CHAPTER 7

Summary and general discussion

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The aim of this thesis was to gain more insight in the neural underpinnings of cognitive control impairments in OCD. Specifically, we wanted to test the hypothesis that reduced top-down control of the dorsal cognitive fronto-striatal circuit over hyperactivated ventral affective fronto-striatal circuits and limbic regions is associated with executive control deficits in OCD. Additionally, we wanted to disentangle state-dependent from trait-related neurocognitive features in order to identify putative endophenotypes. For this purpose we used functional MRI and scanned 46 medication-free adult OCD patients, 19 of their unaffected siblings and 41 matched healthy controls during performance of an emotion regulation, response inhibition and a working memory task. In a follow-up MRI scan session we modulated excitability of the left dorsolateral prefrontal cortex with rTMS to specifically test if the ‘therapeutic’ upregulation of activity of the dorsal circuit results in improved emotion regulation capabilities in OCD patients and if a ‘temporary functional lesion’ of the dorsal circuit impairs emotion regulation in controls. Further, to assess morphometric changes throughout the OCD brain in relation to aging and clinical factors such as co-morbid diagnosis, medication use and symptom dimensions, we compared structural MRI scans of 412 OCD patients with those of 368 control subjects, collected by 6 international sites part of the OCD Brain Imaging Consortium.

In this final chapter, we will discuss the main findings and implications of the research presented in the individual chapters of this thesis. We will subsequently address the implications of the results for our frontal-striatal working model of OCD. We will expand on the role of cognition-emotion interactions and pre-SMA in OCD. Then we will discuss some methodological issues regarding the work presented in this thesis. Lastly, we will make recommendations for future research.

Main findings per chapter:

**Chapter 2: What are consistent changes in regional gray and white matter volume in the OCD brain, and how are these changes related to aging and clinical factors?**

In chapter 2, using a large multi-center sample, we assessed voxel-wise differences in gray matter (GM) and white matter volumes between 412 adult OCD patients and 368 controls. Between-group comparisons were performed on the whole sample as well as an age-matched sample (N=645). We also assessed effects of aging in both groups and the effects of comorbid diagnosis, symptom dimensions, and medication status in the OCD sample. Our main findings were i) smaller volumes of bilateral medial frontal and inferior fronto-insular GM and adjacent white matter, ii) greater GM cerebellar volume, and iii) relative preservation of putamen/insular GM volume with aging in OCD patients compared with controls. These findings were corrected for multiple comparisons across the brain, and were independent from medication-status. In line with the literature in anxiety and depression (Goodkind et al. 2015; Radua et al. 2010),
smaller medial frontal GM and WM volumes were more pronounced in patients with comorbid anxiety disorders and/or unipolar depression, and we hypothesized this is indicative of a common pathophysiological mechanism across affective disorders related to a shared deficit in cognitive control and emotion regulation. In line with our hypothesis, we observed group-by-aging interaction effects in the putamen, which, at an uncorrected level, extended into orbitofrontal cortex and the nucleus accumbens (relative preservation of volume with aging). At the uncorrected level a relative loss of volume with aging was observed in the inferior and middle temporal gyrus, fusiform gyrus and parahippocampal gyrus extending into the thalamus. We also observed reduced volume of the thalamus in patients, this result was apparently driven by the older patients, since it did not survive in the age-matched analysis. We proposed that the relative preservation of orbito-frontostriatal regions in OCD is due to activation-induced neuroplasticity associated with chronic compulsive behaviors or compensatory processes due to cognitive dysfunction. The relative loss of paralimbic parts of the temporal cortex with older age may be related to chronic stress or anxiety, although we cannot rule out medication effects in this data-set. The results of the symptom dimension analyses topologically did not overlap with previous findings (see e.g. van den Heuvel et al. 2009). We hypothesized that results broadly agreed with the notion that the various subphenotypes of OCD have overlapping but also unique neural correlates. However, we should interpret our findings with caution since we cannot exclude the possibility that inconsistent findings are due to methodological issues, such as certain symptom clusters covarying with recruitment at specific sites, comorbidity, medication history, disease onset or severity.

Greater cerebellar GM volume is in line with some but not all previous VBM studies in OCD, and in contrast with the smaller cerebellar volumes reported in disorders often co-morbid with OCD (see Hoppenbrouwers et al. 2008; Phillips et al. 2015). Interestingly, greater cerebellar volume has also been found in offspring of alcoholics, suggesting a role of the cerebellum in compulsive behaviors (Hill et al. 2007). It is thought that the cerebellum integrates cortico-striatal information flow (Middleton and Strick 2000b), and is instrumental for cognition and emotion, as well as for motor control by encoding an internal model of mental representations and essential properties of body parts, respectively (Schutter and van Honk 2005; Ito 2008). We speculated that the observed cerebellar and inferior fronto-insular changes were directly related to cognitive dysfunction and OCD symptomatology, yet this awaits empirical confirmation. Our findings thus partially support the prevailing fronto-striatal models of OCD, but we hypothesized a greater role for the cerebellum in OCD illness models than is currently recognized.

Like most previous VBM reports, we did not observe volume alterations in the amygdala-hippocampal complex in OCD. Recently however, we (Fouche et al. 2016) analysed the same data-set with Freesurfer, which uses putatively superior segmentation
algorithms for subcortical regions, and observed reduced volume of the hippocampus. The results showed that the reduced frontal volumes in the OBIC data-set are at least partially explained by a reduction in cortical thickness, and we additionally observed reduced cortical thickness in parietal and temporal cortex. Further, we recently performed a structural covariance analysis (Subira et al. 2016) in this same data-set. The results showed increased covariance in OCD patients compared with controls between the right centromedial amygdala and the ventromedial PFC (vmPFC), indicating altered connectivity between these regions. Additionally, particularly in older patients increased structural covariance between the bilateral ventral-rostral putamen and the left inferior frontal gyrus (IFG) was found. Overall these combined OBIC results provide additional evidence for structural brain changes in OCD involving fronto-striatal, but also the limbic, parietal and temporal regions and the cerebellum, and emphasize the contribution of altered aging-related trajectories in addition to the hypothesized altered neurodevelopment in OCD. To gain more insight in the significance of altered brain structure in OCD, studies are needed that assess brain structure within-subjects over time during development (in pediatric samples), disease course, and during aging (adult samples).

**Chapter 3: Is emotion regulation impaired in OCD patients and can it be modulated using rTMS?**

It has been thought that reduced top-down control of dorsal frontal-striatal regions over ventral affective fronto-striatal and limbic circuits underlies problems with emotion regulation in psychiatric patient groups including OCD patients (Phillips et al. 2003b; van den Heuvel et al. 2004; Mataix-Cols and van den Heuvel 2006). There has been one recent study showing that attentional deployment can alter emotion processing in OCD (Simon et al. 2014). Yet, cognitive control over emotion using cognitive reappraisal, a strategy employed during cognitive therapy, was never studied in the disorder. We wanted to test whether cognitive reappraisal-related emotion regulation capabilities i) were impaired in OCD patients compared with controls and ii) could be modulated by rTMS. In Chapter 3 we therefore compared functional MRI scans recorded in our sample of unmedicated OCD patients with matched healthy controls while they performed an in-house developed OCD-specific emotion regulation task before (baseline scan session) and after a single-session of rTMS over the left dlPFC (scan 2). Participants processed general fear and OCD-specific stimuli during natural appraisal (‘attend’ instruction) and cognitive reappraisal (‘regulate’ instruction). Given our strong hypothesis the imaging analysis primarily focused on *a priori* regions-of-interests (ROIs) of the dorsal and ventral systems implicated in emotion regulation: the bilateral dlPFC, dorsomedial PFC (dmPFC; medial frontal wall including pre-SMA and dorsal anterior cingulate cortex (ACC)), the amygdala and the rostral ACC. Since it is the first study on reappraisal in OCD and the exploratory nature of our rTMS experiment, results were additionally presented at whole-brain p<.001 uncorrected for multiple comparisons.
In line with previous findings (e.g. Simon et al. 2010, see Rotge et al. 2008 for a meta-analysis), we observed increased self-report distress ratings during processing of both fear and OCD-related stimuli in patients versus controls, combined with increased and/or altered timing of BOLD responses in emotion processing areas including visual cortex, the right amygdala and caudate nucleus. Contrary to our hypothesis, OCD patients seemed able to use cognitive reappraisal as a strategy to down-regulate negative affect (as indicated by the distress ratings) just as well as control subjects for the fear stimuli, and even to a greater extent for the OCD-related stimuli. Although this last finding is probably related to a floor effect due to OCD stimuli not being very salient for control subjects. When implementing cognitive reappraisal however, OCD patients compared with controls showed i) attenuated frontal-parietal activation (including the left dlPFC) and diminished dmPFC - amygdala connectivity (during regulation of general fear stimuli), and ii) augmented dmPFC activation (during regulation of disease-relevant stimuli). Moreover, in patients cognitive reappraisal did not reduce subjective distress to the level reported by controls, nor did it lead to activation of the dlPFC. So although both groups recruited known control regions during emotion regulation (e.g. dmPFC, ventrolateral PFC) and this resulted in down-regulation of rostral ACC responses, patients failed to activate the dlPFC.

Absence of dlPFC activation in the regulate versus attend contrast in OCD patients may be due to a ceiling effect with this region already being maximally recruited during the attend condition, possibly due to regional neural inefficiency in combination with hyperarousal (Simon et al. 2014). The extent to which the dlPFC was activated during emotion regulation was associated with emotion regulation success (i.e. greater distress reduction) in our control sample. The dlPFC, posterior frontal regions including the pre-SMA and the inferior parietal cortex are thought to direct attention to the reappraisal-relevant stimulus features, and to promote the generation of mental representations of affective states, reappraisal goals and of the content of the selected reappraisal used for regulation purposes (Ochsner, Silvers, and Buhle 2012; Morawetz et al. 2016). We interpret the reappraisal-related fronto-parietal hypoactivation and the diminished frontal-limbic connectivity as indicating that patients are unable to control limbic responses over longer stretches of time (Erk et al. 2010). This explanation is however speculative and awaits empirical testing.

We think it is most likely that dmPFC hyperactivation during OCD-regulation could be a compensatory mechanism. The unpublished analysis of the sibling data (Thorsen et al., manuscript in preparation) supports this notion. Namely, preliminary findings indicate that although the subjective distress ratings of the siblings were similar to those of the controls, their brain activation patterns during OCD-related emotion regulation were more similar to patients. We cannot exclude the possibility however that dmPFC hyperactivation in patients versus controls is an indication of the low emotional impact that OCD stimuli had for the control group, or the result of patients
(un)consciously (Moyal, Henik, and Anholt 2014; Ochsner, Silvers, and Buhle 2012) dampening emotional responses with distraction (Sheppes and Levin 2013; Simon et al. 2014; Kanske et al. 2012). We also observed that patients compared with controls reported less use of cognitive reappraisal in everyday life, and that reappraisal use and reappraisal-related recruitment of the bilateral dmPFC and the thalamus were inversely related to symptom severity in patients. Taken together, our findings suggest that only patients that are relatively less affected by the disorder are able to implement reappraisal in everyday-life as well as in the context of the experiment. Based on these results we conclude that reduced cognitive control over emotions is at least a contributing factor in OCD pathophysiology.

We subsequently aimed to assess if high-frequency rTMS over a subject-specific task-related left dlPFC functional hotspot would boost regulatory control in OCD patients, and if low-frequency dlPFC rTMS would decrease emotion regulation capabilities in controls. Contrary to our hypothesis, rTMS did not significantly change subjective distress ratings. In line with our hypothesis and the literature, frontal activation was generally modulated by rTMS in the expected direction. There was an increase of frontal activation after high-frequency rTMS and a decrease in frontal activation after low-frequency rTMS compared to placebo (sham rTMS), but only for the picture viewing contrast (i.e. the baseline condition-independent contrast). On the emotion regulation contrast, however, contrary to our hypothesis, increasing activity of the dlPFC with 10Hz rTMS in patients resulted in reduced emotion regulation-related recruitment of regions of the dorsal (dorsal ACC) and ventral (ventrolateral PFC) cognitive circuit and the limbic circuit (parahippocampal gyrus) in comparison with sham-rTMS (see chapter 3, Table S3.4, uncorrected level). Reducing activity of the dlPFC with 1Hz rTMS in controls, on the other hand, resulted in increased regulation-related recruitment of a limbic region (hippocampus) compared to sham-rTMS. dlPFC rTMS also modulated activity changes over sessions in these circuits, as well as in the cerebellum, and occipito-temporal regions associated with visual processing in opposing fashion (relative reduction of activation in these circuits over sessions after 10Hz, relative increase after 1 Hz). An interpretation of the observed differences in brain activity we observed for the emotion regulation contrast on scan 2 is that inhibition of the dlPFC in controls resulted in increased task-related cognitive effort (increased hippocampal activity) and stimulation of the dlPFC in patients induced increased task-related neural efficiency (with relatively reduced dorsal frontal activity compared to the sham condition). Further, our analyses comparing the change in brain activation over sessions suggested that low-frequent and high-frequent rTMS may have differentially affected the change in emotional saliency network activity in patients and controls. We proposed that dlPFC rTMS affected more automatic (bottom-up) rather than top-down effortful regulatory processes (Gyurak, Gross, and Etkin 2011). We speculate that high-frequency rTMS over dlPFC may thus be beneficial for fear extinction learning, an implicit regulatory process that is known
to be deficient in OCD (Milad et al. 2013). This warrants future (pre)clinical studies investigating the effect of rTMS on fear extinction and exposure therapy in OCD.

**Chapter 4 & 5: Do the neural correlates of response inhibition constitute a candidate endophenotype of OCD?**

Previous studies showed that increased latency of the response inhibition process (i.e. longer stop-signal reaction time; SSRT) and associated widespread changes in gray matter density and frontoparietal white matter integrity constitute putative endophenotypes of OCD (Chamberlain et al. 2005; Menzies et al. 2007; Chamberlain et al. 2007; Menzies, Williams, et al. 2008; Chamberlain and Menzies 2009). In chapter 4 we compared functional MRI scans of our cohort of unmedicated OCD patients, their unaffected siblings and matched healthy controls recorded during the performance of a response inhibition task to assess to what extent these previously described morphological changes were functionally relevant. Given our strong *a priori* hypotheses regarding the regions involved in the task, we applied a ROI approach focusing on the bilateral pre-SMA, IFG, subthalamic nucleus and inferior parietal cortex for the inhibition contrast (comparing successful stop with successful go trials; (Aron and Poldrack 2006; Aron 2011; Hampshire et al. 2010; Mars et al. 2007). Further, we compared the groups on error-related brain activity in the anterior cingulate cortex, a region implicated in increased error-sensitivity in OCD (Fitzgerald et al. 2005). Our choice of ROIs and their location converge with the results of recent meta-analyses on inhibition-related brain activation published after our report (Cieslik et al. 2015; Rae et al. 2014; Cai et al. 2014).

In line with our hypothesis and previous reports (Menzies et al. 2007; Chamberlain et al. 2007) both patients and siblings showed higher SSRT compared with controls, albeit for siblings this did not reach statistical significance. Also, during inhibition both patients (left-lateralized) and siblings (bilateral) showed hyperactivation of the pre-SMA, which may therefore represent a candidate endophenotype of OCD. Moreover, patients showed hypoactivation of the right inferior parietal cortex and IFG compared to both the controls and the sibling group, which possibly contributes to their behavioral deficit. More efficient inhibition (i.e. shorter SSRT) was associated with increased activation of the pre-SMA in patients (left-lateralized) and siblings (right-lateralized), which is in line with studies in healthy subjects and nonhuman primates demonstrating the relevance of the pre-SMA for inhibitory control (see (Aron 2011; Nachev, Kennard, and Husain 2008). In both patients and siblings higher indices of OCD symptoms were associated with lower right pre-SMA activation, suggesting that right-lateralized pre-SMA recruitment is state-dependent. In siblings this was also the case for the right inferior parietal cortex. Contrary to our hypothesis, the groups did not differ in ACC activation during error processing. We did not observe any effect related to co-morbidity on the results. We thus concluded that the observed pre-SMA hyperactivation in OCD
patients and siblings is a task-relevant compensatory mechanism, rather than being related to conflict or error-signals. Bilateral rather than right-lateralized task-related pre-SMA activation in OCD patients and siblings, in the context of left pre-SMA not significantly being recruited by controls, may be an indication of neural inefficiency. Studies in healthy aging suggested that there is a dedifferentiation of lateralized brain responses during task performance, known as the HAROLD model (hemispheric asymmetry reduced in OLD; (Cabeza 2002). This model posits that due to neuronal dysfunction participants are not able to activate networks as selectively or efficiently as participants without the dysfunction, and therefore during task-performance recruit bilateral and/or additional brain regions (Schneider-Garces et al. 2010). We speculated that pre-SMA hyperactivation is due to a spreading or increase of regional brain activity and reflects either regional or network-level inefficient neural processing. We further speculated that this compensation mechanism of upregulation of activation in task-related brain regions is maximally effective in siblings without OC symptoms, but tends to fail in OCD.

We hypothesized that control impairments in OCD would be related to dorsal cognitive network impairments which could be related to dysfunction of the dorsal circuit itself, or to faulty interactions with the ventral/limbic network. To see if the results from chapter 4 were related to changes in functional connectivity (FC) within the dorsal and ventral regions of the inhibition network (Kang et al. 2013; Schlosser et al. 2010), or possibly associated with abnormal connectivity with the amygdala (as main node of interest from the limbic circuit), we performed a connectivity analysis on the same data in chapter 5. The psycho-physiological interaction analysis showed that during inhibition there is more negative coupling between the left IFG and bilateral amygdala in patients and - at a more lenient statistical threshold - in siblings compared with controls. Contrary to our hypothesis, we did not observe changes in FC between any of the other ROIs of the inhibition network. Although there was no relationship with the SSRT, negative coupling of the left IFG with the amygdala in patients and siblings was accompanied by increased task-related activation of the left pre-SMA. The follow-up dynamic causal modeling analysis suggested a condition independent bottom-up influence of the left amygdala on the left IFG in healthy controls, and the more positive the IFG-amygdala coupling was, the better the task performance. In patients, on the other hand, functional coupling between the left IFG and bilateral amygdala was top-down, whereas in siblings it was bidirectional. These findings suggest that bottom-up signals from the amygdala to the IFG during this task are beneficial, possibly through enhancing attention allocation to the stop-signal and increasing processing speed (Davis and Whalen 2001; Schaefer and Gray 2007). We proposed that in patients, and to a lesser extent in siblings as well, the altered connectivity between the IFG and amygdala resulted in inefficient processing within the inhibition network and compensatory hyperactivation of the pre-SMA.
Chapter 6: Do the neural correlates of visuo-spatial working memory constitute a candidate endophenotype of OCD?

In chapter 6 we compared functional MRI scans recorded in our sample of unmedicated OCD patients, siblings and controls while they performed a visuo-spatial Nback working memory task with three increasing working memory load conditions (N1-3) and a baseline (N0). Given our strong a priori hypotheses about the brain regions involved in this task (Owen et al. 2005) we used a ROI approach which included the bilateral dlPFC, pre-SMA/premotor cortex, precuneus, inferior parietal cortex and the dorsal ACC (dACC). We compared brain activation associated with general working memory task performance (N123>N0 contrast) across the groups and additionally looked at task load-dependent changes. In line with our hypothesis and previous studies (e.g. van der Wee et al. 2003), we showed that OCD patients are less accurate than controls and siblings, specifically during trials with the highest working memory load (N3). Also, during general working memory task performance both patients and siblings showed hyperactivation of the left pre-SMA/premotor cortex, dlPFC and precuneus. Similar to the results from chapter 4, upregulation in the task-related network was more extensive in siblings than patients, including all the fronto-parietal ROIs except the right pre-SMA/premotor cortex. Moreover, upregulation in siblings was also greater in amplitude, with siblings showing increased activation of the right dACC and left preSMA compared with OCD patients. In patients, hyperactivation of the left pre-SMA and dlPFC, but not precuneus, was more pronounced in the group of patients with better task performance (which co-occurred with less co-morbidity and higher IQ).

Contrary to our hypothesis and the results from chapter 3 and 4, task-related brain activation in patients was not significantly related to OCD severity. In line with the literature, task-related activation significantly increased with higher working memory load in controls (see Schneider-Garcés et al. 2010), whereas in OCD patients (Koch et al. 2012) and in siblings (a novel finding) activation reached ceiling at N2. This pattern of brain activation plateauing at higher task loads has previously been observed in other samples with a predisposition for psychiatric disease (Callicott et al. 2000; Mannie et al. 2010), and is similar to that seen in healthy aging. It is thought that the aging-related (or in our case hypothesized disorder-related) decrease in neuronal processing efficiency results in limited neural resources that subsequently reach full capacity at lower task-loads. This is known as the Compensation-Related Utilization of Neural Circuit Hypothesis (CRUNCH; Reuter-Lorenz and Cappell 2008). Given our hypothesis about a dorsal-ventral imbalance and the results from chapter 5, we wondered whether aberrant FC with the amygdala was related to deficient task performance and hyperactivations in the left pre-SMA, dlPFC and precuneus. Generalized psycho-physiological interaction analysis showed that patients with OCD - and especially those with low task accuracy, more co-morbidity and lower IQ - had increased positive FC between the amygdala and the left dlPFC (compared to both controls and siblings) and the pre-SMA (compared to
controls only). We speculated that this increased frontal-amygdala connectivity reflected a growing uncertainty about task performance as working memory load increases (Stern et al. 2013). Overall, these data suggest that, like in chapter 4-5 and possibly chapter 3, in OCD patients and to a lesser extent in siblings, frontal task-related recruitment was modulated by its connections with amygdala, and this relates, at least to some extent, to deficient task performance.

**Overall results in relation to the wider literature on OCD and cognitive control**

*The extended frontal-striatal model of OCD: Is there evidence for an imbalance between dorsal and ventral CSTC and limbic circuits in OCD?*

Although probably a highly simplified version of real interactions, our working model predicted that reduced cognitive control and anxiety in OCD were related to 1) hypoactivation of the dorsal circuit, which would lead to a reduction in top-down control over 2) ventral fronto-striatal and limbic regions, that we hypothesized to be hyperactivated and in their turn would negatively influence dorsal circuit functioning (Phillips et al. 2003b; van den Heuvel et al. 2011). The results presented in this thesis were partially in agreement with the predictions of this working model. We indeed observed structural and functional changes in the dorsal and ventral cognitive circuits and limbic circuits in OCD, but the findings do not support a clear-cut dorsal-ventral imbalance. Below I will discuss our findings and relate these to the fronto-striatal model of OCD. See Table 7.1 for an overview of the results of the studies of this thesis subdivided for the different fronto-striatal loops and the limbic circuit. See Figure 7.1 for a summary of the relationship between task-performance, frontal hyperactivations and frontal-amygdala connectivity in the three cognitive control tasks.

To summarize, across the three cognitive control tasks we observed impaired performance in OCD patients (i.e. higher SSRT, lower N-back accuracy, higher distress ratings), whereas in siblings performance was similar to that in controls. In the functional studies, however, the observed direction of activation patterns in these fronto-striatal circuits were not uniform in and showed both trait-related and state-dependent (i.e. effects of task, task-load and disease-load) influences. We found hyper-activation of dorsal cognitive frontal regions during control tasks as a trait feature present in both patients and their unaffected relatives compared with controls (chapter 3-6). The extent of the upregulation of activation in the (nodes of the) task-related dorsal frontal-parietal regions was related to relative preservation of executive performance in both groups (chapter 4,6). However, upregulation to putatively increase functional neural efficiency was state-dependent and only possible to a certain extent. Upregulation was limited as a function of study group: i) hyperactivations during cognitive control tasks were higher in amplitude or extended to additional regions in siblings compared with OCD patients (chapter 4,6); and ii) only in OCD patients compared with controls, but not