Chapter 8

Summary and general discussion

The aims of this thesis were two-fold. In part II, common and distinct aspects of phenomenology and neurobiology in obsessive-compulsive disorder (OCD) and Tourette's Syndrome (TS) were studied from different angles, in order to better understand how they fit in a neurobiological spectrum with limbic and fronto-striatal involvement. More specifically we investigated tic-related OCD, obsessive-compulsive behavior in TS and we directly compared tonic and phasic dopaminergic function between "pure" TS and "pure" OCD. In part III we aimed to increase the understanding of executive function and cognitive control network function as possible neurobiological mechanisms related to OCD. Further, we aimed to test putative neurocognitive endophenotypes for OCD by using a family study design.

Summary

Part II: Comparisons between TS and OCD

In **Chapter 2** we report findings from comparisons between tic-free (n=270) and tic-related OCD patients (n=107) based on longitudinal follow-up data. Tic-related OCD is regarded as a relevant subtype that is present in about 30% of the OCD patients, with a differential clinical presentation and probably partly specific neurobiological underpinnings (Leckman et al., 2010). Some authors suggested that tic-related OCD may have a less favorable outcome (McDougle et al., 1993).

We confirmed a different clinical profile of tic-related OCD, compared with tic-free OCD, with earlier disease onset, male preponderance, increased familiality, more symmetry/ordering symptoms and increased autistic and ADHD traits. OC symptom severity was similar across groups, as were the rates of depressive and anxiety disorders. The two-year course of OCD symptom severity was similar for both subgroups, and this outcome held after correcting for baseline differences in demographic and clinical characteristics. We concluded that the results indeed support the existence of a clinically distinguishable tic-related subtype, with a profile that has commonalities with other neurodevelopmental disorders (early onset, high familial loading, increased autistic and ADHD features). We also concluded that, contradictory to the literature, adult patients with the tic-related subtype do not suffer from more severe OCD, nor does the presence of tics critically affect the natural course of OCD at adult age.

It can be difficult to categorize the repetitive behaviors in TS as complex tics or, alternatively, as OC behavior (Worbe et al., 2010b). About 50% of the TS patients experience OC symptoms, frequently in the symmetry/ordering symptom dimension, also including touching and counting rituals (Cath et al., 2001b). A previous study indicated that different cortico-striatal-thalamo-cortical (CSTC) circuits may be involved in simple versus complex tics and OC behavior (Worbe et al., 2010a). In **Chapter 3** we describe a group of 14 medication-free TS patients suffering from symmetry behavior in comparison with a control group of 10 healthy subjects using a "symmetry" symptom provocation paradigm, while subjects were scanned in a Positron

Emission Tomography (PET) scanner. During provocation of symmetry behavior in TS patients we found increased cerebral metabolism in the anterior cingulate cortex (ACC), supplementary motor area (SMA) and inferior frontal gyrus (IFG). Orbitofrontal (OFC) activity correlated with increased symmetry ratings during symptom provocation in the TS group. This suggests that both motor and limbic circuits are involved in symmetry behavior in TS. SMA activity has been found to precede tics (Neuner et al., 2014) and may relate to an urge to move while limbic activation may indicate that asymmetry stimuli are salient for TS patients. Increased SMA activity in TS patients hints towards a "tic-like" quality of symmetry behavior in TS, but involvement of limbic areas (OFC and ACC, both associated with OCD) suggests similarities with OC behavior. The healthy control group did also experience some sensitivity to the pictures with disordered and asymmetrical objects, but in contrast to the TS patients they did not activate limbic areas, but sensorimotor and cognitive control areas instead. We proposed that healthy controls activate cognitive control areas to suppress the urge to rearrange objects.

The basal ganglia and the CSTC circuits, involved in motor function, cognition and emotional/motivational behavior, are modulated by dopaminergic neurotransmission. Several lines of evidence have suggested increased dopaminergic neurotransmission in OCD and TS, but previous studies did not directly compare the two groups. Dopamine (DA) neurotransmission is dynamic with constant tonic and stimulus-dependent phasic firing. In **Chapter 4** we report the results of a [^{11}C]-raclopride PET imaging study in 12 TS patients without OCD, 12 OCD patients without tics and 12 healthy comparison subjects. We measured striatal $D_{2/3}$ receptor availability at rest and after stimulation with amphetamine, mimicking the phasic dopamine response. In TS and to a lesser extent in OCD $D_{2/3}$ receptor availability at rest was decreased (suggesting increased DA concentrations), but neither group showed increased DA release after stimulation with amphetamine, which was in contrast with previous reports on TS (Wong et al., 2008;Singer et al., 2002). In the TS group DA release in the ventral striatum correlated with an amphetamine-induced increase in tic-severity. Even though we found no evidence for increased amphetamine-induced DA release in TS, we speculate that the phasic DA response is increased during "rest" as TS patients may react with a phasic DA response to stimuli that would not elicit such a response in other subjects. Such a mechanism of increased DA release to "subthreshold" stimuli may explain the increased DA levels at baseline. Amphetamine-induced DA release in TS, although not quantitatively different from controls, may further facilitate tics.

Part III: A family study of OCD

In part II of the thesis we described a family study design, set up to investigate possible neurocognitive endophenotypes of OCD, with a focus on executive function and limbic networks. A group of un-medicated OCD patients in tandem with their

unaffected siblings were compared to a group of healthy comparison subjects with no family history of OCD. In **Chapter 5** we describe response inhibition (measured with the stop-signal task) in which participants needed to inhibit an already initiated response. Patients with OCD had longer stop-signal reaction times (SSRT) than the healthy comparison subjects, indicating impaired response inhibition. Patients with OCD and their siblings, relative to comparison subjects, showed increased activity in the left presupplementary motor area (pre-SMA) during successful inhibition. Decreased activity in the right inferior parietal cortex (IPC) and inferior frontal gyrus (IFG) was found in OCD patients relative to both the comparison subjects and the siblings. In patients and siblings pre-SMA activity correlated negatively with SSRT, suggesting that up regulated activity was beneficial for task execution. We concluded that pre-SMA hyperactivity could be a neurocognitive endophenotype of OCD and may be related to inefficient neural processing within the pre-SMA. Patients with OCD further showed a state-dependent deficit in recruiting right IPC and IFG, which may contribute to their inhibition deficit. In **Chapter 6** results are reported of a visuospatial working memory task with increasing levels of complexity, probing the fronto-parietal circuits. We found decreased task-performance at the most difficult level of the task in patients but not in siblings. Increased fronto-parietal activity (incuding pre-SMA hyperactivity) was present in siblings and to a lesser extent in patients and was related to task-performance. These findings indicate that compensatory fronto-parietal brain activity in OCD patients and their unaffected relatives preserves task performance at low task loads, but for patients it is insufficient to maintain performance at high task loads. OCD patients, compared with siblings and comparison subjects showed increased task-related functional connectivity between frontal regions and bilateral amygdala and this was related to poorer task performance. Fronto-parietal dysfunction may constitute an endophenotype for OCD possibly reflecting neural inefficiency within the fronto-parietal network. Limbic interference may hamper compensatory neural recruitment.

The dual network theory (Dosenbach et al., 2008;Dosenbach et al., 2007) postulates that cognitive control is exerted by a fronto-parietal network (FPN, including DLPFC en IPC) and a cingulo-opercular network (CON including dorsal ACC and insular/opercular cortex). Following increased task-related activity in cognitive control networks in OCD patients and their siblings (**Chapter 5 and 6**), we set out to study the connectivity within and between these networks at rest using resting state functional connectivity (rs-fc) MRI, which is described in **Chapter 7**. During response inhibition and working memory altered coupling between task-related areas and limbic regions was reported (**Chapter 6** and van Velzen et al., 2014;van Velzen et al., 2015). Therefore we also investigated limbic fronto-striatal connectivity, using a seed in the subgenual (sg)ACC within the ventromedial prefrontal cortex (vmPFC). In contrast to our expectations the connectivity patterns of the FPN and CON were similar when comparing patients with healthy controls. FPN connectivity did show an inverse relation

with OC symptom severity in patients. Siblings, however, showed higher connectivity *within* the CON than patients, between the CON and FPN compared to patients and between the FPN and rostral ACC compared with controls. In OCD patients, but not in siblings, hyperconnectivity (compared to controls) was present within the fronto-limbic network, but without a relation to symptom severity. We speculate that the increased within- and between-network connectivity in siblings, but not in patients, could indicate a mechanism of increased cognitive control, possibly related to resilience. None of the observed network alterations can be considered an endophenotype for OCD since differences were present in either patients or siblings, but not in both groups. The pattern however, partially fits the results described in **Chapter 5 and 6**, showing a more prominent neural hyperactivity in siblings than in patients. Results of the three studies are summarized in Table 8.1, including results of the task-related connectivity analysis during the stop-signal task (van Velzen et al., 2015).

Discussion

What is the nature of the relationship between OCD and TS?

In **Chapter 2** we observed a specific clinical profile for tic-related OCD with more symmetry behavior, more males, early onset, increased rates of somatoform (hypochondriasis and BDD), decreased rates of eating disorders and more prominent features of ADHD and autism. Using a latent class approach, Nestadt et al. (2009) differentiated subclasses of OCD based on co-morbidity patterns. Their results showed a 'tic-related' class that also had increased prevalence of impulse control disorders (trichotillomania and pathological skin picking), more males and earlier onset. The 'affective' class in this study had increased rates of symmetry behavior, early onset somatoform disorders and more females, whereas in **Chapter 2** tics were related to somatoform disorders. Differences between studies may be explained by different methods of analysis or differences in patient samples, but interestingly, in the study by Nestadt et al. (2009) tic-disorders also loaded on the 'affective' class, suggesting that clinical heterogeneity exists within tic-related OCD. De Mathis (2013) used a cross-sectional and retrospective design to study co-morbidity in pediatric OCD (mean age at onset at 13 years) from a developmental perspective. The authors defined groups according to their earliest psychiatric diagnosis. They reported an externalizing subtype (OCD was preceded by ADHD or tics), with children developing impulse control disorders and substance abuse around the age of 20, versus an internalizing subtype with separation anxiety disorder as first diagnosis, with increased prevalence of anxiety disorders and anorexia nervosa in early and late adolescence respectively. Our results in adult OCD, were recently corroborated in a pediatric sample of 112 cases, using a cross-sectional design to compare OCD patients without co-morbidity, OCD patients with co-morbid neurodevelopmental disorders (tic disorders and ADHD) and patients with co-morbid internalizing disorders (anxiety, depression, anorexia nervosa). The

"neurodevelopmental" group (comparable to our tic-related OCD group) indeed had more males, earlier onset and increased familiar loading in comparison to the third internalizing group. Moreover, in the neurodevelopmental group increased symmetry behavior was found, whereas increased checking and washing compulsions were found in the internalizing group, with overall comparable OCD severity (Ortiz et al., 2015). In **Chapter 2** we defined tic-related OCD according to the lifetime presence of tics, considering tics a trait mark that may impact the clinical profile independent of current tic presence or tic severity. We found no interaction between tics (or other tic-related co-morbidity patterns) in the two-year course of OCD at adult age. Repeating the analysis while only including the patients with current tics resulted in similar outcomes, and tic severity was not related to outcome. Since the patients in the sample had relatively mild tics, we cannot rule out that in other severe cases tics negatively impact the outcome of OCD. To corroborate our longitudinal findings in adult OCD it would be very interesting to follow-up the pediatric cohort of Ortiz et al. (2015) to test if tics play a similar small role in the course of pediatric OCD. A previous study showed improved outcome in adolescence of pediatric tic-related OCD and the authors suggested that the (neuro) developmental changes that are responsible for the improvement of tics may also have a positive influence on OC symptoms (Bloch et al., 2009). Taken together, these data suggest a spectrum with anxious/internalizing OCD subtypes, affecting more females on the one hand and more impulsive/externalizing (tic-related) subtypes, with male preponderance on the other. The boundaries between subgroups are probably blurred and a two-dimensional spectrum is an oversimplification that does not completely capture the heterogeneity of these disorders.

Mataix-Cols and van den Heuvel (2006) proposed a neurobiological model for OCD based on an imbalance in the CSTC circuits leading to increased *ventral* CSTC activation, associated with anxiety and an increased behavioral drive, and *decreased* dorsal CSTC activation, resulting in reduced control over behavior and emotions. In a previous experiment by van den Heuvel et al (2004) contamination fear was provoked in OCD patients and healthy controls, using a paradigm comparable to the one used in **Chapter 3.** In both of these symptom provocation studies the healthy controls, but not the patients groups recruited the dorsal prefrontal cortex, which was interpreted as exerting top-down cognitive control. This fits the hypothesized deficit of the dorsal fronto-striatal circuits in OCD and related disorders. A recent publication on emotion-regulation showed that dlPFC activity was associated with successful emotion regulation in healthy controls, but not in OCD patients (de Wit et al., 2015). These data lend some support to a relative failure of the dorsal control circuits in OCD and TS. The previously discussed model by Mataix-Cols and van den Heuvel also proposed that TS and OCD belonged to a spectrum with limbic and fronto-striatal involvement in OCD and a shift towards mainly fronto-striatal involvement in TS (Mataix-Cols and van den Heuvel, 2006). OCD with prominent symmetry behavior was hypothesized to be neurobiologically

closer to TS, than OCD with other symptoms. Involvement of sensorimotor CSTC circuits in symmetry behavior has been reported in a structural study of OCD (van den Heuvel et al., 2009). Provocation of contamination anxiety in OCD increased anxiety ratings in patients and elicited amygdala activation (van den Heuvel et al., 2004), but symmetry provocation in TS patients did not increase anxiety, nor did it activate the amygdala (**Chapter 3**). It could thus be speculated that anxiety plays a less prominent role in obsessionality in TS than in OCD. Structural alterations in the motivational CSTC circuits are associated with obsessionality in TS, indicating involvement of limbic circuits (Worbe et al., 2010a), but fMRI studies have also found limbic activation (insula, amygdala, hippocampus) during generation of motor tics (Wang et al., 2011;Neuner et al., 2014). In a small study of 13 TS patients tics were associated with excessive activity in motor pathways, reduced activation in control areas and increased (limbic) hippocampal/amygdala activity. The latter was thought to correspond to premonitory urges (Wang et al., 2011). Neuner et al (2014) tried to disentangle the temporal dynamics of tic-generation in 10 TS patients and showed activation of motor circuits two seconds before tic-onset, followed by paralimbic activity (insula, amygdala) as well as putamen and cerebellar activity at one second before tic-onset and activity in primary sensorimotor areas and thalamus at tic onset. This pattern indeed suggests involvement of several CSTC circuits at different time-points in tic-generation. Taken together, both studies provide evidence that limbic activity may not relate to OC behavior as such, but may be an integrative part of tic-generation, which is in line with viewing symmetry behavior in TS as complex tics. Interpretation of these studies is hampered by inclusion of medicated patients and patients with co-morbid OCD, as well as small sample sizes. Activity in the substantia nigra during spontaneous tics has been described previously (Wang et al., 2011) and in **Chapter 4** we showed a correlation between ventral striatal dopamine release and increased tics. From the above one may conjecture that the limbic component in tic-generation is modulated by dopamine and that it relates to the premonitory urge preceding tics. In conclusion, the findings described in **Chapter 3** suggested involvement of motor and limbic brain areas in symmetry behavior, but our design cannot reliably differentiate the neural correlates of OC behavior from tics, especially since limbic areas also seem to be involved in tic generation. Characterizing TS primarily as a sensorimotor fronto-striatal disorder is probably an oversimplification and the complex of symptoms more likely emanates from sensorimotor hyperactivity, a relative failure of (dorsal) cognitive control *and* increased (ventral) limbic activity. Future studies could investigate both tics and OC-behavior in parallel in the same TS patients, preferably un-medicated. Second direct comparisons between TS patients with and without OC behavior and between TS and OCD patients with OC-behavior could help to increase the understanding of the distinct and overlapping neural pathways that are involved.

A neurocognitive endophenotype of OCD

Deficits in several measures of executive function such as inhibitory control, cognitive flexibility and planning have been identified as candidate endophenotypes of OCD, although in previous studies the unaffected relatives were typically similar to OCD patients or intermediate between patients and controls (Chamberlain et al., 2007;Chamberlain et al., 2008;Menzies et al., 2007;Menzies et al., 2008b;Rajender et al., 2011;Cavedini et al., 2010;Lennertz et al., 2012). Our results confirm altered executive function as a putative endophenotype, but are also partially in contrast with the previous studies, since we found the most prominent differences in the unaffected relatives. Increased neural recruitment with normal performance is suggestive for network inefficiency. Neural recruitment during working memory follows an inverted U-shaped curve with increasing cognitive load. In healthy aging this curve shifts to the left in such a way that older individuals show enhanced recruitment at lower task-loads than a younger control group although their performance remains intact. At higher task loads neural recruitment decreases and performance declines (Schneider-Garces et al., 2010). Non-linear associations between task-load, neural recruitment and performance may explain some inconsistent findings in neuroimaging studies of executive function in OCD, as cognitive loads are different across paradigms (Harkin and Kessler, 2011). When a task is too easy, patients do not need to recruit additional neural resources, but when a task is difficult, they may show increased, decreased, or similar levels of brain activation compared to controls (following the inverted U-curve). Across two executive tasks (assessing response inhibition and visuo-spatial working memory) hyperactivity of the pre-SMA was found in OCD patients and to an even larger extent in their unaffected siblings, suggesting that enhanced recruitment of the pre-SMA during executive tasks represents a neural endophenotype for OCD. A relationship between pre-SMA and task-performance lead to the suggestion that pre-SMA hyperactivity was a compensatory mechanism. Compensatory neural recruitment in OCD was suggested previously by several authors (den Braber et al., 2010;Koch et al., 2012).The results by Koch et al (2012) on working memory in OCD are strikingly similar to ours, with increased activation at lower task loads and a decrease at more difficult levels. The pre-SMA is part of the core network involved in response inhibition (Aron et al., 2007) and has been indicated in OCD patients before (Grutzmann et al., 2014). Failing inhibitory control may be the common mechanism leading to increased pre-SMA recruitment across both tasks. Inefficient working memory in the elderly is thought to result from inadequate suppression of task-irrelevant information (Gazzaley et al., 2005;Schneider-Garces et al., 2010) suggesting that a failure in inhibitory control underlies working memory deficits. Deficits in behavioral inhibition are proposed to be the core neuropsychological deficit (and possible endophentoype) in OCD (Chamberlain et al., 2005). In **Chapter 7** we reported increased cingulo-opercular network connectivity at rest in siblings (compared to patients). This network is associated with suppression of irrelevant information,

which may point towards increased efforts to exert cognitive control (Sadaghiani and D'Esposito, 2015). No evidence was found for alterations in cognitive control network connectivity at rest in patients, but this does not exclude inefficiency of these networks in OCD. Deficits in cognitive control network connectivity may not be present in OCD at rest, but only when engaged in a 'difficult enough' task. On the contrary, in a recent study graph theory was used to show inefficiency of local structural networks and altered modularity of resting state cognitive control networks in patients with OCD and in their unaffected relatives (Peng et al., 2014). Different network properties will be investigated in our own data-set and related to measures of executive function that were also collected.

Fronto-limbic dysfunction

During working memory, altered task-related connectivity between pre-SMA and DLPFC and the amygdala was found in OCD patients. Additional analyses on response inhibition-related changes in functional connectivity showed increased negative coupling in OCD patients and siblings compared to controls between the inferior frontal gyrus and the amygdala (van Velzen et al., 2015). Furthermore, altered resting state connectivity was present between the fronto-limbic sgACC seed and the amygdala and striatum. The direction of the task-related limbic connectivity was different between studies; increased working memory-related *positive* coupling was found between DLPFC and pre-SMA and the amygdala versus increased response inhibition-related *negative* coupling between the IFG and the amygdala and the latter correlated with pre-SMA activity. Since the working memory related alterations in connectivity also related to decreased performance in OCD patients, we speculated that limbic interference hampers adequate compensatory recruitment of fronto-parietal areas, resulting in decreased accuracy. Others have shown that amygdala activation is related to impaired performance in a planning task across several anxiety disorders (OCD, panic disorder and hypochondriasis), even when controlling for state anxiety (van den Heuvel et al., 2011). From the connectivity analyses, presented in this thesis, no causal effects can be deduced and an alternative explanation is that failing task performance in OCD patients triggers a limbic response. The latter was also proposed by Grutzmann et al. (2014) who found increased error-related response in the amygdala and sgACC in OCD patients, compared to a control group, suggesting a stronger affective response towards errors. Interestingly in this study an inverse relation existed between limbic activation and pre-SMA engagement, possibly supporting a role for the pre-SMA in controlling the affective response (Grutzmann et al., 2014). In line with this, van Velzen et al. (2015) suggested that during response inhibition amygdala activity enhances performance in healthy controls (salience of the stop-signal improves attention and performance), but in OCD the stop-signal may have increased saliency which needs to be suppressed. In depressed patients IFG-amygdala interactions during affective regulation were altered

with increased IFG activity causing an increase in amygdala activity. This mechanism was the reverse from what was found in healthy controls, suggesting that the regulation attempts in depressed patients were counterproductive (Johnstone et al., 2007).

Dopamine, Tourette, OCD and executive function

As TS and OCD are considered disorders on the impulsive-compulsive spectrum (Fineberg et al., 2010), deficits in response inhibition are expected in both disorders. Findings on response inhibition in TS, however, have yielded mixed results (see the review in (van Velzen et al., 2014). Initially, response inhibition may be impaired in TS, but continuous activation of cognitive control regions to suppress tics may cause neuroplasticity of these regions and result in increased cognitive control abilities. Preliminary results from our group on the stop-signal task indicated no behavioral differences between TS patients and controls and TS patients did not show an inhibition-related pattern of pre-SMA hyperactivity (nor IFG hypoactivity) that was observed in **Chapter 5** (Fan et al., 2016). We found evidence for increased dopaminergic function in TS and to a lesser extent in OCD (**Chapter 4**). As was mentioned before, dopamine modulates activity in the CSTC pathways and increased dopamine is thought to facilitate behavior and executive function. In a small study with 9 healthy individuals medial prefrontal dopamine signaling increased during response inhibition and related to performance (Albrecht et al., 2014). Since patients with both putative hyper- and hypodopaminergic disorders show altered executive function, it was hypothesized that the relation between executive function and dopamine is probably non-linear following an inverted U-shaped curve (for working memory (Cools and D'Esposito, 2011) and for inhibitory control (van Velzen et al., 2014)), suggesting that disorders on the impulsive-compulsive spectrum with a hyper- or hypodopaminergic state exhibit similar failures in inhibitory control and that this may be (partially) restored with dopamine modulating medication. Dopamine dysregulation has been put forward as a putative endophenotype for OCD because a polymorphism in the dopamine modulatory COMT gene increased prefrontal dopamine levels in patients and unaffected relatives, but not in a control group (Delorme et al., 2010). Since genetic material was collected in all subjects who participated in the imaging studies, a future analysis could investigate the role of genetic polymorphisms related to dopaminergic function and the executive deficits that were found.

Integration of clinical and neurobiological data

The field of psychiatry has been struggling how to best classify its disorders. The underlying genetic pathways and pathophysiology of psychiatric disorders remain largely elusive and it is difficult to define boundaries between disorders that have overlapping symptoms and co-occur within patients. Kendler (2011) conceptualized psychiatric disorders as "species" that have an underlying stable cluster of properties

Table 8.1 Summary of main findings of response inhibition, working memory and resting state (rs) functional connectivity in OCD, siblings (sib) and healthy controls (HC) as described in **Chapters 5, 6, 7**

Brain area		Response inhibition	Working memory	rs connectivity
dlPFC (FPN[a])	task x group	No ROI	*Sib>OCD (L)* *OCD>HC (L)* *Sib>HC (R)*	*Sib>HC* with rACC
	performance	--	Related to accuracy in OCD	--
	disease severity	--	No relation	No relation
IPC (FPN[a])	task x group	*HC>OCD* *Sib>OCD*	*Sib>HC (L+R)*	*Sib>HC* with rACC
	performance	Related to worse performance in OCD	Related to accuracy in HC	--
	disease severity	No relation	No relation	No relation
pre-SMA	task x group	*Sib>OCD>HC (L)* *Sib>HC (R)*	*Sib>OCD>HC (L)*	Not a seed (part of FPN)
	performance	Related to better performance in OCD (L) and sib (R)	Related to accuracy in OCD (L) and HC (R)	--
	disease severity	Inverse relation with Y-BOCS	No relation	--
IFG	task x group	*HC>OCD* *Sib>OCD*	No ROI	Not a seed (part of CON)
	performance	No relation	--	--
	disease severity	No relation	--	--
dACC (CON[b])	task x group	None	*Sib>OCD* *Sib>HC*	*Sib>OCD* - within CON - with FPN
	performance	--	No effect	--
	disease severity	--	No effect	No effect
		Task related connectivity (gPPI)		rs connectivity
Limbic involvement		*OCD + sib>HC* negative coupling between IFG and AMY[c]	*OCD>HC* coupling between pre-SMA and AMY *OCD>sib* coupling between DLPFC and AMY	*OCD>HC* sgACC with AMY/striatum
	disease severity	Not tested	No relation	No relation

[a] The fronto-parietal network seeds (FPN) for the resting state study consisted of bilateral seeds in the dorsolateral prefrontal cortex (dlPFC) and intraparietal sulcus, [b] The cingulo-opercular network seeds (CON) for the resting state study consisted of bilateral seeds in the anterior insula and a seed in the dorsal ACC/dorsomedial prefrontal cortex (dACC), rs= resting state, IPC= inferior parietal cortex, IFG= inferior frontal gyrus, AMY= amygdala, ROI= region-of-interest, pre-SMA= pre-supplementary motor area, [c] van Velzen et al., 2015

that refer to causal genetic, physiological or social mechanisms that interact with each other so that different relative contributions of these properties could lead to or sustain a symptom profile. Those "species" have fuzzy boundaries, indicating that some subjects a more typical (central) and others peripheral, so symptoms may not overlap in all subjects. Van Os (2013) takes a neurodevelopmental perspective and argues that symptoms early in life consist of "a non-specific mixed bag of affective dysregulation, aberrant salience, motivational alterations and anxiety states". These early symptoms follow different pathways, interacting with other symptoms and environmental factors resulting in a range of "more mature" psychiatric phenotypes. Finally from a network perspective, the 'unifying triple network' theory states that dysfunction in or imbalance between three core neural networks (the default mode, salience and central executive network) gives rise to various psychiatric symptoms (Menon, 2011). Additionally, a specific symptom profile may originate from dysfunction in either one of the core networks and dysfunction in each network could stem from several underlying deficits, such as weak intrinsic connectivity, connectivity with areas that are normally not part of the network or impaired communication with the other networks. In other words, one underlying mechanism could develop into different phenotypes and different underlying mechanisms could develop into one phenotype. The first idea suggests (genetic and neurobiological) overlap between different psychiatric disorders and the second idea predicts (genetic and neurobiological) heterogeneity within diagnostic categories, which is probably both true for TS and OC spectrum disorders. This dichotomy is nicely illustrated in an editorial by Licinio and Wong (2013), using the terms pleiotropism (one single factor with multiple effects) and redundancy (one symptom cluster can be caused by many different factors) to explain why, in psychiatry, specific clusters of symptoms do not easily map to distinct biological causes.

Having said that heterogeneity in etiology complicates forming integrative hypotheses, I will use the previous concepts to integrate part I and II of this thesis and speculate on neurobiological pathways underlying the clinical spectrum of OCD and TS (see Figure 8.1). In a very recent review paper van den Heuvel et al. (2015) propose that several functional CSTC circuits contribute to compulsivity; the sensorimotor circuit, the dorsal cognitive circuit (dlPFC, pre-SMA/dmpfc), the ventral cognitive circuit (inferior frontal gyrus), the motivational circuit (orbito-frontal) and limbic circuit (sgACC and

amygdala). Symptoms in TS and OCD seem to arise from an imbalance between different neural networks. Genetic factors may influence impulsive and compulsive traits as well as dopaminergic and serotonergic function both in children and throughout the lifespan. Early in development (heritable) sensorimotor and dopaminergic dysfunction may be associated with impulsivity and tic disorders, but with maturation of the self-regulatory cognitive circuits, at adolescence most patients will be able to control their tics. When, however, self-regulatory control does not mature adequately (for example as a result of stressful events), or when maturation of emotional/motivational circuits is also disturbed (due to early anxiety symptoms or an inhibited temperament), patients will continue to have tics and maybe develop co-morbidities such as OC behavior. Along the way of development, external factors may interact with neural network development (such as immunological events or stressful life events) and circuits interact with each other, leading to a unique pattern of network (dys)function and symptoms. The development of neural networks supporting cognitive control can help to suppress symptoms and sustain better cognitive function. Conversely, dysfunction of the neural circuits of cognitive control will render individuals vulnerable to develop a number of psychiatric problems (Cole et al., 2014). Probably sensorimotor involvement relates predominantly to repetitive behaviors (across disorders) and fronto-limbic circuits relate to anxiety. Loss of cognitive control *and* disturbances in several circuits at the same time may lead to more complex co-morbidity patterns. See Figure 8.1 for a schematic depiction of the ideas mentioned above.

Methodological considerations and limitations

Over two decades of neuroimaging research has provided an expending body of literature and several hypotheses on the pathophysiology of OCD and TS and (more recent) studies showed that other networks beside the CSTC circuits were involved. It has been disappointing, however, that results from imaging studies often cannot be replicated and that inconsistent or sometimes contradicting results exist. A major challenge for the field of neuroimaging will be to bridge these inconsistencies and to form unifying hypotheses and data processing and data analyses protocols. Some methodological issues pertaining to these inconsistencies will be discussed below.

First, neuroimaging studies (especially PET) are costly and time consuming and therefore in the earlier case control designs only a small number of participants (10-15) were included, typically leading to increased risks for false-negative results, or when no correction for multiple comparisons was applied the risk for false-positive results. Publication bias may have favored (possibly false)-positive results from small studies. Larger numbers of participants have been included in the more recent fMRI studies and efforts are made to conduct meta-analyses of existing data as well as to form international collaborations on data sharing and pooling, such as the Enhancing Neuroimaging Genetics through Meta-analysis (ENIGMA) initiative (Thompson et al.,

2015). Hopefully these efforts will reduce type-I and II errors and resolve conflicting results.

Second, as neuroimaging is an evolving field of research, new methods are constantly developed, but relatively little effort is put into a direct comparison between methods and no gold standards for data processing and data analysis exist. This poses a challenge to comparing results from different studies, as different methods for data processing and analysis may produce very different results. Results from more recent studies, that employed more stringent methods to correct for motion-related noise, for example, may not be directly comparable with older studies that have not used similar methods (this also hampers meta-analyses). A comparison between different methods of analysis and the harmonization of preprocessing and noise reduction steps is necessary and may lead to some sort of 'golden standards'.

The third and most difficult methodological issue pertains to clinical heterogeneity of mental disorders. The DSM classification provides an artificial boundary between disorders that does not necessarily follow neurobiological mechanisms. Moreover other factors may influence the neurobiological profile, such as gender, disease duration, (past and present) medication use, variability in symptoms and co-morbid symptoms. In

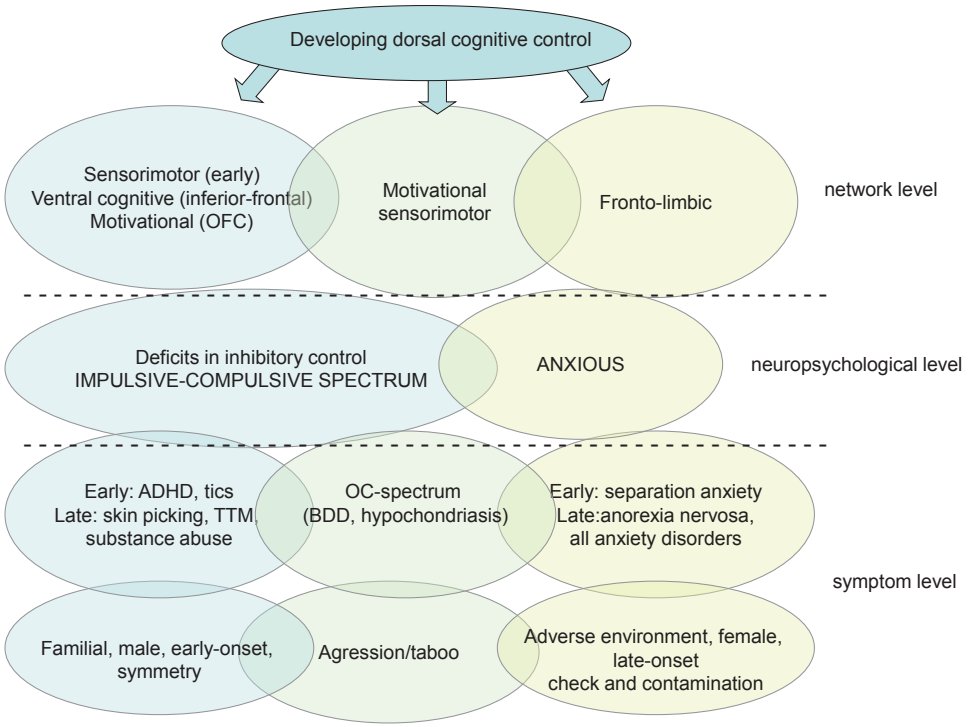

Figure 8.1: Integrating clinical profiles and putative neurobiological mechanisms.

OFC= orbitofrontal cortex, TTM= trichotillomania, BDD= body dysmorphic disorder

this thesis, only un-medicated patients were studied and groups were matched for age, gender and educational level. Including only un-medicated patients, however, may have biased the sample towards a less severely affected group. Furthermore, the included patients were a mix of early-onset and late-onset, familiar and non-familiar OCD, with different symptom and co-morbidity profiles, also including some tic-related OCD patients. Across imaging studies it is likely that, due to coincidence, patients are included with different disease profiles and disease stages, which may contribute to inconsistent results between studies. Although we performed post-hoc analyses to test if results remained significant after excluding patients with co-morbidity, the numbers were not large enough to investigate differential neural correlates for these different groups. Large datasets such as in the ENIGMA initiative can be used to investigate the influence of subtypes, co-morbidity and medication use, but are still bound to classification according to the current criteria. Due to pleiotropism and redundancy, diagnoses based on classification of clinical symptoms do probably not correspond with homogeneous neurobiological mechanisms.

The National Institute of Mental Health (NIMH) has developed the framework of Research Domain Criteria (RDoC) aiming to better integrate neuroscience and psychiatry. In this approach several levels (from genes to symptoms) of research are hierarchically ordered, starting at the micro level with genetic mechanisms, and advancing through molecular mechanisms, neural networks and neuropsychological function to clusters of symptoms. The intermediate level of neural network function in health and disease was proposed as a starting point for investigations. Molecular and genetic contributions to these networks can then be disentangled as well as the correspondence to symptom clusters. When the pathophysiological mechanisms are sufficiently clarified, a new diagnostic system could be based on etiology instead of symptom clusters (Cuthbert, 2014). Studying symptoms and neural mechanisms across disorders (**Chapter 2, 3 and 4**) may also advance new classification schemes. Criticism on the RDoC approach has pointed out that this framework (and the allocation of NIMH funding) favors the biological view on psychiatric disorders and tends to neglect the social and cultural context in which symptoms appear (Kirmayer and Crafa, 2014). Moreover the implementation of RDoC as a replacement for DSM classification in research was considered premature, as the assumptions underlying it are deemed disputable and the proposed measures to replace clinical criteria lack validity (Peterson, 2015).

The fourth and last methodological issues concerns the fact that most neuroimaging studies use a cross-sectional design that is not suitable for determining causal effects, as only correlations can be established. Therefore changes between patients controls can be a consequence of the disorder, compensatory efforts to support functioning despite of symptoms, have a causal relation with the disease or potentially be associated with another variable that co-exists with the patient group (Mataix-Cols and van den Heuvel,

2006). Using a family study design helps to differentiate between state and trait aspects, twin study designs may further increase knowledge on genetic and environmental factors and longitudinal studies will support inferences on causality.

Future directions

Following from the above several suggestions for future research can be made. Longitudinal studies starting in childhood (or even before birth) should include comprehensive measures on family history, genotyping and environmental stressors as well as validated assessments of neuropsychological development and psychiatric symptoms. Within such a study children at risk for neuropsychiatric disorders could be identified when their parents or siblings are affected with tics and or OCD, or alternatively children with at risk neuropsychological profiles or sub threshold symptoms could be identified. By following these children over time, genetic and environmental factors contributing to vulnerability and resilience can be studied in concert. Relevant neuropsychological measures specifically related to distinct but interacting networks concern, among others, inhibitory control (related to the ventral cognitive CSTC circuit), working memory (related to the dorsal cognitive CSTC circuit), reward learning (related to the ventral affective CSTC circuit), emotion regulation (related to the fronto-limbic circuit), and habit formation and motor processing (related to the sensorimotor CSTC circuit). Moreover, it would be very interesting to apply neurobiological knowledge in an attempt to create new classifications of psychiatric disorders, instead of following DSM criteria related to symptoms. For example, profiling a patient by possible genetic and environmental contributions as well as neuropsychological dysfunction could result in a personalized diagnosis and interventions.

Conclusions

OCD and TS are neurodevelopmental disorders that show overlap in phenomenology and neurobiology, and that may be considered as disorders in the same (impulsive-compulsive) spectrum. Dysfunction of multiple neuronal networks (sensorimotor, limbic, ventral affective, dorsal and ventral cognitive circuits) may be present with different contributions from each network in individual patients, resulting in heterogeneous clinical profiles. In addition, imbalance between these networks may depend on the (emotional) state and disease-stage of patients. Enhanced recruitment of cognitive control networks may partially compensate for network dysfunction/inefficiency and possibly protects against developing symptoms. The maturation and (dys)function of neuronal networks is complex as it is influenced by genetic and environmental factors and modulated by several neurotransmitters. To further elucidate the underlying neurobiology, TS and OCD should be studied from a neurodevelopmental perspective, starting in childhood and in comparison with other obsessive-compulsive related disorders (as classified in DSM-5), related neurodevelopmental disorders such as ADHD and autism, and to

anxiety disorders in order to better understand the relative contribution from brain circuit (dys)function to neuropsychological deficit and symptom clusters. Neuroimaging is one approach to further investigate these disorders by contributing to the development of an etiology-based classification system of psychiatric symptoms.