G Matthews. Fetal programming of hypothalamo pituitary adrenal function: prenatal stress and glucocorticoids. *The Journal of physiology*, 572(1):31–44, 2006.

[11] Mary L. Schneider, Colleen F. Moore, Gary W. Kraemer, Andrew D. Roberts, and Onofre T. DeJesus. The impact of prenatal stress, fetal alcohol exposure, or both on development: Perspectives from a primate model. *Psychoneuroendocrinology*, 27(1-2):285–298, 2002.

[12] Vivette Glover, T. G. O'Connor, and Kieran O'Donnell. Prenatal stress and the programming of the HPA axis. *Neuroscience and Biobehavioral Reviews*, 35(1):17–22, 2010.

[13] Oliver P. Love and Tony D. Williams. Plasticity in the adrenocortical response of a free-living vertebrate: The role of pre- and postnatal developmental stress. *Hormones and Behavior*, 54(4):496–505, 2008.

[14] Carmine M. Pariante and Stafford L. Lightman. The HPA axis in major depression: classical theories and new developments. *Trends in Neurosciences*, 31(9):464–468, 2008.

[15] Vivette Glover. Annual research review: Prenatal stress and the origins of psychopathology: An evolutionary perspective. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 52(4):356–367, 2011.

[16] Arnaud Charil, David P. Laplante, Cathy Vaillancourt, and Suzanne King. Prenatal stress and brain development. *Brain Research Reviews*, 65(1):56–79, 2010.

[17] Mary Jane West-Eberhard. *Developmental plasticity and evolution*. Oxford University Press, Inc, 2003.

[18] Marco Del Giudice. Fetal programming by maternal stress: Insights from a conflict perspective. *Psychoneuroendocrinology*, 37(10):1614–1629, 2012.

[19] Peter D. Gluckman, Mark A. Hanson, and Hamish G. Spencer. Predictive adaptive responses and human evolution. *Trends in Ecology and Evolution*, 20(10):527–533, 2005.

[20] Keith M. Godfrey, Karen A. Lillycrop, Graham C. Burdge, Peter D. Gluckman, and Mark A. Hanson. Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. *Pediatric Research*, 61(5 PART 2 SUPPL.):31–36, 2007.

[21] Patrick Bateson, Peter Gluckman, and Mark Hanson. The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. *Journal of Physiology*, 592(11):2357–2368, 2014.

[22] Waddington CH. The epigenotype. *Endeavour*, (1):18–20, 1942.

[23] J R Morris. Genes , Genetics , and Epigenetics : A Correspondence. 293(August):1103–1106, 2001.

[24] David Ratel, Jean Luc Ravanat, François Berger, and Didier Wion. N6-methyladenine: The other methylated base of DNA. *BioEssays*, 28(3):309–315, 2006.

[25] Benedikt Hallgrímsson and Brian K. Hall. *Epigenetics: Linking Genotype and Phenotype in Development and Evolution*. 2010.

[26] T. H. Bestor. The DNA methyltransferases of mammals. *Human Molecular Genetics*, 9(16):2395–2402, 2000.

[27] Catherine Jensen Peña, Catherine Monk, and Frances A. Champagne. Epigenetic effects of Prenatal stress on 11β-Hydroxysteroid Dehydrogenase-2 in the Placenta and fetal brain. *PLoS ONE*, 7(6):1–9, 2012.

[28] Richelle Mychasiuk, Nichole Schmold, Slava Ilnytskyy, Olga Kovalchuk, Bryan Kolb, and Robbin Gibb. Prenatal bystander stress alters brain, behavior, and the epigenome of developing rat offspring. *Developmental Neuroscience*, 33(2):159–169, 2011.

[29] Angela M. Devlin, Ursula Brain, Jehannine Austin, and Tim F. Oberlander. Prenatal exposure to maternal depressed mood and the MTHFR C677T variant affect SLC6A4 methylation in infants at birth. *PLoS ONE*, 5(8):2–9, 2010.

[30] S. Frazer, K. Otomo, and A. Dayer. Early-life serotonin dysregulation affects the migration and positioning of cortical interneuron subtypes. *Translational Psychiatry*, 5(9), 2015.

[31] Mark S Ansorge, Mingming Zhou, Alena Lira, René Hen, and Jay A Gingrich. Early-Life Blockade of the 5-HT Transporter AltersEmotional Behavior in Adult Mice. *Science*, 306(5697):879–881, 2004.

[32] Connie J. Mulligan, Nicole C. D'Errico, Jared Stees, and David A. Hughes. Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. *Epigenetics*, 7(8):853–857, 2012.

[33] K. M. Radtke, M. Ruf, H. M. Gunter, K. Dohrmann, M. Schauer, A. Meyer, and T. Elbert. Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Translational Psychiatry*, 1(7):e21–6, 2011.

[34] Tim F. Oberlander, Joanne Weinberg, Michael Papsdorf, Ruth Grunau, Shaila

Misri, and Angela M. Devlin. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*, 3(2):97–106, 2008.

[35] Vasilis G. Moisiadis and Stephen G. Matthews. Glucocorticoids and fetal programming part 2: Mechanisms. *Nature Reviews Endocrinology*, 10(7):403–411, 2014.

[36] Thin Vo and Daniel B. Hardy. Molecular mechanisms underlying the fetal programming of adult disease. *Journal of Cell Communication and Signaling*, 6(3):139–153, 2012.

[37] E. Ron De Kloet, Marian Joëls, and Florian Holsboer. Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*, 6(6):463–475, 2005.

[38] Yvonne M Ulrich-lai and James P Herman. Neural Regulation of Endocrine and Autonomic Stress Responses. *Nature reviews Neuroscience*, 10(6):397–409, 2009.

[39] S Levine. Infantile Experience and Resistance to Physiological Stress. *Science*, 126:405, 1957.

[40] Chris Murgatroyd and Dietmar Spengler. Epigenetic programming of the HPA axis: Early life decides. *Stress*, 14(6):581–589, 2011.

[41] Thorsten Braun, John R. Challis, John P. Newnham, and Deborah M. Sloboda. Early-life glucocorticoid exposure: The hypothalamic-pituitary-adrenal axis, placental function, and longterm disease risk. *Endocrine Reviews*, 34(6):885–916, 2013.

[42] A. S. Clarke, D. J. Wittwer, D. H. Abbott, and M. L. Schneider. Longterm effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. *Developmental Psychobiology*, 27(5):257–269, 1994.

[43] A. C. Huizink, D. M. Dick, E. Sihvola, L. Pulkkinen, R. J. Rose, and J. Kaprio. Chernobyl exposure as stressor during pregnancy and behaviour in adolescent offspring. *Acta Psychiatrica Scandinavica*, 116(6):438–446, 2007.

[44] Thomas G. O'Connor, Yoav Ben-Shlomo, Jon Heron, Jean Golding, Diana Adams, and Vivette Glover. Prenatal anxiety predicts individual differences in cortisol in preadolescent children. *Biological Psychiatry*, 58(3):211–217, 2005.

[45] Barbara M. Gutteling, Carolina De Weerth, and Jan K. Buitelaar. Maternal prenatal stress and 4-6 year old children's salivary cortisol concentrations pre- and postvaccination. *Stress*, 7(4):257–260, 2004.

[46] Patricia A. Brennan, Rebecca Pargas, Elaine F. Walker, Paula Green, D. Jeffrey Newport, and Zachary Stowe. Maternal depression and infant cortisol: Influences of timing, comorbidity and treatment. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 49(10):1099–1107, 2008.

[47] Kerry Ann Grant, Catherine McMahon, Marie Paule Austin, Nicole Reilly, Leo Leader, and Sinan Ali. Maternal prenatal anxiety, postnatal caregiving and infants' cortisol responses to the still-face procedure. *Developmental Psychobiology*, 51(8):625–637, 2009.

[48] Sonja Entringer, Robert Kumsta, Dirk H. Hellhammer, Pathik D. Wadhwa, and Stefan Wüst. Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. *Hormones and Behavior*, 55(2):292–298, 2009.

[49] B. R. Mueller and T. L. Bale. Sex-Specific Programming of Offspring Emotionality after Stress Early in Pregnancy. *Journal of Neuroscience*, 28(36):9055–9065, 2008.

[50] Rasoul Alikhani-koopaei, Fatemeh Fouladkou, Felix J Frey, Brigitte M Frey, and Fatemeh Alikhani-koopaei, Rasoul Fouladkou. Epigenetic regulation of 11β-hydroxysteroid dehydrogenase type 2 expression. *October*, 114(8):1146–1157, 2004.

[51] J Newell-Price. Proopiomelanocortin gene expression and DNA methylation: implications for Cushing ' s syndrome and beyond. *Journal of Endocrinology*, 177:365–372, 2003.

[52] Maria G. Leavitt, Graham W. Aberdeen, Marcia G. Burch, Eugene D. Albrecht, and Gerald J. Pepe. Inhibition of fetal adrenal adrenocorticotropin receptor messenger ribonucleic acid expression by betamethasone administration to the baboon fetus in late gestation. *Endocrinology*, 138(7):2705–2712, 1997.

[53] Christopher L. Coe, Marian Kramer, Boldizsár Czéh, Elizabeth Gould, Alison J. Reeves, Clemens Kirschbaum, and Eberhard Fuchs. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile Rhesus monkeys. *Biological Psychiatry*, 54(10):1025–1034, 2003.

[54] Florian Rakers, Vilmar Frauendorf, Sven Rupprecht, Rene Schiffner, Sabine J. Bischoff, Michael Kiehntopf, Petra Reinhold, Otto W. Witte, Harald Schubert, and Matthias Schwab. Effects of early-and late-gestational maternal stress and synthetic glucocorticoid on development of the fetal hypothalamus-pituitary-adrenal axis in sheep. *Stress*, 16(1):122–129, 2013.

[55] Vasilis G. Moisiadis and Stephen G. Matthews. Glucocorticoids and fetal programming part 1: Outcomes. *Nature Reviews Endocrinology*, 10(7):391–402, 2014.

[56] Kristin Bergman, Pampa Sarkar, Thomas G. O'Connor, Neena Modi, and Vivette Glover. Maternal stress during pregnancy predicts cognitive ability and fearfulness in infancy. *Journal of the American Academy of Child and Adolescent Psychiatry*, 46(11):1454–1463, 2007.

[57] Elysia Poggi Davis and Curt A. Sandman. Prenatal psychobiological predictors of anxiety risk in preadolescent children. *Psychoneuroendocrinology*, 37(8):1224–1233, 2012.

[58] Natalie Grizenko, Yasaman Rajabieh Shayan, Anna Polotskaia, Marina Ter-Stepanian, and Ridha Joober. Relation of maternal stress during pregnancy to symptom severity and response to treatment in children with ADHD. *Rev Psychiatr Neurosci J Psychiatry Neurosci J Psychiatry Neurosci*, 333333(111):10–6, 2008.

[59] Jiong Li, Jørn Olsen, Mogens Vestergaard, and Carsten Obel. Attention-deficit/hyperactivity disorder in the offspring following prenatal maternal bereavement: A nationwide follow-up study in Denmark. *European Child and Adolescent Psychiatry*, 19(10):747–753, 2010.

[60] et al. Khashan, A.S., Abel, K.M., McNamee, R. Higher risk of offspring schizophrenia following antenatal exposure to serious adverse life events. *Arch. Gen. Psychiatry*, 65(2):146–152, 2008.

[61] Dennis K Kinney. Prenatal Stress and Risk for Schizophrenia. *International Journal of Mental Health*, 29(4):62–72, 2001.

[62] D. Q. Beversdorf, S. E. Manning, A. Hillier, S. L. Anderson, R. E. Nordgren, S. E. Walters, H. N. Nagaraja, W. C. Cooley, S. E. Gaelic, and M. L. Bauman. Timing of prenatal stressors and autism. *Journal of Autism and Developmental Disorders*, 35(4):471–478, 2005.

[63] Abigail L. Fowden, Juan Li, and Alison J. Forhead. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proceedings of the Nutrition Society*, 57(01):113–122, 1998.

[64] V. Gitau, R., Cameron, A., Fisk, N. M., & Glover. Fetal exposure to maternal cortisol. *The Lancet*, 352(9129):707–708, 1998.

[65] S Mesiano. The endocrinology of human pregnancy and fetal-placental neuroendocrine development. In *The endocrinology of human pregnancy and fetal-placental neuroendocrine development. In: Strauss JF, Barbieri R, editors. Yen and Jaffe's reproductive endocrinology: Physiology, pathophysiology, and clinical management*, chapter hashasfjh, page 952. 2017.

[66] Tallie Z. Baram and Carolyn G. Hatalski. Neuropeptide-mediated excitability: A key triggering mechanism for seizure generation in the devloping brain. *Trends in Neurosciences*, 21(11):471–476, 1998.

[67] Elizabeth C. Cottrell. Prenatal stress, glucocorticoids and the programming of adult disease. *Frontiers in Behavioral Neuroscience*, 3(15):113–128, 2009.

[68] Hideo Uno, Steve Eisele, Akiko Sakai, Steve Shelton, Eva Baker, Onofre DeJesus, and James Holden. Neurotoxicity of glucocorticoids in the primate brain. *Hormones and Behavior*, 28(4):336–348, 1994.

[69] L Seress, H Abrahám, T Tornóczky, and G Kosztolányi. Cell formation in the human hippocampal formation from mid-gestation to the late postnatal period. *Neuroscience*, 105(4):831–843, 2001.

[70] H J Romijn, M J Hofman, and A Gransbergen. At what age is the developing cortex of the rat comparable to that of the full term newborn baby? *Early Hum.Dev.*, 26:61–67, 1991.

[71] Chunmei Zhao, Wei Deng, and Fred H. Gage. Mechanisms and Functional Implications of Adult Neurogenesis. *Cell*, 132(4):645–660, 2008.

[72] P S Eriksson, E Perfilieva, T Bjork-Eriksson, A M Alborn, C Nordborg, D A Peterson, and F H Gage. Neurogenesis in the adult human hippocampus. *Nat Med*, 4(11):1313–1317, 1998.

[73] C Golgi. Sulla Fina Anatomia Degli Organi Centrali Del Sistema Nervoso. *Riv. Sper. Fremiat. Med. Leg. Alienazione Ment.*, 11:72–123, 1885.

[74] Hideo Uno, Lon Lohmiller, Carol Thieme, Joseph W. Kemnitz, Michael J. Engle, Ellen B. Roecker, and Philip M. Farrell. Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. I. Hippocampus. *Developmental Brain Research*, 53(2):157–167, 1990.

[75] T. Szuran, E. Zimmermann, and H. Welzl. Water maze performance and hippocampal weight of prenatally stressed rats. *Behavioural Brain Research*, 65(2):153–155, 1994.

[76] C. Schmitz, M. E. Rhodes, M. Bludau, S. Kaplan, P. Ong, I. Ueffing, J. Vehoff, H. Korr, and C. A. Frye. Depression: Reduced number of granule cells in the hippocampus of female, but not male, rats due to prenatal restraint stress. *Molecular Psychiatry*, 7(7):810–813, 2002.

[77] V. Lemaire, M. Koehl, M. Le Moal, and D. N. Abrous. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences*, 97(20):11032–11037, 2000.

[78] Valerie Lemaire, Stephanie Lamarque, Michel Le Moal, Pier Vincenzo Piazza, and Djoher Nora Abrous. Postnatal Stimulation of the Pups Counteracts Prenatal Stress-Induced Deficits in Hippocampal Neurogenesis. *Biological Psychiatry*, 59(9):786–792, 2006.

[79] Anna Rita Zuena, Jerome Mairesse, Paola Casolini, Carlo Cinque, Giovanni Sebastiano Alemà, Sara Morley-Fletcher, Valentina Chiodi, Luigi Giusto Spagnoli, Roberto Gradini, Assia Catalani, Ferdinando Nicoletti, and Stefania Maccari. Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. *PLoS ONE*, 3(5), 2008.

[80] A. Fujioka, T. Fujioka, Y. Ishida, T. Maekawa, and S. Nakamura. Differential effects of prenatal stress on the morphological maturation of hippocampal neurons. *Neuroscience*, 141(2):907–915, 2006.

[81] V.G. Barros, M. Duhalde-Vega, L. Caltana, A. Brusco, and M.C. Antonelli. Astrocyte-neuron vulnerability to prenatal stress in the adult rat brain. *Journal of Neuroscience Research*, 83(5):–, 2006.

[82] Marta Weinstock, Erlena Matlina, Gilmor I. Maor, Haim Rosen, and Bruce S. McEwen. Prenatal stress selectively alters the reactivity of the hypothalamic-pituitary adrenal system in the female rat. *Brain Research*, 595(2):195–200, 1992.

[83] R. Dufoix, A. Charil, D.P. Laplante, T. Paus, J. Pruessner, and S. King. Prenatal maternal stress from a natural disaster predicts hippocampus volumes in males at age 11: Project Ice Storm. *International Journal of Developmental Neuroscience*, 47(May 2016):12, 2015.

[84] Claudia Buss, Elysia Poggi, Babak Shahbaba, Jens C Pruessner, Kevin Head, and Curt A Sandman. Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. 109(20), 2012.

[85] Lisa M. Shin, Scott L. Rauch, and Roger K. Pitman. Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Annals of the New York Academy of Sciences*, 1071:67–79, 2006.

[86] Mark W. Gilbertson, Martha E. Shenton, Aleksandra Ciszewski, Kiyoto Kasai, Natasha B. Lasko, Scott P. Orr, and Roger K. Pitman. Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nature Neuroscience*, 5(11):1242–1247, 2002.

[87] Tryphena Humphrey. The development of the human amygdala during early embryonic life,. *The Journal of Comparative Neurology*, 132(1):135–165, 1968.

[88] Martin H. Teicher, Susan L. Andersen, Ann Polcari, Carl M. Anderson, Carryl P. Navalta, and Dennis M. Kim. The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience and Biobehavioral Reviews*, 27(1-2):33–44, 2003.

[89] Shaul Feldman and J. Weidenfeld. The excitatory effects of the amygdala on hypothalamo-pituitary- adrenocortical responses are mediated by hypothalamic norepinephrine, serotonin, and CRF-41. *Brain Research Bulletin*, 45(4):389–393, 1998.

[90] Michael R Trimble and Ludger Tebartz Van Elst. The amygdala and psychopathology studies in epilepsy. *Annals of the New York Academy of Sciences*, 985:461–468, 2003.

[91] Michał Kraszpulski, Patricia A Dickerson, and A K Salm. Prenatal stress affects the developmental trajectory of the rat amygdala. 3890, 2009.

[92] A. K. Salm, Michelle Pavelko, E. Marshall Krouse, Wendy Webster, Michał Kraszpulski, and Dale L. Birkle. Lateral amygdaloid nucleus expansion in adult rats is associated with exposure to prenatal stress. *Developmental Brain Research*, 148(2):159–167, 2004.

[93] I. Zohar and M. Weinstock. Differential Effect of Prenatal Stress on the Expression of Cortiocotrophin-Releasing Hormone and its Receptors in the Hypothalamus and Amygdala in Male and Female Rats. *Journal of Neuroendocrinology*, 23(4):320–328, 2011.

[94] A. Qiu, T. T. Anh, Y. Li, H. Chen, A. Rifkin-Graboi, B. F.P. Broekman, K. Kwek, S. M. Saw, Y. S. Chong, P. D. Gluckman, M. V. Fortier, and M. J. Meaney. Prenatal maternal depression alters amygdala functional connectivity in 6-month-old infants. *Translational Psychiatry*, 5(2), 2015.

[95] T. Fujioka, Y. Sakata, K. Yamaguchi, T. Shibasaki, H. Kato, and S. Nakamura. The effects of prenatal stress on the development of hypothalamic paraventricular neurons in fetal rats. *Neuroscience*, 92(3):1079–1088, 1999.

[96] E. Kanitz, W. Otten, M. Tuchscherer, and G. Manteuffel. Effects of prenatal stress on corticosteroid receptors and monoamine concentrations in limbic areas of suckling piglets (Sus scrofa) at different ages. *Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medicine*, 50(3):132–139, 2003.

[97] David K. Anderson, Reuben W. Rhees, and Donovan E. Fleming. Effects of prenatal stress on differentiation of the sexually dimorphic nucleus of the preoptic area (SDN-POA) of the rat brain. *Brain Research*, 332(1):113–118, 1985.

[98] Anja C Huizink, Eduard J H Mulder, and Jan K Buitelaar. Prenatal Stress and Risk for Psychopathology: Specific Effects or Induction of General Susceptibility? *Psychological Bulletin*, 130(1):115–142, 2004.

[99] Randolph M. Nesse and Phoebe C. Ellsworth. Evolution, Emotions, and Emotional Disorders. *American Psychologist*, 64(2):129–139, 2009.

[100] Thomas G. O'Connor, Jonathan Heron, Jean Golding, and Vivette Glover. Maternal antenatal anxiety and behavioural/emotional problems in children: A test of a programming hypothesis. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 44(7):1025–1036, 2003.

[101] Karen Markussen Linnet, Søren Dalsgaard, Carsten Obel, Kirsten Wisborg, Tine Brink Henriksen, Alina Rodriguez, Arto Kotimaa, Irma Moilanen, Per Hove Thomsen, Jørn Olsen, and Marjo-Riitta Jarvelin. Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. *The American journal of psychiatry*, 160(6):1028–1040, 2003.

[102] PETER S. JENSEN, DAVID MRAZEK, PENELOPE K. KNAPP, LAURENCE STEINBERG, CYNTHIA PFEFFER, JOHN SCHOWALTER, and THEODORE SHAPIRO. Evolution and Revolution in Child Psychiatry: ADHD as a Disorder of Adaptation. *Journal of the American Academy of Child & Adolescent Psychiatry*, 36(12):1672–1681, 1997.

[103] M W De Vries. Temperament and infant mortality among the Masaii of East Africa. *American Journal of Psychiatry*, 141(October):1189–1194, 1984.

[104] Jonathan Williams and Eric Taylor. The evolution of hyperactivity, impulsivity and cognitive diversity. *Journal of The Royal Society Interface*, 3(8):399–413, 2006.

[105] Anja C. Huizink, Pascale G. Robles De Medina, Eduard J.H. Mulder, Gerard H.A. Visser, and Jan K. Buitelaar. Psychological Measures of Prenatal Stress as Predictors of Infant Temperament. *Journal of the American Academy of Child and Adolescent Psychiatry*, 41(9):1078–1085, 2002.

[106] Ben R.H. Van Den Bergh and Alfons Marcoen. High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8- and 9-year-olds. *Child Development*, 75(4):1085–1097, 2004.

[107] S. Park, J. M. Lee, J. W. Kim, D. Y. Cho, H. J. Yun, D. H. Han, J. H. Cheong, and B. N. Kim. Associations between serotonin transporter gene (SLC6A4) methylation and clinical characteristics and cortical thickness in children with ADHD. *Psychological Medicine*, 45(14):3009–3017, 2015.

[108] Nina H. van Mil, Régine P.M. Steegers-Theunissen, Marieke I. Bouwland-Both, Michael M.P.J. Verbiest, Jolien Rijlaarsdam, Albert Hofman, Eric A.P. Steegers, Bastiaan T. Heijmans, Vincent W.V. Jaddoe, Frank C. Verhulst, Lisette Stolk, Paul H.C. Eilers, André G. Uitterlinden, and Henning Tiemeier. DNA methylation profiles at birth and child ADHD symptoms. *Journal of Psychiatric Research*, 49(1):51–59, 2014.

[109] T. F. Oberlander, R. J. Bonaguro, S. Misri, M. Papsdorf, C. J.D. Ross, and E. M. Simpson. Infant serotonin transporter (SLC6A4) promoter genotype is associated with adverse neonatal outcomes after prenatal exposure to serotonin reuptake inhibitor medications. *Molecular Psychiatry*, 13(1):65–73, 2008.

[110] Wei Zhang, Khushmand Rajendran, Jacob Ham, Jackie Finik, Jessica Buthmann, Kei Davey, Patricia M. Pehme, Kathryn Dana, Alexandra Pritchett, Holly Laws, and Yoko Nomura. Prenatal exposure to disaster-related traumatic stress and developmental

trajectories of temperament in early childhood: Superstorm Sandy pregnancy study. *Journal of Affective Disorders*, 234(April 2017):335–345, 2018.

[111] Isaac f.M. Marks and Randolph M. Nesse. Fear and fitness: An evolutionary analysis of anxiety disorders. *Ethology and Sociobiology*, 15(5-6):247–261, 1994.

[112] Andrew A Bartlett, Rumani Singh, and Richard G Hunter. Neuroepigenomics in Aging and Disease. 978:145–166, 2017.

[113] Yann Quidé, Anke B. Witteveen, Wissam El-Hage, Dick J. Veltman, and Miranda Olff. Differences between effects of psychological versus pharmacological treatments on functional and morphological brain alterations in anxiety disorders and major depressive disorder: A systematic review. *Neuroscience and Biobehavioral Reviews*, 36(1):626–644, 2012.

[114] Michael D. De Bellis, B. J. Casey, Ronald E. Dahl, Boris Birmaher, Douglas E. Williamson, Kathleen M. Thomas, David A. Axelson, Karin Frustaci, Amy M. Boring, Julie Hall, and Neal D. Ryan. A pilot study of amygdala volumes in pediatric generalized anxiety disorder. *Biological Psychiatry*, 48(1):51–57, 2000.

[115] Michael P. Milham, Allison C. Nugent, Wayne C. Drevets, Daniel S. Dickstein, Ellen Leibenluft, Monique Ernst, Dennis Charney, and Daniel S. Pine. Selective reduction in amygdala volume in pediatric anxiety disorders: A voxel-based morphometry investigation. *Biological Psychiatry*, 57(9):961–966, 2005.

[116] American Psychiatric Association. *Handboek voor de classificatie van psychische stoornissen (DSM-5)*. 2014.

[117] Lori A. Zoellner, Barbara O. Rothbaum, and Norah C. Feeny. PTSD not an anxiety disorder? DSM committee proposal turns back the hands of time. *Depression and Anxiety*, 28(10):853–856, 2011.

[118] Steven L. Lima and Lawrence M. Dill. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68(4):619–640, 1990.

[119] Rachel Yehuda, Amanda Bell, Linda M. Bierer, and James Schmeidler. Maternal, not paternal, PTSD is related to increased risk for PTSD in offspring of Holocaust survivors. *Journal of Psychiatric Research*, 42(13):1104–1111, 2008.

[120] F. Rice, G. T. Harold, J. Boivin, M. Van Den Bree, D. F. Hay, and A. Thapar. The links between prenatal stress and offspring development and psychopathology: Disentangling environmental and inherited influences. *Psychological Medicine*, 40(2):335–342, 2010.

[121] Edward D. Barker and Barbara Maughan. Differentiating early-onset persistent versus childhood-limited conduct problem youth. *American Journal of Psychiatry*, 166(8):900–908, 2009.

[122] Thomas Huebner, Timo D. Vloet, Ivo Marx, Kerstin Konrad, Gereon R. Fink, Sabine C. Herpertz, and Beate Herpertz-Dahlmann. Morphometric brain abnormalities in boys with conduct disorder. *Journal of the American Academy of Child and Adolescent Psychiatry*, 47(5):540–547, 2008.

[123] Stephanie H.M. Van Goozen, Walter Matthys, Peggy T. Cohen-Kettenis, Jan K. Buitelaar, and Herman Van Engeland. Hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in disruptive children and matched controls. *Journal of the American Academy of Child and Adolescent Psychiatry*, 39(11):1438–1445, 2000.

[124] Klaus-Peter Lesch, D Bengel, Armin Heils, S Z Sabol, B D Greenberg, S Petri, J Benjamin, C R Müller, D H Hamer, and D L Murphy. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science (New York, N.Y.)*, 274(5292):1527–31, 1996.

[125] N. Maconochie, P. Doyle, S. Prior, and R. Simmons. Risk factors for first trimester

miscarriage - Results from a UK-population-based case-control study. *BJOG: An International Journal of Obstetrics and Gynaecology*, 114(2):170–186, 2007.

[126] Rachel L. Copper, Robert L. Goldenberg, Anita Das, Nancy Elder, Melissa Swain, Gwendolyn Norman, Risa Ramsey, Peggy Cotroneo, Beth A. Collins, Francee Johnson, Phyllis Jones, and A M Meier. The preterm prediction study: maternal stress is associated with spontaneous preterm birth at less than thirty-five weeks' gestation. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *American journal of obstetrics and gynecology*, 175(5):1286–92, 1996.

[127] Ivy L. Pike. Maternal stress and fetal responses: Evolutionary perspectives on preterm delivery. *American Journal of Human Biology*, 17(1):55–65, 2005.