General discussion
9.1 General discussion

The aim of the current thesis was to investigate the neural mechanisms underlying neuroticism to gain insight into why individuals scoring high on this personality trait are more vulnerable to develop psychopathology. To this end, we examined differences in brain function in relation to neuroticism with functional magnetic resonance imaging (fMRI) during rest as well as particular emotion processing tasks. As prior studies have shown that neuroticism is moderately heritable (≈50%), (Boomsma et al., 2000; Canli, 2008; Distel et al., 2009; Flint, 2004; Hansell et al., 2012; Riese et al., 2009), we examined whether specific genes moderate the association between neuroticism and brain function.

9.2 Summary of the findings

In this section of the general discussion, we summarize and discuss the findings of Chapters 2-8. In 2001, the first fMRI study on neuroticism was performed by Canli et al. to investigate whether neuroticism is associated with brain activation in response to emotional stimuli. Subsequently, a variety of studies have been performed that examined different cognitive-emotional processes using a diverse set of paradigms. However, the results of these studies were largely inconsistent and hardly had been integrated to construct a model of the underlying neural mechanisms of neuroticism. Until then, no meta-analysis had been performed that provided a quantitative summary of the literature. Therefore, the aim of the study in Chapter 2 was to perform a meta-analysis on neuroimaging studies investigating emotion processing in association with neuroticism, to examine the consistency of findings across studies. Significant results were only observed for the contrast (negative>neutral) in association with neuroticism, not for the contrast (positive>neutral). In high compared to low neurotic individuals, we found higher activation in brain regions related to fear learning (e.g. hippocampal-parahippocampal complex) and emotion processing/regulation (e.g. frontal and cingulate regions), and lower activation in brain regions related to the anticipation of aversive stimuli (e.g. anterior/posterior cingulate cortex and striatum). These findings were integrated in a model of emotion processing in neuroticism. In this model, we proposed that negative biases in information processing together with a more active fear learning system, may hamper adaptive associative learning in individuals scoring higher on neuroticism, leading to impairments in the prediction and anticipation of aversive events. Consequently, this may produce feelings of uncertainty in these individuals, requiring increased cognitive control. In conclusion, we suggest that abovementioned cognitive-emotional processes and corresponding neural correlates may contribute to the increased emotional reactivity and negative emotionality observed in individuals with high scores on neuroticism compared to individuals with low scores.
Using a fear learning paradigm, a recent study has investigated the relationship between neuroticism and functional connectivity between i) the amygdala and hippocampus and ii) the amygdala and prefrontal regions in healthy, 14-year-old adolescents (Tzschoppe et al., 2014). The authors found increased functional connectivity between these specific brain regions in association with neuroticism during the acquisition phase of fear learning, which may indicate increased emotion processing and consolidation of learned fear associations in individuals scoring higher on neuroticism. This is in line with our meta-analytic finding of a more active fear learning system in high compared to low neurotic individuals. For future research, it would be of interest to also investigate functional connectivity patterns in association with neuroticism during the anticipation of aversive stimuli (thereby making a clear distinction between the fear learning and anticipation phase) and emotion regulation. Furthermore, as proposed in Chapter 2, research is needed into the causal and bidirectional relationships between the different cognitive-emotional processes found in our meta-analysis and associations with neuroticism on a behavioral, psychological and neurobiological level.

As described in Chapter 1, the majority of research on neuroticism has primarily been focused on processes related to fear learning, anticipation of aversive stimuli and emotion processing/regulation. In the current thesis, we focused on three other key cognitive-emotional processes related to neuroticism that lack investigation, that is, i) the use of worry as a coping mechanism, ii) emotional reactivity to unfairness and iii) sensitivity to criticism. First, prior research has shown that the application of maladaptive coping strategies is one of the reasons that individuals scoring higher on neuroticism express heightened emotional reactivity and experience more negative emotions (for a review, see Suls and Martin, 2005). Excessive worry is such a strategy and neuroticism has been shown to be directly related to increased worrying or to processes that negatively reinforce, initiate or extend the use of worry as a coping mechanism (Blair and Blair, 2012; Borkovec et al., 2004; Bringmann et al., 2013; Hale et al., 2010; Matthews et al., 2000; Mennin et al., 2005; Muris et al., 2005; Roelofs et al., 2008). Nonetheless, the relationship between neuroticism and the tendency to worry has not been studied extensively, specifically not with fMRI. Hence, the aim of the study in Chapter 3 was to investigate the neural correlates of state worry in association with neuroticism. We found that individuals scoring higher on neuroticism demonstrated increased worrying based on questionnaire, task and imaging results. The questionnaire results showed that, in daily life, high neurotic individuals rated their worry episodes as more excessive and uncontrollable in comparison to low neurotic individuals (trait worry). This propensity was also observed in our task results of experimentally-induced worry, that is, high neurotic individuals indicated to have generated more worry-related thoughts after the presentation of a worry-inducing sentence than low neurotic individuals (state worry). The neuroimaging results demonstrated that, during worry, neuroticism was associated with lower activation in the retrosplenial and visual cortex. Based on the connectivity patterns of both brain regions during worry and theories related to worry, we proposed that these regions may be
implicated in autobiographical specificity and visual mental imagery, respectively. These findings possibly indicate that, during worry, high compared to low neurotic individuals tend to recollect memories in an overgeneral way (Sumner, 2012; Svoboda et al., 2006) and reduce the visualization of emotional events (Borkovec et al., 2004; Holmes and Mathews, 2010). In the literature, both processes have been related to the cognitive avoidance of emotional distress, which is in line with the proposed function of worry in the theory of Borkovec et al. (2004). In conclusion, neuroticism may be associated with the use of worry as a coping mechanism to temporarily release stress. However, in the end, it may make individuals scoring higher on neuroticism more vulnerable for the development of psychopathology, because emotions are not regulated optimally (Borkovec et al. 2004).

As of yet, this is still the first fMRI study to investigate the neural correlates of state worry in association with neuroticism. For this reason, our findings need to be replicated and further validated. In addition, it would be interesting to calculate graph measures, such as efficiency, to investigate the functional integration and segregation of information processing during worry in high compared to low neurotic individuals. For instance, it is possible that information is processed differently in terms of efficiency in the default mode network during worry, specifically in individuals scoring higher on neuroticism (see the section Future research for a more extended description). Moreover, it would be of interest to investigate the relationship between neuroticism and worry in daily life, using ambulatory experience sampling methods (Suls and Martin, 2005), to examine when and how often individuals scoring higher on neuroticism apply worry as a coping mechanism and how this influences their mood.

Second, previous studies have shown that high compared to low neurotic individuals report more interpersonal conflicts, leading to increased levels of stress (Bolger and Schilling, 1991; Bolger and Zuckerman, 1995; Gunthert et al., 1999). It has been proposed that high neurotic individuals are emotionally more reactive to these types of stressors, because they i) tend to apply maladaptive interpersonal coping strategies (Gunthert et al., 1999), ii) display more avoidance and revenge motivations in response to transgressions and iii) are less forgiving (Brose et al., 2005; Maltby et al., 2008). These findings indicate that individuals scoring higher on neuroticism compared to individuals scoring lower experience more problems in dealing with interpersonal conflicts, which may impact their decision-making in these situations. For this reason, the aim of the study in Chapter 4 was to investigate the association between neuroticism and brain activation during the perception of social norm violations and social decision-making in the Ultimatum Game (UG), specifically in response to unfair offers. We found that high compared to low neurotic individuals did not reject more unfair offers, neither did they show differential brain activation patterns during the proposal of unfair offers. However, during the acceptance of unfair offers, high neurotic individuals did show lower activation in the right dorsal striatum, previously involved in the formation of stimulus-action-reward associations (Balleine et al.,
motivation and arousal (Miller et al., 2014; Takeuchi et al., 2014). The findings suggest that both high and low neurotic individuals recruit brain regions signaling social norm violations in response to unfair offers. However, when it comes to decision-making, it seems that neural circuitry related to reward and motivation is altered in individuals scoring higher on neuroticism, when accepting an unfair offer. In conclusion, high compared to low neurotic individuals may experience more negative affect in response to social norm violations - possibly due to maladaptive coping - and therefore, show decreased reward responsiveness during the acceptance of unfair offers.

Thus far, this is still the first study that investigated the association between neuroticism and brain activation during the perception of social norm violations and social decision-making in the UG. In future research, it would be of interest i) to collect behavioral and autonomic measures during the task to investigate emotional responses to offers (Pillutla and Murnighan, 1996; van't Wout et al., 2006; Xiao and Houser, 2005) and ii) to offer a greater range of offers (Kirk et al., 2011), since high and low neurotic individuals may make similar decisions for offers on the extremes, but different ones for offers in the middle (i.e. the ‘grey’ area). Furthermore, it would be interesting to investigate the relationship between neuroticism and unfairness inflicted by a significant other (not a stranger) in a more interpersonal context (less economic) using fMRI. For instance, an emotional memory task can be applied, wherein individuals are instructed to relive transgressions involving friends. Notably, a quantitative meta-analysis on emotional memory tasks wherein the rejection of a significant other was relived, has shown the involvement of brain regions (e.g. anterior insula and anterior cingulate cortex) previously related to uncertainty, rumination, emotional craving and the mental representation of significant others and interpersonal relationships (Cacioppo et al., 2013), which are all concepts that have been associated with neuroticism (for a review, see Ormel et al., 2013a).

Third, it has been shown that high compared to low neurotic individuals are more self-critical (Clara et al., 2003) and are highly sensitive to criticism by others (Watson et al., 1994). Alterations in the processing of criticism have been observed in several psychiatric disorders (Blair et al., 2008; Hooley et al., 2009) and are associated with increased risk of relapse and poor clinical outcomes in, for example, depression and anxiety disorders (Hooley et al., 2012). Hence, both neuroticism and sensitivity to criticism are clinically relevant concepts. However, their association has not been investigated extensively, particularly not with fMRI. Therefore, the aim of the study in Chapter 5 was to investigate the effect of criticism on functional brain connectivity in association with neuroticism, using a novel resting-state paradigm. In this paradigm, we presented participants with three standardized critical remarks through headphones in which the investigator urged the participant, with an increasingly agitated tone, to please lie still in the scanner (independent of whether they were lying still or not). During criticism, we found that high compared to low neurotic individuals showed higher functional connectivity between
the prefrontal/fronto-temporal cluster and the lateral PFC, a brain region previously implicated in cognitive control and reappraisal (Ochsner et al., 2002; Ochsner and Gross, 2005; Ochsner and Gross, 2008). Furthermore, in these high neurotic individuals, we found lower functional connectivity between the prefrontal cluster and several default mode brain regions and between the amygdala/hippocampal cluster and several frontal regions. The findings may suggest that, in high compared to low neurotic individuals, information is processed less efficiently in circuits related to self-reflective (Buckner et al., 2008) and emotion (Etkin et al., 2011) processing during criticism, requiring increased cognitive control of frontal circuits as a compensatory mechanism (Ochsner and Gross, 2005). In conclusion, during criticism, individuals scoring higher on neuroticism may need greater regulatory efforts in order to gain cognitive control over negative emotions than individuals scoring lower, possibly explaining their sensitivity to negative social-evaluation (Watson et al., 1994).

A recent study on self-criticism in relation to neuroticism is in line with our results (Doerig et al., 2013). The authors found higher activation in several brain regions during the processing of self-critical adjectives (compared to neutral words and non-self-referential adjectives) that converged with regions we selected as seed regions in our study, that is, the amygdala-hippocampal complex, anterior insula, default mode brain regions and an extended network of bilateral frontal brain regions (incl. the lateral prefrontal cortex). This latter network was suggested, by the authors, to be involved in top-down emotion regulation through the cognitive reappraisal or suppression of negative emotional stimuli, such as negative images of the self. Furthermore, they found higher activation in a brain region part of this network (i.e. right superior frontal cortex) in association with neuroticism, indicating that high compared to low neurotic individuals require more frontal activation to achieve the same amount of cognitive control. For future research, it would be interesting to investigate the relationship between criticism, self-criticism and neuroticism in daily life, using ambulatory experience sampling methods (Suls and Martin, 2005) to examine i) contexts in which high neurotic individuals feel criticized or criticize themselves, ii) the feelings they experience during such situations and for how long and iii) the kind of regulation strategies they apply. Moreover, as proposed in Chapter 5, it would be of interest to investigate how connectivity dynamically changes (see the section Future research for a more extended description) when individuals receive criticism to examine interactions between brain networks involved in emotion processing and cognitive control, and how long changes in connectivity last and differences herein in relation to neuroticism.

Besides investigating differences in brain functioning during certain cognitive-emotional processes, we were interested in investigating the underlying functional network organization in association with neuroticism. Findings from structural connectivity studies sparked this interest by demonstrating decreased white-matter integrity in multiple fiber tracts interconnecting various brain regions (Bjørnebekk et al., 2013; Xu and Potenza, 2012). Hence, the aim of the study
in Chapter 6 was to investigate whether there is also evidence for an alteration of whole-brain functional connectivity in association with neuroticism. We found that the functional network organization of high compared to low neurotic individuals is organized less optimally with regard to efficient information processing and shows signs of functional disconnectivity. Furthermore, we demonstrated that subnetworks related to emotion and salience processing play a more prominent role in the network organization of high neurotic individuals, while subnetworks related to sensory(-motor) functions and cognitive control play a less prominent role. These findings may indicate that the ‘neurotic brain’ processes information less cost-efficiently (Achard and Bullmore, 2007) and is less cognitively controlled (Doucet et al., 2011; Kinnison et al., 2012; Laird et al., 2011). In conclusion, during rest (i.e. when the brain is in default mode), high compared to low neurotic individuals may process information on salient emotional stimuli more efficiently, while they process information less efficiently in subnetworks that cognitively control the impact of such stimuli. This may impose constraints on and bias cognitive-emotional processing during task performance and social interaction (Menon, 2011), which may lead to emotional instability in individuals with higher levels of neuroticism (Ormel et al., 2013a).

One other graph theory study has been performed on neuroticism (Gao et al., 2013) as described in Chapter 1. Two network measures in this study were also calculated in our study, that is, global and local efficiency (both were calculated on the whole-brain for binary graphs by Gao et al.). We found a positive correlation between neuroticism and global efficiency, and a negative correlation between neuroticism and local efficiency. However, this was not found by Gao et al. (2013) (for a description of their results, see Chapter 1, section Resting-state imaging). There can be a number of reasons for this discrepancy, specifically a difference in gender distribution of the sample (women only versus men and women), age range of the sample (18-25 yrs versus 17-36 yrs), ethnicity of the sample (Dutch versus Chinese), applied neuroticism questionnaire (NEO-PI-R versus EPQ-R), scan duration (10 min versus 8.5 min) and several aspects of the analysis method (e.g. seed regions from Power et al., 2011 versus AAL seed regions). Hence, more studies should be performed to replicate and further validate our results. Additionally, for future research, it would be interesting to investigate the relationship between structural and functional connectivity and between network measures and behavioral vulnerabilities (e.g. maladaptive forms of emotion processing and emotion regulation) in association with neuroticism (see the section Future research for a more extended description).

Finally, we performed two genetic imaging studies. In the first study in Chapter 7, we investigated the alleged association between the serotonin transporter polymorphism (5-HTTLPR) and amygdala activation. Prior research has shown that carrying the S-allele compared to carrying two copies of the L-allele is associated with reduced transcriptional efficacy of the serotonin transporter (5-HTT) gene and explains inherited variance in neuroticism and other anxiety-related traits (Lesch et al., 1996). Higher amygdala activation in response to
negative emotional stimuli (e.g. fearful facial expressions) was proposed as an underlying neural mechanism for the latter. A recent meta-analysis on the association between 5-HTTLPR and amygdala activation revealed a significant result (Hedge g = + 0.35, p = 0.03) (Murphy et al., 2013). However, this meta-analytic association was no longer significant (g = + 0.20, p = 0.06), when unpublished studies were included and the meta-analysis was updated with our relatively high-powered sample (n=120). In conclusion, the association between 5-HTTLPR and amygdala activation is either much smaller than previously thought or conditional on other factors, such as the experience of adverse life events (Caspi et al., 2010). This study underlines the importance of repeatedly updating meta-analyses. This holds especially for the quantitative integration of published findings regarding 'hot topics', wherein positive findings are largely based on studies with small sample sizes and publication bias cannot be ruled out.

In the second study in Chapter 8, we investigated the triadic interplay between 5-HTTLPR/COMT, functional brain network organization and neuroticism. It has been shown that neuroticism is moderately heritable, that is, approximately 50% of the variance can be explained by genetic factors (Boomsma et al., 2000; Canli, 2008; Distel et al., 2009; Flint, 2004; Hansell et al., 2012; Riese et al., 2009). Two polymorphisms that have been related to neuroticism and emotion processing are the 5-HTTLPR and COMT (for reviews, see Bevilacqua and Goldman, 2011; Canli, 2008; Domschke and Dannlowski, 2010; Hariri and Holmes, 2006; for a meta-analysis on COMT, see Mier et al., 2010). Previous studies have typically investigated associations between neuroticism-related traits (e.g. phobic proneness), specific genes (e.g. 5-HTTLPR) and brain activation in limbic areas during the processing of fearful stimuli (for a review, see Domschke and Dannlowski, 2010). However, recently, it was proposed that psychopathology probably does not arise from dysfunctional activation in a few specific brain regions during a particular task, but from alterations in the functional integration and segregation of neural circuits (i.e. disrupted connectivity) (for reviews, see Buckholtz and Meyer-Lindenberg, 2012; Fornito and Bullmore, 2012; Meyer-Lindenberg, 2012; Tost et al., 2012; for a research article, see Fornito et al., 2011). In the current study, we indeed found an altered functional network organization in genetic risk carriers (5-HTTLPR: S/S, Lg/Lg, S/Lg, S/La, Lg/La; COMT rs4680: Val/Val and rs165599: Met/Met) compared to genetic non-risk carriers (5-HTTLPR: La/La; COMT rs4680: Met/Met and Val/Met, and rs165599: Val/Val and Val/Met) for both polymorphisms. Specifically, the findings showed that subnetworks related to salience and visual processing play a more prominent role in the functional network organization of genetic risk carriers, than subnetworks related to cognitive control. As a consequence, genetic risk carriers compared to non-risk carriers may perceive the world as more threatening and express heightened emotional reactivity to negative events, due to reduced cognitive control (for a review, see Jonassen and Landro, 2014 and Ormel et al., 2013a). Furthermore, COMT (not 5-HTTLPR) moderated the association between neuroticism and the functional network organization. In the genetic risk group, neuroticism was associated with lower efficiency coefficients in salience and sensory(-motor) subnetworks and relatively more
connections between the fronto-parietal control subnetwork and other functional subnetworks. This effect was not observed in the genetic non-risk group. In the genetic risk group, these findings may indicate that high compared to low neurotic individuals show impairments in the prediction and anticipation of salient events, requiring increased cognitive control to process the sensory overload (Gradin et al., 2011; for a review, see Palaniyappan and Liddle, 2012). In conclusion, the findings of altered topology of specific functional subnetworks may help explain why risk carriers of the 5-HTTLPR and COMT (scoring higher on neuroticism) experience difficulties in emotion processing and are more prone to develop psychopathology.

This study is an illustration of the added value of using connectomic measures in genetic imaging research, because more is learned about the underlying neural mechanisms and the way information is integrated and segregated. This in contrast to the investigation of genetic effects on brain activation in specific regions during a particular task. However, the results should be replicated and further validated, since this is the first study to investigate the triadic interplay between 5-HTTLPR/COMT, functional brain network organization and neuroticism. For future research, it would be of interest to investigate the relationship between connectomic measures and genetics using a multivariate approach to concomitantly examine their overall effect (i.e. explained variance) on the network organization in association with neuroticism (see the section Future research for an extended description).

### 9.3 Integration of the findings

In this section of the discussion, I propose an attempt to integrate abovementioned findings and conclusions to guide future research. In Figure 1, I show a tentative hierarchical model (i.e. arrows indicate consequence) of the different cognitive-emotional processes and their corresponding neural correlates found in the studies of the current thesis. Herein, I assume that i) altered processes and brain connectivity during rest (i.e. when the brain is in default mode) impose constraints on and bias processes and brain activation/connectivity during task performance (Menon, 2011), ii) during task performance, alterations in processes and activation/connectivity patterns may lead to alterations in other processes and activation/connectivity patterns. During rest, we observed that affective and salience subnetworks have a more prominent role in the network organization of individuals scoring higher on neuroticism compared to individuals scoring lower (Chapter 6). I propose that this may underlie and cause the expression of negative biases in information processing (Chan et al., 2007) and a more active fear learning system in high neurotic individuals, leading to difficulties in adaptive associative learning (Suls and Martin, 2005) (for a model including these processes, see the meta-analysis in Chapter 2). It is possible that due to this, high neurotic individuals perceive their environment as more threatening and distressing than low neurotic individuals (Suls and Martin, 2005; Watson
et al., 1994). Hence, individuals scoring higher on neuroticism may experience problems with the prediction and anticipation of aversive stimuli, giving rise to increased levels of stress and feelings of uncertainty (McEvoy and Mahoney, 2012; Suls and Martin, 2005) (for a model, see the meta-analysis in Chapter 2). High neurotic individuals may use worry as a coping mechanism (Chapter 3) in order to gain cognitive control over these feelings of uncertainty, since it is often applied to i) prevent future negative outcomes, ii) prepare for the worst and iii) solve problems related to upcoming negative events (Borkovec et al., 2004). Genetic variation in the COMT (rs4680-rs165599) polymorphism may partially explain these difficulties in the anticipation of aversive stimuli, as we observed less efficient processing in subnetworks related to this process and dopamine functioning (Bromberg-Martin et al., 2010; Hauser et al., 2014; Winton-Brown et al., 2014) in high compared to low neurotic individuals in the genetic risk group (Chapter 8). Furthermore, we observed that individuals scoring higher on neuroticism compared to individuals scoring lower show i) less efficient processing in cognitive control subnetworks during rest (Chapter 6), ii) increased activation in brain regions related to emotion regulation during emotional tasks (Chapter 2) and iii) increased connections between the fronto-parietal control subnetwork and other functional subnetworks during rest, moderated by genetic variation in the COMT (rs4680-rs165599) polymorphism (Chapter 8). This specific pattern - e.g. lower functioning of the fronto-parietal control subnetwork and higher activation in brain regions that are part of this subnetwork during emotional tasks - has also been observed in association with trait anxiety (for a review, see Sylvester et al., 2012). The authors of this review proposed that individuals with high trait anxiety may need additional cognitive control in order to regulate emotions compared to individuals with low trait anxiety (Sylvester et al., 2012). Therefore, the findings in the current thesis may indicate that individuals scoring higher on neuroticism show less efficient cognitive control over (negative) emotions than individuals scoring lower. This has also been proposed in the review of Ormel et al. (2013a) on the biological and psychological basis of neuroticism. As a consequence, high compared to low neurotic individuals may experience more negative emotions that potentially spill over into the next time period (e.g. next three hours), due to poor emotion regulation (Suls and Martin, 2005). In addition, these findings may explain why high neurotic individuals often apply maladaptive coping strategies, such as worry (Chapter 3) or escape-avoidance strategies (Lee-Baggley et al., 2005; Watson and Hubbard, 1996), as their regulatory capacity is limited. Indeed, worry has been argued to be a consequence of the ineffective processing and regulation of emotions (Blair and Blair, 2012; Mennin et al., 2005). Moreover, I propose that these difficulties in emotion processing and regulation may be an explanation for the decreased reward responsiveness during the acceptance of unfair offers (Chapter 4) and increased sensitivity to criticism by others (Chapter 5) in high compared to low scoring individuals on neuroticism. Results from Chapter 7 are not included in the model in Figure 1, since neuroticism was not investigated in that study.

To come back, the aim of the current thesis was to investigate the neural mechanisms
underlying neuroticism to gain insight into why individuals scoring high on this personality trait are more vulnerable to develop psychopathology. In the current thesis, we found that affective, salience, default mode and fronto-parietal control subnetworks show altered information processing in high compared to low neurotic individuals. Notably, these functional subnetworks have also been found to be disrupted in, for example, depression, anxiety disorders, autism, schizophrenia and dementia (for a review, see Menon, 2011). Sylvester et al. (2012) proposed that different patterns of between- and within-network connectivity lead to different clinical symptoms. The findings of the current thesis - that is, higher functioning of the affective and salience subnetwork and lower functioning of the default mode and fronto-parietal subnetwork in association with neuroticism (Chapter 6) - are in line with findings from studies on high trait anxiety and anxiety disorders, as briefly mentioned in the previous paragraph with regard to the fronto-parietal control subnetwork (for a review, see Sylvester et al., 2012). This is also consistent with our meta-analytic findings that show a more active fear learning system, impaired anticipation of aversive stimuli and less efficient emotion processing/regulation in high compared to low neurotic individuals (Chapter 2). In conclusion, alterations in the topology of the whole-brain and specific functional subnetworks in association with neuroticism may underlie and cause a cascade of problems in higher cognitive functions important for adaptive behavior. The findings show the greatest resemblance to findings obtained from anxiety research. However, I would like to emphasize that it is not my intention to equate neuroticism to fear/anxiety, rather I propose that heightened emotional reactivity and emotional instability are the core elements of neuroticism. Yet, feelings of fear/anxiety may play a causal role in relation to these latter two elements and explain a large part of the variance. Abovementioned findings may elucidate why high neurotic individuals are more vulnerable to develop psychopathology, specifically affective disorders.
In this section of the general discussion, I give some suggestions for future research. First, it would be of interest to investigate the assumptions made in the former section, namely that alterations in processes and neural correlates during resting-state have an influence on processes and neural correlates during task performance, which themselves have an influence on other processes and neural correlates during task performance (see Figure 1). One can investigate whether graph measures (e.g. efficiency) calculated on the whole-brain or specific functional subnetworks during resting-state explain variance in behavioral results and brain functioning of i) the whole-brain (e.g. graph measures), ii) specific functional subnetworks (e.g. graph measures) or iii) regions that are part of a subnetwork (e.g. activation, connectivity or graph measures) during...
task performance.

Second, structural connectivity studies on neuroticism, using diffusion tensor imaging (DTI), have shown extensive decreases in white matter integrity in multiple fiber tracts interconnecting different brain regions (Bjørnebekk et al., 2013; Xu and Potenza, 2012). In line with this, we showed an altered functional network organization and signs of functional disconnectivity in individuals scoring higher on neuroticism (Chapter 6). An interesting idea would be to investigate the relationship between structural and functional connectivity in the same sample, to examine whether a loss of white matter integrity underlies the observed alterations in functional connectivity associated with neuroticism. Indeed, studies have shown that resting-state functional connectivity is strongly related to the underlying structural connectivity architecture (van den Heuvel et al., 2009) and that the strength of structural connections predicts the strength of functional connections derived from rs-fMRI (Honey et al., 2009).

Third, we calculated network measures on rs-fMRI data in the current thesis. For future research, it would be interesting to study the relationship between network measures calculated on the whole-brain and different functional subnetworks, such as efficiency, and behavioral vulnerabilities, such as maladaptive forms of emotion processing and emotion regulation (Bullmore and Sporns, 2012), during tasks. For instance, the worry (Chapter 3) and criticism (Chapter 5) task, that we applied in the current thesis, would be suitable for this purpose.

Fourth, we investigated the functional network organization on the basis of the whole resting-state scan (10 min) in the current thesis. However, it is also important to investigate smaller time spans, since it has been shown that both the strength and the directionality of functional connections changes over the course of minutes or even seconds. This is called ‘dynamic connectivity’ (for a review, see Hutchison et al., 2013). As the human brain is built hierarchically and functional subnetworks can be segregated in smaller functional subnetworks (e.g. the anterior and posterior part of the default mode subnetwork, Allen et al., 2014), it would be interesting to investigate how functional connectivity changes between these smaller subnetworks and with that, cooperation between them over time in different contexts (e.g. receiving criticism, Chapter 5) (Park and Friston, 2013). Prior research has shown that these dynamic cooperations follow a reliably recurring pattern of functional connectivity over time and hence, can be separated in a number of stable states (Allen et al., 2014). In future research, during rest as well as emotional tasks, one can investigate in which states individuals scoring higher on neuroticism dwell more and for how long. For instance, it may be possible that high compared to low neurotic individuals switch less between states and dwell longer in states wherein emotion and salience subnetworks play a more prominent role, than cognitive control subnetworks. Furthermore, transitions between states can be investigated and differences herein in relation to neuroticism.

Fifth, in the current thesis, we used a univariate approach for our graph analyses. However, it is known that univariate analyses do not capture the full complexity of brain networks (Simpson
et al., 2013). Multivariate approaches are currently being developed to investigate the complex dependence structure of networks and the effects of multiple network measures on the network organization (Simpson et al., 2013). Extensions are even developed to investigate dynamic or longitudinal networks in a multivariate way, called ‘doubly multivariate’ (Simpson et al., 2013). Besides investigating network measures, one can also include several important behavioral (e.g. task performance), psychological (e.g. questionnaire or experience sampling data) and genetic (e.g. genome-wide association (GWA) data) variables in multivariate analyses to concomitantly investigate their overall effect (i.e. explained variance) on the network organization in association with neuroticism (for a survey, see Simpson et al., 2013). Furthermore, network measures can be calculated on multimodal imaging data (e.g. MRI, fMRI, DTI, electroencephalography; EEG, magnetoencephalography; MEG) and included in multivariate analyses (Sui et al., 2013).

A specific class of multivariate statistical techniques is classification techniques (McIntosh and Misic, 2013). Network measures, such as efficiency and modularity, can be used as features in a machine learning classification method to discriminate individuals scoring higher on neuroticism from individuals scoring lower (Guo et al., 2012). Specifically, network measures calculated for both high and low neurotic individuals can be used as input for classifiers (i.e. classification algorithms) that ‘learn’ which network measures are important for distinguishing both groups. Subsequently, a classifier can be applied on data from an independent test sample to assess its performance in distinguishing high from low neurotic individuals based on ‘knowledge learned’ from the training sample. When the classification accuracy is high (i.e. significantly above chance level), one can examine which network measures have contributed to the discrimination between high and low neurotic individuals to learn more about the underlying neural mechanisms of neuroticism (for reviews on the method and its application, see Pereira et al., 2009 and Orrù et al., 2012, respectively). Furthermore, it would specifically be of interest to use this approach in a longitudinal study to predict the transition to a psychiatric disorder by using data of individuals scoring high on neuroticism at baseline who made or made not the transition to a disorder at follow-up (Orrù et al., 2012). This may better answer the question which neural mechanisms underlying high neuroticism make individuals more vulnerable to develop psychopathology.

Besides new analysis techniques, I suggest that our results should be replicated in a sample selected from the general population. Studies in the current thesis have been limited to high functioning students with a relatively high IQ that, possibly due to that reason, were able to compensate for (a part of) their difficulties in cognitive-emotional functioning related to neuroticism. However, by selecting a homogenous sample, we controlled for several important confounders, such as gender, age, education level and ethnicity, which increased our power.
9.5 Clinical implications

In this section of the discussion, I make some suggestions for clinical implications of research on neuroticism. Previous research has shown that it is difficult to accomplish a reduction of neuroticism levels, presumably because it is a stable trait and moderately heritable (Ormel et al., 2013a). However, there is evidence that neuroticism scores can change due to (life) experiences and are malleable (for a review, see Barlow et al., 2014 and Ormel et al., 2012; for a research article, see Jeronimus et al., 2013 and Riese et al., 2014). In the current thesis, we found that individuals scoring higher on neuroticism show alterations in emotion processing, salience processing and cognitive control compared to individuals scoring lower (Chapter 6 and Figure 1). These neural alterations may impose constraints on and bias information processing in high neurotic individuals, possibly leading to cognitive-emotional problems in daily life (e.g. Chapter 2, 3, 4, and 5) that need to be counterbalanced or attuned. There are two possible ways in which neuroticism can be clinically relevant. First, high neuroticism may be used as a risk marker to prevent individuals of making a transition from a healthy state to a clinical state. Specifically, the 5%-10% highest scorers on neuroticism have a high risk of developing psychopathology, that is, 45%-61% of these individuals develop a psychiatric disorder at one-year follow-up (Cuijpers et al., 2010). Preventive treatments may focus on increasing resilience in individuals scoring high on neuroticism to help them cope with negative experiences and protect themselves from developing clinical symptoms (Skodol, 2010). Resilience is associated with a well-differentiated and integrated sense of self (e.g. self-esteem, self-confidence, self-efficacy, a positive future orientation), strong reciprocal interpersonal relationships (e.g. sociability, emotional expressiveness and empathy), and adaptive coping strategies (e.g. problem-focused coping) (for a description on the resilient personality, see Skodol, 2010). Interventions that increase resilience may protect individuals scoring higher on neuroticism from developing a psychiatric disorder by increasing their abilities to cognitively control negative emotions (Skodol, 2010).

Second, it may make sense to try to reduce neuroticism levels in individuals with affective disorders (e.g. depression and anxiety disorders) to target putative and fundamental processes underlying these disorders (for a review, see Barlow et al., 2014). Prior studies have shown that i) affective disorders have a number of commonalities, due to high rates of comorbidity, broad treatment response across comorbid disorders and shared neurobiological mechanisms and that ii) a latent structure, called ‘neuroticism’, underlies the development of these disorders (Barlow et al., 2014). With the emergence of the DSM-III (1980), this latent structure was classified in a number of specific depressive and anxiety disorders (Barlow et al., 2014). According to Brown and Barlow (1995), a problem with this specificity is that current treatments are effective in reducing disorder specific symptoms, but do not lead to a reduction of general personality-related liabilities, such as neuroticism, that leaves patients vulnerable for relapse or the development of comorbid disorders. To this end, Barlow et al. (2011) have developed the unified protocol (UP)
for transdiagnostic treatment of affective disorders to alter negative reactivity patterns to emotions and subsequent avoidant coping, characteristic of high neurotic individuals. The first results seem promising in reducing neuroticism and disorder specific symptoms, but more research is required (Barlow et al., 2014). It is possible that UP decreases the observed biases in emotion and salience processing in high compared to low neurotic individuals, possibly leading to more adaptive coping styles (Barlow et al., 2014). It would be of interest to investigate graph measures calculated on the different functional subnetworks, such as local efficiency and the participation coefficient (as in Chapter 6), in individuals scoring high on neuroticism before and after resilience or UP treatment.

9.6 Conclusion

The aim of the current thesis was to investigate the neural mechanisms underlying neuroticism to gain insight into why individuals scoring high on this personality trait are more vulnerable to develop psychopathology. To this end, we conducted a series of meta-analytic, neuroimaging and genetic imaging studies. During rest (i.e. when the brain is in default mode), we found that the network organization of high compared to low neurotic individuals is organized less optimally with regard to efficient information processing and shows signs of functional disconnectivity. In addition, we demonstrated that subnetworks related to emotion and salience processing play a more prominent role in the network organization of high neurotic individuals, while subnetworks related to sensory(-motor) functions and cognitive control play a less prominent role (Chapter 6). This may impose constraints on and bias subsequent information processing during task performance (Menon, 2011), possibly leading to a range of problems in cognitive-emotional processing (e.g. Chapter 2, 3, 4, and 5). Furthermore, we found that genetic markers, namely 5-HTTLPR and COMT, have an impact on the functional network organization, also in interaction with neuroticism (Chapter 8). In short, possible answers to why high compared low neurotic individuals are emotionally more reactive to negative events or experience more negative emotions, may already be found in the basic network organization of the brain. Specifically, alterations in processes and neural correlates during resting-state may set in motion a chain of reactions, affecting other processes and neural correlates during task performance. Indeed, in the current thesis, connectivity and connectomic analyses have particularly been fruitful and should be further explored in future research. The findings may help explain why high compared to low neurotic individuals have an increased risk of developing psychopathology and may increase the knowledge for developing treatments that will prevent these individuals from transiting from a healthy state to a clinical state.