Chapter 9

General discussion

Genetic variance enables us to respond to our changing environment.
Aim of the dissertation
The aim of this dissertation was to examine genetic and environmental risk factors for the development of depressive symptoms in adolescent boys and girls. The research described in this dissertation was part of the TRacking Adolescents’ Individual Lives Survey (TRAILS), a large prospective study of adolescents from the general population. The effect of several factors on adolescents’ depressive symptoms were studied within the total TRAILS sample (n = 2127): the influence of stressful life events (SLEs), gender, parental depressive symptoms (PDS), the val/met polymorphism in the BNDF gene, the polymorphic site in the promoter region of the serotonin transporter gene, and early life stress. Other factors were studied in a focus sample (n = 715) selected from the TRAILS population: effects of PDS, gender, menstrual cycle phase, oral contraceptive use and SNPs in the mineralocorticoid and glucocorticoid receptor genes on physiological responses to a standardised psychosocial stress test (Groningen Social Stress Test – GSST).

Methodological strengths
The studies presented in this dissertation have several methodological strengths. First, data comes from a large population sample of adolescents. The prospective design of the TRAILS study enabled us to correct for pre-existing depressive symptoms and assess the influence of stressful experiences occurring between measurement waves. Second, we used information on adolescent depressive symptoms coming from multiple sources (parent- and self-report), which decreased the risk of inflated associations due to shared method variance. Third, we studied gene-environment interactions by associating biologically plausible genes with (endo)phenotypes of depression instead of gene hits from whole genome scans, which do not always underlie biologically (known) pathways. Fourth, the use of a standardised social stress test (GSST) allowed us to study physiological responses to stress in a large group of adolescents under a controlled environmental condition. Finally, contrary to most studies that examine gene-environment interactions in mixed samples, we studied interactions separately for boys and girls. We showed that it is important to consider gender-specific effects, because interactions between polymorphic genes and stressful environments were found to be different between the genders.

In the first section of this chapter the main findings are interpreted and discussed while considering the possible methodological limitations of our studies. As is often the case in science, more questions were raised than answered. In the second part of this chapter theoretical suggestions are given that might be useful in finding the answer to these, and other, questions regarding the aetiology of depressive behaviour.
DISCUSSION OF THE MAIN FINDINGS

Adolescent girls are more sensitive to the depressogenic effect of stressful life events than adolescent boys

Consistent with our expectations and the current literature (e.g. Hankin et al., 1998), girls reported more depressive symptoms than boys in early adolescence (Chapter 3). This gender difference was not present at age 11. In both boys and girls we found a significant relationship between experienced stressful life events and depressive symptoms. It should be noted that the gender difference in depressive symptoms in early adolescence (age 13) was due to a decrease of depressive problems in boys, rather than an increase among girls, compared to preadolescent levels (age 11). The lack of increase in depressive problems among girls in our sample might suggest that boys became less sensitive to stressful events (SLEs) while girls’ sensitivity to events did not change. However, the latter is not likely, since Oldehinkel et al., (2008) showed in the same sample that the association between parental divorce and depressive problems increased with age in girls. Among girls, the relationship between SLEs and depressive symptoms was stronger than in boys. This suggests that girls become more sensitive to the depressogenic effects of SLEs than boys during the transition from childhood to adolescence, as suggested by previous studies (Silberg et al., 1999; Cyranowski et al., 2000). This might be due to the influence of increasing levels of female sex hormones on emotional and physiological responses to psychosocial stress (e.g. Angold et al., 1998).

A consideration with regard to the measurement of adolescent symptoms and stressful life events must be made here. Adolescents’ depressive symptoms were based on the Child Behavior Check List (CBCL) and Youth Self Report (YSR) Depressive Problems scale, which was not developed to assess depressive problems according to DSM-IV criteria. Instead, it was constructed on the basis of expert ratings of the original, empirically derived CBCL and YSR scale items. Consequently, the items do not represent one-to-one counterparts with all DSM-IV criteria. Moreover, the amount and severity of stressful life events might be exaggerated, since individuals with depressive problems have a tendency to over-report the number as well as the severity of stressful life events (Brewin et al., 1993). Additionally, a possible limitation concerns the severity score of the stressful life events, which was based on self-report rather than interview ratings. The latter take into account contextual information and can give a more objective measure of the severity of the event (Brown and Harris, 1978).
The physiological stress response: adolescents versus adults

Similar gender difference

Gender differences in the response to stress have been reported in adults (reviewed by Kudielka and Kirschbaum, 2005) but not in children (Buske-Kirschbaum et al., 1997). We examined the psycho-physiological response to a standardised social stress test (GSST) in a large group of adolescents. We measured the response of the HPA axis by increases in cortisol collected from saliva (Chapters 3, 5 and 6), and the response of the autonomic nervous system (ANS) by increases in heart rate (Chapter 6). Our study found the same gender difference in adolescence as has been reported in adults: higher cortisol responses in boys and higher heart rate responses in girls. Different appraisal of the social stress tests in boys and girls is not likely, since no differences in subjectively experienced stress were present (Chapter 3).

Possible differences in cortisol levels

The cortisol levels found in our adolescent sample were lower than those found in adults. This might raise questions regarding the stressfulness of the GSST. There are differences between the protocols of the Trier Social Stress test, used in most studies of adults, and our Groningen Social Stress Test. The main difference concerns the amount of social evaluation: a panel of three judges (TSST) versus one experimenter and a video camera (GSST). But even if this difference led to a lower stress appraisal compared to the TSST, we see no reason to label this difference as a limitation of our research. Individual differences in stress responses to mild stressors may be just as important to investigate as the responses to more extreme stressors. Our GSST protocol induced significant cortisol responses, as well as meaningful between-group differences. Evaluative threat and uncontrollability, the combination that activates the HPA axis most strongly (Dickerson & Kemeny, 2002), are both present in the GSST. Another possible limitation associated with the lower cortisol responses is that the GSST was the participants’ final task during an experimental session, and we cannot be certain that previously completed tasks did not influence our findings. However, we do not expect systematic bias in the associations, because the GSST was by far the most stressful element of the laboratory session (Oldehinkel, unpublished data).

The lower cortisol levels in adolescents could also be explained by age differences in sensitivity to psychosocial stress. Increased expression of glucocorticoid receptors in the adolescent brain, as shown by Perlman and colleagues (2007), can result in a faster feedback of the HPA, which could have resulted in lower levels of cortisol after the GSST. It seems very plausible that maturational changes in the brain, and the intense social interactions that need to be learned during
adolescence, make adolescents respond differently to psychosocial stress than children and adults.

**The stress-sensitivity of adolescent girls is influenced by parental depressive symptoms**

In Chapters 4 and 5, parental history of depressive symptoms was used as a marker of genetic load for depression in offspring. The parental depressive symptom (PDS) score was based on the absence or presence of self-reported lifetime symptoms. The three-way interaction between gender-by-PDS-by-SLE was not significant. This indicates that the moderating effect of PDS was not different between boys and girls. In both boys and girls, the depressogenic effect of SLEs increased due to presence of PDS. In Chapter 5, we examined cortisol responses to the GSST in adolescent boys and free-cycling girls. PDS was associated with cortisol responses in daughters but not in sons, which suggests a moderating effect of PDS on stress reactivity in daughters.

Although PDS was considered as a marker of genetic load in our studies we cannot ignore the possible environmental consequences of parental depressive behaviour such as increased family stress, adverse parenting and low levels of social support (e.g. Cohen and Wills, 1985; Beardslee et al., 1996; Pilowsky et al., 2006). Adolescent offspring of depressed parents had more depressive symptoms after SLEs than adolescents without depressed parents (Chapter 4). This can be explained by higher levels of family stress associated with the depressive problems of the parents. However, as we showed in Chapter 4, the moderating effect of PDS was not explained by family environmental factors. In Chapter 5, the effect of PDS on daughters’ cortisol responses was also not due to girls’ experienced life stress.

A possible explanation for why experienced life stress did not mediate the effect of PDS on daughters’ responses is related to the generality of the lifetime stress measure that was used. This measure might be too heterogeneous to have a unidimensional effect on the cortisol response. As shown by Lupien et al. (2009), childhood stressors can lead to both over- and under-secretion of cortisol later in life, depending on the nature of the stressors. Childhood experience of both severe (Elzinga et al., 2008) and mild stress (Gunnar et al., 2009) were found to be associated with blunted cortisol responses to social stress paradigms later in life. In adults, hypocortisolemia was associated with experience of stress in childhood, whereas hypercortisolemia was associated with more recent experience of stress (Miller et al., 2007). Hypocortisolemia can be caused by down-regulation of the HPA axis to protect the organism from high levels of cortisol in persistently stressful environments (Fries et al., 2005). In future studies we will examine the cortisol reactivity profiles of girls at familial risk for depression and take into account
experienced stress in different childhood periods. Because we showed in Chapter 7 that the depressogenic effect of stress was influenced by genotype of the 5-HTTLPR, we will take this, as well as the genotypes of the other candidate genes, into account.

Research from our own group showed that in the same sample, parental psychopathology was genetically transmitted to offspring (Ormel et al., 2005). The increased sensitivity to SLEs might be, in part, a consequence of a genetic component (Chapter 3). Stress and depression-related vulnerability genes are likely to be equally transmitted to sons and daughters. Despite this, we only found an effect of PDS on daughters’ cortisol response, and not on sons’ (Chapter 5). This might relate to the study of Silberg et al., (1999) who showed that genetic factors become more pronounced in adolescence in girls than in boys. Gotlib and colleagues (2008) showed that 5-HTTLPR genotype influenced the cortisol response in adolescent girls at familial risk for depression. Girls with the ll and sl allele displayed no cortisol response to the stress tests, while girls with the ss genotype displayed the characteristic peak response. In Gotlib’s study the frequencies of the short and long allele were not different between girls at low and high familial risk. Preliminary analyses in our own sample showed also no differences in allele frequencies between adolescents at high and low familial risk for depression. Nor did we find such differences for the tri-allelic variance in the 5-HTTLPR (la, lg, s) or for SNPs in the BDNF, MR and GR gene (Bouma, unpublished data). Depression is a mental disorder involving the interplay of allelic variants of multiple genes that can influence one another (epistasis). Future studies should try to take these effects into account but in order to this properly we need more advanced statistical modelling methods.

Three methodological limitations must be considered while interpreting the results regarding parental depression (Chapters 4 and 5). First, the measure of parental depressive symptoms did not directly reflect DSM-IV criteria for depression. Second, results from Chapters 4 and 5 are not totally comparable, since PDS were assessed differently at T1 and T3. During the parent interview at T1, the mother most often reported about her own depressive symptoms and of those of the biological father. At T3, psychopathology of both biological parents was assessed by means of self-report questionnaires. Not surprisingly, the correlations between mothers’ depressive symptoms reported at T1 and T3 were higher (kappa = .57) than for fathers (kappa = .43). Third, parents’ retrospective ratings of the stressfulness of their child’s life might not be the most reliable measure, since we do not know if parents rate the same events equally as stressful as the children do. In addition, ratings of stressfulness could be confounded by the parents’ current depressed mood.
Given the gender differences in adolescent responses, questions might arise as to whether effects of maternal and paternal depression are different for adolescent boys and girls. We explored this possibility but found no gender differences in the association between maternal and paternal depressive symptoms and cortisol responses to the GSST (Bouma, unpublished data).

**Influence of sex hormones on the physiological stress response**

In this dissertation we showed that, just as in adults, HPA (Chapters 3, 5 and 6) and cardiovascular responses (Chapter 6) to a standardised social stress test (GSST) differed according to gender, suggesting a regulating role for sex hormones (estrogen, progesterone and testosterone) on the stress response system. Chapter 6 revealed that associations between genetic variants in the MR and GR genes and indices of the stress response system differed according to gender and OC use.

**Influence of female and male sex hormones: findings from animal studies**

Animal studies can provide insights into the underlying mechanisms between sex hormones and the stress response. Most studies have focused on the HPA axis and not on the cardiovascular system. Although studies on adolescent female rats are scarcer than studies on adolescent male rats, findings indicate that sex differences in HPA activity do emerge during adolescence and are associated with sex hormones. Female sex hormones can directly affect corticoid receptors in the hypothalamus and amygdala (e.g. Turner, 1997; Chrousos et al., 1998; Levine, 2002) and indirectly regulate neurotransmitter systems involved in the control of HPA function (e.g. Bigeon and McEwen, 1982). For example, high estrogen levels increased the expression of the serotonin transporter (McQueen et al., 1997). Progesterone can diminish HPA axis feedback by binding to corticoid receptors (Svec, 1991) and increasing the rate of cortisol dissociation from these receptors (Rousseau et al., 1972). Estrogen have also complex regulatory effects on cortisol receptors, resulting in up and down regulation of stress responsiveness, depending on type of receptor and region of the brain (e.g. Pfeiffer et al., 1991; Burgess and Handa, 1992; Handa et al., 1994). Moreover, estrogen stimulates the production of Cortisol Binding Globuline (GBC) (Moore et al., 1978), while testosterone seems to diminish the stress response (Viau, 2002). How these mechanisms are translatable to humans is not entirely clear.

**Influence of fluctuating hormone levels during the menstrual cycle**

Although we found no significant effect of follicular or luteal phase during the menstrual cycle on the cortisol response (Chapter 4), the direction was similar to that found in adult women. That is, higher cortisol responses are measured in the luteal phase than in the follicular phase (Altemus et al., 1997; Kirschbaum et al.,
1999; Wolf et al., 2001; Rohleder et al., 2003). The lower levels of cortisol in the follicular phase may be due to increased production of CBG in the follicular phase (Moore et al., 1978), when estrogen levels are high (Fox, 1999). A possible reason for the lack of significant effect of cycle phase on cortisol responses to stress is that the adolescent menstrual cycle is not as stable as the cycle of adult women. Lower levels, or a different ratio, of sex hormones in adolescent girls compared to adult women could influence the HPA axis response to stress differently. The lack of effect of menstrual cycle phase could also be due to the way we determined cycle phase. This was done by self-report of the last menstruation and length of cycle (excluding girls with no regular cycle), and not via more objective measures, such as serum levels of estrogen and progesterone.

**Influence of oral contraceptive use**

In Chapter 4 we examined the influence of oral contraceptive (OC) use on the cortisol response to the GSST. Compared to free-cycling (FC) girls, OC-users displayed a blunted cortisol awakening response (CAR), comparable to the attenuated CAR found in adults (Pruessner et al., 1997). In addition to a blunted CAR, we found high pre-test cortisol values in the OC-users during the morning session of the experiment, which suggests a slower increase and decrease of cortisol levels after waking. Although blunted cortisol responses have been found in adult women (Wolf et al., 2001; Rohleder et al., 2003), it is remarkable that girls using OC in our adolescent sample showed hardly any response to the GSST. The lack of response in the OC users during the morning sessions could be explained by the high pre-test levels of cortisol, which prevented a further increase. However, these high pre-test levels in the morning cannot explain the absence of a cortisol response in the afternoon sessions, when pre-test levels were comparable to those of boys and free-cycling girls. Furthermore, it is unlikely that the GSST was not stressful for the group of OC-users, since we observed no differences in subjective reports of stress between free-cycling girls and OC-users (Bouma et al., 2009). The slightly different finding regarding OC use in adult women (blunted response) and adolescent girls (absent response) might relate to the duration of OC use and the time to adjust to the effects of OC intake. Synthetic hormones can have direct as well as indirect effects by influencing girls’ natural levels of sex hormones (Wiegraz et al., 2003). To further explore the influence of OC use on the stress response, future studies should include measures of corticoid-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and cortisol-binding globuline (CBG). These measures can indicate whether CRH and ACTH production in the brain are altered or if free-cortisol, detectable in saliva, is reduced because of increased levels of CBG. In addition to this, longitudinal research is needed to elucidate the effects of oral contraceptive use on HPA axis functioning, especially because long-term effects of OC use are unknown (Ott et al., 2008).
Inconclusive evidence for the influence of polymorphic candidate genes underlying the stress reactivity endophenotype for depression

In Chapter 6, we examined whether polymorphisms in the mineralocorticoid and glucocorticoid receptor gene were associated with HPA axis and cardiac autonomic responses to the GSST in adolescent boys, girls using oral contraceptives (OC), and free-cycling (FC) girls. Previous studies in adults examined only associations in OC using women and men. We found significant associations between two SNPs (GR 9 beta and MR -2G/C) and stress reactivity indicators. We showed that the nature of these associations depended on gender and use of oral contraceptives. Our sample size was large (> 500) for this kind of research, and we had sufficient power to detect differences, which decreases the chance of false-negative results (Ioannidis, 2005).

Our results support the suggestion by DeRijk et al. (2008) that cardiac autonomic responses do not seem to be affected by GR variants but by MR variants, at least for the -2G/C SNP in OC-users. We could not replicate previous findings with regard to GR Bcll and cortisol responses (Wüst et al., 2004; Kumsta et al., 2006) and MR I180V and heart rate responses in men (DeRijk et al., 2006). Besides differences in age, different experiences of early life stress could explain the inconsistencies between our study and previous ones. Early life stress might have different programming effects on stress reactivity later in life depending on genetic variance of the MR and GR receptors. In future studies we will take experience of early life stress into account and re-examine the association between corticoid receptor SNPs and cortisol and heart rate.

We did not assess serum cortisol measures and ACTH levels because continuous intravenous blood sampling was considered too stressful for the adolescents, and was not logistically feasible in our study group. Although this was a well thought out decision, it is a limitation that our results cannot be fully comparable with other studies that did assess serum cortisol and ACTH. Further study is needed to determine how sex hormones interfere with different corticoid receptors and how this influences the HPA axis and cardiac autonomic nervous system.

Stress in early childhood influences the depressogenic effect of stressful life events in adolescent girls

The hypothesis postulated by Brown and Harris (2008) that early life stress might modulate gene-environment interactions later in life is confirmed by our results, presented in Chapter 7. This study showed that in girls who experienced mild stress early in life, the s/lg allele of the 5-HTTLPR was associated with fewer depressive symptoms after experience of SLE in adolescence than the l allele. In Chapter 8 we examined the epistatic effect of the s and lg allele of the 5-HTTLPR.
gene and the *met* allele of the BDNF in interaction with several childhood stress indicators (age 0 - 11) on a sum score of depressive symptoms between age 11 and 16. We found no main effects and no interaction effects. At first glance, the results of Chapter 7 and 8 might seem contradictory, but they can be explained by differences in statistical modelling and duration, timing and type of childhood stress. First, in Chapter 8 we did not account for gender-specific interactions because, although our sample size was large (> 1200), we did not have sufficient power to detect a gender-by-gene-by-gene-by-environment interaction. Second, the stress measure in Chapter 7 concerned stress between age 0 and 5, while Chapter 8 concerned stress between age 0 and 11.

**Programming effects of early life stress**

Programming effects on stress response systems by early experiences have a critical period in early life because of plasticity of the brain (Andersen and Teicher, 2008; Lupien et al., 2009). In rodents, unpredictable stress such as maternal separation (Pryce et al., 2002) increased the HPA axis response to stress later in life (Plosky et al., 2005). The assumed mechanism behind these results involves decreased negative-feedback due to decreased glucocorticoid receptor expression in the hippocampus and frontal cortex (Meaney et al., 1996), possibly due to epigenetic mechanisms. The programming effect of severe stress contrasts with that of neonatal handling (the short separation of pups and mother) (Levine et al., 1967), which was associated with decreased responses of the HPA axis to stress later in life (Meaney et al., 1988).

**Epigenetic effects of early life stress**

There is some evidence that experiences in early life can permanently alter the function of the stress system in rodents. The offspring of mothers who were deprived of maternal care had increased methylation of genes involved in the stress response (Francis et al., 1999; Weaver et al., 2004), which suggests transmission of epigenetic effects of stress. Persistent changes in HPA axis functioning due to stress experience was also suggested in humans (Heim and Nemeroff, 1999). Some evidence for this comes from a recent study in which epigenetic regulation of the GR receptor was found in the brain of adult men who were exposed to childhood adversity (McGowan et al., 2009). The studies described in Chapters 7 and 8 suggest that stress experience in early childhood is especially important in shaping the outcome of genetic variance in response to stress in adolescent girls.

**Inoculation effects of mild stress**

Mild stressful experiences early in life can provide resistance to later psychosocial adversity (Rutter, 1987; O'Leary, 1998) by regulating the physiological response to
stress. Thus mild stress, such as small stressors and minor hassles, might have stress inoculation effects. In contrast, extreme aversive events such as childhood abuse and severe neglect are associated with development of psychopathology. Extreme stress might push individuals, regardless of underlying genetic variance, into a negative trajectory of maladaptive physiological and emotional responses to stress, as well as increased risk for mental disorders. The exact mechanism behind inoculation stress needs further attention. Studying interactions between genetic variants and mild but common life stressors has more relevance for public mental health, since these mild stressors generate a larger attributable risk for the general population than do severe, but rare, experiences.

**Supportive environment**

Girls with the SS genotype appear to be protected from depressive symptoms after stress in adolescence if they had experienced some stress in early childhood (Chapter 7). This could be explained by the inoculation hypothesis, but it might also be explained by the supportiveness of their early environment. A study in adolescent girls suggested a beneficial effect of the homozygous low functioning allele (ss / slg / lglg) genotype in low-stress environments compared to girls with the high functioning (LaLa) genotype (Eley et al., 2004). Kaufman and colleagues (2004) showed that social support decreased the amount of depressive problems in maltreated children, even in those with the ss allele. In addition, Taylor et al. (2006) showed that young adults with the ss genotype had more depressive symptoms than those with other genotypes in the presence of childhood adversity. However, those with the ss genotype had the fewest symptoms when they experienced a supportive early environment or recent positive experiences. These studies suggest that although our response to environmental challenges is to a large extent influenced by our genetic make-up, improving the quality of our environment can improve our mental health. Unfortunately, we have no indication of the social support experience in the early environment of our TRAILS participants. We have some information about social support in adolescence of which the effect will be examined in future studies.

**Phenotypic plasticity**

The S allele is associated with increased reactivity to psychosocial stress (e.g. Heinz et al., 2007; Gotlib et al., 2008; Munafò et al., 2008) but is not consistently associated with the depressogenic effect of stress (Risch et al., 2009). Inspired by Ellis and Boyce (2005), Belsky et al. (2009) suggest that the variance in genes associated with depression should not be seen as predisposing for vulnerability factors, but rather for plasticity. Individuals with certain alleles are responsive to positive as well as negative environments (Ellis and Boyce, 2005; Belsky et al., 2009). With regard to the 5-HTTLPR, the low functioning allele (s or lg) might be
more responsive to any environment, either severe or mildly stressful, than the la alele. In a situation of severe stress (and/or low social support) this can result in non-adaptive responses to stress later in life and increased risk for psychopathology, as shown by Caspi et al. (2003). However, in a mild stressful situation this could result in adaptive responses to social stress in adolescence because of inoculation effects (Chapter 7). Since timing and severity of experienced life stress and levels of social support are not always accounted for in studies, this might explain the absence of a clear direction of 5-HTTLPR on the depressogenic effect of life stress (Risch et al., 2009).

To conclude, brain regions such as the hippocampus, amygdala and prefrontal cortex are especially sensitive to environmental influences in early childhood and adolescence. The programming effect of early life stress in interaction with genetic factors may depend on severity, timing and nature of the experienced stressors. This is not often accounted for in genetic association studies, which could explain the inconsistent results. In addition, experience of positive life events and social support may counterbalance the negative effects of psychosocial stress and risk for depression, which could have also obscured the associations.

**Main conclusion: adolescent girls are more sensitive to the depressogenic effect of social stress than adolescent boys**

The studies in this dissertation examined several risk factors for depressive symptoms in adolescence. We found significant main and interaction effects in girls but not in boys. From this we can conclude that females are the sensitive sex; they are more sensitive to psychosocial stress and depressed mood than males. The gender-specific results of the studies presented in this dissertation suggest interactions between female sex hormones and genetically based differences in glucocorticoid receptors and the serotonin transporter. Although studies in animals have indicated several mechanisms in which female sex hormones can influence the response to stress, exact mechanisms on how sex hormones influence brain and behaviour in humans is still poorly understood (Paus et al., 2008). Further research is necessary to determine whether estrogen and progesterone levels can affect the serotonin transporter and corticoid receptors differently according to genotype. This could provide a mechanism for understanding how genetic risk factors for the depressogenic effect of stress become more evident in adolescent girls, as suggested by Silberg et al., (1999).

However, the gender-specificity of higher sensitivity to stress and depression cannot be fully explained by female sex hormones. Animal studies have shown that in males and females, the difference between the responses of the HPA axis in adolescent and adult rats was not only explained by circulating sex hormone levels
(Romeo et al., 2006; Viau et al., 2005; McCormick and Mathews, 2007) but also by changes in maturation of the brain during the adolescent period. Gender differences in brain maturation are also present in human adolescence (Andersen and Teicher, 2008). In addition to biological changes, both boys and girls face dramatic psychosocial changes during adolescence (e.g. parental, peer, and romantic/sexual relationships). Boys cope with psychosocial stress differently than girls; they show their pain or frustration in a more external way (e.g. Hoffman and Su, 1997; Eschenbeck et al., 2007). In boys, we found no associations between polymorphic candidate genes and (endo)phenotypes of depression. This might be due to the lower prevalence of depressive symptoms in adolescent boys, which have not yielded enough power to detect associations between genetic variance and (endo)phenotypes of depression.

More research is needed to understand the interplay between genes and environment in the development of depressive symptoms in adolescent girls. This dissertation showed that associations between polymorphic gene variants and (endo)phenotypes for depression were dependent on gender, OC use and early life stress, but the underlying mechanisms are still largely unknown. In the next section, directions for further research are given.

DIRECTIONS FOR FURTHER RESEARCH

As in most research, our study raised more questions than it answered. In formulating new hypotheses and interpreting past, present, and future results, a theoretical framework can be useful. According to Mayr (1961), one of the 20th century’s leading evolutionary biologists, behaviour can be investigated at a proximate and at an ultimate level. Proximate explanations relate to how environmental stimuli and mechanisms inside an individual result in behaviour or disease. Ultimate explanations describe why proximate processes are expressed as they are; they explain the function or adaptation of the process. Niko Tinbergen (1963), influenced by Mayr, said that behaviour could only be fully understood when researchers consider phylogeny (evolutionary history of a species), ontogeny (individual development within a species), underlying biological mechanisms, and adaptive value of the behaviour. These four levels might be very useful in psychiatric epidemiological research, since phylogeny and adaptive value are not often considered in understanding mental disorders. Considering notions such as reproductive disadvantage, evolutionary age, or adaptive value of certain gene variants can inform us about the why of behaviour and sickness. In the next section, considerations on the ultimate level are presented for depressive
behavior. Finally, I aim to give suggestions for future research on proximate levels inspired by these ultimate considerations.

**Considerations on depressive behaviour on the ultimate level**

*Depressive behaviour and adaptive value*

Evolutionary psychiatrists are still puzzled over whether human depression is a by-product of maladaptive responses to stress or an adaptive behaviour in itself (Nettle, 2004). Since the 1970s, several theories arose to explain the stable presence of depressive behaviour in humans, but none of them seems to provide a conclusive answer. The high preponderance of depressive behaviour in women compared to men evokes the questions: ‘Why are women more likely to display depressive behaviour than men?’ and ‘Is there a higher adaptive value for women in being more sensitive to psychosocial stress than there is for men?’ Before trying to answer these questions we should acknowledge that gender differences in behaviour result from a deeply embedded and highly coordinated biology, evolved to serve the gender-specific functions that are necessary for reproduction and survival (e.g. Buss and Smitt, 1993). From an evolutionary perspective, depressive behaviour can be seen as taking shelter and staying out of danger (Nesse, 2000). Such behaviour might have had a higher impact on survival in women than in men, since during our evolutionary past women were mostly involved in childbearing and child caring, whereas men were the providers of food, material resources, and protection. Depressive behaviour might have been selected for in women over time and selected against in men. The question of whether increased sensitivity to psychosocial stress is more adaptive in women than in men is difficult to answer. The increasing need for affiliation in adolescent girls (Cyranowski et al., 2000) is most likely related to sexual maturation and their role as child caregivers. For this reason, women might place higher value on communion and intimacy in relationships. This is consistent with findings in our adolescent TRAILS sample; girls were more sensitive to the loss of relationships with peers and romantic partners than adolescent boys (Bakker et al., in press).

*Evolutionary age of variance in depression-related genes*

A recent review concluded that mental disorders with high heritability and severe reproductive disadvantages, such as schizophrenia and autism, are likely the result of more recent and rare genetic variants. Conversely, common mental illnesses with mild reproductive disadvantage and moderate heritability might be due to interactions between common genetic variants of older evolutionary origin and environmental exposure to stress (Uher, 2009). This seems very likely for depression. Adaptive responses to stress, such as learning to avoid detrimental situations in the future, increase the likelihood of survival. Moreover, these stress response systems are present throughout the mammalian kingdom and depressive
behaviour after stress, (i.e., decreased play activity, decreased appetite and weight, decreased motor activity, sleep disturbance), is observed in many animals, such as rodents, monkeys and pigs (reviewed in McKinney & Bunney, 1969). This suggests an old evolutionary age of genetic variants implicated in the response to stress and in depressive behaviour.

Adaptive value of variance in depression related genes
So far, the gene hunt for depression has not yield clear results for one or more genes (Lopéz-León et al., 2008; Bosker et al., 2010). This makes sense, since genes associated with depression are not specific ‘depression’ genes but are likely related to psychosocial stress responses in general. Additionally, polymorphic candidate gene studies are not conclusive in their findings that one allele is always associated with a ‘bad’ outcome. (this dissertation; Doornbos et al., 2009; Grabe et al., 2009 versus Caspi et al., 2003). And why would it be? From an evolutionary perspective, the developmental plasticity of the stress response system suggests adaptive value (Boyce and Ellis, 2005). Genetic variance is present in the human gene pool because it enables us to react to our (psychosocial) environment. Genetic variance in the genes underlying these systems results in adaptive or maladaptive outcomes in the face of stress, and might to a large extent be dependent on programming effects by the early environment. The challenge for science is to unravel how environmental stimuli interact with common genetic variants to result in adaptive stress responses (coping with stress) or depressive behaviour. The next section states some of the questions that need to be addressed to reach this goal.

Focus points for future research on proximate levels
What is the functionality of allelic variants in genes underlying the response to psychosocial stress and depressive behaviour?
Multiple polymorphic genes have been associated with physiological responses to stressors and vulnerability for mental disorders. However, no straightforward associations were found; the same allelic variant can associate with both vulnerability and resilience for depression after psychosocial stress. In order to understand these interactions, we first need to understand the functionality of these allelic variants. This is difficult to study in animals because similar (orthologous) genetic variants between animals and humans are often absent or not yet identified. Functionality is currently examined by the expression of genetic variants in cell cultures. A disadvantage to this method is the labour intensity and the fact that cell lines do not reflect the exact cell environment within the human body. New techniques might be needed to study the functional consequences of common genetic variants more effectively. So far, genetic variants can only be associated
with (endo)phenotypes of depression. No causal inferences can be made until the functional consequence of these gene-products is clearly understood.

**Are programming effects of early stress different according to genetic variance?** 
Programming effects caused by early environment can prepare the individual to cope with similar environments later in life. It is tempting to speculate that some genetic variants respond more strongly to early environmental conditions than others. This could be due to direct effects of the gene products resulting from these variants, but might also be mediated by epigenetic processes. At the moment, attempts are being made to understand the mechanisms and outcome of epigenetic processes. Future research should explore the hypothesis that programming effects might differ according to polymorphisms in our DNA.

**What is the role of female sex hormones in sensitivity to stress and depression?** 
Research in animals and in humans (including this dissertation) suggests that interactions between sex hormones and polymorphic gene products in pathways leading to depression can be different for boys and girls. Future research should focus on understanding the underlying mechanisms behind the different effects of psychosocial stress on males and females. Considering how gender-specific functions evolved to increase survival and reproduction might guide our research questions and help to interpret the findings.

**Conclusion**
Where ultimate explanations of behaviour might bring us new hypotheses, proximate explanations are needed for the prevention and treatment of depression. The link between the appraisal of psychosocial stress and depressive behaviour starts in the brain, where altered neurotransmission in the hippocampus, prefrontal cortex, hypothalamus and amygdala is associated with maladaptive responses to stress. Therefore, neurological imaging techniques might be useful to see how environmental factors influence the brain in individuals with a certain genetic make-up. Since depression is a consequence of common genetic variants and psychosocial stress, we need to understand the functionality of allelic variants in genes underlying endophenotypes for depression. In order to study the multiple interactions of multiple genetic variants and a large variety of environmental stimuli, we might need new statistical methods. We also need large samples to assure sufficient power, samples to replicate our findings and longitudinal settings to examine changes over the human life span. Cooperation between research groups should be encouraged in order to accomplish this. The research described in this thesis highlights the possible programming effect of mild stress in early life, which can explain the hyper- and hypo-responses of the stress system to psychosocial stress later in life. Additionally, special attention must be paid to the nature of the
stressor, because this can influence the direction of the stress response. Since female gender is a strong predictor of stress-related mental disorders, we emphasize the importance of accounting for gender in future studies on underlying mechanisms. The consideration of our evolutionary past and the underlying biology in behavioural differences between men and women can help with the interpretation of both previous and future findings. Focusing on the functionality of allelic variants, programming effects of early life stress, and gender-related risk factors will improve our understanding of the aetiology of depression and will enhance our ability to prevent this common but highly impairing mental disorder.