Summary and general discussion
SUMMARY AND GENERAL DISCUSSION

The primary purpose of this thesis was to investigate structural and functional brain abnormalities of Complex posttraumatic stress disorder (Complex PTSD) in adult female patients who experienced repeated child sexual and/or physical abuse. Child physical abuse and rape in women are far more likely to result in posttraumatic stress disorder (PTSD) than any type of trauma (10-20%) (Breslau et al., 1998), with risks up to 50% (Kessler et al., 1995). According to the Diagnostic Statistical Manual DSM-IV-TR (APA, 2000), customary PTSD symptom clusters are: 1) intrusive symptoms (flashbacks, nightmares), 2) avoiding and numbing of feelings, and 3) hyperarousal (sleep and concentration problems, irritability, increased alertness and startle responses). Child sexual and/or physical abuse is associated with psychiatric symptoms that extend beyond DSM-IV-TR 'simple' PTSD (Green et al., 2000). This syndrome has been brought under the heading of 'PTSD with associated features' in the DSM-IV-TR and is also known by clinicians as 'Complex PTSD' or 'Disorders of Extreme Stress Not Otherwise Specified' (DESNOS) (Ford, 1999; Herman, 1992; Pelcovitz et al., 1997; Zlotnick et al., 1996). Complex PTSD consists - apart from 'simple' PTSD symptoms - of the following symptom domains: I) problems in affect regulation (e.g. alteration between rage and affective emptiness, and impulsivity), II) dissociative symptoms (inability to remember certain periods of life, losing track of time, 'spacing out', feeling unreal), III) problems in self-perception (feelings of guilt and shame), IV) cognitions of the perpetrator (idealizing, revenge), V) relationships (inability to trust), VI) somatization and VII) loss of future perspectives. Complex PTSD is associated with high co-morbidity, especially depressive and dissociative disorders on DSM-IV-TR Axis I and borderline personality disorder (BPD) on Axis II (Ford & Kidd, 1998).

Neurobiological correlates of PTSD have been studied extensively in the last decades. PTSD is associated with structural and functional abnormalities in limbic structures, in particular the amygdala, a region associated with appraisal of danger and (conditioning of) fear responses, and hippocampus, a key structure associated with declarative memory and contextualization of fear responses (Elzinga & Bremner, 2002; Sapolsky, 2000). A meta-analysis of 21 structural imaging studies on adults with chronic PTSD (Karl et al., 2006) revealed a significantly smaller left amygdala volume and smaller hippocampus volumes that decreased with PTSD severity. Furthermore, regulating cortical structures, such as the prefrontal cortex (PFC), orbitofrontal cortex (OFC) and the anterior cingulate cortex (ACC), which are associated with fear extinction and emotion regulation are thought to be critically involved in the pathophysiology of PTSD (Rauch et al., 2006). ACC volume was found to be reduced in PTSD (Chen et al., 2006; Corbo et al., 2005; Kasai et al., 2008; Kitayama et al., 2006; Yamasue et al., 2003;
Kitayama et al., 2006; Rauch et al., 2003; Woodward et al., 2006) and the ACC volume appeared to be inversely related to PTSD symptom severity (Yamasue et al., 2003). According to fear conditioning models, PTSD is characterized by an exaggerated response of the amygdala to threat related stimuli, whereas activation is decreased in the medial PFC, OFC, and ACC as well as in the hippocampus (Rauch et al., 2006). This model has been confirmed by neuroimaging studies with symptom provocation as well as with cognitive activation paradigms (Bremner et al., 1999a; Bremner et al., 1999b; Bremner et al., 2003b; Bremner et al., 2003a; Bremner et al., 2004a; Driessen et al., 2004; Hendler et al., 2003; Protopopescu et al., 2005; Rauch et al., 2000; Semple et al., 2000; Shin et al., 1997; Shin et al., 1999; Shin et al., 2005). However, empirical data have been contradictory: functional imaging studies have shown decreased as well as increased activation in the hippocampus and PFC, ACC and OFC in PTSD (for review, see (Francati et al., 2007)). It is not clear to which extent trauma type and comorbidity, may affect these results. Complex PTSD patients have hardly been studied. Regrettably, there are far less imaging studies on child abuse than on adult trauma patients. Moreover, in these few imaging studies on child abuse PTSD patients it is not reported if patients fulfill criteria for Complex PTSD or for dissociative disorder or a personality disorder. This results in a gap of knowledge on this clinically important group with chronic disease courses, high risk of suicidal behavior and considerable use of (acute) general and psychiatric health services. This thesis investigates for the first time neurobiological correlates in a well-diagnosed Complex PTSD population.

The second purpose of this thesis was to investigate whether these abnormalities could be normalized by stabilizing treatment, according to the clinical guidelines for complex trauma related disorders. Clinical importance of the syndrome of Complex PTSD is its adverse effect on treatment outcome (Ford & Kidd, 1998): symptom severity of PTSD and presence of comorbid disorders is associated with high drop-out rates (McDonagh et al., 2005) and decreased efficacy of exposure (Foa et al., 2000) and Eye movement desensitization and reprocessing (EMDR) (Van Der Kolk et al., 2007). Comorbid axis II diagnoses call for more structured treatment (Cloitre et al., 2010; Lau & Kristensen, 2007) and predict worse outcome (Baars et al., 2011). Few studies have investigated neural correlates of effective therapy. To study neural correlates of effective stabilizing treatment in Complex PTSD, we screened patients for participation in a brain imaging study from our larger randomized control trial (RCT, n = 71) on the effectiveness of a stabilizing group treatment based on psycho-education and cognitive behavioral therapy for Complex PTSD (Dorrepaal et al., 2012b). Included patients (n = 33) and controls (n = 30) performed an emotional memory task and an emotional selective attention (Stroop) task while lying in a magnetic resonance imaging (MRI) scanner.
and responding by pushing a button box. We assessed: 1) psychological symptoms during scanning (state dissociation, subjective distress), 2) behavioral data (error rates, reaction times), 3) structural MRI (gray matter density), and 4) functional (Blood Oxygenation Level Dependent or BOLD response, which is a measure of neural cell activation) MRI outcome data. Baseline scans of patients were compared to the scans of non-trauma-exposed healthy controls, to find out if findings could be related to Complex PTSD. Next, patients were randomly assigned to treatment as usual (TAU) or to a stabilizing group treatment next to TAU and were all scanned both before treatment and after a treatment period of six months, in order to rule out time effects. We hypothesized that functional brain abnormalities in child abuse related Complex PTSD patients would be similarly to ‘simple’ PTSD based on the fear conditioning model. In addition, we hypothesized that - as was found in ‘simple’ PTSD - volumes of the hippocampus, amygdala and anterior cingulate cortex (ACC) would be diminished in Complex PTSD patients compared to controls. Finally, we hypothesized that functional abnormalities in this neural network could be normalized after treatment.

7.1. Summary of findings
Before starting our RCT, we performed a pilot functional MRI study using an emotional memory task in a sample of female outpatients with Complex PTSD (n = 9) and controls (n = 9) (Chapter 2). We aimed to investigate declarative memory function and medial temporal lobe activation and to test organizational and methodological issues; most importantly we wanted to know if it is possible for these complex and anxious patients to endure a brain scanning procedure. The main conclusion of this pilot study was that patients - despite high anxiety and dissociation levels - were able to comply with scanning procedures. We expected to have much drop-out during the diagnostic assessments and potentially threatening scanning procedure. Therefore, we were careful to not overtax the participants, for example by leaving interesting endocrinology measures out of the study, and to take much time in supporting them in preparation to and during the scanning procedure. Based on the fear conditioning model in ‘simple’ PTSD, we hypothesized that Complex PTSD patients would show worse declarative memory function with a preference for negative words relative to neutral words, and that this would be reflected by an increased amygdala response and a decreased hippocampus response in patients compared to controls. As was hypothesized, Complex PTSD patients showed impaired declarative memory for neutral words with a preservation of memory of negative words compared to controls. However, deep successful encoding of negative words was associated with an increased BOLD response in the left hippocampus, extending to the parahippocampal and fusiform gyrus in patients. In addition, both correct recognition of deeply encoded negative words and false alarms
were associated with increased activation in the left hippocampus. This is at odds with the findings in 'simple' PTSD and the fear conditioning model in which mainly decreased hippocampus activation had been found. It possibly reflects increased effort to adequately maintain memory performance and/or increased contextual coupling of (triggering) words with personal traumatic memories.

In Chapter 3 we aimed to further investigate declarative memory function in a new and larger sample of patients (i.e. the subsample from our RCT) with child abuse related Complex PTSD (n = 33) compared to non-trauma-exposed healthy controls (n = 30), extending the previous pilot study. Specifically, we wanted to investigate how symptom severity and comorbidity affect neurocognitive functioning in Complex PTSD. We simplified and adapted the fMRI emotional memory task to increase power and extended our regions of interest to the prefrontal cortex (PFC), considering its role in extinction and emotion regulation. Complex PTSD patients reacted relatively fast on negative compared to neutral words during encoding, which was correlated with higher subjective fear ratings in the scanner. BOLD response in the left posterior hippocampus was increased during successful encoding of negative words, which was a replication of the findings of our previous pilot study. Apart from that, we found increased BOLD responses in the left ventral and dorsal anterior cingulate cortex (ACC) extending to the dorsomedial PFC, which may indicate difficulty with dividing attention away from trauma related material. From the fear conditioning model it had been expected to find decreased BOLD responses in the ACC and hippocampus. Contrary to expectations as well, Complex PTSD patients showed enhanced BOLD responses in the left OFC/ventrolateral PFC during recognition. This is associated with impaired response inhibition, resulting in a tendency for more false alarms to negative words. These are presumably due to memory intrusions. Severity of child abuse and severity of depressive symptoms were positively correlated to activation in the ventral ACC and left posterior hippocampus, respectively, supporting attribution of complex trauma and comorbidity on axis I to the results.

In this same sample (i.e. the subsample from our RCT) (Chapter 4) we investigated if child abuse related Complex PTSD is associated with regional reduced brain volumes as compared to healthy controls. Patients were found to have reduced gray matter density - indicating volume loss - in the right hippocampus and ACC compared to controls as was expected from 'simple' PTSD studies. However, patients showed decreased OFC volumes as well, a brain region that is associated
with affect regulation, and this is more in line with imaging studies on borderline personality disorder (BPD). In addition, severity of impulsivity and anger - both borderline personality symptoms - were negatively correlated with hippocampus and/or OFC volume, indicating influence of comorbidity - now axis II - again.

Next, we investigated in this same subsample from our RCT, whether functional brain abnormalities associated with selective attention would normalize after effective treatment, using a classic and emotional Stroop task during functional MRI (Chapter 5). At baseline, Complex PTSD patients showed greater Stroop interference for trauma words compared to neutral and a trend for incongruent compared to congruent words. This was coupled with increased activation in the dorsal ACC and left anterior insula in the classic Stroop. After treatment, classic Stroop interference decreased, specifically in those patients receiving psycho-educational and cognitive behavioral stabilizing group treatment next to TAU. Moreover, in patients treated with stabilizing group treatment next to TAU, we found decreased activation - which we suggest may be interpreted as “normalized” - in the dorsal ACC and left anterior insula for the classic Stroop contrast, possibly reflecting increased selective attention and lower emotional arousal after treatment. This indicates that the interventions may have contributed to more cognitive control over Complex PTSD symptoms.

Finally in Chapter 6, we systematically reviewed 20 neuroimaging treatment outcome studies on 216 mainly ‘simple’ PTSD patients exposed to adult trauma and a minority of child abuse related PTSD patients treated with pharmacotherapy or psychotherapy and 88 WL, TAU or placebo control patients. In functional imaging studies, the strongest evidence came from 6 functional RCTs using symptom provocation after psychotherapy. After pharmacotherapy increased hippocampus volume has consistently been found in adult and child abuse related PTSD. Although not extensively studied, this was not the case after psychotherapy. During symptom provocation, adult trauma PTSD patients showed increased activation in the rostral/ventral ACC, superior frontal gyrus, OFC and hippocampus and decreased activation in amygdala and lateral PFC after psychotherapy and pharmacotherapy. These findings fit in the glucocorticoid-stress hypothesis and fear conditioning model. Child abuse related PTSD patients however, had a distinct functional activation pattern, showing decreased rostral ACC, OFC and superior frontal gyrus activation after treatment and no change on the level of the hippocampus, amygdala and lateral PFC.
7.2. Methodological considerations

Our study has both strengths and limitations. An important strength is the fairly large baseline sample size for imaging (33 Complex PTSD subjects and 30 controls) compared to other cross-sectional neuroimaging studies. It should be noted however, that this is less than half of the original RCT sample (n = 71), from which we excluded mainly patients who used other than SSRIs or low dose benzodiazepines (tricyclic antidepressants, antipsychotics), patients who feared the scanning procedure, and patients with specific MRI exclusion criteria (head trauma, metal implants, etc). Important to note is that the baseline sample for imaging (n = 30) tended to have a lower baseline score on PTSD severity (p = 0.054) than the overall RCT sample (n = 71), but there were no significant differences on severity of Complex PTSD, depressive and dissociative symptoms. This possibly lower PTSD score could be the reason for the finding that the (psychological) effectiveness was somewhat stronger in our subsample for imaging (n = 33) than in the overall RCT (n = 71). It seems that this subsample is a little less severe than the overall RCT sample and therefore we have to be careful not to generalize the imaging results to the total group. Another limitation is the relatively small sample for imaging and treatment outcome (16 patients); those are the patients who performed both a baseline and second fMRI. This further drop-out/exclusion after the baseline MRI was due to following reasons: 4 dropped out the RCT, 4 refused the second MRI, 3 started antipsychotics or mood stabilizers and 2 patients were pregnant before the second MRI. The treatment outcome sample for imaging (16) did not differ significantly, though, from the baseline sample for imaging (33) on severity of PTSD, Complex PTSD, depressive and dissociative symptoms and as such can be generalized to the baseline imaging group.

Furthermore, our study is one of few studies with extensive assessment of comorbidity on Axis I and II of DSM-IV-TR and with a diagnostic interview for Complex PTSD (SIDES). We consider this systematic assessment as a necessary requirement to investigate differences between PTSD and Complex PTSD. In order to investigate a representative sample, we included patients with suicidal and self-injurious behavior and/or high dissociation scores - while DID was excluded for reasons of treatment indication. This ‘reality matching’ sample increases generalizability. For the same reason we allowed the use of stable SSRIs and low dose benzodiazepines. Employing very few exclusion criteria, we added findings to an identified gap of knowledge on this child abuse related Complex PTSD population.
We used event related functional MR-imaging, which has advantages over e.g. PET imaging. PET imaging is restricted to block designs, in which all events - correct and false - are analyzed together. Our event-related fMRI studies provided the possibility of post-hoc selection of correct and false trials, such as we did in analyzing our memory task data (Chapter 2 and 3). However, separating correct from false trials reduces the number of observations and inherently reduces power. For that reason, we used a block design in our Stroop treatment out com study (Stroop task, Chapter 5).

We used mainly emotional imaging paradigms (emotional variants of a declarative memory task and of a selective attention/Stroop task, or other examples are: symptom provocation by trauma scripts or angry faces) and therefore especially limbic and medial frontal areas will be activated. Using cognitive task paradigms (e.g. executive function tasks as the Tower of London or a working memory task, e.g. N-back), would show more dorsal frontal cortex activation and resting state studies would activate default networks.

We used voxel based morphometry (VBM) and no manual tracing for our structural study. Although VBM is an objective and comprehensive assessment of regional anatomical differences throughout the brain (Ashburner & Friston, 2000; Ashburner & Friston, 2001), it has its limitations as well. False positive or false negative VBM findings cannot be completely ruled out, especially with regard to the detection of structural abnormalities in very small brain regions (Wright et al., 1999). Additionally, VBM may be biased against finding group differences in areas that are spatially complex (Davatzikos, 2004). And finally, we cannot rule out the possibility that the abnormalities detected by VBM in our study reflected group differences in the shape of brain structures rather than their volume (Corbo et al., 2005).

We excluded current alcohol or drug dependence or abuse for the imaging part of the RCT, because it is generally known that this could affect regional brain structure. It is possible that we included patients with a history of substance abuse or dependence, because we did not request to refrain from alcohol or drug use prior to entry into the study. The allowed use of medication (SSRIs or benzodiazepines, no other psychoactive drugs) can be a potential limitation as well, although our patients still fulfilled criteria for Complex PTSD while using this medication and our main results did not differ between medicated and non-medicated patients. Recent data from our group have indicated that brain activation during
emotion processing and cognitive task performance is similar in medicated and non-medicated patients with depressive disorders and/or anxiety disorders (van Tol et al., 2011) and volumetric differences were not attenuated in BPD patients being treated with psychotropic medications (Ruocco et al., 2012).

Because of the characteristics of our (female) child abuse related Complex PTSD population, results cannot be straightforwardly generalized to other PTSD populations. Because we chose to include a non-trauma-exposed healthy control group, we cannot exclude the possibility that baseline differences were related to trauma exposure rather than to PTSD diagnosis per se. Furthermore, the frequent comorbidity of e.g. major depressive disorder (MDD) makes it difficult to disentangle the effects of different axis I diagnoses.

7.3. Complex PTSD and role of comorbidity?

Although we did not directly compare ‘simple’ PTSD patients with Complex PTSD patients, we found some evidence that structural and functional brain abnormalities in Complex PTSD in the present studies differ from the findings in the literature which are mainly on ‘simple’ PTSD. Complex PTSD patients showed some similarities with the literature on ‘simple’ PTSD patients: decreased hippocampus volume and decreased ACC volume (Chapter 4). However, we found more differences:

- Increased (instead of decreased) left posterior hippocampus and ACC/mPFC activation during encoding of negative words (Chapter 3)
- Decreased (medial) OFC volume (Chapter 4) and increased (lateral) OFC/VLPFC activation during falsely recognizing negative words (Chapter 3)
- No increased amygdala activation (Chapter 3), or decreased amygdala volume compared to healthy controls (Chapter 4).

Complex PTSD is associated with high comorbidity on Axis I (depressive disorders and dissociative symptoms) and Axis II (borderline personality disorders). Unfortunately, PTSD imaging studies neither assess Complex PTSD with a Structured Interview for Disorders of Extreme Stress (SIDES) nor report associated features of PTSD as described in the DSM-IV-TR. Comorbid disorders on Axis I are reported sometimes, but in general measures of Axis II diagnoses are absent. We performed extensive detailed assessments of Complex PTSD and of comorbidity (e.g. 64 % depressive disorders; 33-42 % borderline personality disorder). Our Complex PTSD sample was too small to directly compare different diagnostic groups within our sample with sufficient power. Therefore, we tried to address complexity of PTSD indirectly via severity of trauma and severity of comorbid symptoms, exploring correlations of trauma and symptom severity
with functional and structural imaging data. We used the SIDES as a diagnostic interview for the assessment of the disorder and not as a severity measure of Complex PTSD, to be able to compare severity with other studies on child abuse related PTSD and because there are no imaging studies - as far as we know - in which the SIDES has been used. We found that:

• Severity of child abuse was negatively correlated with hippocampus volume (Chapter 4, Table 4).
• Severity of child abuse was negatively correlated with ACC volume (Chapter 4) and positively correlated with ventral ACC activation (Chapter 3), meaning the more severe the abuse the smaller the volume and the higher the activation.
• Severity of depressive symptoms positively correlated with left posterior hippocampus activation during successful encoding of negative words (Chapter 3).
• Severity of state dissociation (measured by the Clinician Administered Dissociative State Scale, CADSS) positively correlated with bilateral hippocampus activation during successful encoding of negative words.
• Severity of impulsivity and anger - both symptoms from the borderline personality severity index - negatively correlated with respectively left posterior hippocampus and OFC volume (Chapter 4).

7.3.1. Comorbid depressive and dissociative symptoms
Brain volume reductions of the hippocampus and ACC are presumably not specific for PTSD. In Complex PTSD, reduced hippocampus and ACC volumes were not associated with severity of depressive symptoms (as measured by the Beck Depression Inventory or BDI) or with severity of dissociative symptoms (DES) (Chapter 4, Table 4). Major Depressive Disorder (MDD), just as PTSD, is associated with smaller hippocampal volume, especially after multiple episodes (Videbech & Ravndal, 2004). Interestingly, a comparison of MDD patients with child abuse versus non-exposed MDD patients revealed hippocampal volume loss in the trauma-exposed group only (Vythilingam et al., 2002). In our study, hippocampus volume was correlated with severity of child abuse (See Chapter 4, Table 4) and was found to be reduced in disorders highly associated with child abuse, such as borderline personality disorder (BPD) (Ruocco et al., 2012) and dissociative identity disorder (DID) (Ehling et al., 2007; Vermetten et al., 2006). Hippocampal volume was correlated with severity of dissociative symptoms in child abuse patients with PTSD and/or a dissociative disorder (Stein et al., 1999) and in DID patients (Ehling et al., 2007). Reduced ACC volume has similarly been found in MDD, but only after three or more episodes (Yucel...
et al., 2008), and in BPD as well (Hazlett et al., 2005; Tebartz et al., 2003). Thus, volume loss in the hippocampus and ACC are specific for PTSD, but may be specific for trauma exposure or represent a feature of different kinds of (early) trauma related psychiatric disorders and is associated with the level of dissociation.

Although volumes of the hippocampus and ACC were found to be decreased in Complex PTSD (Chapter 4), activation in these areas was increased (Chapter 2, 4, 5). This combination of smaller volume and higher activation is supposed to indicate a compensatory mechanism (van den Heuvel et al., 2005a). It is not said that this overcompensation leads to improved functioning of these structures. We suppose that increased activation in our study does not reflect efficiency but merely increased (inefficient) effort. Increased hippocampal and ACC activation as found in our Complex PTSD population has also been found in MDD (Drevets et al., 2008; Krause-Utz et al., 2012; Videbech et al., 2002; Werner et al., 2009b) and in PTSD patients with comorbid depressive disorder (Lanius et al., 2007). In our Complex PTSD population, left (para) hippocampal activation during encoding of negative words was correlated with severity of depressive symptoms, and not severity of PTSD symptoms (Chapter 3, Figure 3). In PTSD patients, comorbidity of a depressive disorder may account for relatively less activation in limbic areas (amygdala, insula) and increased ACC activation (Lanius et al., 2007) (Kemp et al., 2007). Comorbid dissociative symptoms were associated with increased dorsal ACC activation as well during script-driven imagery together with increased insula and decreased parahippocampal activation in patients with BPD and comorbid PTSD (Ludascher et al., 2010). It appears that in Complex PTSD patients, depressive and dissociative symptoms counteract PTSD effects. Depressive and dissociative symptoms are associated with less arousal, and we suppose that predominance of depressive or dissociative symptoms can be seen as the most severe stage of Complex PTSD, as exhaustion after a chronic course, in which interventions need to be more extensive while having less effect. Similarly, it has been found that in PTSD with comorbid MDD, HPA-axis sensitivity is in line with MDD patients and not with PTSD (See also 7.4). It may be argued, that comorbidity of depressive disorders in our sample is inherent to the Complex PTSD diagnosis and probably reflects severity of Complex PTSD. Severity of dissociative symptoms, as measured by the Dissociative Experiences Scale (DES), was not significantly correlated with functional brain abnormalities in Complex PTSD (Chapter 3, Figure 3). However, focusing on the imaging event itself, state dissociation (measured by the Clinician Administered Dissociation State Scale or CADSS) was positively correlated with bilateral hippocampus activation during successful encoding of negative words (Chapter 3, note 1).
7.3.2. Comorbid borderline personality disorder

Apart from hippocampus and ACC volume decreases, we found reduced volume of the medial OFC in Complex PTSD (Chapter 4, Table 2), a structure that is associated with response inhibition, extinction of conditioned fear, emotion regulation (Milad & Rauch, 2007; Milad et al., 2007; Phelps et al., 2004), and impulsivity (Silbersweig et al., 2007). Patients with borderline personality disorder (BPD) show both structural (Chanen et al., 2008; Tebartz et al., 2003), and functional (Silbersweig et al., 2007; Soloff et al., 2003) OFC abnormalities, which have not been described in PTSD as far as we know. These results suggest that neural correlates of Complex PTSD are more severe than in ‘simple’ PTSD. Complex PTSD patients showed increased activation in the lateral OFC (Chapter 3, Table 2), during an emotional declarative memory task, which may reflect attempts to inhibit false positive errors. This is supported by our findings that patients showed increased levels of subjective fear during imaging and faster reaction times to negative words, resulting in higher false alarms to negative words. False alarms could be interpreted as intrusive errors, with trauma related stimuli activating autobiographical memories confounding task performance (Whalley et al., 2009). The increased OFC activation was extending into the left ventrolateral PFC (including Broca’s area), a region found to be activated in most memory studies, more in successful encoding than in successful recognition (Spaniol et al., 2009). The left ventrolateral PFC has been implicated in semantic rather than declarative memory, stressing the importance of processing the meaning of the stimuli and the interdependence of encoding and retrieval processes (Rugg et al., 2002; Rugg et al., 2003).

Severity of impulsivity and anger, as measured by the borderline personality severity index (BPDSI), negatively correlated with hippocampus and/or (medial) OFC volume (Chapter 4, Table 4). Both impulsivity and anger are clinically relevant symptoms of Complex PTSD and are characteristics for BPD as well. Treatment outcome in PTSD patients was found to be negatively affected by anger severity (Cloitre et al., 2004; Feeny et al., 2002; Foa, 2000; Orth et al., 2008). Symptoms of impulsivity (e.g., self-mutilation and suicide efforts) tend to resolve more quickly than affective symptoms (e.g. anger regulation), which may represent more enduring aspects of the disorder (Zanarini et al., 2007). It has been suggested that Complex PTSD contains an externalizing subgroup (with predominantly anger and impulsivity, e.g. aggression towards others or self-injurious behavior as in BPD) and an internalizing subgroup (with predominantly sub-assertiveness and avoidance; cluster C personality features) (Dorrepaal et al., 2012a). A post-hoc analysis on our data revealed that patients with both Complex PTSD and BPD showed smaller gray matter density in the dorsal ACC and OFC than
patients with Complex PTSD and a cluster-C personality disorder, implying that the externalizing subgroup had more severe abnormalities than the internalizing subgroup. These results should be interpreted with care, however, because of the small sample size of this post-hoc analysis.

7.4. Neurobiological models

7.4.1 Fear conditioning model and the glucocorticoid-stress hypothesis

Posttraumatic stress disorder (PTSD) is one of most studied anxiety disorders, as it involves a clearly defined etiology and its pathogenesis has been explained from a glucocorticoid-stress model, providing a link to animal research (Sapolsky, 2000; Toffanin et al., 2011). According to this model, excessive and/or prolonged stress may result in Hypothalamic-pituitary-adrenal (HPA) axis dysregulation due to increased secretion of glucocorticoids. Increased binding of glucocorticoids to its receptors results in impaired neurogenesis and leads to cell loss (Sapolsky, 2000) or reversible dendritic retraction (Conrad, 2008), eventually damaging the hippocampus and prefrontal areas. We found decreased hippocampus and ACC volumes in Complex PTSD, similar to findings in ‘simple’ PTSD. The hippocampus and ACC have a high density of glucocorticoid receptors (GRs) which makes these structures particularly sensitive to the influence of high circulating cortisol levels. GRs in the ventral ACC are likely to have a key role in the negative feedback mechanism of cortisol secretion and impaired ACC function may contribute to cortisol hypersecretion during stress (Drevets et al., 2008). Evidence for recovery of the hippocampus by neural cell growth has been found in animal studies (McEwen, 2001) but also in humans treated for Cushing’s disease, a disorder involving pathologically increased glucocorticoid secretion due to a pituitary adenoma, and very recently in depressive disorder (Boldrini et al., 2012). Therefore, the glucocorticoid-stress model may explain, at least in part, functional and morphological changes in stress-related neuropsychiatric disorders and their recovery. In healthy subjects, increased cortisol secretion ceases after terminating the stressor, but hypercortisolemia tends to be prolonged in depressive disorders. In contrast, baseline cortisol is reportedly decreased in PTSD, specifically in PTSD after child abuse (Yehuda & Seckl, 2011) and after physical and sexual abuse (Meewisse et al., 2007). Along with decreased cortisol levels in PTSD one would expect “recovery” from hippocampus atrophy just as seen in treated Cushing disease, but we suppose this is not the case because emotional triggers still give rise to transient cortisol increases in PTSD (Elzinga et al., 2003).

In contrast to our expectations based on the fear conditioning model, we did not find increased amygdala responses in patients compared to controls while this is a core feature of abnormal fear conditioning (Ledoux, 2002). Actually, patients showed an interference effect on trauma words as well as amygdala activation,
but this was also present in controls and therefore did not result in significant differences. At first, we supposed that Complex PTSD patients may have over-reactive amygdala, but that our design was not sensitive to detect it: employing a verbal paradigm instead of a visual or imagery instruction paradigm decreases the chance of provoking intrusions and measuring an increased amygdala reaction (Costafreda et al., 2008). However with the same memory and Stroop paradigm increased amygdala reactions have been found in other anxiety disorders in our lab (van den Heuvel et al., 2005b). Second, our inclusion criteria may have been responsible for missing this finding: the use of SSRIs in over half of our population could have dampened amygdala activation (Lanius et al., 2010b). However, as explained above, brain activation during emotion processing and cognitive task performance is similar in medicated and non-medicated patients with depressive disorders and/or anxiety disorders (van Tol et al., 2011). Third, it may be argued amygdala activation would not only occur during trauma or negative stimuli but also during neutral or baseline stimuli, resulting in ceiling effects and confounding group by task interaction analyses. We performed post-hoc analyses (not reported in this thesis) of early versus late responses in the blocks with trauma words, but there was no evidence for decreased habituation of amygdala reactivity in patients. Interestingly, in a meta-analysis on functional neuroimaging in different anxiety disorders, increased amygdala activation was more frequently observed in social and specific anxiety disorders than in PTSD (Etkin et al., 2005). Furthermore, in more Complex populations, e.g. in patients with borderline personality disorder, there is amygdala hyper-reactivity to emotional faces, but it is negatively correlated to state dissociation (Krause-Utz et al., 2012). It is possible that dissociative symptoms are a survival strategy helping to down-regulate excessive amygdala activation. Also, in chronic forms of PTSD there could be habituation or exhaustion of the amygdala, resulting in flattened feelings or feelings of emptiness. Thus, maybe we did not miss an over-reactive amygdala, but possibly patients with Complex PTSD do not show over-reactivity in this brain structure (anymore).
Because of the more frequent comorbidity with personality disorders and the relationship with emotion regulation and specifically anger regulation, ongoing this study we expected to find more abnormalities on the level of the insular cortex instead of the amygdala (McHugh et al., 2012; Fusar-Poli et al., 2009), which was indeed found using our Stroop task. Complex PTSD patients showed increased emotional Stroop interference for trauma related words compared to controls. Next to increased dorsal ACC activation compared to controls in the classic Stroop contrast, there was increased activation in the left anterior insula. The insula is associated with experiencing complex, intense emotions such as anger and disgust (Wright et al., 2003). The anterior insula is connected to ventral ACC regions generating emotional awareness (Taylor et al., 2009), and is part of a ventral attention network (Eckert et al., 2009). Increased anterior insula activation may therefore indicate high arousal during task performance.

Apart from the absent amygdala activation, the present study demonstrates functional abnormalities in Complex PTSD in the frontolimbic brain circuit in opposite direction to that predicted by the fear conditioning model: increased activation in the hippocampus, ACC and prefrontal regions. This fear system response could be understood as over-compensation of a failing or exhausted fear circuit, due to early, chronic, repeated trauma exposure. A similar - opposite - pattern has been found on the level of the HPA-axis, which influences hippocampus and prefrontal functioning importantly. In MDD decreased suppression results in high basal stress hormone (cortisol) levels; in contrast in PTSD, increased HPA-axis suppression results in low baseline cortisol blood levels although alternating with repetitive short-lasting increases triggered by intrusions. In borderline personality disorders - with highly prevalent abuse histories and high comorbidity with (Complex) PTSD - no HPA-axis changes have been found. However, splitting the group in two, BPD patients with comorbid PTSD and MDD determine the direction of HPA-axis suppression (Wingenfeld 2007), although an earlier study identified severity of child abuse to be decisive (Rinne et al., 2002). Disentangling effects of comorbidity from type of trauma is to be continued.

As such, our results indicate that the fear conditioning model of PTSD falls short in explaining the neurophysiologic correlates of memory function as found in the present Complex PTSD population. Results of the present study demonstrate functional abnormalities in Complex PTSD in the frontolimbic brain circuit also implicated in fear conditioning models, but generally in the opposite direction, which may be explained by trauma severity and severity of comorbid disorders in Complex PTSD.
7.4.2 An emerging neurobiological model: a dissociative subtype of PTSD

Not only fear regulation but problems in regulating a diversity of emotions represent the first symptom domain of Complex PTSD. It consists of being overwhelmed by emotions (fear, anger, sadness) without having sufficient capacity to soothe oneself leading to self-destructive behavior, suppression of anger or in contrast acting-out of aggression, risk-taking behavior and impulsivity and problems in sexual behavior. This is combined with the second symptom domain of Complex PTSD: dissociation, which is an adaptive defense mechanism in response to high stress or trauma experience characterized by memory loss and a sense of disconnection from oneself or one’s surroundings. It helps patients to endure high levels of stress and is as such advantageous on the short term, but leads to certain negative consequences on the longer term: patients are less alert to upcoming danger, miss crucial practical and social information, leading to personal and relational problems. High stress will lead evolutionarily to fight or flight responses, but also to a freezing reaction when the alarm system is overwhelmed, just like the little rabbit which is blinded by the headlights of a car and paralyzed for a moment. Complex PTSD is characterized by high levels of dissociation. The problem with dissociation, or the excessive modulation of affect, is that emotions will be therapeutically unattainable giving rise to the need of treating dissociative symptoms (e.g. by stabilization techniques) before trauma processing (e.g. by prolonged exposure, imaginary rescripting or EMDR) can be performed.

From a neurobiological point of view different subtypes of PTSD have been described (Lanius et al., 2010c). A dissociative subtype is characterized by an over-modulation of emotions, while the more common non-dissociative or intrusive subtype involves under-modulation of emotion leading to re-experiencing and hyperarousal symptoms. Several independent imaging studies revealed a dissociative subtype of PTSD patients showing increased frontal activation (ACC/ medial PFC) along with decreased limbic activation during exposure to a traumatic script, and an intrusive subtype with decreased frontal and increased limbic activation (Hopper et al., 2007; Kemp et al., 2009; Lanius et al., 2002; Lanius et al., 2010c; Ludascher et al., 2010). According to this model, patients can show both over- and under-modulation at different time points (e.g. in DIS, see (Reinders et al., 2006)), but patients with prolonged traumatic experiences such as child abuse or chronic combat trauma show mostly chronic symptoms of dissociation (Lanius et al., 2010c). In the present highly dissociative population, we found increased frontal activation and absent amygdala activation. In our memory paradigm, we found a positive association between severity of state dissociation (measured by the CADSS) and bilateral hippocampus activation during successful encoding of negative words. The only odd finding is the
increased insula (limbic) activation found in our Stroop fMRI study, which would refer to an intrusive or non-dissociative subtype. Anterior insular activation was found to be positively correlated with re-experiencing symptoms (Hopper et al., 2007) in a mainly non-dissociative type I trauma PTSD population and insula function is described as mediating the neural representation of somatic aspects of emotional states. On the other hand, (more posterior) insular activation has been positively correlated with dissociation scores in BPD patients with PTSD during traumatic scripts and increased insula activation resembles a super-suppression of affective states during dissociation (Ludascher et al., 2010). It seems that in our patients at the same time a dissociative as well as an intrusive response has been observed, reflecting a dissociative but simultaneously - or quickly alternating to - a hyper-aroused state. In a sense dissociation could be seen as a regulatory strategy used to endure extreme arousal by inhibiting limbic activation through frontal hyper-activation, inhibition of limbic system however not being always complete.

From all different studies presented here on the neurobiological correlates of PTSD, we conclude the central neural substrate of Complex PTSD being the network/feedback loop of the limbic system (insula), hippocampus and the prefrontal cortex, anterior cingulate and orbitofrontal cortex. Trying to unravel influences of comorbidities (“Is it a mood disorder? An anxiety disorder? Is it a disturbance of anger? Is it a bipolar disorder or a personality disorder”) does not always help us further in very complex patients. There is a need to look more dimensionally - over diagnoses or “trans-diagnostic approach” (Elzinga, BM, oral communication) - and try to see commonalities in a “posttraumatic depressive disorder”, a “posttraumatic stress/anxiety disorder” or a “posttraumatic personality disorder” and the concept of Complex PTSD as a way to integrate very different reactions to prolonged interpersonal traumatic experiences. For treatment purposes, it is less important which prior diagnosis has been established, but if there is an indication for trauma related psychotherapy and in what form.

In Complex PTSD the same network of emotion regulation seems to be involved as in PTSD, although in an over-modulated/compensatory way, because of a long-lasting effect of early and prolonged trauma (and possibly of the associated neglect). Reductions in volumes of the amygdala, hippocampus and ACC in PTSD have been attributed to both environmental (i.e., stress) and genetic factors. Evidence from a human twin study suggests that ventral ACC volume losses would be acquired or stress induced, via binding of the stress-hormone cortisol to densely present glucocorticoids receptors in this structure (glucocorticoid-stress hypothesis) (Kasai et al., 2008). This is supported by our finding that severity of child abuse was negatively correlated to ACC volume. Child abuse was
also positively correlated to ventral ACC activation. Particularly early life stress, during a ‘window of susceptibility’, may have profound and enduring effects on the regulation of stress later in life. Because of the rapid development of the human hippocampus in early childhood, we suppose that in child abuse which is associated with increased cortisol levels at the time of the trauma, damage has already been done in early childhood. It has been shown that the risk for adult PTSD is higher (e.g. in veterans or abused women) if there has been child abuse as well (Clancy et al., 2006; Weisbart et al., 2008). A history of child abuse is related to increased neuro-endocrine stress reactivity, which is further enhanced when additional adult trauma is experienced (Heim et al., 2002). There is also evidence that different brain regions have unique periods of heightened sensitivity to the effects of early stress (Teicher et al., 2006). Although hippocampal volume was not different in a direct comparison of a small sample of child abuse PTSD patients with patients after a single adult trauma (Bonne et al., 2008), the above-mentioned meta-analysis (Karl et al., 2006) revealed that child abuse had a greater impact on hippocampus volume than adult trauma.

We assume that as a neural substrate of Complex PTSD, apart from the hippocampus, the anterior cingulate structure is central, together disturbing an emotional network linked to processing of threat. High circulating levels of stress hormone at the time of the early trauma could have damaged its volume. Later in life, when by exhaustion basal cortisol levels are low, repeated cortisol upsurges during triggers of traumatic material can put the ACC in an overdrive, and this is associated with difficulty to divert attention away from trauma related stimuli (Carter & van Veen, 2007) or, in other words, absorbed by traumatic material or ‘dissociated’ from daily life. From a twin study it is supposed that ACC atrophy is related to stress and not a pre-existing risk factor that could increase the vulnerability to develop PTSD (Kasai et al., 2008). With regard to hippocampal atrophy in PTSD the evidence is mixed. On the one hand, a twin study points at hippocampal atrophy as a genetically determined risk factor to develop PTSD rather than as a result of stress (Gilbertson et al., 2002). This could explain why only 50% of people develop PTSD after prolonged child abuse. On the other hand, exposure to chronic stress and cortisol has been shown to damage the hippocampus in prospectively designed animal studies (Sapolsky, 1996). Compatible with emerging evidence for neural plasticity (Sapolsky, 2003), successful pharmacotherapy for PTSD was associated with enlargement of the hippocampus (Vermetten et al., 2003).
7.5. Treatment effects

As said above, being alternatively dissociative and extremely aroused makes it difficult to learn, or to profit from therapies which rely on learning, such as cognitive behavior therapy. It makes sense to focus firstly on strategies to regulate emotions in a more balanced way. Although extinction learning - basis for exposure therapies - involves mainly the ventromedial PFC to regulate increased limbic responses (Phelps et al., 2004), emotion regulation by cognitive restructuring or “re-appraisal” relies on the dorsolateral PFC to limbic activation (Ochsner et al., 2002), while dorsomedial PFC is important for self-related processing and the dorsal ACC for monitoring the need for (further) cognitive control as an alarm or control system for the dorsolateral PFC to be successful in reappraisal. “Suppression” is another cognitive emotion regulating strategy, which also relies on activation of the dorsolateral PFC, but although it decreases experience of negative affect, it does not result in decreased amygdala and insula responses (Goldin et al., 2008).

Anxiety and mood disorders are increasingly studied and neurobiological treatment effects are revealed. For example, in PTSD increased bilateral hippocampus volume after treatment can be seen as “normalization”, because it changes in the direction of healthy controls and is correlated with psychological improvement. Because of its supposed role in memory function, hippocampal growth would be expected to be related to memory improvement, but this was not (yet) confirmed. Improved function of the hippocampus however, can be measured as better contextualization (less generalization) of fear responses and this is a function more directly involved in clinical symptoms of intrusions and dissociation. Exposure and EMDR, but also stabilizing treatments aim at better contextualization or less generalization of fear responses. Interestingly, larger pre-treatment (para-) hippocampal volume predicts better response to psychotherapy in ‘simple’ PTSD (Nardo et al., 2010).

In our Complex PTSD patients, we found decreased activation of the anterior insula and dorsal ACC after six months of treatment (Thomaes et al., 2012). Furthermore, in a regression analysis we found that clinical improvement measured with the CAPS was associated with this decreased activation in dorsal ACC and anterior insula. We suggest that these changes point to normalization of dorsal ACC and insula activation after stabilizing group treatment, which may reflect fewer response conflicts or improved selective attention, and lower emotional arousal. It has been debated if the Stroop paradigm measures automatic attention processes or rather strategic processes of effortful avoidance of threat cues (Bar-Haim et al., 2007; Buckley et al., 2000), possibly indicating greater cognitive control following treatment in the current study.
The treatment effect over time was mainly driven by the experimental group: only patients, who received the stabilizing group treatment based on psycho-education and cognitive behavioral therapy, showed decreased bilateral dorsal ACC and left anterior insula activation after treatment, while TAU patients did not.

Interestingly, in ‘simple’ PTSD decreased ventral ACC activation predicted better response to cognitive behavioral therapy (CBT) including exposure (Bryant et al., 2008a), and this may imply that Complex PTSD patients - with high ACC activation - will not favorably respond to this form of treatment. In a RCT (Peres et al., 2007) in adult patients with (partial) PTSD and mixed trauma, significantly increased activation was observed following psychotherapy in the left anterior cingulate, together with decreased activation in the left amygdala after treatment. Decreased amygdala activation predicted better response to CBT (exposure and cognitive restructuring) (Bryant et al., 2008a) and increased amygdala volume predicted better response to EMDR (Nardo et al., 2010). We did not find differences of amygdala responses in patients with Complex PTSD compared to controls, or pre- compared to posttreatment.

7.6. Future research
More research is needed in complex populations. Patients with Complex PTSD or personality disorders are not different in willing and being able to participate adequately from patients with short term psychiatric diagnoses. On the contrary, because of their life long burden, they are motivated to do even invasive research to help to unravel the mysteries of their scars, hoping this will lead to better treatments. The high preparedness to participate in a complex study as the one presented, suggests it is not necessary to exclude these Complex PTSD patients.

More uniform diagnosing complexities of trauma is needed. Studies from experts in the Netherlands (Langeland, Draijer, Boon, Hart and others, LCVT, 2008), have implemented a set of diagnostic instruments, including trauma and neglect measures, (Complex) PTSD measures, dissociation measures, personality measures and quality of life measures. Abroad the same debate is going on, focused on the validity of the SIDES and implications for the DSM-V-TR (Resick et al., 2012a; Bryant, 2012; Goodman, 2012; Resick et al., 2012b; Wolf et al., 2012).

Future research should aim to further investigate the role of trauma, PTSD and severity of (comorbid) depressive disorders, dissociative disorders and borderline personality disorders by directly comparing diagnostic groups. In future studies we would like to include a trauma-exposed comparison group to determine whether reported findings are related to trauma-exposure or (Complex) PTSD or
both. Also we would like to directly compare PTSD with and without comorbid psychopathology or 'simple' with Complex PTSD. We would like to perform head-to-head comparisons of non-trauma-exposed healthy controls, trauma-exposed non-PTSD controls, trauma-exposed PTSD patients, trauma-exposed Complex PTSD patients, trauma-exposed and non-trauma-exposed borderline personality disorder patients, exposed and non-exposed bipolar disorder patients, exposed and non-exposed depressive disorder patients and exposed and non-exposed patients with OCD or other anxiety disorders.

Additionally, it is important to investigate the impact of emotional neglect, which is nearly always involved in prolonged child abuse. A quadrant is proposed to indicate the influence of child sexual and physical abuse on the x-axis and of emotional neglect on the y-axis (Draijer N, 2003; Draijer & Langeland, 2009): the more child sexual and physical abuse, the more severe the PTSD or trauma related disorders, ranging from 'simple' PTSD to Complex PTSD with dissociative disorders NOS to dissociative identity disorder at the extreme; and the more emotional neglect, the more severe personality disturbances and less self-soothing capacities. This quadrant could guide treatment choices, for example to decide whether stabilization treatment aimed at affect regulation should precede trauma focused interventions or vice versa.

I stress the importance of investigating emotion regulation problems non-categorically over diverse disorders. In depressive and bipolar disorders, in different anxiety disorders, such as OCD, and in bipolar disorders, emotional dysregulation is presenting in different ways than in a population with personality disorders, dissociative disorders and with PTSD. Still it is necessary to look in a dimensional way for overlapping features, specifically in case of a history of child abuse. Studying predisposing factors for the course of psychiatric disorders developing after child abuse and predictive factors for effects of treatment ('profiling', see (Beekman et al., 2012)) would be directed to different themes: 1) vulnerability genes predisposing to development of psychiatric disorders, 2) severity of trauma and abuse as well as emotional neglect, 3) temperament or emotion regulation capacities and its neurobiological correlates, and 4) chronicity or recurrence of psychiatric episodes.

Neuroimaging is a fundamental research area which is maybe difficult to directly implement, but it can help constructing an enriched vocabulary to comprehend psychiatric disorders and its development. This may strengthen the credibility of therapeutic interventions and improve psycho-education for medicines, psychotherapists and - most importantly - patients. Because increased knowledge implies increased power to change behaviors.
In further analyses of our RCT data we plan to study whether there are treatment effects on declarative memory and if it is associated with activation changes in the hippocampus, and if hippocampal volume increases after successful psychotherapy. There is also a need for conducting a study with a visual - instead of verbal - material, to see whether increased amygdala responses will be found in Complex PTSD and to study emotion regulating strategies. Most treatment imaging effects studies used symptom provocation paradigms. Future studies should use more cognitive and resting paradigms to fill these gaps. We recommend studying if hippocampal volume can increase with psychotherapy - as with pharmacotherapy - in both adult trauma and child abuse and more complex symptoms.

Also, we stress the importance of studying treatment options for this specific Complex PTSD group and its effects on brain activation. The ZONMW grant we received for this study was aimed to build bridges between (fundamental) research and clinical practice. The ultimate goal with our neuroimaging studies is to investigate if treatment effect is associated with improvement on a neural level, to refine clinical diagnosis and to predict treatment outcome in Complex PTSD which would help in clinical decision making and individualize treatments to the needs of the patient. This seemed a highly long-term and perhaps unrealistic goal, but is with all studies in this field becoming nearer/ more realistic.

Furthermore, studies could focus on new imaging techniques such as real-time MRI (Hampson et al., 2012) and promising treatment options, such as trauma focused stabilizing treatment for Complex PTSD, neurofeedback (Linden, 2006), transcranial magnetic stimulation (Pallanti & Bernardi, 2009) and deep brain stimulation (Langevin et al., 2010), to unravel neurophysiologic underpinnings of brain abnormalities during recovery of trauma related disorders.

Last but certainly not least, prevention is better than cure. Prevention of child abuse is an important but ultimately difficult challenge. Secretly in family homes in private, or secretly organized in day-nurseries, which cruelly affected recently an amazing number of children and their families in Amsterdam, prevention of child abuse is at least for a part still illusive. As clinicians and welfare workers we need to implement the guidelines on domestic violence and child abuse (in the Netherlands: ‘Code huiselijk geweld en kindermishandeling’): to detect early signs of abuse, consult colleagues directly in case of doubt and consult the advice agencies on child abuse (in the Netherlands: ‘Advies- en Meldpunt Kindermishandeling: AMK’), and try to support families and organize supportive care to prevent further abuse.
7.7. Concluding remarks

We performed structural and functional imaging studies in an understudied population of patients with Complex PTSD with a history of chronic, repeated and interpersonal traumatic experiences in childhood. Our imaging studies revealed relevant neurobiological differences of these child abuse related Complex PTSD patients with adult trauma related PTSD. There is evidence that the fear conditioning model is not sufficient to explain neural correlates of Complex PTSD and there is evidence for a dissociative subtype of PTSD. Differences were found in brain structure and brain function related to emotional memory, attention and emotion regulation. We think that treatments need to be tailored specifically to the need of these patients who show high rates of comorbid mood, anxiety and personality disorders. Patients improved with a stabilizing group treatment based on psycho-education and cognitive behavioral therapy and this correlated with improvements in brain function.