The sensitive sex
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Major depression is a complex mental disorder caused by interactions between genetic and environmental risk factors. Understanding the underlying mechanisms of these interactions will help to treat or prevent this disease more effectively in the future. Depressive problems increase during adolescence to a greater extent in girls than in boys, a gender difference that remains throughout adulthood. In this dissertation six studies are presented that explore several risk factors involved in the development of depressive symptoms in a large sample of adolescent boys and girls. The first section of this chapter describes the risk factors and biological dysfunctions associated with depressive symptoms. The second and third section of this chapter describes how candidate genes and biologically plausible (endo)phenotypes of depression can be studied. At the end of this chapter, the outline of the dissertation is presented.

**DEPRESSION**

**Prevalence**
Depression is an important global public health issue because it is one of the four leading causes of disease burden throughout the world (Murray et al., 1996; Ustun et al., 2001; Wong and Lincinio, 2001). It has relatively high lifetime prevalence and is associated with substantial disability (e.g. Demyttenaere et al., 2004; Hyman et al., 2006). Depression affects individuals’ emotions, thoughts, sense of self, behaviour, interpersonal relations, physical functioning, biological processes, work productivity and overall life satisfaction (e.g. Ormel et al., 2008). Lifetime prevalence (age 18-65) of major depression in the Netherlands was found to be 20% in men and 30% in women (Kruijshaar et al., 2005). Women are at higher risk to develop a depressive episode than men (Kessler et al., 1993; Nolen-Hoeksema, 2001). The incidence of depression increases dramatically from 1% in preadolescence to 17 to 25% by the end of adolescence, with a greater prevalence in girls than in boys (e.g. Verhulst et al, 1997; Hankin et al., 1998; Angold et al., 2002). Because this gender difference emerges around age 14 (Hankin et al., 1998; Wade et al., 2002), it is likely that several gender-specific hormonal and social developmental factors associated with adolescence play a role (Larson and Ham, 1993; Ge et al., 2001; Angold and Costello, 2006).

**Description of symptoms**
According to the DSM-IV, Major Depressive Disorder (MDD) is characterized by symptoms such as mood disturbances, irritability, low self esteem, feelings of hopelessness, guilt and worthlessness, sleep disorders, fatigue, low energy, concentration problems, weight loss or gain, recurring thoughts of death and decreased interest in pleasure stimuli (sex, food and social interactions). These
symptoms must be consistent for a period of at least two weeks and should represent a change from the person's normal mood. In addition, social, occupational, educational or other important functioning must be negatively impaired by the change in mood (DSM IV, 2000). But even without meeting the criteria for MDD, depressive symptoms can cause serious impairments and are a risk factor for depressive episodes later in life (e.g. Lewinsohn, 1999; Pine et al., 1999).

**Neurobiology**
The aforementioned symptoms involve altered neurotransmission in the prefrontal and cingulated cortex, the hippocampus, the amygdala and the hypothalamus (Nestler et al., 2002). Evidence for this comes from brain imaging studies, in which differences in the activation of these areas were found between depressed patients and healthy controls (e.g. Drevets, 2000). These brain areas operate in connected circuits involving glutamatic, GABAergic, dopaminergic, norepineprine and serotoninergic neurotransmission. The cortex and hippocampus are involved in the cognitive deficits in depression such as learning and memory impairment, feelings of guilt, hopelessness, worthlessness and suicidal thoughts. The nucleus accumbens and amygdala are involved in the regulation of emotional responses and memory of aversive events. Altered neurotransmission in these areas is associated with decreased drive and reward for pleasure activities, anxiety and reduced motivation. The hypothalamus is involved in sleep problems, loss of appetite, energy and interest in sex. Although the exact mechanism needs far more clarification in humans (Andersen and Teicher, 2008), female sex hormones are likely to influence the neurobiology of depression and may, in part, explain the higher prevalence of depression in women.

**Environmental risk factor: psychosocial stress**
Epidemiological studies showed substantial genetic and environmental influences in the risk to develop depression (Sanders et al., 1999; Fava and Kendler, 2000). Experiences of stressful life events (SLEs) or chronic psychosocial stress are the most important environmental risk factors for depression (e.g. Brown & Harris, 1989; Kendler et al., 1993; Ge et al., 1994; Kessler et al., 1997; Ormel et al., 2001; Tennant, 2002). The term stress was first used by Hans Selye (1936) to refer to physical demands of the body in response to a stressor. Stressors can be defined as: ‘environmental events or chronic conditions that objectively threaten the physical and/or psychological health or well-being of individuals in a particular age in a particular society’ (Grant et al., 2003). The psycho-physiological stress response is necessary to cope with the physical demands of a stressful situation (McEwen, 2000). Stress is only problematic when there is a discrepancy between the demands of the situation and the ability to cope with it. Additionally, a
psychosocial situation is only stressful when it is appraised as such (Lazarus, 1966), and cognitive processes are central in determining whether a situation is potentially threatening, constitutes harm or loss, or is benign. Intense, persistent or uncontrollable stress can lead to maladaptive responses (Selye, 1936), which are associated with a number of somatic and mental health risks (e.g. Heim et al., 2000). Not all people become depressed in the face of stress, because large individual differences exist in responses to stress and subsequent development of depressive symptoms. The depressogenic effect of psychosocial stress depends on the frequency, duration, severity and multiplicity of the stressor, gender, personality factors, available social support and genetic predisposition (e.g. Brown and Harris, 1978; Kendler et al., 1993; Kendler et al., 1995; Ormel et al., 2001; Kendler et al., 2003).

Genetic risk factors
Based on twin studies, the heritability of depression was found to be about 40 to 50 percent (e.g. Kendler et al., 1993; Sullivan et al., 2000; Kendler et al., 2006). Adoption studies also provide some support for the role of genetic factors in depression (e.g. Wender et al., 1986). Most of the published genetic association studies of mood disorders have focused on functional polymorphisms (DNA sequence variations that alter the expression and/or functioning of the gene product) in genes. Because genetic factors seem to modify the association between life events and depression (e.g. Kendler et al., 1995; Silberg et al., 1999), plausible candidate genes for depression are involved in the functioning of the physiological response to stress and neurotransmission systems (Nestler et al., 2002; Levinson, 2006). However, the gene-hunt for depression has not yield clear results for one or more genes as indicated by two large meta-analysis (Lopéz-León et al., 2008; Bosker et al., 2010). And this makes sense since genes associated with depression are not specific ‘depression’ genes but are likely related to psychosocial stress responses in general. Additionally, studies on polymorphisms (genetic variants) in candidate genes are not conclusive that a certain allele is always associated with a ‘bad’ outcome (this dissertation; Doornbos et al., 2009; Grabe et al., 2009 versus Caspi et al., 2003).

Familial risk of depression
Depression runs in families (e.g. Goodman and Gotlib, 1999; Hammen et al., 2004; Verhagen et al., 2008), and having a parent with a history of depression is a strong predictor for depressive problems in offspring (Weissman et al., 1997; Pilowsky et al., 2006; Schreier et al., 2006). The relative risk (RR) (ratio of risks to first-degree relatives of probands with depression versus the general population) is 2 to 3 (e.g. Maier et al., 1992). The familial risk for depression is most likely a consequence of interactions between transmitted vulnerability genes and family factors. Lack of
family closeness, poor communication, absence of supportive relationships, parental rejection and lack of emotional warmth have all been found to be prevalent in families with depressed parents (e.g. Davies and Windle, 1997; Beardslee et al., 1998; Meares et al., 2000; Jaser et al., 2005). Poor family life might increase the impact of stressful events even further because low social support might increase the depressogenic effect of stress (Cohen and Will, 1985). The genetic transmission of risk for depression may run partly through altered functioning of the physiological stress system (e.g. Frederenko et al., 2004; Wüst et al., 2004). Additionally, negative affectivity, a temperament dimension encompassing frustration and fearfulness, reflects the tendency to experience negative emotions when confronted with environmental challenges (Rothbart et al., 2000). Ormel et al. (2005) showed that the effect of parental psychopathology on offspring psychopathology was partly mediated by offspring temperament, suggesting that a depression-vulnerable temperament may be genetically transmitted from parent to child.

**PHYSIOLOGICAL RESPONSE TO STRESS**

Stress evokes a rapid response of the sympathetic nervous system (SNS) and a slower, longer response of the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky et al., 1986). The activation of the sympathetic system results in the release of catecholamines (adrenaline and norepinephrine), which increases heart rate and blood pressure and accelerates respiration. Activation of the HPA axis results in the release of cortisol from the adrenal cortex. Cortisol converts fat into glucose, inhibits the immune system and is implicated in memory processes. The stress response is regulated by a negative feedback system in which cortisol binds to mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in the brain. There is a difference between the activation of the stress response system towards physical and psychosocial stress (Herman et al., 2005). Physical stress, indicated by pain signals or cytokines, is recognized from somatic and visceral pathways. Psychosocial stress activates anticipatory processes, which are under the control of the hippocampus, amygdala and prefrontal cortex. The research described in this dissertation focuses only on psychosocial stressful stimuli such as stressful life events or chronic stressful situations. The physiological response to stress can be influenced by many factors: type, intensity and duration of a psychosocial stressor, functional outcome of genes in underlying biological pathways, gender, early life experiences, smoking, presence of depressive symptoms, physical exercise, body mass and certain types of medication use.
Gender differences in response to psychosocial stress have been found in adults (reviewed in Kudielka and Kirschbaum, 2005; Wang et al., 2007; Kelly et al., 2008), but not in pre-adolescent children (Buske-Kirschbaum et al., 1997; Tout et al., 1998). Adult men displayed higher cortisol responses to psychosocial stress paradigms than women, while women displayed higher heart rate increases than men. Whether adolescent boys and girls respond differently to psychosocial stress has not been examined in a large population-based sample. Studies in both animals and humans indicate that estrogen and progesterone can influence the response to psychosocial stress (Altemus et al., 1997; Kirschbaum et al., 1999; Rohleder et al., 2003; McCormick and Mathews, 2007). Although the mechanism is not entirely clear, it seems that sex hormones interact with receptors in the hypothalamus and the amygdala, influencing the emotional and physiological response to psychosocial stress (Chrousos et al., 1998; Levine, 2002; Andersen and Teicher, 2008).

THE DEPRESSOGENIC EFFECT OF STRESS

In depressed patients, abnormal functioning of the HPA axis has often been reported, and could, in part, be normalised by antidepressant medication that targets serotonergic neurotransmission (e.g. Holsboer, 2001). Prolonged HPA axis activation in response to severe or chronic stress is associated with both hyper- and hypocortisol secretion in response to subsequent stressors (Miller et al., 2007; Nestler et al., 2002; McEwen and Wingfield, 2003). It has been hypothesised that increased (hyper) cortisol responses are due to more recent stress experiences, while decreased (hypo) responses are a consequence of childhood stressors (Miller et al., 2007). Hypocortisolemia might be a consequence of protective down-regulation of the HPA axis in response to stress early in life (Fries et al., 2005; Tarullo and Gunnar, 2006). Support for this has been found with regard to severe as well as mild stress experience in childhood (e.g. Elzinga et al., 2008; Gunnar and Donzella, 2009).

Increased sensitivity in adolescence

Adolescence is the period between childhood and adulthood (age 10 - 22) in which developmental pathways are set in motion or become established (e.g. Ferdinand et al., 1995). This period is characterised by major biological, psychological and social challenges and opportunities (e.g. Greenfield et al., 2003; Nelson et al., 2005; Paus et al., 2008). Brain parts that play a role in affective and cognitive regulation undergo functional and anatomical reorganization during adolescence through increased myelination and pruning of existing synaptic networks (Giedd et
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This occurs all over the brain but is concentrated in the prefrontal cortex (PFC) (Gogtay et al., 2004). The PFC regulates the response of the hypothalamus and amygdala to psychosocial stress (Lewis and Todd, 2007). Perlman and colleagues (2007) showed that the adolescent brain is more sensitive to stress than in adulthood as illustrated by increased expression of glucocorticoid receptors in the PFC of adolescents compared to adults and children. This supports the idea that during adolescence, the learning and memory of psychosocial experiences carries greater importance (Rudolph and Hammen, 1999; Steinberg and Morris, 2001). Increased sensitivity and responsiveness to negative interpersonal interactions can result in the development of depressive symptoms which increase with the onset of adolescence (e.g. Petersen et al., 1993; Hankin et al., 2006). For example, rejection by romantic partners or peers is a strong predictor for inducing depressive symptoms (Furman et al., 2003). Additionally, a social network is important as a buffer against stressors (Cohen and Wills, 1985), and since parents become less important during adolescence, adolescents have to actively form new networks with peers (Steinberg and Morris, 2001).

Gender difference

The effect of psychosocial stress appears to be particularly strong in adolescent girls (Pine et al., 1999), as demonstrated by the female preponderance of depression that emerges in adolescence (Agold et al., 1998; Cyranowski et al., 2000; Hankin and Abrahanson, 2001; Kessler et al., 2003). Female sex hormones increase due to maturation of the reproductive system (Sisk and Foster, 2004), and these hormones influence brain functions (McEwen, 2001). The evaluation of stress in the brain seems to be different during adolescence in boys and girls (e.g. McClure et al., 2004). Additionally, females tend to rely more on social support than males (Rudolph and Hammen, 1999; Cyranowski et al., 2000; Taylor et al., 2000; Stroud et al., 2009). Girls ruminate more on emotional problems than boys, who seek distraction instead (Kovacs et al., 2003). Additionally, genetic risk factors for major depression were found to be higher in women than in men (Kendler et al., 2006). The increased sensitivity in adolescent girls might be due to stronger gene-environment interactions in girls than in boys. A longitudinal study of female twins found that genetic factors increased the risk for depression in reaction to SLEs, but only after and not before puberty (Silberg et al., 1999). This suggests an interaction between genetic factors and factors associated with the onset of adolescence, such as increasing levels of female sex hormones. Changes in circulating female sex hormones during adolescence are often implicated as exerting direct or potentiating effects on the central nervous system that relate to disturbances in mood (Susman et al., 1987). After stressful life events, high levels of estrogen have been found to mediate depressive symptoms (Brooks-Gunn and Warren, 1989). This is supported by animal studies, estrogen and progesterone can affect the
function of corticoid receptors in the brain of female rats (e.g. Weisser and Handa, 2009), while no effects of androgens were found in male rats (Handa et al., 1994). However, although the mechanism is not clear, it is suggested that testosterone has antidepressant potency in human males (Pope et al., 2000).

In conclusion
Depression is a complex mental disorder, caused by interactions between vulnerability genes and environmental stressors. Psychosocial stress, such as chronic stress or stressful life events, is the most important environmental risk factor for a depressive period. Heritability of depression is moderate and seems to involve multiple genes underlying the function of several biological systems. Serotonergic neurotransmission, learning and memory processes and emotional and physiological responses to stress are often altered in people with depressive symptoms. Presence of parental depressive problems can be considered a proxy for the presence of transmitted vulnerability genes in a stressful or non-supportive family environment. The increase in depressive symptoms in adolescence is associated with increased social experiences with peers and maturation of the brain and reproductive system. Increasing levels of sex hormones influence the adolescent brain and responses to psychosocial stress. The risk to develop depressive symptoms after psychosocial stress is higher for women and emerges in adolescence, which may, in part, be explained by the effects of female sex hormones. The studies presented in this dissertation examine main effects and interaction effects of environmental stressors and genetic variation in genes involved in functioning of the stress system and neurotransmission in the brain on adolescents’ development of depressive symptoms. The next section explains how genetic effects in depression can be studied.

GENETIC VARIANCE AND ASSOCIATION STUDIES

Genetic variance
Genetic variance is a consequence of sexual reproduction, a process in which genes from the father and mother are mixed to create new combinations. Genetic variance is essential for evolution because it enables the possibility to adapt to new environments. General information concerning DNA, genes and expression of genes is given in Box 1. Genetic variation exists on several levels. This dissertation deals only with the following genetic variants: Variable Number of Tandem Repeats (VNTR) and Single Nucleotide Polymorphisms (SNP). VNTRs consist of repeats of several nucleotides that can vary between individuals and between alleles of a gene. A SNP consists of one nucleotide substitution and is the most abundant form of DNA variation in the human genome. There are about 10 million common SNPs
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(International HapMap Consortium, 2005). Both SNPs and VNTRs can be found everywhere in the DNA, and when they influence gene expression they are referred to as functional polymorphisms. In the field of genetic epidemiology there are several approaches to study the role of genetic variance in the aetiology of diseases (Caspi and Moffit, 2006). One of these approaches incorporates information from the environment and assumes that both environment and genes influence the susceptibility for a disorder. This approach fits well for development of depression, in which multiple genes with several allelic variants seem to interact with one another and with environmental risk factors.

**Association studies of endophenotypes and candidate genes**

Gene-gene (GxG) and gene-environment (GxE) interactions can best be studied by association analyses. Association studies test whether a particular allele in a candidate gene and a trait co-occur above change level, given the frequency of the allele and the distribution of the trait in the population (e.g. McCaffery et al., 2007). A valid association must have two prerequisites: the candidate gene product must be involved in a plausible biological pathway, and genetic variance must result in differences in development and/or function of the trait of interest (Hattersly and McCarthy, 2005). Endophenotypes refer to phenotypes that are present somewhere between a candidate gene and the disease (Gottesman and Shields, 1973) and are useful in identifying traits of complex diseases such as depression. Significant associations between genetic variants and endophenotypes are easier to detect because they explain a larger part of the variance in the endophenotype than in the disease phenotype. This is due to the fact that the endophenotype is only influenced by a subset of the underlying genes for the disease. Endophenotypes can be identified by psychological, cognitive, neuroanatomical and biochemical measures. Hastler and colleagues (2004) proposed several endophenotypes in the search for depression-related genes.

**Considerations in statistical testing of associations**

GxG and GxE can be modelled by incorporating all statistical interaction terms between two genetic predictors, or between one genetic and one environmental predictor, into a regression analysis (Cordell, 2002). Effect sizes of individual genes in complex diseases are most likely very small. It is expected that a genetic variant explains about 2% of the variance in a complex disease (Nolte et al., 2009). To detect a small effect size with a power of a minimum of 80%, the sample must be of considerable size. For example, to detect a medium effect (f = 0.25, Cohen, 1998) of a gene (allele frequency = 0.4) at α = 0.05, a sample size of 258 is needed. To detect a gene-environment interaction in which the environmental exposure is 30%, a sample size of 1,200 is needed (calculations from Nolte et al., 2009). For example, to detect a medium effect (f = 0.25, Cohen, 1998) of a gene
(allele frequency = 0.4) at $\alpha = 0.05$, a sample size of 258 is needed. To detect a gene-environment interaction in which the environmental exposure is 30%, a sample size of 1,200 is needed (calculations from Nolte et al., 2009).

**Box 1. Basics on DNA, genes and gene-expression**

**DNA and genes**
Deoxyribonucleic acid (DNA) contains the genetic instructions for all living organisms. DNA consists of four types of nucleotides (Adenine, Tyrosine, Cytosine and Guanine) and is organised in two long molecules that together form the characteristic double helix structure. DNA is organised within chromosomes in the nucleus of each cell. Genes are the basic unit of heredity and are translated into gene products (mostly proteins), which are involved in the development and function of organisms.

**Gene expression**
A gene has coding sequences (exons) that determine what the gene does, and non-coding sequences (introns) that are involved in regulation of gene expression. During transcription (see figure) both exons and introns are transcribed into a RNA (ribonucleic acid) molecule. This messenger RNA is transported out of the nucleus and into ribosomes where the RNA is translated. Each codon, consisting of three nucleotides, is translated into amino acid, which are the building block of proteins. This process is simplified illustrated in Figure 1.

**Figure 1. From gene to protein**

**Genotype and phenotype**
While a gene is the basic instruction for a protein, an allele is a variant of that instruction. All individuals receive one allele from their father and one from their mother. Alleles can differ slightly due to processes such as recombination and mutation. These small differences can result in different gene-products (e.g. receptors), which influence the function and survival of the individual. When both alleles of a gene are exactly the same, the genotype of this gene is referred to as homozygous. When a gene has two different alleles, it is referred to as heterozygous. While genotype refers to individuals’ genetic hardware, their phenotype describes behaviour and physical appearance and is the outcome of the genotype in a particular environment. ‘Environment’ must be understood in the broadest sense: developmental factors in the womb (e.g. smoking or malnutrition of the mother) and experience of psychosocial stress can all influence the cellular environment with consequences for gene expression.
Plausible candidate genes for depression are those involved in the functioning of the physiological response to stress and neurotransmission systems (Nestler et al., 2002; Levinson, 2006). This dissertation examines the influence of SNPs in the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) genes, the 5-hydroxytryptamine-linked-polymorphic region (5-HTTLPR) in the serotonin transporter gene and a SNP in the brain-derived neurotrophic factor (BDNF) gene. Physiological responses to stress are considered an endophenotype of depression and will be indicated by cortisol and heart rate responses to a standardised social stress test. Additionally, depressive symptoms after experience of psychosocial stress are considered as a phenotype of depression and the influence of GxE and GxG interactions between stressful life events and allelic variance in the BDNF and serotonin transporter gene will be examined. The (endo)phenotypes and the candidate genes that will be examined in this dissertation are presented in Figure 2.

**Figure 2.** (Endo)phenotypes between candidate genes and depression (adapted from Hastler et al., 2004).
Brain-derived neurotrophic factor

The brain-derived neurotrophic factor (BDNF), which is encoded by the BDNF gene, is involved in the growth, differentiation and survival of neurones and synapses (Huang and Reichardt, 2001). It is most active in brain areas involved in learning and memory, such as the hippocampus and the cortex. Depression has been associated with impaired learning and memory (Videbech and Ravnkilde, 2004), and decreased serum levels of BDNF were found in depressed patients (Karege et al., 2002). In rats, exposure to stress and glucocorticoids in the brain decreased the expression of the BDNF gene (Smith et al., 1995), while antidepressants and exercise increased BDNF expression (Russo-Neustadt et al., 2000). A functional (G/A) SNP in the BDNF gene results in the conversion of the amino acid Valine into Methionine (val66met) (Egan et al., 2003). The met allele results in lower expression of the BDNF protein than the val allele (Bath et al., 2006) and is associated with reduced hippocampal volume in healthy people (Szesko et al., 2005; Bueller et al., 2006) and in patients with major depression (Frodl et al., 2007). The met allele is also associated with impaired memory in healthy people (Hariri et al., 2003; Pezewas et al., 2004).

Glucocorticoid receptor and mineralocorticoid receptor

Stress causes an increase in the level of circulating corticosteroids (cortisol and corticosterone) by activation of the HPA axis (Box 2). In the brain, corticosteroids bind to the high affinity mineralocorticoid receptor (MR) and the lower affinity glucocorticoid receptor (GR), which suppresses the transcription of CRF and ACTH (Reul and de Kloet, 1985). The balance between occupation of GR and MR receptors influences the response of serotonergic neurons in the hippocampus (Porter et al., 2004), and imbalanced occupation has been associated with deregulation of the stress response (de Kloet et al., 1998). Allelic variation in the genes coding for the GR and MR receptor determines the availability and efficiency of the receptors (Russcher et al., 2007), which influences functioning of the HPA axis (Wüst et al., 2004). SNPs in both the GR and MR genes have been associated with cortisol and heart rate responses to stress (e.g. Wüst et al., 2004; DeRijk et al., 2006) and with depression (e.g. van Rossem et al., 2006; van West et al., 2006; Hage et al., 2009). The associations with cortisol were found to be gender-specific (Kumsta et al., 2007; Ising et al., 2008). The influence of two SNPs in the GR (Bcl and 9beta) and in the MR gene (-2G/C and I180V) will be examined in this dissertation. Detailed information on these SNPs will be given in Chapter 6.
Serotonin transporter
Communication between the amygdala, hippocampus and the HPA axis involves serotonergic neurotransmission (Box 3). Abnormalities in mood, such as those seen in depression, are associated with hypofunctioning of the serotonergic system. Decreased neurotransmission can be a consequence of depletion of serotonin, increased serotonin transporter function or decreased 5HT1A receptor function (Arango et al., 2001). Increasing extracellular serotonin levels can improve mood, as shown by antidepressant treatment (e.g. Schildkraut 1956). The serotonin transporter (SLC6A4) is involved in the termination of serotonergic neurotransmission. Selective serotonin re-uptake inhibitors (SSRIs) are antidepressants that block the serotonin transporter and increase signal transmission between neurons.

A VNTR in the promoter region of the serotonin transporter gene (SLC6A4), consists of a 43bp insertion or deletion resulting in a short (s, lesser expression) or a long (l, greater expression) version of the gene (Heils et al., 1996). A single nucleotide substitution (A>G, rs25531) located immediately upstream of the 5-HTTLPR (5-hydroxytryptamine-linked-polymorphic region) creates an lg allele that is functionally equivalent to the s allele because of reduced transcription via the
creation of a suppressor-binding site (Kraft et al. 2005; Wendland et al., 2006; Zalsman et al., 2006). Caspi and colleagues showed that the short allele was associated with a higher incidence of depression after psychosocial stress (Caspi et al., 2003), although a recent meta-analysis could not confirm this initial finding (Risch et al., 2009). Figure 5 shows a graphical representation of the 5-HTTLPR in the serotonin transporter gene.

Box 3. Serotonergic neurotransmission

Serotonin (5-HT) is involved in the regulation of sleep, wakefulness, feeding behaviour, mood and sexual activities. Serotonin is unable to pass the blood-brain barrier and is synthesized from L-tryptophan in the brain. After synthesis, serotonin is stored in vesicles in the axon terminal. Release of serotonin is controlled by the 1A (5HT1A) auto-receptor present in the pre-synaptic axon terminal. After release, serotonin can bind to several post-synaptic receptors (1A,1B,1D,1F, 2A, 2C, 3). Extracellular serotonin is taken back from the synapse into the presynaptic neuron by the serotonin transporter, which terminates the signal transmission. Serotonin is re-used or degraded by the enzyme monoamine oxidase A (MAOA).

Figure 4. Serotonergic neurotransmission
Serotonin (5-HT) is involved in the regulation of sleep, wakefulness, feeding behaviour, mood and sexual activities. Serotonin is unable to pass the blood-brain barrier and is synthesized from L-tryptophan in the brain. After synthesis, serotonin is stored in vesicles in the axon terminal. Release of serotonin is controlled by the 1A (5HT1A) auto-receptor present in the pre-synaptic axon terminal. After release, serotonin can bind to several post-synaptic receptors (1A,1B,1D,1F, 2A, 2C, 3). Extracellular serotonin is taken back from the synapse into the presynaptic neuron by the serotonin transporter, which terminates the signal transmission. Serotonin is re-used or degraded by the enzyme monoamine oxidase A (MAOA).
In this dissertation several environmental and genetic risk factors for development of depressive symptoms in adolescence are examined. More specifically: the influence of parental depressive symptoms (PDS), stressful life events (SLE), polymorphic candidate genes and gender are studied in a large adolescent sample (n = 2127). In a smaller sample (n = 715), the effects of PDS, polymorphic candidate genes and gender on the cortisol and heart rate response to a standardised social stress test (Groningen Social Stress Test - GSST) are studied.

Chapter 2 describes the selection procedure and characteristics of the study samples and data collection procedures. Chapter 3 concerns adolescents’ cortisol responses to the GSST and the effects of gender, oral contraceptive use and menstrual cycle phase. Chapter 4 describes a study in which the influence of gender and parental depressive symptoms (PDS) on the relationship between SLEs and adolescent depressive symptoms was examined. In Chapter 5 a follow-up study is presented in which the effect of PDS on adolescents’ cortisol responses to the GSST is explored. In Chapter 6, the association between SNPs in the GR and MR gene and cortisol and heart rate response to the GSST is examined. Chapter 7 concerns a study in which a gene-environment interaction effect of 5-HTTLPR and adolescent stress on depressive symptoms was examined. In this study we accounted for differences in childhood stress experience, because early

Figure 5. The 5-HTTLPR site in the serotonin transporter gene. Long and short refer to the presence or absence of the 43bp insertion/deletion polymorphism in the transcriptional control region TCR (light grey). This figure was generated based upon information presented by Ohara et al., (1998) and Kraft et al., (2005).
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experiences may modify the depressogenic effect of stress later in life. In Chapter 8, a gene-gene-environment interaction effect is examined between the BDNF val/met, the 5-HTTLPR and childhood stress on adolescent depressive symptoms. Finally, in Chapter 9, the main findings of the dissertation studies are presented and discussed and directions for future research are given. A schematic representation of the associations that are examined in the six research chapters is presented in Figure 6.

**Figure 6.** Schematic representation of the associations examined in Chapter 3 to 8.