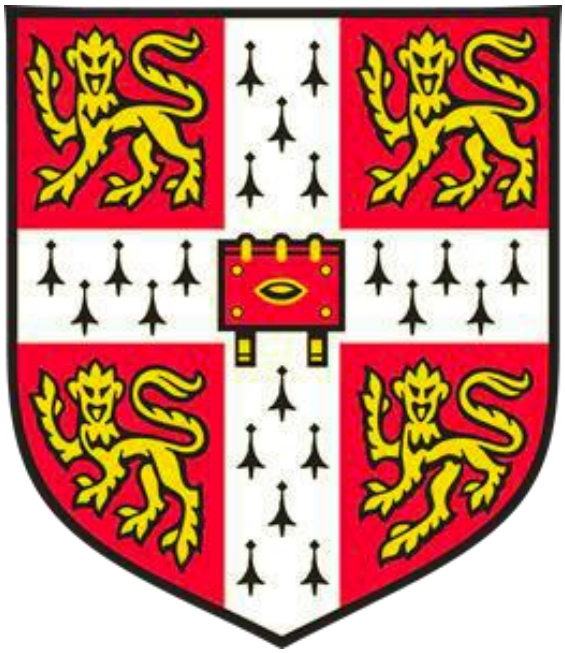
Neuropsychological and biological mechanisms of checking in OCD and clozapine induced Schizo-OCS



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Declaration

I hereby declare that this thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text, and that it is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text. This dissertation does not exceed the prescribed word limit for the Degree Committee for the Faculty of Biology.

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Abstract

The introductory **Chapter 1** reviewed several possible explanations of compulsive behaviour as manifested especially in Obsessive Compulsive Disorder (OCD), and schizophrenia, following treatment with the second generation antipsychotic medication clozapine. Particular focus is placed on compulsive checking behaviours and their relationship to current theories of compulsivity based on the hypothesis of imbalance between the goal-directed and habit systems and aberrant prediction-error learning.

Chapter 2 describes experimental attempts in this thesis to measure human checking behaviour in the laboratory. Initially, a previously published test of checking was employed which however failed to show significant increases in OCD. I then designed a new testing procedure to measure checking, based on perceptual decision-making under a time constraint. This was administered together with other cognitive tests to patients with OCD, clozapine treated schizophrenia patients without and with obsessive compulsive symptoms, and a healthy volunteer group. In general, there were no major differences compared to controls, although patients with schizophrenia performed worse. In a second study, contingency degradation learning and checking were measured using a second variant of the task in which there was no time constraint. However, significant increases in checking behaviour were shown in another group of OCD patients compared with healthy volunteers.

In **Chapter 3**, after a review of previous findings in OCD using the Magnetic Resonance Spectroscopy (MRS) technique at 7T, the same participants employed in the last checking study, were subjected to MRS scans to measure GABA, Glutamate (Glu), Glutamine (Gln), and NAA in three areas of brain including the Anterior Cingulate Cortex (ACC), Supplementary Motor Area (SMA) and the Visual Cortex. The most important findings were in the ACC, where significantly higher levels of Glu and Gln and lower levels of GABA and GABA:Glu ratio were found in OCD patients compared to the healthy group.

In **Chapter 4**, the relationship between the behavioural results from chapter 2 and the neurometabolites measured with MRS in our OCD and healthy participants in chapter 3 was examined. The major findings were: 1) Higher ACC GABA/Glu ratio was related to superior accuracy of decision-making as well as increased checking on the checking task in OCD patients. 2) Checking was negatively correlated with SMA Glu in the healthy group but not in OCD. Moreover, in a test of goal directed behaviour and habit learning based on contingency degradation, a positive relationship was evident between performance and the ACC GABA/Glu ratios in patients for full degradation of the task contingencies. A similar positive relationship was observed for healthy volunteers for GABA/Glu ratios in SMA for partial degradation of the contingencies.

Chapter 5 discusses neuropsychological interpretations of our findings in relation to the symptomatology of OCD and schizophrenia, together with their implications for understanding the role of the anterior cingulate cortex in decision-making and compulsive behaviour.

1. Chapter 1: General Introduction

1.1. Clinical features of Obsessive Compulsive Disorder

Obsessive Compulsive Disorder (OCD) is a highly debilitating psychiatric disorder affecting 2.5% - 3% of the population (Ruscio et al., 2010). The average age of onset is 19.5 years (Fenske & Petersen, 2015). However, many cases go undetected, mostly due to the embarrassment associated with the nature of the symptoms (e.g. inappropriate sexual thoughts or compulsive behaviours) or are undertreated; the average time to seek treatment is 11 years (Pinto et al., 2006). The disorder onset follows a bimodal distribution, with a peak at approximately 10 years of age and a second one in the 20s (Geller et al., 1998; Geller et al., 2006; Hemmings et al., 2004) which may indicate distinct clinical, etiological and biological factors (Robbins et al., 2019). Twin studies estimate different heritability rates of OC symptoms in children (45% to 65%) and adults (27% to 47%) with OCD (Van Grootheest et al., 2005). Strong evidence exists for familial aggregation of OCD, with an increased risk for OCD and obsessive compulsive behaviours amongst first-degree relatives of individuals with OCD (Browne et al., 2014; Fyer et al., 2005; Grabe et al., 2006; Pauls et al., 2014). Complementarily, neurocognitive endophenotypes exist in unaffected relatives of patients with OCD, showing similar cognitive and neural abnormalities (Menzies et al., 2007).

OCD is characterised by *obsessions*- recurrent intrusive thoughts, impulses or images that are unwanted and anxiety provoking and *compulsions*- ritualistic mental or physical actions that a person feels compelled to perform, described by patients as ego-dystonic- being excessive and senseless (American Psychiatric Association, 2013). The clinical phenotype of OCD is very heterogeneous and can differ completely from one patient to another which may complicate treatment decisions (McKay et al., 2015). Several studies tried to reduce this heterogeneity to a few main symptom categories (Cullen et al., 2007; Denys et al., 2004; Feinstein et al., 2003; Mataix-Cols et al., 2002) using the commonly used Yale-Brown Obsessive Compulsive Scale (Y-BOCS) symptom checklist (Goodman et al., 1989) consisting of more than 50 obsessions and compulsions. According to a meta-analysis including 21 studies and 5,124 patients, a four factor structure could explain a large proportion of heterogeneity in clinical symptoms of OCD. These factors were: 1) *symmetry*: symmetry obsessions, ordering and counting compulsions; 2) *forbidden thoughts*: aggression, sexual, religious and somatic obsessions, and checking compulsions; 3) *cleaning*: contamination obsession and cleaning compulsions; and 4) *hoarding:* hoarding obsessions and compulsions (Bloch et al., 2008). OCD symptom dimensions seem to have specific neural substrates (Mataix-Cols et al., 2004; Phillips & Mataix-Cols, 2004) and remain stable over time, rarely shifting to another dimension (Mataix-Cols et al., 2002). The question remains whether these different groups are different subtypes or different disorders. This is particularly the case for

hoarding as some have suggested it to be a different disorder due to its absence of anxiety, or an alternative perspective could be that it is OCD with purely compulsive behaviour, e.g. hoarding (Robbins et al., 2019). However, a recent study in a multinational cohort of 1366 OCD patients across life span, suggested a larger number of symptom dimensions than classically assumed, with different primary and sub-dimensions (Cervin et al., 2021). Using the 87 questions on the YBOCS symptom check list, and factor and network analysis techniques, the authors reduced the OCD symptoms into 8 parsimonious dimensions of 1) *hoarding*, which was separate from other dimensions and not central at all in the network analysis, 2) *somatic/body focus*, linked to incompleteness dimension and associated with eating disorders, 3) *superstition*, more common in adults than children, showing superstitious fears and behaviours, and giving significance and meaning to numbers and colours, 4) *transformation*: fear of becoming someone else and gaining unwanted characteristics, associated with body focus and disturbing thoughts dimensions and more common in children and adolescents with OCD, 5) *separation/loss*, with a history of separation anxiety disorder, and was not previously described as an OCD symptom, 6) *disturbing thoughts*, consisting of harm/checking, and forbidden thought sub-dimensions, 7) *incompleteness*, divided into 3 sub-dimensions of accuracy, mental/perceptual factors, and Not Just Right behaviours, and 8) *contamination*, divided into separate sub-dimensions of having fear of illness (disease concern) and disgust (dirt/cleanness). The incompleteness and disturbing thoughts appeared as the most central dimensions, with the most unique interconnections to the other dimensions, and could possibly represent core OCD phenotypes (Cervin et al., 2021).

There are different theories about what mechanisms could underly the OCD symptoms such as anxiety, cognitive inflexibility and inability to regulate thoughts, lack of cognitive control which could lead to an inability to control compulsive behaviour, or a disruption in balance between the goal directed and habitual systems, where OCD may manifest due to excessive habit formation. A different approach also considers impairments in meta-cognition and decision making related to aberrant processing of uncertainty (Robbins et al., 2019). It is crucial to understand which of these hypotheses are correct in order to design better treatments for OCD.

Although OCD has a lifelong persistence, there are treatment options that can significantly improve the symptoms, however, not all treatments work for all patients. Cognitive Behavioural Therapy (CBT) with elements from *in vivo* Exposure and Response Prevention (ERP) is a first line of psychotherapeutic treatment in OCD. During this form of treatment patients are confronted with situations or items that would provoke their symptoms and they are encouraged to resist performing their compulsions (Meyer, 1996; Rasmussen & Eisen, 1997). First line of pharmacological therapy involves regulating the serotonin (5-hydroxytryptamine, 5-HT) levels. There is ample evidence supporting the efficacy of selective serotonin reuptake inhibitors (SSRIs) in the treatment of OCD such as fluoxetine, sertraline, fluvoxamine, paroxetine, and citalopram (Eddy et al., 2004; Koran et al., 2007; Skapinakis et al., 2016;

Soomro et al., 2008). Patients with more severe symptoms could be prescribed with the tricyclic drug clomipramine, or augmenting SSRI's with an atypical antipsychotic medication such as dopamine (DA) receptor blocking agents (Fenske & Petersen, 2015; Dold et al., 2015; Robbins et al., 2019). In patients responsive to conventional treatments however, the most effective outcome is usually achieved by combining the ERP and SSRI treatments (Foa et al., 2005; Jenike, 2001). Neuromodulation approaches may also be employed to treat severe treatment resistant patients. Although invasive, amongst all neuromodulation techniques, OCD patients seem to benefit the most from the deep brain stimulation targeting regions within the frontostriatal network such as ventral capsule/striatum, sub-thalamic nucleus, anterior limb of the internal capsule and nucleus accumbens (Bias et al., 2014; Denys et al., 2010; Vicheva et al., 2020).

OCD symptoms can also present themselves in other psychiatric disorders such as schizophrenia. One important subgroup is schizophrenia patients with obsessive compulsive symptoms (OCS) or an OCD diagnosis as a consequence of their antipsychotic medication. The next chapter will describe this important patient group.

1.2. Clinical features of clozapine induced OCS in schizophrenia

Schizophrenia is a disabling psychiatric disorder affecting one percent of the general population (Schultz et al., 2007). It is characterised by symptoms such as hallucinations, delusions, poor planning, disorganised communication, reduced motivation, and blunted affect and around two-thirds of the individuals with schizophrenia continue having persisting or fluctuating symptoms despite receiving treatment (Saha et al., 2005). Patients with schizophrenia often present comorbidities with other psychiatric disorders such as substance abuse disorder, anxiety disorder, depression, and obsessive compulsive disorder. They have a 12.5 fold risk of having comorbid OCD with an average prevalence of 25% for Obsessive Compulsive Symptoms (OCS) and 23% for OCD (Buckley et al., 2009). OCS can precede, or occur in the prodromal stage, or during the first episode, with approximately 10% of first episode psychosis cases also presenting OCS (Hagen et al., 2013). Additionally, OCS can also occur after the initiation of antipsychotic medication.

The development of obsessive-compulsive symptoms as a consequence of Second Generation Antipsychotics (SGAs), particularly via clozapine treatment, which has the most complex binding properties in psychopharmacology (Stahl, 2000), with mainly anti-serotonergic (especially 5-HT2A, 5-HT1A and 5-HT2C- receptors) and anti-dopaminergic (especially the D2 receptor) mechanisms (Meltzer et al., 2008), is commonly reported in a substantial proportion (20-70%) of schizophrenia patients (Beduin et al., 2012; Fonseka et al., 2014; Doyle et al., 2014; Mukhopadhaya et al., 2008; Schirmbeck & Zink, 2012). Schizophrenia patients with obsessive-compulsive symptoms (schizo-OCS)

have a more negative prognosis, such as higher severity of positive and depressive symptoms (Biria et al., 2019; Sa et al., 2009), reduced social functioning (Tonna et al., 2015) and a greater suicidal risk (Szmulewicz et al., 2015). Both individuals with schizophrenia and OCD show similar neuropsychological dysfunctions such as difficulties in different aspects of executive functioning, memory, and attention, especially attentional set-shifting (Abramovitch et al., 2013; Bowie & Harvey, 2006; Goldberg & Green, 2002). At the neural level, there are functional and structural changes related to clozapine treatment of psychosis.

Several studies have found volume reductions in the basal ganglia, mainly the caudate nucleus, and increased grey matter volume in the left frontal lobe in participants on SGAs when compared to those on First Generation Antipsychotics (FGAs; Chamberlain et al., 2008). Although similarities in brain abnormalities have been found between OCD and schizophrenia participants treated with SGAs, e.g. in orbitofrontal dysfunction (Garcia et al., 2015; Schirmbeck et al., 2015), it remains unclear what exactly causes individuals experiencing schizophrenia on clozapine to develop OCS and, more specifically, the neurobiological differences between schizophrenia participants on clozapine, with and without OCS, and individuals with OCD.

When it comes to their clinical presentation, OCD obsessions and schizophrenia delusions may be difficult to distinguish, making the identification of OCS in schizophrenia more challenging. One essential criterion for differentiating them is the higher level of insight in OCD/OCS, with OCS being ego-dystonic, and unwanted, and the awareness that the obsessions or rituals are irrational. Whereas insight is typically lacking for delusional thoughts (De Haan et al., 2015). When comparing the schizophrenia patients with and without OCS, schizo-OCS patients, the severity of schizophrenia symptoms such as the positive, and depressive symptoms are higher in the schizo-OCS patients (Biria et al., 2019; Seng et al. 2018), and found to be correlated with the obsessive compulsive symptoms (Gürcan et al., 2021). The schizo-OCS patients also seem to exhibit worse cognitive flexibility as measured by increased number of errors during the extradimensional set-shifting of the CANTAB IED task, higher obsessive compulsive scores as measured by YBOCS, higher depression scores, and a trend towards increased motor tics (Patel et al., 2010).

With regard to the obsessive compulsive symptoms in schizophrenia patients, studies suggest predominant checking behaviour over other types of compulsions (Grover et al., 2015). Additionally, excessive checking behaviour becomes exacerbated over time, as prevalence increases from 25% for people treated during the first 5 years of clozapine, to over 50% of those treated more than 10 years (Fernandez-Egea et al., 2018). Co-administration of clozapine with the partial dopamine agonist/antagonist aripiprazole (De Bartolomeis et al., 2015) appears to be very effective at reducing the obsessive-compulsive symptomatology induced by clozapine in schizophrenia patients (Englisch et

al., 2009; Eryılmaz et al., 2013; Glick et al., 2008). However the mechanism of action responsible for its effectiveness is still unknown. It is possible that the dopamine receptor antagonism works by reducing the activity of ACC (Volkow et al., 2000). In OCD, the ACC appears to be overactivated during error monitoring paired with enhanced Error Related Negativity (ERN), elicited by the erroneous responses (Carrasco et al., 2013; Endrass et al., 2008; Endrass & Ullsperger, 2014; Nieuwenhuis et al., 2005), and in response to reward prediction error signals as measured by functional MRI studies when adjustment of behaviour was needed (Hauser et al., 2017a; Murray et al., 2019). Interestingly, this overactivity in OCD is modulated by dopamine (Murray et al., 2019), and in line with this finding, others have reported a decrease in ERN amplitude in schizophrenia patients after antipsychotic treatment with the D2 dopamine antagonist haloperidol (De Bruijn et al., 2006; Zirnheld et al., 2004). Thus, perhaps the increased and decreased symptomatology in schizophrenia patients is a consequence of the opposing roles that clozapine and aripiprazole may have in the modulation of ACC activity, respectively. Treatment with SSRI's could also potentially ameliorate OCS in schizophrenia (Poyurovsky et al., 1999; Stryjer et al., 2012).

The clozapine dose employed was found to be higher in schizo-OCS group compared to the schizophrenia patients on clozapine without any OC symptoms (Biria et al., 2019). Additionally, several studies demonstrated a positive correlation between the severity of OCS and the clozapine dose (Biria et al., 2019) and plasma levels (Fernandez-Egea et al., unpublished observations, personal communication; Gürcan et al., 2021; Kim et al., 2020; Lin et al., 2006; Mukhopadhaya et al. 2009; Reznik et al., 2004; Schirmbeck et al. 2011). However, when it comes to the clozapine treatment duration, the findings are more mixed with some studies reporting a positive correlation between the duration of treatment and OCS (Lin et al., 2006; Schirmbeck et al., 2011), while another recent study did not find any relationship (Kim et al., 2020).

1.3. Neural correlates of OCD

The most prominent neuroanatomical model of obsessive compulsive disorder (OCD) suggests abnormal functioning of cortico-striato-thalamo-cortical (CSTC) pathways (Saxena & Rauch, 2000), involved in motor, cognitive, affective and motivational processes (Cummings, 1993; Groenewegen & Uylings, 2000). According to this model, tracts from frontal cortical areas travel to striatum, and then project through direct and indirect pathways to thalamus, and travel back to frontal areas. This hypothesis is supported by numerous studies showing functional and structural alterations in patients with OCD (Robbins et al., 2019). The cortical pathway includes Anterior Cingulate Cortex (ACC), ventromedial prefrontal cortex (vmPFC), dorso-lateral prefrontal cortex (dlPFC), and lateral-orbitofrontal cortex (OFC). The striatal pathway consists of Nucleus Accumbens (NAc), Caudate

nucleus, and putamen. Caudate and putamen together are part of the dorsal striatum, whereas, the ventral striatum includes NAc, and parts of the caudate nucleus (Milad & Rauch, 2012). Moreover, a recent meta-analysis has identified the most commonly used seeds in resting state functional connectivity studies in OCD to be the striatum (putamen, caudate, and NAc), thalamus and ACC, highlighting the importance of these areas for OCD symptomatology (Liu et al., 2022).

The direct CSTC pathway is a positive feedback loop, leading to initiation and continuation of behaviour. It sends an excitatory glutamatergic signal is to striatum, then striatum gamma-amino-butyric acid (GABA)-ergic neurons project to internal globus pallidus, which leads to disinhibition of thalamus, and in turn an increased excitation of frontal cortex. Whereas, the indirect pathway acts as a negative feedback loop, important for inhibition and switching of behaviour. In the indirect pathway, an enhanced inhibition of thalamus leads to a decreased excitation of the frontal areas. It starts by striatum sending inhibitory projections to external globus pallidus and the subthalamic nucleus, and in turn increased excitation of the internal part of the globus pallidus, which increases the inhibition of the thalamus. Impairments within these pathways, and an imbalance between inhibition, initiation and a flexible switching between behaviour can lead to abnormalities observed in OCD symptoms (van den Heuvel et al., 2016). Targeting the NAc using deep brain stimulation has been proven effective in reducing obsessive and compulsive symptoms, anxiety and depression in treatment refractory patients with OCD (Denys et al., 2010). NAc receives projections from the ACC, which also has been successfully targeted by cingulotomy to improve OCD symptoms in severe patients (Dougherty et al., 2002; Rauch et al., 2001).

The anatomy and neurochemistry of these pathways were well established in the past 3-4 decades (Alexander et al., 1986), and formed the basis for early studies showing abnormal metabolism in orbito-frontal cortex (OFC) and caudate nucleus (Baxter et al., 1987; Saxena et al., 1999), and ACC (Perani et al., 1995; Swedo et al., 1989) in OCD using positron emission tomography (PET). The recent advances in neuroimaging have helped us understand more about the structural and functional connectivity of these regions. Resting state functional connectivity studies have found an altered functional connectivity between basal ganglia and cortical pathways in OCD. More specifically, a reduced functional connectivity between the caudate and vmPFC, and increased connectivity between ventral striatum to dlPFC (Harrison et al., 2009; Vaghi et al., 2017a). The early models of CSTC pathways considered OFC having a homogeneous function and structure, however, later studies have made us aware of two primary distinct sub-regions within the OFC, such as the lateral OFC and medial OFC (lOFC, mOFC respectively; Milad & Rauch, 2012). These areas seem to play different roles in cognitive and reward processing. For example, the lateral OFC seems to be involved in ritualised behaviour in response to punishment and danger, whereas the medial OFC may be relevant for emotion regulation

and processing of reward (Kringelbach & Rolls, 2004). There is also ample evidence in humans and rodents, showing the role of vmPFC, and mPFC in the regulation of fear (Graham & Milad, 2011).

There are resting state, and functional task related, and symptom provocation studies providing evidence of the diverse functions and deficits of these subregions in OCD (Robbins et al., 2019). While in a resting state, reduced functional connectivity between lateral PFC and dorsal striatum was observed in patients with OCD, whereas an enhanced connectivity was found between vmPFC, and medial PFC regions and the ventral striatum (Apergis-Schoute et al., 2018; Harrison et al., 2009; Vaghi et al., 2017a). Additionally, within the CSTC pathways, the vmPFC was hyperconnected to the dACC through the thalamus (Apergis-Schoute et al., 2018). A recent meta-analysis also reported a hyperconnectivity between the ACC and vmPFC, caudate and putamen, whereas, the connection between dlPFC and ACC was reduced (Liu et al., 2022). However, it is important to note that vmPFC may also be involved in large-scale brain networks in OCD patients, as this area was hyperconnected to regions outside of the CSTC pathways, such as the temporal and occipital lobes, cerebellum and motor areas (Apergis-Schoute et al., 2018).

Reversal learning, an important measure of cognitive flexibility, is associated with hypoactivation of lateral OFC in OCD. During an affective reversal learning task, when change in behaviour was required after punishment, OCD patients showed deficits in their behaviour which was associated with reduced activity of the lateral OFC (Remijnse et al., 2006). This finding was later confirmed in both OCD patients and their first-degree relatives (Chamberlain et al., 2008). In contrast, during fear reversal learning, in an aversive setting, OCD patients failed to differentiate the safe from the threatening stimulus after the reversal and showed hyperactivation of vmPFC (Apergis-Schoute et al., 2017). Similar results were found when patients had to learn to avoid aversive stimuli (Gillan et al., 2015a). A reversed pattern of hyperactivation of lateral OFC and hypoactivation of medial OFC was found in a symptom provocation study, after presenting OCD related images or videos to patients (Banca et al., 2015a).

OCD pathophysiology likely represents dysfunctional network interactions rather than disruptions within individual structures. Abnormalities within the direct and indirect projections of the cortico-striato-thalamo-cortical pathways seem to underlie the diverse set of symptoms observed in OCD, caused by abnormal inhibitory and excitatory projections. This is considering the importance of motivational, avoidance, goal-directed and habit-directed behaviours in OCD which require a balance and flexibility in initiation and inhibition of behaviours, and the role of CSTC pathways in these processes. A detailed literature review on PET and Magnetic Resonance Spectroscopy (MRS) studies in OCD will be discussed further in Chapter 3.

1.4. Cognitive characteristics of Obsessive Compulsive Disorder

1.4.1. Cognitive flexibility

Cognitive flexibility can be defined as shifting one's attention from one concept or stimulus to another in response to changing rules or demands in the environment (Dajani & Uddin, 2015). It is one of the three posited executive functions besides updating of working memory and inhibitory response control, the processes that regulate thought and action (Friedman et al., 2006). Cognitive flexibility can be seen as a form of learning, and machine learning theorists proposed a distinction between 'exploration versus exploitation' to define the balance needed between these two states, in order to adapt successfully to the changing environment and optimise learning (Cohen et al., 2007; Kaelbling et al. 1996). It is then no surprise to see a repetitive pattern of thoughts (obsessions) and actions (compulsions) in presence of cognitive inflexibility in OCD patients. However, this type of learning seems to be different than the executive functions required to switch from one task to another. Researchers have studied the latter function (executive switching) using tasks such as the Trail-Making Test (TMT; Lezak et al., 2004) which requires switching in response to changing feedback. For example, during the TMT, participants are supposed to alternate drawing lines between letters and numbers. OCD patients are shown to be slower on the test compared to healthy controls (Ozcan et al., 2016). Another similar test is the Wisconsin Card Sort Task (WCST), which requires sorting cards according to a changing rule (e.g. shape or colour) which can be learnt by trial and error. OCD patients typically make more errors on the WCST (Bucci et al., 2007; Okasha et al., 2000; Tükel et al., 2012). The CANTAB Intra-Extra Dimensional Set Shift (IED; Owen et al., 1991; Roberts et al., 1988) is the computerised analogue of the WCST and can be used to measure cognitive flexibility, more specifically the set-shifting ability (rule acquisition, extra-dimensional set-shifting and reversal learning). Participants are instructed to choose the correct alternative from two options, either two single (early stages), or compound stimuli (later stages). Each stimulus has two dimensions of lines or shapes. After choosing one of the stimuli, feedback is provided to help learn the current rule. Once the current rule is learnt and a correct answer is given repeatedly for a certain number of times, the rule changes and the participant is tested on the ability to learn the new rule (reversal learning). In the next stage of the task, a compound stimuli is presented and the correct dimension must be found through trial and error (Intra-Dimensional Shift, IDS), also followed by a reversal stage. The final and most relevant stage is the Extra-Dimensional Shift (EDS) stage, where participants are presented with new compound stimuli, and for the first time the previously irrelevant stimulus category or dimension (e.g. line) becomes relevant, and the previously relevant one (e.g. shape) becomes irrelevant. Thus during the EDS stage, participants must learn to shift attention away from a previously learnt rule (relevant stimulus dimension) and direct it towards a previously irrelevant stimulus dimension, as opposed to the IDS stage, where they had to pay attention to the previously relevant stimulus dimension when new stimulus exemplars are presented.

These different types of cognitive flexibility have shown to have their own neural circuits in both marmosets, with lateral PFC relevant for EDS, and the OFC important for reversal learning (Dias et al., 1996), and humans, with vmPFC relevant for EDS, lateral OFC for reversal after positive feedback, and medial OFC for the reversal after punishment (Hampshire & Owen, 2006). The dissociation between these aspects of cognitive flexibility is also apparent from studies finding separate neural deficits in different patient groups. For example, patients with lateral frontal lobe damage seem to be impaired on EDS, but not IDS (Owen et al., 1991), whereas, frontal lobe dementia patients, presented difficulty with reversal learning (Rahman et al., 1999).

Amongst findings on cognitive inflexibility in OCD, the EDS deficit is the most robust finding (Chamberlain et al., 2007; Chamberlain et al., 2021; Vaghi et al., 2017), meaning patients keep selecting the previously important rule, instead of shifting attention towards the new rule. At a neural level, Vaghi et al. (2017) reported an aberrant connectivity of the fronto-striatal connectivity, such as reduced functional connectivity between ventrolateral prefrontal cortex and dorsal caudate nucleus , which was associated with worse a EDS performance. Cognitive inflexibility may be found across other conditions whose symptoms may indicate repetitive or compulsive behaviour. For example, patients with first episode psychosis, showed impairments during the early discrimination stage, reversal learning and ED shifting (Leeson et al., 2009). Schizophrenia patients also demonstrated deficits in reversal, compound stimuli dissociation, and ED shifting (Elliot et al., 1995). In addition to the previous impairments in schizophrenia, Pantelis et al. (1999) also found IDS deficits. Interestingly, schizo-OCD patients behaved closer to OCD patients than schizophrenia, showing a selective impairment during ED shift only (Patel et al., 2010).

In conclusion, ED shift deficits are the main source of cognitive inflexibility in OCD and schizo-OCD, however, other related disorders of compulsivity often show a different pattern of deficits.

1.4.2. Habit-directed and goal-directed systems

Actions are suggested to be regulated by two distinct systems. A goal-directed system that relies on strengthening the link between stimulus in the environment, action/response and it outcome (S-R-O), and a habit system, insensitive to the outcome, and relying on formation of a direct link between Stimulus-Response (S-R). The goal-directed system is mediated by the knowledge about contingency between an action and its outcome and controls actions in order to obtain specific desirable outcomes. On the other hand, habits can occur automatically by perceiving the stimulus in the context, even if the outcome is no longer associated with it (Balleine & O'Doherty, 2010; Verplanken, 2018). The balance between these two systems is required for reward learning behaviour. While the goal-directed system

is more accurate, it needs more effort and attention, as opposed to the easy and automatic execution of habits. Both systems are needed to regulate behaviour, the goal-directed system is required for new learning, whereas the habit system can be helpful for gaining control after a prolonged training (Dickinson et al., 1995). OCD symptoms have been conceptualised as maladaptive habits (Graybiel & Rauch, 2000), being ritualistic in nature without a clear purpose or goal. Both behavioural and neural evidence support the existence of these two distinct systems.

At behavioural level, two behavioural tasks, outcome devaluation and contingency degradation, are commonly used to study the extent of overreliance on habitual over the goal-directed system. These tasks are based on sensitivity to change in value of an outcome. In the outcome devaluation task, participants are instructed to select different stimuli that would gain them a reward. In order to do so successfully, they need to flexibly update which stimuli are not yielding valuable outcomes anymore. When a devalued option is persistently selected, the behaviour is considered as not goal-directed and under the control of the habit system (Dickinson & Balleine, 1994). Gillan et al. (2011), demonstrated the overreliance on habits in OCD patients due to a deficit in the goal-directed system. Patients continued selecting the devalued stimuli under both appetitive and aversive conditions (Gillan et al., 2011). In contingency degradation task, the association between an action and outcome is tested by modifying the contingencies. Participants are instructed to respond to an stimuli and to rate how much their actions were predictive of the outcome. In some conditions, actions can predict outcome with a high probability, whereas in other conditions, the outcome still occurs without the need to perform an action. Learning this contingency and performing a goal-directed behaviour allows to gain the reward (outcome), without performing the action. Habitual responses are measured if an instrumental behaviour, trained in a previous phase, is still continued after degrading the contingency between the action and outcome. OCD patients tend to keep responding even after degrading the relationship between action and outcome, and despite having intact action-outcome knowledge (Vaghi et al., 2019).

The neural evidence also points towards two distinct pathways underlying the two goal-directed and habit systems. The medial PFC and caudate nucleus seem to be responsible for goal-directed behaviour as they showed increased and decreased activity corresponding to selecting the valued and devalued options respectively (Valentin et al., 2007). Whereas putamen seems to underlie the habit system activity in both humans (Tricomi et al., 2009) and rodents (Yin et al., 2004). In a symptom provocation study (triggering obsessions and compulsions by showing provocative images or videos), OCD patients showed a deactivation of medial PFC and caudate nucleus, regions associated with the goal-directed system, and hyperactivity of the subthalamic nucleus and putamen, which were associated with habit system. These findings provide neural evidence for two dissociate pathways for each system, and the role of the habit system in triggering OCD compulsions (Banca et al., 2015). Caudate nucleus receives main cortical projections from ACC and in line with previous findings, inactivation and hyperactivation

of ACC or reduced excitatory input from ACC to caudate, all led to deficits in goal directed behaviour in marmoset monkeys, using the contingency degradation paradigm (Duan et al., 2021).

Although compulsions have been hypothesised to result from the aberrant formation of, and control over habits, to date, this hypothesis has been tested only indirectly by observing impairments in goal-directed behaviour (Banca et al., 2015; Banca et al., 2016; Gillan et al., 2015a; Gillan et al., 2015b; Vaghi et al., 2019). The implicit assumption that if the behaviour is not goal-directed, it must be habitual, is questionable, as it is a "zero-sum game" (Robbins & Costa, 2017). Failure in updating the value in goal-directed tasks, shows a deficit in the first stage of habit formation representing a more cognitive stage. Whereas, a distinct neural pathway may be at play to strengthen the link between stimulus and response, the autonomous stage, being autonomous from the goal (Robbins et al., 2019). It is also important to note the difference between autonomous and automatic behaviour, as skilled behaviour, can be automatic and yet goal-directed (Robbins & Costa, 2017).

To conclude, OCD symptoms arise from an imbalance between the goal-directed and habit-directed controls. However, to make claims about the involvement of each system specifically, better study designs are required to measure habit formation directly rather than implicit assumptions based on deficits in the goal-directed system, as without knowing the precise involvement of the habit system, we cannot assume their involvement to be a "zero-sum game".

1.4.3. Compulsive checking and underlying mechanisms

1.4.3.1. Inflated sense of responsibility/Harm avoidance

Cognitive behavioural models of obsessive compulsive disorder state that the intrusive thoughts act as cognitive stimuli and are experienced by the clinical as well as the healthy population (Rachman, 1976, 1993; Salkovskis, 1985, 1999). They suggest that the cognitive response to these intrusive thoughts in OCD patients are typically linked to increased beliefs of harm to themselves or others and increased feelings of responsibility. These beliefs would then increase feelings of discomfort and anxiety and lead in turn to neutralising and anxiety reducing compulsive behaviours such washing or checking (Salkovskis et al., 2000). Although there are different types of neutralising behaviours identified in OCD, several authors have suggested that increased sense of responsibility is particularly important for inducing checking behaviour (Ladouceur et al., 1995; Lopatka & Rachman, 1995; Rachman 2002). Additionally, obsessive compulsive checkers (and not non-checkers) seem to have an increased perception of responsibility for harm and this may lead to a higher need to rectify the situation to reduce the potential harm (Foa et al., 2002).

In a study investigating the impact of responsibility on checking behaviour, OCD, anxiety disorder and healthy control participants were instructed to classify pills from a large pot into smaller pots according to their colours. Participants were allocated to 'low' versus 'high' responsibility conditions with their action having no influence versus fatal impact on patients' lives after taking the pills. OCD patients within the high responsibility condition carried out more checking compared with the low responsibility group. The authors concluded that responsibility plays a causal role in checking behaviour, however, only in OCD patients. The increased responsibility did not impact checking behaviour in anxiety and healthy groups (Arntz et al., 2007). Mantz et al. (2019) studied the role of increased feelings of responsibility and threat in paediatric samples with OCD, anxiety disorder and healthy controls. The results showed that overall children with OCD spent more time completing the task and checked significantly more. Whereas within the OCD group, higher threat/responsibility feelings led to more checking and spending more time to complete the task, compared to the low threat condition (Mantz et al., 2019). Even without modifying responsibility feelings, OCD patients seem to report a higher trait and state responsibility for harm than anxiety disorder patients, and checked for a longer duration on an in vivo 'stove checking' experiment in a functional kitchen (Bucarelli & Purdon, 2016).

According to the cognitive theory of compulsive checking in OCD patients, the checking behaviour is maintained by a heightened sense of responsibility and reduced memory confidence. In a study by Radomsky & colleagues (2020) nine OCD patients, with checking behaviour performed for at least one hour a day, underwent 12 CBT sessions mainly focused on reducing the inflated responsibility beliefs, recalculating the beliefs about harm and modifying the negative beliefs about memory confidence. After the 12 sessions, responsibility beliefs were reduced along with increased memory confidence. These improvements significantly predicted the duration of checking behaviour (Radomsky et al., 2020). However, since the therapy targeted both inflated responsibility beliefs and memory confidence at the same time, it would be difficult to draw conclusions for each aspect separately. Although inflated responsibility seems to have a role in checking behaviour in OCD, in non-clinical population these findings were not replicated in sub-clinical high checkers (Cougle et al., 2007;), nor in healthy students, by manipulating the responsibility feelings (Grisham et al. ,2014).

Summary

Based on the available literature, we can conclude that an inflated sense of responsibility may be related to increased checking behaviour, both in adults and children with OCD whether they are checkers or non-checkers in real life. However, the inflated responsibility beliefs may be an OCD trait with no impact in other populations such as healthy individuals or anxiety disorder. It is not yet clear if an inflated responsibility belief is sufficient to induce checking, or whether there is an interplay with other clinical or cognitive aspects of OCD symptoms. The next sections discuss the role of anxiety, intolerance of uncertainty, memory deficits, or meta-cognition on checking.

1.4.3.2. Anxiety

In the studies above, although inflated responsibility was studied in anxiety disorder with no relation to checking, this does not indicate what the role of anxiety might be on OCD checking compulsions. Clair et al., (2013) suggest that anxiety has no impact on checking by studying OCD checkers versus non-checkers. What differentiated the two groups was the increased number of checks for non-anxiety provoking stimuli in OCD checkers, as compared with the anxiety provoking ones (Clair et al., 2013). In another study, when the stimuli were provocative images commonly associated with contextual cues in OCD suggesting the possibility of a harmful event occurring, OCD patients, followed by a subclinical checking group experienced increased anxiety, and indicated higher obsessive thoughts and urges to check after seeing the images, compared to a healthy control group with no checking tendencies (Brooks et al., 2018). Moretz & McKay (2009) found a small but significant relationship between the trait anxiety and checking behaviour in a non-clinical sample ($r = 0.18$; Moretz & McKay, 2009).

Summary

OCD patients experience increased sensitivity to harm and elevated feelings of responsibility, both of which may in turn be accompanied by feelings of anxiety. However, anxiety alone does not seem to be sufficient to explain checking behaviour. It may however have a mediating role in the relationship between inflated responsibility and threat overestimation or intolerance of uncertainty. Better study designs would be required to capture this mediatory effect in OCD patients. The next section will discuss the role of intolerance of uncertainty on checking compulsions.

1.4.3.3. Intolerance of Uncertainty

Pathological doubt and intolerance of uncertainty have been described as hallmark features of OCD patients, especially so in OCD checkers (Dar, 2004). OCD patients seem to be more sensitive to uncertainty than the healthy population (Tolin et al., 2003). Their obsessive thoughts usually relate to uncertainty pertaining to possible negative outcomes and often lead to compulsive actions aimed at reducing feelings of uncertainty (American Psychiatric Association, 2013). This intolerance towards uncertainty may lead to reassurance seeking behaviour such as repeated checking. However, ironically, this repeated checking may in turn lead to even more uncertainty (Boschen & Vuksanovic, 2007; Dek, van den Hout, Giele, & Engelhard, 2010; Radomsky, Gilchrist, & Dussault, 2006), which thus maintains the typical checking behaviour observed in OCD patients.

To study the impact of mild uncertainty on checking behaviour, Toffolo et al. (2013) divided healthy volunteers into two groups of high versus low obsessive compulsive symptoms (OCS) according to their Obsessive Compulsive Inventory-Revised scores (OCI-R; Foa et al., 2002). Participants were

instructed to find a visual target, which in some trials was absent (uncertain trials) and in some trials was present (certain trials). Checking behaviour was measured by assessing search time and the number of fixations/checks using an eye-tracking device. High OCS individuals checked more and longer, as compared with the low OCS individuals, but only in uncertain trials (Toffolo et al., 2013). The previous study was repeated in OCD patients using the same paradigm, compared with anxious and healthy controls. OCD patients checked more than both groups in certain and uncertain conditions, however, with a larger effect in the uncertain trials. No differences in checking behaviour were reported between the anxious and healthy control groups (Toffolo et al., 2016).

Summary

Uncertain situations increase checking in both OCD patients and healthy individuals with higher OC tendencies, but not in anxiety patients or healthy participants. However, these few studies do not confirm whether intolerance of uncertainty is sufficient to induce checking, or whether other cognitive constructs such as memory or meta-cognition could play a direct or mediating role on checking. In the next section, the impact of memory and meta-cognition on checking will be discussed.

1.4.3.4. Memory performance

Memory functioning has been one of the most widely studied cognitive constructs in OCD, mainly because of the intuitive hypothesis that memory impairments could be the main mechanism underlying compulsive rituals. For example, people may check as a consequence of their inability to encode or retrieve the memory of their past actions**.** However, the memory literature in OCD is swamped by inconsistent findings, which only recently have been put together within a comprehensible framework.

Several authors support the theory of a general memory dysfunction underlying compulsive checking in OCD, given that OCD checkers have been shown to be impaired on various memory tasks. For example, a general reduction of the patients' verbal and visuospatial working memory span was linked to checking behaviour in OCD (Jaafari et al., 2013). Beyond working memory, other authors (Savage et al., 2000) including a meta-analysis from Woods et al (2002) further confirmed impairments in episodic memory as well (specifically in verbal free and cued recall and recall of actions) in checkers as compared to non-checkers (Woods et al., 2002). Prospective memory, a type of memory required to retain, recollect and carry out timely and in an appropriate context intentions and plans, has also been shown to be impaired in subclinical checkers (Cuttler and Graf, 2007, 2008, 2009) and in patients with OCD (Harris et al., 2010; Yang et al., 2015). Nevertheless, such memory deficits were not observed in other studies. Moritz et al (2009) assessed nonverbal and verbal memory accuracy and confidence across different time-points and found similar performance between OCD patients and normal controls

on all variables measured (Moritz et al., 2009a). Nakao et al., (2009) did not observe significant group differences between verbal and visual memory span backward performances, but found task-related different brain activity between patients and controls (Nakao et al., 2009). Other authors found no general memory impairments but this intact behavioural pattern changed when they manipulated the task to include distractors or misleading information (Ciesielski et al., 2007; Harkin and Kessler, 2009) or to be of higher complexity/cognitive demand (van der Wee et al., 2003).

The inconsistent findings and the latter recognition that task manipulations delivered differential results led researchers to conclude that one needs to take into account the confounds related to different methodologies (including task difficulty/complexity), different nature of the stimulus (for example Clair et al., (2013) found that memory performance varied as function of the anxiogenic nature of the stimulus) or different characterization of the population sample. Several specialists in the field have expertly organised this literature (Woods et al., 2002; Greisberg and McKay, 2003; Olley et al., 2007**;** Kalenzaga et al., 2020, 2020; Persson et al., 2021) and concluded that the memory impairments observed in OCD checkers (clinical and subclinical) are secondary to executive dysfunction, thus not the main drive for checking compulsions. In fact, when executive demands are controlled (obviously in conjunction with task demands), the effect size differences between visual and verbal memory tasks and the impact of binding complexity and memory load in OCD patients are neglected (Persson et al., 2021). Therefore, memory impairments emerge when the tasks require executive or organisational abilities of OCD patients (which have been established to be diminished in OCD). A recent meta-analysis has confirmed such conclusions and further developed and validated a Classification System (EBL) that provides a coherent and unified explanation for the memory impairments in OCD as well as the disparate findings (Persson et al., 2021). This model basically identifies the executive function (E), the binding complexity (B, i.e. chunking of complex information), and the memory load or capacity (L) as the three main interactive dimensions that predict and classify working memory deficits in compulsive checking (Harkin and Kessler, 2011). The recent meta-analysis has now quantified how each of these dimensions moderate memory performance in OCD and concluded that executive function is the driving mechanism behind the EBL's impact on OCD memory performance (Persson et al., 2021).

Summary

Poor memory performance is unlikely to cause repeated checking in OCD. Memory impairments in OCD seem to emerge rather from ancillary deficits in executive functioning.

1.4.3.5. Reality monitoring

Another aspect of a putative memory deficit is reality monitoring, which refers to the possible confusion as to whether an action has indeed been performed or only imagined. The resulting uncertainty would trigger obsessive thought that would lead to compulsive checking. In other words, the memory of the action is available but the person is unable to determine whether the action was actually performed or simply imagined.

In a subclinical sample, Zermatten et al (2006) found that checkers (participants with higher checking subscores on the OCI) confused actions they had performed with actions they saw the experimenter performing more often than non-checkers. Such number of confusions significantly correlated with checking symptoms (Zermatten et al., 2006). Zermatten et al (2008) further suggested that the identification of a memory as real could be dependent on how vivid and detailed is the memory. They tested this hypothesis and shown that checkers' memories of past events were less vivid and had fewer visual kinaesthetic and spatial details than non-checkers (Zermatten and Van Der Linden, 2008; Zermatten et al., 2008).

The above findings were however not observed in several other studies, which reported similar reality-monitoring abilities in clinical, subclinical checkers and non-checker controls (Merckelbach and Wessel, 2000; Hermans et al., 2003; Cougle et al., 2008; Moritz et al., 2009b). Despite rejecting a global source-monitoring deficit hypothesis, some of these authors have reported checker's poorer confidence in memory and highlighted memory confidence and meta-cognitive beliefs as a likely cognitive marker that may underly compulsive checking. This will be discussed in the next section**.**

Summary

Repeated checking does not seem to be triggered by deficits in source-monitoring.

1.4.3.6. Meta-cognition and cognitive confidence

As per recent conclusions, the hypothesis that compulsive checking could be driven by memory deficits or flawed reality monitoring has now been discounted. However, it remains plausible that checkers, despite not having memory impairments per se, may suffer from memory distrust and/or low confidence in their cognitive capabilities, which could lead to similar behavioural impairments (Hermans et al., 2008a). Meta-cognition refers to the individual's awareness in their own abilities. In contrast to the habit hypothesis, meta-cognitive model of OCD suggests that compulsions are the consequence of overestimating the credibility of intrusive thoughts (Myers & Wells, 2005; Rachman, 1993). Despite an intact judgement of the link between action and outcome, excessive actions have been observed in

OCD patients after degrading this link in a contingency degradation task (Vaghi et al., 2019), and dissociated from subjective confidence from outcome in a predictive inference task (Vaghi et al., 2017c). The latter findings show a dissociation between action and subjective reports in OCD, indicating a deficit in meta-cognition by performing an excessive action in presence of intact subjective beliefs. A different type of meta-cognition in OCD was reported by Hauser et al. (2017c), where high-compulsive individuals (without an OCD diagnosis), showed reduced ability to monitor their own performance compared to low compulsive subjects and showing a higher decision threshold. Banca et al., (2015a) showed similar results in OCD patients performing a perceptual discrimination task, where patients needed to accumulate more evidence and presented a higher threshold for decision making.

Meta-memory and cognitive confidence has received a lot of attention from OCD researchers and many studies have tested the link between compulsive checking and pathological doubts driven by the lack of confidence in one's own memory, attention and perceptual capabilities. Despite some failures to reproduce the findings (Moritz et al., 2009b, 2009a), it is fairly consistent to observe lower memory (and overall cognitive) confidence in patients with OCD (irrespective of their OCD subtype) as compared to healthy controls (Dar, 2004; Hermans et al., 2003, 2008b; Jennings et al., 2011; Karadag et al., 2005; Taylor and Purdon, 2016; Tolin et al., 2001; Zitterl et al., 2001). Such finding has been consistently observed in studies using either neutral and OCD-related (including idiosyncratic) stimuli. Additionally, studies which manipulated beliefs about control have shown that OCD patients report lower estimates of control as compared to healthy subjects (Gillan et al., 2014) and moreover that people with higher beliefs of losing control check more than those with lower beliefs of losing control (Gagné and Radomsky, 2017). Altogether, there seems to be reliable evidence supporting the cognitive confidence hypothesis of OCD and the notion that repetitive checking is motivated by the need to reduce uncertainty in one's own cognition.

Numerous authors have suggested, on the other hand, that repetitive checking paradoxically increases not only the uncertainty about the checked targets, but also the mnemonic characteristics and general confidence (Tolin et al., 2001; Rachman, 2002; van den Hout and Kindt, 2003; Hermans et al., 2008b). It is then hypothesised that the pathological checking symptomatology is maintained by a vicious cycle in which cognitive distrust triggers checking but the checking itself causes more doubts, anxiety and ironically increases meta-memory problems, which promotes further checking. This hypothesis has now been corroborated by several studies in non-clinical controls, clinical controls and individuals with OCD (Coles et al., 2006; Radomsky et al., 2006; Boschen and Vuksanovic, 2007; Dek et al., 2010; Boschen et al., 2011; Medway and Jones, 2013; Giele et al., 2015; Linkovski et al., 2016; Toffolo et al., 2016). A recent exhaustive meta-analysis further confirmed that repeated checking has large effects on decreasing memory confidence, vividness and detail; and in a much lesser extent, it produces small reductions in memory accuracy (van den Hout et al., 2019). Van de Hout and colleagues explain this

phenomenon by the increased familiarity with the stimulus that is consequent of repetition: the more the stimulus becomes familiar, the less perceptual processing occurs, diminishing memory vividness and detail and consequently memory confidence and rendering the behaviour an automatic routine (Boschen et al., 2011; Dek et al., 2014, 2015; van den Hout et al., 2019). A recent study, however, using an adaptation of the original Van de Hout's Virtual Gas Stove Checking paradigm has questioned whether the checking component is really necessary to produce memory distrust (Burns et al., 2020). Unexpectedly, they replicated previous results of increased memory distrust across repeated trials even when the checking component was eliminated. An alternative explanation to the familiarity account previously described is that simply observing the stimulus over repeated trials builds up proactive interference that causes memory distrust (Burns et al., 2020).

There has been a few studies that used a psychological treatment targeting metacognitive beliefs. Some have found that, after psychological intervention, OCD patients significantly decrease their maladaptive beliefs (both about feelings of responsibility and memory distrust) as well as the number and time spent on checking behaviours (Alcolado and Radomsky, 2016; Radomsky et al., 2020). Nevertheless, other studies failed to demonstrate any effect of psychological intervention at improving cognitive confidence or reducing checking behaviours (Fitzgerald et al., 2011; Jennings et al., 2011). Belayachi and Van der Linden (2017) have suggested that these divergent results could be indicative that cognitive confidence may be an indirect factor contributing to checking behaviours rather than a major drive for compulsions (Belayachi and Linden, 2017).

Summary

Despite the lack of consensus about the mechanisms underlying memory distrust, there is considerable evidence to conclude that this is a common phenomenon amongst OCD patients. However, it is unclear whether cognitive confidence deficits are specific to OCD. In fact, despite the clear evidence for lower memory confidence in OCD patients as compared to healthy controls, the findings are mixed when comparing to other clinical groups, which suggests that such dysfunctional beliefs are likely transdiagnostic across anxiety disorders (Ouellet-Courtois et al., 2018) and thus not the main drive for compulsions. Similarly, repeated checking does not seem to be triggered by memory impairments per se or by a global deficit in source-monitoring either. It is likely that all these cognitive components (illusions of memory, reality monitoring, memory confidence) are interconnected, as well as with others such as inflated responsibility, intolerance of uncertainty, etc and somehow indirectly contribute for the OCD symptomatology. However, it is unlikely that they are the main mechanism underlying compulsions on their own.

1.4.3.7. Neurobiological mechanisms underlying checking

OCD is a clinically heterogeneous disorder with complex symptom dimensions that are presumably mediated by distinct neural systems, responsible for distinct emotional and cognitive processes. One way to measure the brain activity underlying checking behaviour is by using the functional magnetic resonance imaging (fMRI) while participants perform checking in the scanner. However, since checking has been proven to be difficult to provoke under experimental conditions, several studies used symptom provocation paradigms to measure the brain response to checking. Additionally, investigators used symptom provocation techniques to test the hypothesis that the major symptom dimensions of OCD might be subserved by partially distinct neural systems.

One example is the study by Mataix-Cols et al. (2004) who studied the response to pictures showing objects associated with checking behaviour (e.g. electric appliances, stove, door, purse) versus a group of neutral pictures (e.g. furniture, nature scene, and household items). They reported activity in distinct brain areas in response to the objects provoking checking compulsions in OCD, such as greater activations in the putamen, thalamus, and dorsal cortical areas such right ACC, OFC, and bilateral subgenual ACC. When they tried to provoke washing and hoarding symptoms, each time they measured increased activity in various different regions in OCD patients than the ones measured for checking objects. Such as the left precentral gyrus and right OFC for hoarding and the ventromedial prefrontal regions and right caudate nucleus in response to washing symptom provocation (Mataix-Cols et al., 2004). Murayama et al. (2013), investigated the differential neural activation during symptom provocation in groups of OCD checkers and washers, comparing them to healthy controls. They reported that OCD checkers when compared to healthy control subjects, showed hypoactivation in the left caudate and left ACC (BA24). They also found a positive correlation between YBOCS total score and left ACC activity during symptom provocation. Whereas hyperactivation in several bilateral cortico-cerebellar regions and a positive correlation between YBOCS symptom severity and the bilateral fronto-temporal gyrus were found for OCD washers (Murayama et al., 2013). This hypoactivation in caudate and ACC seems to be contradictory to the increased activity found by other studies in OCD patients in general, and in OCD checkers. The authors suggest that this may be due to the way they contrasted the conditions: they first contrasted the checking versus the neutral blocks within groups and then compared the results between groups. If the ACC activity in OCD was high in neutral phase already, the contrast with the checking block will be small and they could wrongly conclude hypoactivation in OCD once compared with the same values in healthy volunteers. A better study design would have been to compare the same contrast within their two OCD groups to rule out the hyperactivation of ACC during the neutral phase as a default ACC activity in OCD patients.

In alignment, Ravindran et al., (2020) compared the neural activity and connectivity in patients with checking and washing compulsions, and healthy controls. Participants were presented with an emotion provocation paradigm using neutral versus anxiety provoking pictures related to contamination/washing, checking and hoarding. The aim was to study how sensory information is integrated into front striatal areas and what were the differences between the different subtypes. The authors found that the OCD checkers showed elevated activity in the dorsal and medial posterior cingulate cortex and stronger connectivity between posterior cingulate gyrus and motor cortices in response to emotion provocation compared to both the OCD washing subtype and healthy volunteers (Ravindran et al., 2020).

In addition to the fMRI studies, one study measured the electrophysiological response of single neurons to checking and doubt while patients were performing a checking paradigm under surgery (Burbaud et al 2013). They performed single unit electrophysiological recordings in 10 OCD patients, 9 of which were checkers. They recorded 87 individual subthalamic nucleus (STN) neurons continuously during checking behaviours exhibited during a task based on a delayed matching-to-sample paradigm where participants had an option to check their answers. Results revealed that checking led to improved performance and was associated with increased firing rates of subthalamic neurons when recording their firing rates between trials with checking compared to trials without checking (Burbaud et al., 2013). As deep brain stimulation in the subthalamic nucleus improved OCD symptoms, the authors selected this area to look at checking behaviour and the reaction of these neurons. Despite the interesting results collected in challenging circumstances while patients underwent surgery, we still do not know if this region would behave in a similar way in healthy volunteers during checking. Additionally, checking here seemed to be functional, improving patients' performance, It would be interesting to know how these neurons would fire in response to not goal-directed/non-functional checking observed in OCD. Lastly, we do not know of course how other regions may have been involved in this behaviour. as the behaviour of neurons in other regions could not be studied.

Summary

Most studies have used symptom provocation techniques to study neural activity underlying checking behaviour. Despite some differences in findings, a common theme in the functional imaging work is aberrant activity of the cingulate cortex.

1.5. Aims of this thesis

A substantial proportion of schizophrenia patients on clozapine develop obsessive compulsive symptoms (Beduin et al., 2012; Fonseka et al., 2014; Doyle et al., 2014; Mukhopadhaya et al., 2008; Schirmbeck et al., 2012). The schizo-OCS patients tend to have a worse prognosis such as reduced social functioning (Tonna et al., 2015), higher positive and depressive symptoms (Biria et al., 2019; Sa et al., 2009), and a greater suicidal risk (Szmulewicz et al., 2015). It is thus important to understand the cognitive and clinical characterisations of schizo-OCS patients compared with clozapine treated patients that do not develop OCS, and OCD patients. Detecting the similarities and differences between the schizo-OCS, OCD and schizophrenia patients without OCS can shed light on the nature of the cognitive deficits that may underlie the obsessive compulsive symptoms.

Checking compulsions are one of the main obsessive compulsive symptoms observed in both OCD (Fontenelle et al., 2006) and schizo-OCS patients (Grover et al., 2015; Fernandez-Egea et al., 2018). Thus, this thesis aimed to understand the obsessive-compulsive symptoms, in particular checking behaviour and its underlying brain correlates. To achieve this goal:

1) Several attempts have been made in the first three studies to measure checking under various experimental conditions and in relationship to different cognitive and clinical measures:

- **Study 1**: the Observing Response Task (Morein-Zamir et al., 2018) was administered to OCD patients and healthy controls to measure checking in a laboratory setting. A higher rate of observing/checking was predicted in OCD patients.

- **Study 2**: no excessive checking was measured in OCD patients in Study 1, thus, a new checking paradigm was developed and administered to OCD, schizo-OCS, and schizophrenia patients without OCS (on clozapine) and healthy control subjects. This study aimed to (a) characterise the cognitive and clinical features of clozapine induced OCS in schizophrenia patients, and (b) discover the similarities and differences between patients with OCD and schizo-OCS with regard to their obsessive and compulsive, and clinical symptoms compared with 'pure' schizophrenia patients on clozapine and healthy subjects. Schizophrenia patients were not included in the next 3 studies due to recruitment difficulties caused by the pandemic.

- **Study 3**: the checking task instructions were modified to remove the time limit, which was hypothesised to underlie the absence of excessive checking in OCD and schizo-OCS patients in the previous study. Increased and dysfunctional checking in OCD patients compared to healthy controls was expected after removing the time limit.

2) One of the limitations of most MRS studies in OCD patients is the strength of their magnetic fields (all between 1 to 3 Tesla; see a detailed literature review in section 3.1). Thus, a more powerful study using a 7T MRS scanner was conducted as part of this thesis to measure changes in neurometabolites in OCD patients more reliably:

- **Study 4:** This study aimed to characterise the neurochemical abnormalities in OCD patients in ACC and SMA, two brain regions important for obsessive compulsive symptoms (Carrasco et al., 2013; D'Urso et al., 2016; Endrass et al., 2008; Gowda et al., 2019; Hauser et al., 2017a; Hazari et al., 2016; Mukherjee et al., 2021; Murray et al., 2019). In line with the higher activation in these areas in OCD patients, increased Glu and reduced GABA levels have been predicted in both these regions.
- **Study 5:** the final study of this thesis aimed to investigate the relationships between checking, habit versus goal directed behaviours, clinical symptoms and neurometabolite levels in ACC and SMA in both OCD and healthy subjects.

2. Chapter 2: Measuring compulsive checking in the laboratory context

2.1. Study 1 (ORT: OCD vs HV)

Checking compulsions are one of the most frequent symptoms reported in OCD patients (Fontenelle et al., 2006). Researchers have tried using objective computer tasks to measure checking in a laboratory setting. However, their findings are mixed, with some studies finding excessive checking in OCD patients on perceptual decision making tasks, while others could not replicate the results (Strauss et al., 2020).

Eagle et al. (2014) designed a novel operant paradigm, Observing Response Task (ORT), to examine the cognitive processes involved in checking in rodents. They defined checking as seeking information/observing, which was initially functional (using the information from observing to reach positive consequences), and became dysfunctional (excessive information seeking with no positive consequences) after treatment with quinpirole. The authors also tested the impact of uncertainty, by omitting an expected reward, on checking behaviours and found that all rats increased both functional and non-functional observing regardless of the quinpirole treatment, confirming the involvement of uncertainty in checking behaviour (Eagle et al., 2014). Interestingly, similar results were found in the human version of the ORT, where patients with OCD could check to reduce uncertainty, indicating the translational value of the task (Morein-Zamir et al., 2018). Study 1 describes a pilot study, where we tried to replicate the increased observing in OCD patients compared to healthy control subjects.

2.1.1. Methods

2.1.1.1. Participants

This study included 21 OCD patients and 16 healthy control subjects that were fluent in English and matched for relevant demographic variables (**Table.2.1**). This study was approved by the East of England - Cambridge South Research Ethics Committee (REC 16/EE/0465). All volunteers gave written consent before beginning testing and received monetary compensation for taking part in the study. All patients met the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria

for OCD as determined by a detailed interview with a clinician- but no other Axis one mental disorders including dementia, psychosis, major depressive disorder, bipolar disorder, Tourette's syndrome, attention deficit-hyperactivity disorder, autistic spectrum disorders and eating disorders. Patients with a Yale- Brown Obsessive Compulsive Scale (YBOCS, Goodman et al., 1989) higher than 12 according to Lewin et al. (2011) were enrolled in the study. Healthy controls had no current or past psychiatric disorders as determined by a screening interview including the Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1998) and the Montgomery- Asberg Depression Rating Scale (MADRS; Montgomery and Asberg, 1979). For all participants, excessive drug or alcohol use, neurological deficits or head injury were exclusion criteria. All but three patients were medicated. Out of the 18 medicated patients, 10 were prescribed only with SSRIs (4 fluoxetine, 4 Sertraline, 2 Escitalopram), 6 were treated with adjunct antipsychotics, and 2 were on a number of medications including SSRIs, benzodiazepine, pregabalin and antipsychotics. Two OCD patients were missing most questionnaires including IQ (NART), OCI, YBOCS, MADRS, and STAI, one patient was missing IU, two healthy volunteers were missing STAI. Out of all participants, only 4 from the OCD group were smokers. Lastly, all participants had normal or corrected-to normal vision and hearing.

Table.2.1 Means and standard deviations of control and OCD patient group characteristics.

Characteristics	Measure	Controls (n = 16)	OCD (n = 21)	*t*	*p*
		M (SD)	M (SD)		
Age	Years	40.8 (13.58)	39.3 (12.02)	- 0.36	0.72
Gender	M:F	6:10	10:11		
Verbal IQ	NART	118.29 (4.3)	118.4 (4.87)	- 0.07	0.93
Education	Years	15.38 (3.47)	14.62 (3.32)	0.66	0.50
Obsessions and Compulsions	YBOCS	---	23.31 (5.87)		
Depression	MADRS	1.18 (1.47)	10.78 (5.54)	6.71	$p < 0.001$
State anxiety	STAI-S	30 (6.3)	41.1 (6.87)	4.75	$p < 0.001$
Trait anxiety	STAI-T	29.35 (6.27)	54.26 (8.88)	8.95	$p < 0.001$
Uncertainty intolerance	IU	46.8 (13.62)	84.1 (19.4)	6.48	$p < 0.001$
Obsessions and Compulsion	OCI-R	13.43 (17.22)	60.42 (27.92)	5.85	$p < 0.001$

NART: National Adult Reading Test, YBOCS: Yale-Brown Obsessive Compulsive Scale, MADRS: Montgomery-Asberg Depression Rating Scale (MADRS), STAI-S: State Trait Anxiety Inventory-State; STAI-T: State Trait Anxiety Inventory-Trait; IU: Intolerance of Uncertainty; OCI-R: Obsessive Compulsive Inventory-Revised.

2.1.1.2. Questionnaires

The following measures were collected for all participants:

- State/Trait Anxiety Questionnaire (Spielberger et al., 1983): standardized self-report measure of general anxiety

- Obsessive-Compulsive Inventory Revised (Foa et al., 2002): standardized self-report measure of obsessive-compulsive symptoms
- Intolerance of Uncertainty Scale (Carleton et al., 2007): standardized self-report measure on how much uncertainty can be tolerated
- National Adult Reading Test (Nelson H., 1982) to provide an estimate of verbal intelligence

2.1.1.3. Stimuli and procedure

The Observing Response Task which has previously been studied in both OCD and non-clinical high observers (Morein-Zamir et al., 2018), and rodents (Eagle et al., 2014) was employed to study checking behaviour. Participants were seated in front of a 13 inch touch screen SAMSUNG laptop in a comfortable viewing distance with the experimenter always present. Before beginning the task, the sound volume was adjusted to 16 to have the same volume level in all participants. They were then presented with the outline of two shapes (a green triangle and a purple circle) on two sides of the screen (**Figure.2.1.a**), with a counterbalanced location for the shapes. They were told that throughout the task, only one of the shapes/sides would be active and pressing their corresponding key ("m" for the figure on the right and "z" for the figure on the left) would lead to a reward of 50 pence, and choosing the inactive side would lead to a loss of 50 pence. A 50 pence coin would appear below the rewarded side and within an orange frame as a reward, accompanied by an uplifting sound (**Figure.2.1.d**). As punishment, a cross-out coin in red frame would appear below the wrong/inactive side, accompanied by an aversive sound (**Figure.2.1.e**). Participants were instructed to earn as much money as possible. Centrally below both figures, they would see a counter on the screen showing how much money they have earned so far in front of the text "Total Earned". The instructions also informed participants that they would receive a proportion of their earnings in cash, which in practice was always £5. After a training phase with only one shape, participants were presented with both shapes with an observing cue present for 1 minute, shifting as the active side location changed. Finally, the cue was removed and replaced by an observing key (letter "t" on the keyboard) which allowed the reproduction of the cue in exchange for a £0.05 cost. This stage lasted for 10 minutes, and was then followed by an extinction stage where the observing key was removed. In this study, only the first phase with observing is reported which measured checking behaviour at baseline.

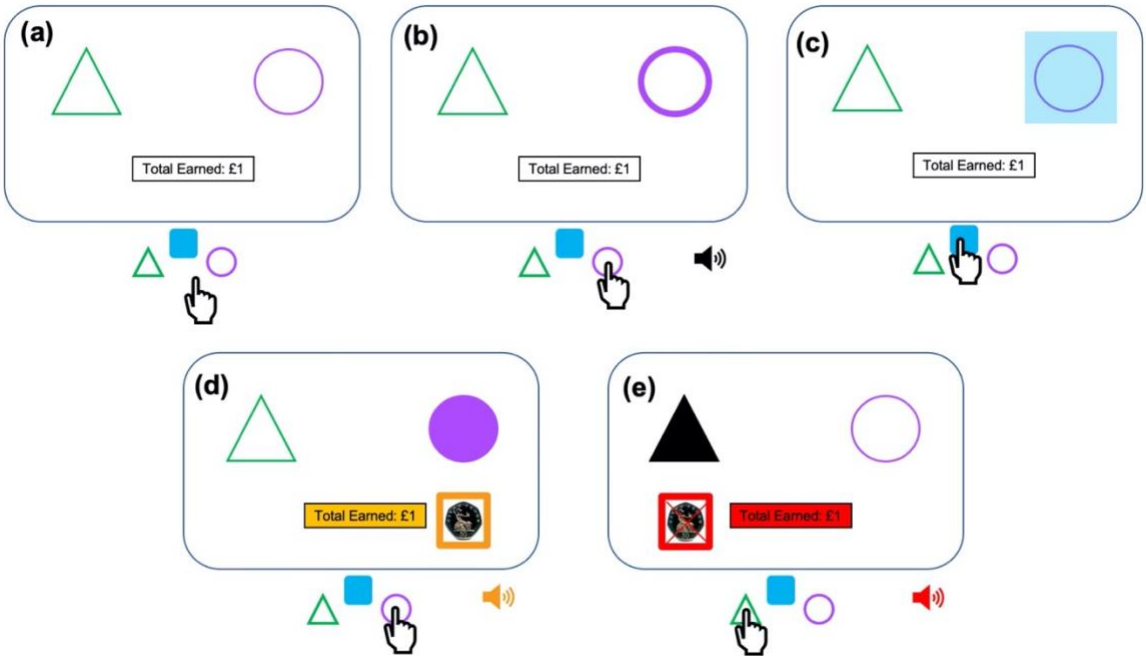


Figure.2.1. This figure is reproduced from Morein-Zamir et al. (2018) and is a schematic representation of the free operant observing task and its procedure. At any given time only one figure/side is active, while the other figure/side is inactive. The active side yields rewards while the inactive side leads to punishment (winning or losing 50 pence, hearing an uplifting or aversive sound respectively). (a) Participants can press either of the two keys to select the active side and earn money (b) The outline of the shape becomes briefly thicker to indicate the selected side and is accompanied by a click sound. (c) Participants can press the blue observing key to check which side is currently active, resulting in a light blue square appearing behind the figure on the active side for 1.5s. (d) Rewards are conveyed via the following: the outline of the shape on the active side is filled, an orange framed 50 pence coin is shown, £ 0.50 is gained, and an uplifting sound is played. (e) Punishments are conveyed via the following: the outline of the shape on the inactive side is filled in black, a red outlined and cross-out 50 pence coin appears on screen below the inactive side, £0.50 is lost, and an aversive sound is played.

2.1.2. Results

An independent sample t-test was performed to compare the median observing rates per minute as the main measure of checking between groups (**Figure.2.2**), with no significant differences found (t = -0.69, p = 0.49). OCD patients scored significantly higher on state and trait anxiety (STAI-S and STAI-T), intolerance of uncertainty (IU), depressive (MADRS) and obsessive and compulsive symptoms (OCI-R)- **Table.2.1** reports these findings in detail. The associations between checking and YBOCS total score, OCI-R total and sub-scale scores, IU total score were investigated using Pearson's correlation coefficient r. Again, as opposed to the significant correlations reported by Morein-Zamir et al., (2018), no significant relationship between these clinical scales and checking/observing on the task were found.

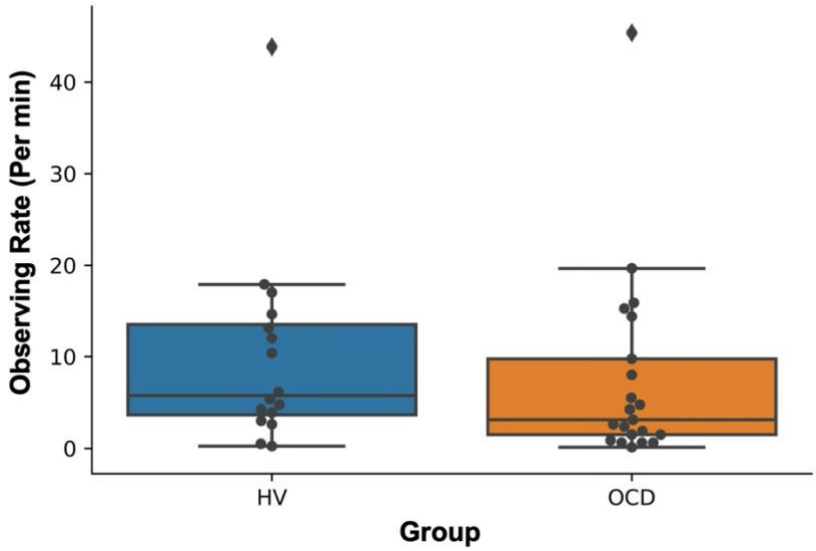


Figure.2.2 Boxplots showing the median observing (checking) rate per minute in healthy volunteers (blue) and OCD patients (orange) at baseline with the observing key present

2.1.3. Discussion

Checking behaviour was studied in OCD and healthy controls using the Observing Response Task. Despite previous findings in human participants by Morein-Zamir et al. (2018), we did not replicate the increased checking rate, nor the positive correlations between checking and obsessive-compulsive symptoms and intolerance of uncertainty. The OCD group did not show excessive checking behaviour compared to healthy controls and their observing rates were not correlated with any of the clinical measures. Although it is difficult to explain this inability to replicate the previous results, it could possibly be due to a different context in which the previous experiment took place (leaving the instructions sufficiently vague to increase uncertainty and anxiety in patients and in turn encouraging checking). However, without designing a more controlled study with more conditions using different instructions, this hypothesis cannot be tested. Thus, the following chapter describes the results of a newly designed checking paradigm that tried to measure more factors in 4 blocks, with different variations, to more systematically analyse checking behaviour and its underlying motivations.

2.2. Study 2 (IVT-version-1: HV vs OCD vs schizo-OCS vs schizophrenia)

Individuals with schizophrenia on clozapine have usually been resistant to treatment with typical or first-generation antipsychotics (Whitney et al., 2015). This only effective drug however also causes OCS or even OCD in a substantial number of these patients (Beduin et al., 2012; Fonseka et al., 2014; Doyle et al., 2014; Mukhopadhaya et al., 2008; Schirmbeck et al., 2012). Despite the high prevalence of clozapine induced OCS in schizophrenia and its negative consequences and prognosis such as higher severity of positive and negative symptoms (Sa et al., 2009), reduced social functioning (Tonna et al., 2015) and a greater suicidal risk (Szmulewicz et al., 2015), no studies have investigated the neuropsychological characterization of this important patient group. Hence, the cognitive problems and their underlying cognitive mechanisms are still unknown. We propose to address these points and provide a deeper understanding of the neuropsychological basis of clozapine-induced OCS in schizophrenia. Additionally, we hope to gain greater insight into the nature of potentially distinct behavioural and cognitive deficits underlying obsessive compulsive symptoms between primarily OCD, schizophrenia patients treated with clozapine with OCS.

Previous studies reported predominant checking over other types of compulsions in both schizo-OCS (Grover et al., 2015; Fernandez-Egea et al., 2018) and OCD patients (Fontenelle et al., 2006). However, we could not replicate the checking behaviour measured by Morein-Zamir et al (2018) in the context of our lab setting in study 1, which was possibly caused by an increase in anxiety due to vague task instructions. Thus, I revised and developed a new checking paradigm based on perceptual decision making. Amongst all checking paradigms, only the ones requiring perceptual decision-making seem to reliably induce checking behaviour in OCD (Arntz et al., 2007; Jafari et al., 2013; Rotge et al., 2008; Toffolo et al., 2016). This could be caused by the reliance on data gathering and trusting one's own sensory modalities, as opposed to the reasoning and deliberate thinking during the task (Strauss et al., 2020). Study 2 uses the newly developed checking paradigm, called the Image Verification Task (IVT) to measure checking behaviour in OCD, schizophrenia with and without OCS on clozapine, and a healthy group. Additionally, to further specify the distinctive behavioural and neuropsychological characteristics of our clinical populations, we investigated the cognitive measures that could possibly contribute to compulsive checking and are known to be impaired in schizophrenia. Therefore, computerised tests were employed to assess attention, processing speed, visuospatial perception, visual memory, working memory performance and cognitive flexibility in our participants.

Study 2 aimed to:

- Characterise the cognitive and clinical features of schizophrenia with and without OCS (caused by clozapine).

- Discover the similarities and differences between patients with OCD and schizo-OCS with regard to their obsessive and compulsive symptoms.

We aimed to test the following hypotheses:

H1: Patients with OCD and schizo-OCS will display excessive checking that is dysfunctional, or not goal-directed (does not improve performance on the task).

H2: Checking is not due to working memory deficits, but rather due to excessive habit formation associated with the anti-serotoninergic properties of clozapine in schizo-OCS group, and a deficit in goal-directed system in OCD.

H3: Clozapine dosage and schizophrenia symptoms (positive and depressive symptoms) will be related to checking in schizo-OCS but not the schizophrenia group without OCS.

H4: Intolerance of uncertainty has an impact on checking behaviour on the IVT in both OCD and schizo-OCS groups but not the other two groups.

H5: Despite sensitivity to punishment, excessive checking will occur in both OCD/schizo-OCS groups.

2.2.1. Methods

2.2.1.1. Participants

This study included 30 healthy volunteers, 31 OCD patients, and clozapine treated schizophrenia patients, 21 of whom showed Obsessive Compulsive Symptoms (OCS) and 15 schizophrenia patients without OCS. All participants were fluent in English and were matched for age and verbal IQ, besides schizophrenia groups, the remaining two groups were also matched for gender. **Table.2.2** shows the demographic and clinical characterisation of all groups. This study was approved by the East of England - Cambridge South Research Ethics Committee (REC 16/EE/0465) for OCD and healthy volunteers and the Cambridge and Peterborough NHS Foundation Trust (REC 18/EE/0073) for patients with schizophrenia. All volunteers gave written informed consent before beginning the testing and received monetary compensation for taking part in the study.

Patients met the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for OCD or schizophrenia as determined by a detailed interview with a clinician- but no other Axis-I mental disorders for OCD patients. Whereas in schizophrenia patients with OCS (Schizo-OCS), 3 had comorbidities with depression, 1 had a diagnosis of Asperger syndrome, 1 schizoaffective disorder and 1 with dyslexia. In the pure schizophrenia group, 1 had an emotionally unstable personality disorder,

and 2 had comorbid Generalised Anxiety Disorder (GAD). All OCD patients had a primary diagnosis of OCD. Anxiety and depressive symptoms were present in all patients, however OCD participants with comorbid major depressive disorder or GAD were not included in the study. Schizophrenia patients with a Yale- Brown Obsessive Compulsive Scale (YBOCS, Goodman et al., 1989) higher than 6 were enrolled in the study in the OCS group. This also included patients with milder symptoms to make recruitment easier. The same threshold was used in patients with OCD. Healthy controls had no current or past psychiatric disorders as determined by a screening interview including the Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1998) and the Montgomery-Asberg Depression Rating Scale (MADRS; Montgomery and Asberg, 1979). For all participants, excessive drug or alcohol use, neurological deficits or head injury were exclusion criteria. For the schizophrenia group, only 10 were treated solely with clozapine, the rest were on a combination of medications. Thirteen were on clozapine and SSRIs, 9 on clozapine, SSRI and aripiprazole, 3 on clozapine and aripiprazole, 1 patient was on clozapine, sulpiride and clomipramine. In the OCD group, all but 9 patients were medicated. Out of the 23 medicated patients, 19 were prescribed only with SSRIs, 3 were treated with adjunct antipsychotics, and 1 was on clomipramine and propranolol.

Table.2.2 Means and standard deviations of descriptive group characteristics.

Characteristics	Controls (n = 30) M (SD)	OCD (n = 31) M (SD)	Schizo-OCS (n = 21) M (SD)	Schizophrenia (n = 15) M (SD)	F/t	*p*
Age (Years)	40.36 (12.37)	35.78 (13.5)	44.1 (10.74)	46.7 (11.52)	14.46	0.094
Gender (M:F)	14:16	13:19	17:4	13:2		
Verbal IQ (NART)	116.44 (6.03)	116.96 (4.69)	117 (6.1)	113.77 (9.21)	1.02^{K}	= .79
Education (Years)	17.16 (3.19)	15.62 (3.13)	NA	NA	-1.91	= .06
YBOCS total score	NA	21.31 (5.51)	15.33 (4.56)	NA	4.09	< .001
Depression (MADRS)	2.33 (3.96)	9.43 (7.98)	9.95 (4.12)	5.33 (6.16)	33.47^{K}	< .001
State anxiety (STAI-S)	26.93 (6.22)	40.06 (11.24)	43.38 (11.8)	38.40 (11.87)	31.04^{K}	< .001
Trait anxiety (STAI-T)	32.33 (8.79)	54.61 (10.38)	51.33 (9.09)	42.6 (12.76)	47.72^{K}	< .001
IOU	47.17 (20)	74.50 (22.53)	76.09 (23)	59.93 (17.39)	27.55^{K}	< .001
OCI (total score)	8.37 (9.84)	58.62 (28.18)	53.14 (32.5)	24.6 (16.63)	56.67^{K}	< .001
OCI-washing	1.47 (2.7)	11.09 (10.3)	7.1 (7)	2.92 (3.19)	51.16^{K}	< .001
OCI-checking	1.47 (2.11)	11.81 (7.69)	12.48 (8.08)	5.27 (3.81)	19.87	< .001
OCI-doubting	0.40 (0.92)	5.56 (3.14)	4.81 (3.32)	1.73 (1.3)	56.28^{K}	< .001
OCI-ordering	1.80 (2.37)	6.41 (4.34)	5.38 (4.69)	2.6 (3.24)	24.50^{K}	< .001
OCI-neutralising	0.83 (1.19)	7.25 (4.49)	7.24 (4.66)	3.07 (3.24)	49.50^{K}	< .001
OCI- hoarding	1.07 (1.54)	2.97 (2.98)	3.9 (2.74)	3.67 (3.4)	29.95^{K}	< .001
OCI-obsessing	1.27 (1.81)	13.53 (6.91)	11.86 (7.87)	5.27 (5.13)	50.62^{K}	< .001
Digit span forward	10.4 (1.93)	11.68 (3.11)	10.24 (2.44)	10.73 (2.53)	1.74	= .16
Digit span backwards	7.17 (2.09)	8.13 (2.62)	6.05 (1.9)	6.07 (2.28)	4.52	< .05
IED completed stages	8.66 (1.32)	8.58 (0.79)	7.1 (2.72)	8.27 (0.936)	10.87^{K}	= .01
IED PRE-EDS errors	7.66 (6.26)	6.03 (1.9)	14.62 (9.76)	9.33 (4.03)	8.78	< .001
IED EDS errors	6.24 (7)	9.1 (9.45)	16.14 (10.79)	15.33 (10.05)	6.09	< .001
SWMBE	7.46 (8.81)	9.58 (9.37)	15.38 (8.93)	14.67 (9.11)	10.80^{K}	= .01
Clozapine dose	NA	NA	309.52 (93)	305.77 (103)	0.11	0.91
Clozapine duration	NA	NA	17.63 (7.88)	18.92 (7.31)	-0.45	0.65
PANSS-Positive	NA	NA	13.33 (9.54)	6.53 (6.32)	-2.57^{U}	= .01
PANSS-Negative	NA	NA	10 (7.66)	6.6 (7.33)	-1.34^{U}	= .18
PANSS-Disorganised	NA	NA	7.61 (5.1)	4.80 (4.98)	-1.65^{U}	= .11
PANSS-Excited	NA	NA	3.14 (1.82)	3.13 (3.38)	-0.009^{U}	= .99
PANSS-Motor	NA	NA	1.85 (1.38)	1.46 (1.45)	-0.80^{U}	= .42
PANSS-Depression	NA	NA	3.71 (2.59)	1.86 (1.84)	-2.49^{U}	= .01
PANSS-Anxiety	NA	NA	5.23 (3.71)	2.80 (2.70)	-2.27^{U}	< .05
AIMS	NA	NA	0.81 (0.39)	1 (1.41)	0.507^{U}	= .67

NART: National Adult Reading Test, YBOCS: Yale-Brown Obsessive Compulsive Scale, MADRS: Montgomery-Asberg Depression Rating Scale (MADRS), STAI-S: State Trait Anxiety Inventory-State; STAI-T: State Trait Anxiety Inventory-Trait; IU: Intolerance Of Uncertainty; OCI: Obsessive Compulsive Inventory, IED: Intra-Extra Dimensional Set Shift, ED: extradimensional shift, SWMBE: SWM Between Errors: The number of times the subject incorrectly revisits a box in which a token has previously been found, PANSS: Positive and Negative Symptoms Scale, AIMS: Abnormal Involuntary Movement Scale. F/t: F-test and t-test were calculated for variables that were available for four versus two groups respectively. "U" and "K" stand for Mann-Withney U and Kruskal-Wallis tests respectively, in case of non-normal/inhomogeneous data.

The following data were missing: 1 CANTAB Intra-Extra Dimensional Set Shift (IED; Owen et al., 1991; Roberts et al., 1988), and 2 CANTAB Spatial Working Memory (SWM; Owen et al., 1990a) in healthy control group, 2 Verbal IQ (NART), 1 digit span and STAI, 1 IED and SWM in OCD patients; and 1 Verbal IQ for schizophrenia patient without OCS, and 2 Verbal IQ's from schizophrenia patients with OCS. The latter 2 missing Verbal IQ's in schizophrenia patients was due having dyslexia diagnoses. Education years were only compared between OCD and healthy control group as this information was not available for most schizophrenia patients. Besides 6 schizophrenia patients without OCS, and 6 with OCS, no one else including the healthy and OCD groups was a smoker. Lastly, all participants had normal or corrected-to normal vision and hearing.

2.2.1.2. Outcomes and measures

The following measures were collected for all participants:

- State/Trait Anxiety Questionnaire (Spielberger et al., 1983): standardized self-report measure of general anxiety with 20 items for state and trait subsets each using a 4-point scale ranging from 1 (= Not at all) to 4 (= Very much so).
- Obsessive-Compulsive Inventory (Foa et al., 1998): standardized self-report measure of obsessive-compulsive symptoms compromising 6 subscales: washing, checking, ordering, hoarding, obsessional thinking, and mental neutralising. Participants have to rate how much distress each experience has caused them in the past rating on a 5-point scale from 0 (= Not at all) to 4 (= A lot). There are 42 questions in this version which would lead to a score ranging from 0 to 126, with higher scores indicating a higher severity of obsessive-compulsive symptoms.
- Intolerance of Uncertainty Scale (Buhr and Dugas, 2002): standardized self-report measure on how people react to uncertainties of life, with 27 items with answers ranging from 0 (= Not at all characteristic of me) to 5 (= Entirely characteristic of me).
- National Adult Reading Test (Nelson H., 1982) to provide an estimate of verbal intelligence

The following were collected only in schizophrenia groups:

- The Abnormal Involuntary Movement Scale (AIMS; Guy, 1976): to measure involuntary movements
- The Positive and Negative Symptoms Scale (PANSS; Kay et al., 1987) is a measure of symptom severity in schizophrenia performed through a detailed interview with a psychiatrist to quantify the positive and negative symptoms. It is a 36 items scale, divided in 3 domains of positive (7 items), negative (7 items) and general symptoms (12 items). Each item is scored from 1 (=absence) to 7 (=extreme severity), 3 being the threshold for clinical significance. Although the sum of 36 items, or three subscales can be used, it also has received several

criticisms as the negative items also contained cognitive measures. A better and more recent approach is to use factor analysis. Seven subscales (factors) including Positive, Negative, Excited, Depressive, Motor, Disorganized and Anxiety were calculated according to Emsley et al. (2003) and used to correlate with checking behaviour.

- Clozapine dosage, duration of treatment, habits such as smoking (cigarettes per day) were also collected for schizophrenia patients. Smoking influences the working of clozapine and usually leads to an increase of the dose. Thus the number of cigarettes smoked per day will be used in correlations with clozapine dose, as a covariate.

2.2.1.3. Stimuli and procedure

Cognitive flexibility and attention

The CANTAB Intra-Extra Dimensional Set Shift (Owen et al., 1991; Roberts et al., 1988) was used to assess cognitive flexibility, more specifically the set-shifting ability (**Figure 2.3.a**) which tests rule acquisition and reversal learning. It was administered on a touch-screen tablet with a total duration of 7 minutes. The task features visual discrimination between colour-filled shapes and white lines, and shifting and flexibility of attention. The test is well validated in individuals experiencing OCD (Chamberlain et al., 2007; Vaghi et al., 2017) and schizophrenia (Leeson et al., 2009; Pantelis et al., 1999) and is a computerised analogue of the Wisconsin Card Sorting test.

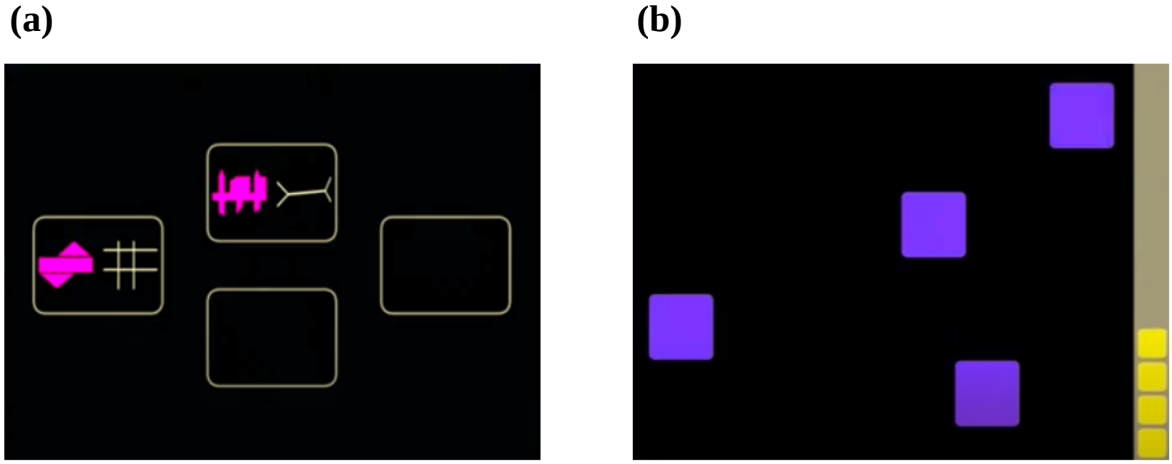


Figure.2.3 (a) A screenshot of the CANTAB IED set shifting task with shapes in purple and lines in white. The rule changes from shapes to lines at the extradimensional set shift stage. **(b)** The CANTAB SWM task where 4 tokens had to be found by selecting the boxes and using a process of elimination. After finding each token, they had to be used to fill up the empty column on the right side of the screen. The number of boxes gradually increases and can reach a maximum of 12.

Spatial and Verbal working memory

Participants were additionally tested on CANTAB self-ordered Spatial Working Memory task (Owen et al., 1990a) in which they perform a sequence of responses on a touch sensitive screen to detect the location of 'reward' tokens testing spatial working memory and executive functioning (**Figure.2.3.b**). The SWM was administered on a touch-screen tablet with a total duration of 4 minutes. Moreover, the capacity of verbal working memory was determined using the forward and backwards digit task from the WAIS-III (Wechsler, 1997a) to have measures of verbal short term and working memory, and executive functioning. Subjects had to repeat series of digits of increasing length, read by the researcher. Digit span forward consisted of 8 x 2 digits, whereas digit span backwards had 2 sets of 7 digits. If a participants made a mistake for both digits within a set, the test would stop.

Checking behaviour

As no reliable computerised behavioural task was available to measure checking accurately, a new checking paradigm was designed and developed. I have designed the Image Verification Task, a touch screen task with four blocks measuring different aspects of cognition and decision-making that may influence checking behaviour. It is conceptually simple yet perceptually difficult enough to increase doubt and checking, especially so in patients with OCD or OCS. Participants are instructed to inspect two black and white drawings of objects, displayed one after another and decide if the two objects are the same or different (**Figure.2.4**). They are instructed to be as accurate as possible and finish the task in the limited time that they have- both accuracy of answers and the overall speed are important measures. Before giving an answer, participants are given the chance to go back and view/examine the images again as many times as they like, as an explicit measure of checking. There are differences in size, angle or shapes/types of the objects presented. Each object presentation is separated by a white image, all three presented for 1 second with an interstimulus interval of 800ms. They must then rate how confident they are about their answer on a scale with 4 choices ranging from "not confident at all", "not very confident", "fairly confident", and "very confident". After a pilot study and observing that participants choose the neutral/middle option more readily, this option was removed to encourage more consideration and comparison and thus to select a less neutral choice that reflects their true confidence.

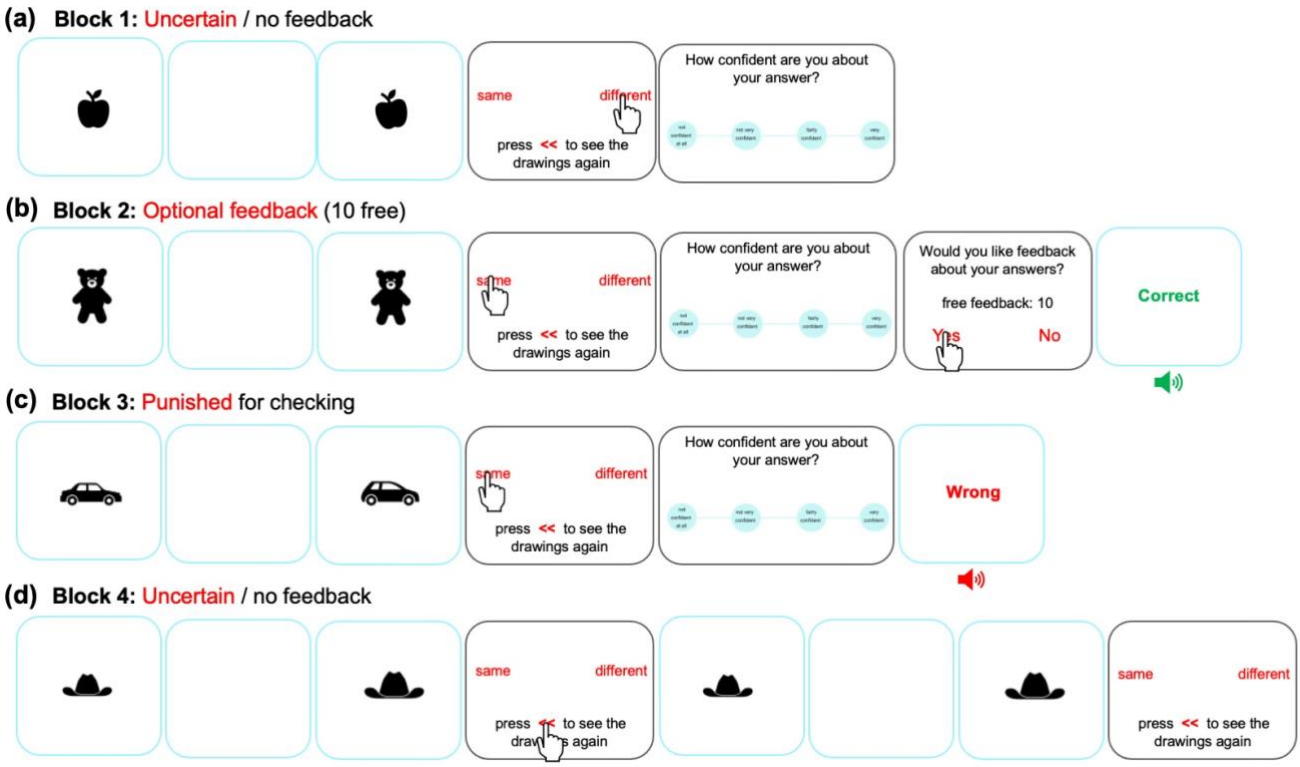


Figure.2.4 is a schematic representation of the four IVT blocks, where two black and white drawings of objects are displayed one after another and must be compared to decide if they are the same or different, with a chance to check the images before giving an answer by pressing on the red << sign. A confidence rating is followed after every answer with 4 choices between "not confident at all", "not very confident", "fairly confident", and "very confident". The blue frames last for 1 second with an interstimulus interval of 800ms. The black frames will remain on the screen until an answer is given. **(a)** Block one is a high uncertainty block with no feedback provided. In this example, the stimuli are different in angle. **(b)** Block 2 offers optional feedback, the first 10 for free and an additional trial as a cost for any extra feedback asked after the free feedbacks are used. A counter is shown on the screen for participants to know how many free feedbacks they have left. This is an additional more implicit measure of checking. In this example, the two stimuli are exactly the same in size, angle and shape. The correct feedback is provided in green colour accompanied with an uplifting sound **(c)** In Block 3 checking is punished by adding one additional trial to this block for every time someone presses the checking sign on the screen. Feedback is also provided for all trials. An example of two different shapes/object is depicted here. The wrong feedback is provided in red colour accompanied with an aversive sound **(d)** Block 4 is exactly the same as the first block including the stimuli presented. This allows testing the habit hypothesis for dysfunctional checking. The two hats in this example are different in Size. Pressing on the red << sign repeats the presentation of the stimuli and allows checking of the drawings before giving an answer.

In the **first Block** (**Figure.2.4.a**) of the task no feedback is provided to study how people behave under uncertainty. In **Block 2** (**Figure.2.4.b**) feedback is optional, after each answer, participants are asked whether they would like to receive feedback. After using the first 10 'free' feedbacks, a 'price' must be paid in the form of one additional trial for every additional feedback asked. This enables studying a more implicit form of checking that requests feedback. In **Block 3** (**Figure.2.4.c**) feedback is always provided, however, checking is punished by adding one additional trial per checking. Feedback is provided in the form of written words "wrong" in red and "correct" in green accompanied with an aversive and uplifting sound respectively. In block 3 differences in checking behaviour under punishment are studied. For blocks with punishment (2 and 3), participants are made aware of the explicit consequence of receiving punishment (doing more trials), which is staying in the lab for longer. Lastly, **Block 4** (**Figure.2.4.d**) is exactly the same as block 1 including the stimuli, and the habitual versus goal-directed properties of checking can be tested. All blocks have 45 trials, starting with 5 practice runs.

2.2.1.4. Analysis of demographic and behavioural data

Univariate ANOVA was applied to assess group differences in descriptive data, clinical measures and task performance on IED and SWM tasks. More specifically, the errors made before (IEDEPRE) and during (IEDEEDS) the extra-dimensional set shift stage, the number of times subjects incorrectly revisited a box in which a token has previously been found (SWMBE) as a measure of cognitive flexibility and spatial working memory performance respectively. In case the equal variances &/or normality distribution could not be assumed, a Kruskal-Wallis test was used instead of an ANOVA. For variables such as the years of education, YBOCS, PANSS and AIMS that were available only in two groups, an independent sample t-tests was used in case of a normal distribution and equal variances and otherwise a Mann-Whitney U test was used. To compare the clozapine dose between the two schizophrenia groups a ANCOVA (analysis of covariance) was used with number of cigarettes smoked as covariate.

To examine checking behaviour and its related cognitive and behavioural aspects as collected by the IVT task, three orthogonal contrasts were conducted using three separate repeated measure ANOVAS (analysis of variances) for each variable. The contrasts were between: 1) healthy volunteers versus the three patient groups 2) OCD versus two schizophrenia groups, and 3) schizophrenia with OCS versus schizophrenia without OCS. The average checking rates, accuracy of answers, and confidence ratings were used as dependent variables, repeated across blocks within subjects, and were also compared between groups. In case the homogeneity or normality assumptions were not met for the mixed ANOVA, a robust ANOVA (Mair & Wilcox, 2021) was instead applied. In addition to interactions between groups and blocks, main effects were calculated using a Univariate ANOVA to determine if the between groups or within subject factors were significantly different. Kruskal Wallis test was used

for group/block effects of not normally distributed data. In case of significant main effects, *post hoc* multiple comparisons were performed using a Bonferroni correction. Tukey's multiple comparison test was used when equal variances could be assumed, otherwise Games-Howell test was used to learn where specifically the differences between groups/blocks resided. If the sphericity condition for repeated measures was not satisfied, a Greenhouse-Geisser correction was applied. Moreover, to study associations between task performance and clinical measures and questionnaire scores, Pearson correlation coefficients, r, were calculated for each group separately, and in case of non-normally distributed data a Spearman correlation coefficient r_s was used. Since smoking has an impact on the working of clozapine, and smokers are usually on higher doses, a partial correlational analysis was performed to look at the correlation between clozapine dose and checking in schizophrenia patients, with the number of cigarettes smoked per day as a covariate. In the case of significant correlations, Fisher's Z-test was performed to compare the correlations across groups. R studio Version 1.2.5033, and package WRS2 were used to perform the robust mixed ANOVA. SPSS version 28 (SPSS IBM) was used for the rest of the analysis.

2.2.2. Results

A series of analyses of variances (ANOVAs) were performed between groups. **Table.2.2** shows all these comparisons, their mean, standard deviations and their significance levels. Multiple comparison tests were then performed for variables that displayed a significant variation between groups. **Table.2.3** shows the mean differences and *p* values for all significant multiple comparisons.

2.2.2.1. Demographic and clinical scales

Besides age, and verbal IQ, all the other ANOVA tests performed yielded significant variation between the 4 groups, such as depressive symptoms ($F = 10.06$, $p < 0.001$) which was higher in patients than healthy volunteers ($p < 0.001$), and higher in two OCD/schizo-OCS groups compared to pure schizophrenia ($p < 0.001$). Regarding the significant state anxiety differences ($F = 13.42$, $p < 0.001$), patient groups were once again driving the differences and scored higher than the healthy group ($p < 0.001$), but there were no differences in anxiety between OCD and schizophrenia patients. However, the Schizo-OCS group scored higher than the schizophrenia group ($p < 0.05$). Similarly, there were significant group differences for the trait anxiety between groups ($F = 28.33$, $p < 0.001$), this difference was caused by again all patients being more anxious than healthy controls ($p < 0.001$), and OCD patients being much more anxious than the pure schizophrenia group ($p < 0.001$). Despite the state anxiety differences between the two schizophrenia groups, with regard to trait anxiety despite the slight increase in schizo-OCS patients, there were no significant differences between groups. The next significant group difference was for intolerance of uncertainty scores ($F = 11.01$, $p < 0.001$), for which again all

patients had higher scores than healthy controls ($p < 0.001$), and the two OCD/schizo-OCS groups being more intolerant of uncertainty than the pure schizophrenia group ($p < 0.001$). The OCI total scores were different between groups ($F = 27.36$, $p < 0.001$), higher in patients than healthy group ($p < 0.001$), and higher in OCD and schizo-OCS patients than the pure schizophrenia group ($p < 0.001$). With regard to the OCI checking subscale, OCD and schizo-OCS group showed significantly higher scores than the remaining two groups ($p < 0.001$). **Table.2.2** shows the group means/SD and group comparisons for all OCI subscales, the direction is similar for all of them with OCD and schizo-OCS group scoring higher than healthy and schizophrenia groups. With regard to the differences between the two schizophrenia groups, the schizo-OCS group displayed higher positive ($U = -2.57$, $p = 0.01$), depressive ($U = -2.49$, $p = 0.01$), and anxiety ($U = -2.27$, $p = 0.03$) scores as measured by PANSS. There were no differences between two groups for duration of clozapine treatment ($t = -0.45$, $p > 0.65$), and clozapine dose (using cigarettes per day as covariate in an ANCOVA ($F = 0.011$, $p = 0.91$)).

2.2.2.2. Cognitive flexibility and working memory

Although no differences were found for digit span forward scores, significant differences in variance were found after performing an ANOVA between groups for digit span backward ($F = 4.52$, $p < 0.05$), with the schizo- OCS ($p < 0.01$) and the pure schizophrenia group ($p < 0.05$) performing worse than healthy controls, and OCD patients performing *better* than both healthy ($p < 0.01$), and both schizophrenia groups ($p < 0.001$). With regard to the CANTAB spatial working memory task, the only differences found were for the total number of errors on the task (SWMTE, $F = 4.55$, $p < 0.01$), and the between search errors which are the number of times the subject incorrectly revisits a box in which a token has previously been found (SWMBE, $F = 3.98$, $p < 0.01$). In both cases, the schizophrenia groups performed worse than both OCD ($p < 0.001$), and healthy volunteers ($p < 0.001$). Although OCD patients performed worse than controls on both these measures, the difference did not reach significance. Groups also displayed significant differences in the number of stages reached on the IED task ($F = 4.67$, $p < 0.01$), with schizo-OCS patients reaching significantly less stages than healthy, OCD and pure schizophrenia groups ($p < 0.001$). There were no differences between the remaining three groups. The number of errors pre extra-dimensional set-shift ($F = 8.78$, $p < 0.001$) were also different between groups, with the two schizophrenia groups performing worse than OCD and healthy volunteers ($p < 0.001$). The final difference was the number of errors made during the extradimensional set-shift ($F = 6.09$, $p < 0.001$), for which both schizophrenia groups performed worse than OCD and healthy groups ($p < 0.001$), and schizo-OCS worse than pure schizophrenia group ($p < 0.001$). Again, despite OCD patients performing worse than healthy controls, this did not reach significance.

Table.2.3 Multiple comparison of means – Tukey / Games Howell *post hoc* tests

Characteristics	Group 1	Group 2	Mean-difference	*p*-adjusted
Depression (MADRS)	HV	OCD	7.1208	0.0
	HV	Schizophrenia	3.0167	0.0065
	HV	Schizo-OCS	-7.636[G]	0.0
	OCD	Schizo-OCS	-0.515[G]	*0.90*
	Schizophrenia	Schizo-OCS	-4.619[G]	0.0
State anxiety (STAI-S)	HV	OCD	-13.131[G]	0.0
	HV	Schizophrenia	-11.467[G]	0.0
	HV	Schizo-OCS	-16.448[G]	0.0
	Schizophrenia	Schizo-OCS	4.981	0.0185
Trait anxiety (STAI-T)	HV	OCD	-22.28[G]	0.0
	HV	Schizophrenia	-10.267[G]	0.0
	HV	Schizo-OCS	-19[G]	0.0
	OCD	Schizophrenia	-12.0129	0.0
	Schizophrenia	Schizo-OCS	-8.7333[G]	0.0
IOU	HV	OCD	-27.3333[G]	0.0
	HV	Schizophrenia	-12.7667[G]	0.00009
	HV	Schizo-OCS	-28.9286[G]	0.0
	OCD	Schizophrenia	-14.5667	0.0001
	Schizophrenia	Schizo-OCS	-16.1619[G]	0.0001
OCI-R (total score)	HV	OCD	50.2583	0.0
	HV	Schizophrenia	-16.2333[G]	0.0001
	HV	Schizo-OCS	-44.7762[G]	0.0
	OCD	Schizophrenia	34.025[G]	0.0
	Schizophrenia	Schizo-OCS	-28.5429[G]	0.0
Digit span backwards	HV	OCD	0.9624	0.0055
	HV	Schizophrenia	-1.1	0.0123
	HV	Schizo-OCS	-1.119	0.0032
	OCD	Schizophrenia	-2.0624	0.0
	OCD	Schizo-OCS	-2.0814	0.0

Characteristics	Group 1	Group 2	Mean-difference	*p*-adjusted
IED completed stages	HV	Schizo-OCS	-9.901[G]	0.0
	OCD	Schizo-OCS	-1.4854	0.0
	Schizophrenia	Schizo-OCS	-1.1714	0.0001
IED PRE-EDS errors	HV	Schizophrenia	9.092	0.0
	HV	Schizo-OCS	9.9015	0.0
	OCD	Schizophrenia	6.2366	0.0001
	OCD	Schizo-OCS	7.0461	0.0
IED EDS errors	HV	Schizo-OCS	6.9639	0.0
	OCD	Schizophrenia	3.3011	0.0031
	OCD	Schizo-OCS	8.5868	0.0
	Schizophrenia	Schizo-OCS	5.2857	0.0
SWMBE	HV	Schizophrenia	-7.202[G]	0.0
	HV	Schizo-OCS	-7.917[G]	0.0
	OCD	Schizophrenia	-5.086[G]	0.003
	OCD	Schizo-OCS	-5.800[G]	0.0

HV: Healthy Volunteers; MADRS: Montgomery-Asberg Depression Rating Scale (MADRS), STAI-S: State Trait Anxiety Inventory-State; STAI-T: State Trait Anxiety Inventory-Trait; IU: Intolerance Of Uncertainty; OCI-R: Obsessive Compulsive Inventory-Revised, IED: Intra-Extra Dimensional Set Shift, ED: extradimensional shift, SWMBE: SWM Between Errors: The number of times the subject incorrectly revisits a box in which a token has previously been found. "G" stands for Games Howell test for non-parametric data.

2.2.2.3. Checking behaviour

Three repeated measure ANOVA's were performed for the three contrasts between 1) healthy volunteers and patients, 2) OCD and schizophrenia groups, and 3) schizophrenia with versus without OCS. There were no main effects of age nor verbal IQ on any of the measures. Complementary to the mixed ANOVAs, multiple comparison tests (using Bonferroni correction) were used to uncover the pair-wise differences. Three OCD patients did not check at all on any of the blocks. After the testing session they mentioned they had found out checking was being measured and they therefore tried to suppress checking behaviour. The analysis was repeated with and without them and since their exclusion did not impact the findings, the original results including these 3 patients are reported in this thesis.

Healthy controls versus patients

For contrast 1 (**Figure.2.5**), no interactions between groups and blocks were found for any of the measures. There were main effects of blocks for: 1) checking ($F = 28.06$, $p < 0.001$), where everyone

checked significantly less from block 1 with no feedback to block 3 where they were punished for checking (F = 71.73 $p < 0.001$), and this decrease also continued to block 4 (F = 26.86, $p < 0.001$); 2) accuracy (F = 8.781, $p < 0.001$), with all groups performing significantly better from first to the final block (F = 9.874, $p < 0.001$); 3) confidence (F = 32.19 $p < 0.001$), with the largest decrease from block 1 with no feedback to block 3 where feedback was provided (M = 0.256, SE = 0.04, $p < 0.001$). No main effects of group were found when considering checking rates. However, significant group main effects were found for accuracy (F = 8.915, $p < 0.001$), and confidence (F = 3.99, $p < 0.04$) with healthy volunteers performing better than patients and having a higher confidence rating. After performing multiple comparisons, it appeared however, that OCD patients had a comparable performance to that of the healthy group ($p > 0.05$), and schizophrenia patients were both performing worse than healthy ($p < 0.001$) and OCD participants ($p < 0.001$). OCD and pure schizophrenia patients also had a lower confidence than the healthy volunteers ($p < 0.05$, for each comparisons), but not significantly different from schizo-OCS group.

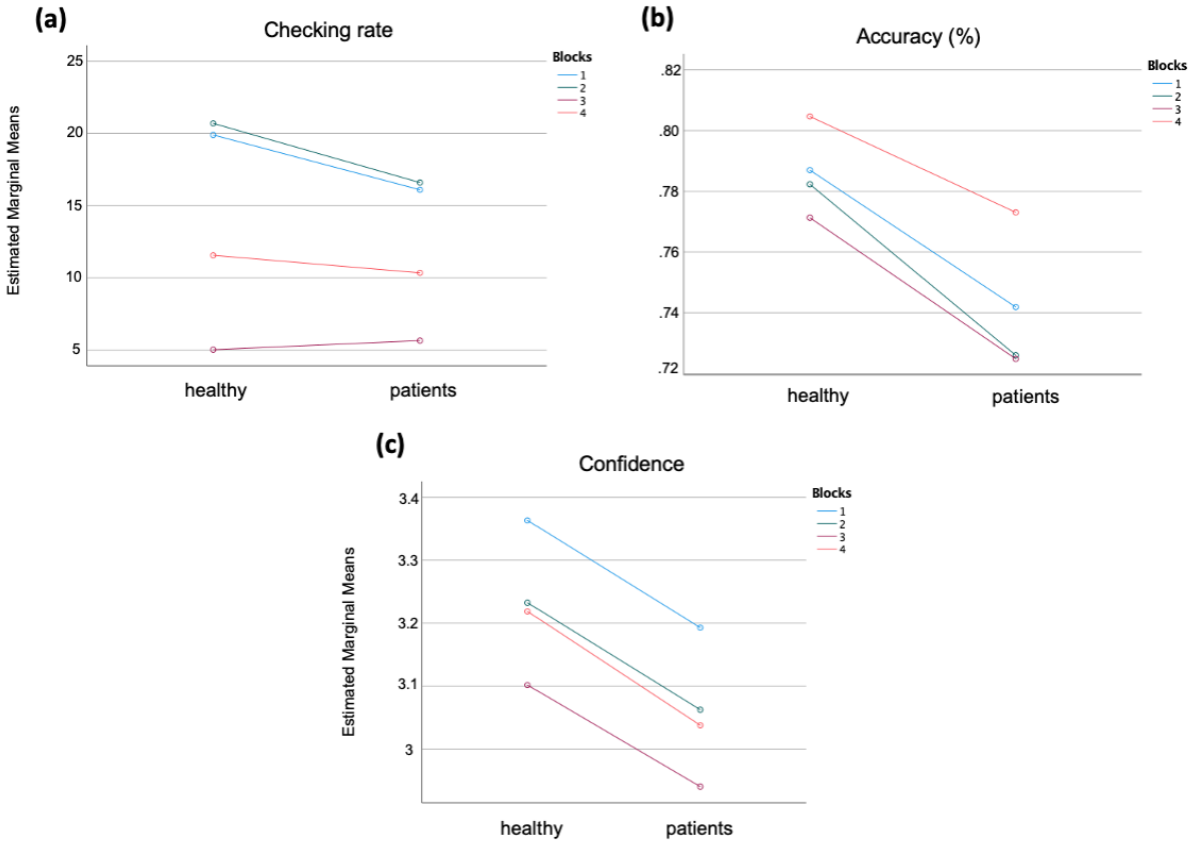


Figure.2.5 depicts the Estimated Marginal Means for the first contrast between healthy and patient groups for **(a)** checking rate, **(b)** accuracy of answers (%), and **(c)** confidence ratings.

OCD versus Schizophrenia

For contrast 2 (**Figure.2.6**), besides checking (F = 3.43, p = 0.009) none of the measures showed significant interactions for block and group. The interaction for checking behaviour was due to the increased checking in OCD in block 2, whereas in schizophrenia group block 1 had the highest checking rate. Within subjects, significant main effects of block were found for: 1) checking (F = 5.97, p = 0.017), where everyone checked less from 1st to 3rd block (M = 10.73, SE = 1.56, p < 0.001); 2) accuracy of responses (F = 12.88, p < 0.001), becoming more accurate from first to last block (M = -0.031, SE = 0.009, p = 0.004); 3) confidence (F = 21.352, p < 0.001), with a decrease in confidence after block 1 to block 3 (M = 0.256, SE = 0.04, p < 0.001). These finding were similar to the previous contrast. There were no group effects for confidence. Whereas OCD and schizophrenia groups differed significantly in accuracy of responses and checking behaviour with OCD patients performing better (F = 29.21, p < 0.001), and checking more than the schizophrenia patients, especially so in blocks 1 and 2 (F = 5.79, p < 0.006; see **Figure.2.6.a**).

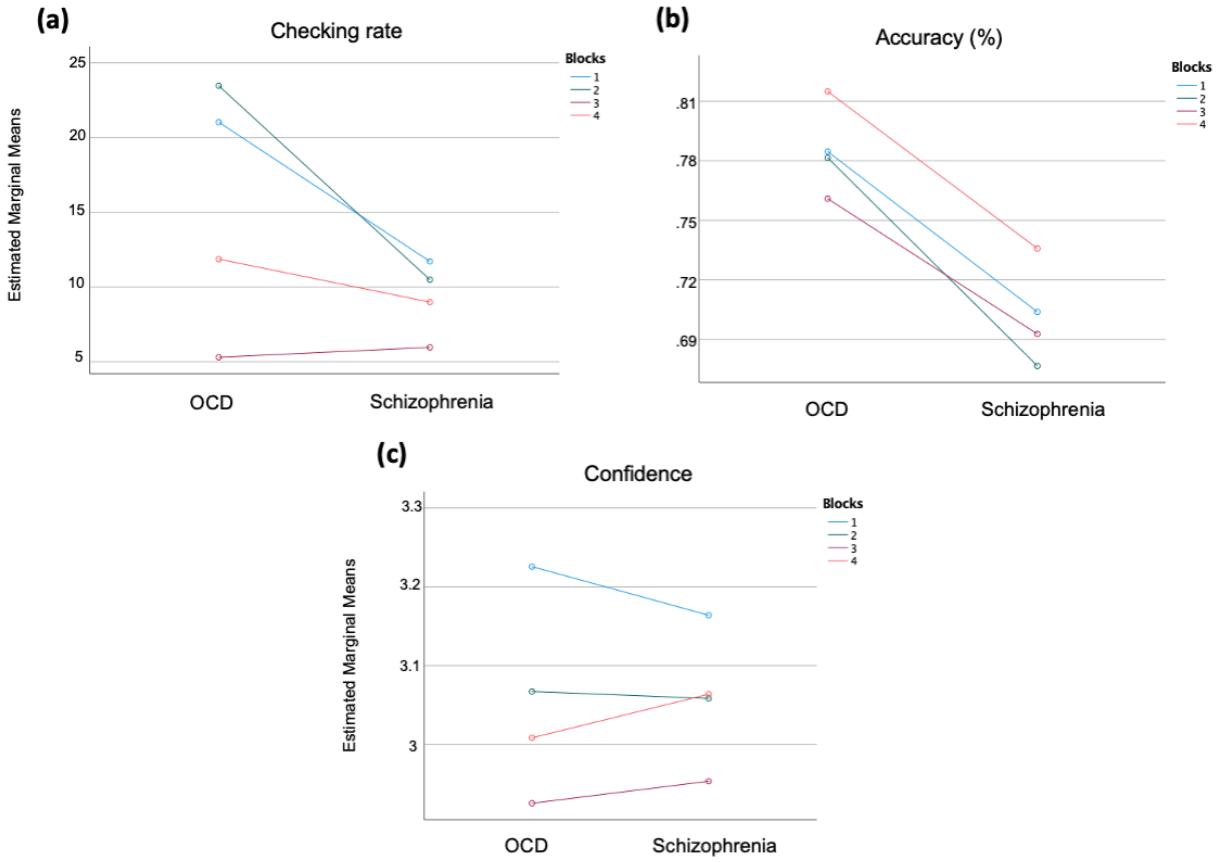


Figure.2.6 depicts the Estimated Marginal Means for the second contrast between OCD and Schizophrenia groups for **(a)** checking rate, **(b)** accuracy of answers (%), and **(c)** confidence ratings.

Schizo-OCS versus Schizophrenia

In last contrast (**Figure.2.7**), the two schizophrenia groups were compared against one another. The only interaction between blocks and groups was for checking in blocks 2 and 3 (F = 5.49, $p < 0.001$; see **Figure.2.7.a**), with schizo-OCS group checking less in block 4 than the pure schizophrenia group, who checked more than schizo-OCS in block 2. There was only a trend for the main effect of checking (F = 2.6, p = 0.07), with reduced checking from first to the third block in both groups (F = 6.95, p = 0.01). There was a main effect of block on accuracy (F = 4.14, p = 0.01), and confidence (F = 3.166, p = 0.03) with accuracy increasing in the last block, and confidence significantly decreasing from 1st to 3rd block in both groups when punishment is introduced (F = 6.95, p = 0.01). However, similar to checking, the pure schizophrenia group again increased their confidence ratings when feedback and punishment for checking were removed in block 4 (F = 6.95, p = 0.01). No group main effects for checking, accuracy, and confidence were found across blocks between the schizophrenia with and without OCS groups.

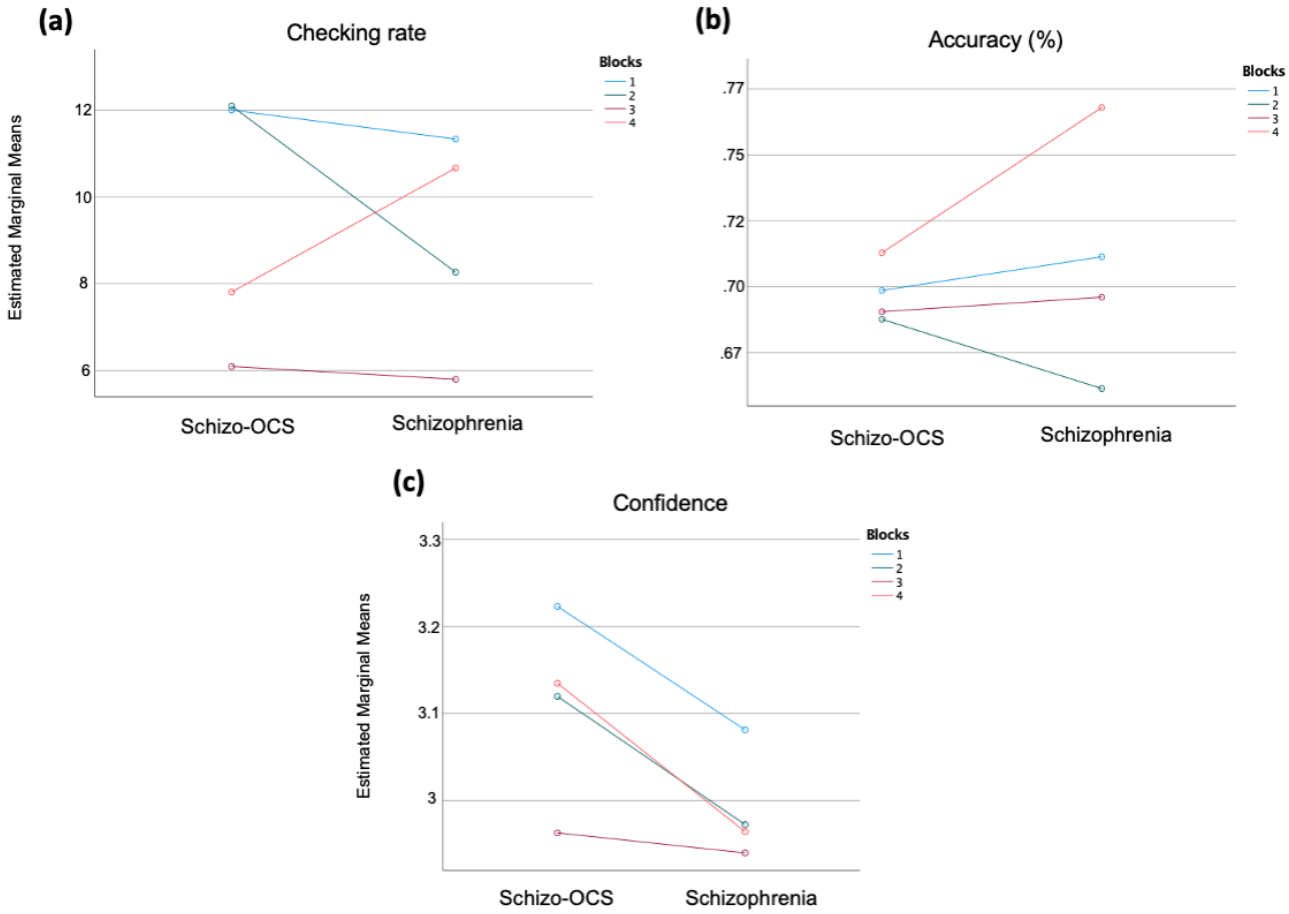


Figure.2.7 depicts the Estimated Marginal Means for the third contrast between Schizophrenia with OCS (Schizo-OCS) and 'pure' schizophrenia groups for **(a)** checking rate, **(b)** accuracy of answers (%), **(c)** confidence ratings, and **(d)** the variation in the finger press location on the screen.

IVT checking, accuracy and confidence correlations

Accuracy of answers (correctly reporting whether drawings were the same or different) and checking were significantly and positively correlated with each other in OCD (r_s = 0.61, p = 0.0002) and pure schizophrenia in block 1 with high uncertainty (r_s = 0.79, p = 0.0004), this was not the case for schizo-OCS and healthy groups. The difference between these correlations in the two schizophrenia groups was significant (z = 2.079, p < 0.01), and OCD and healthy volunteers (z = 1.93, p = 0.02), but not between OCD and schizo-OCS (p = 0.08). **Figure.2.8** depicts these relationships in all groups. However, performance in healthy volunteers was significantly higher than the two schizophrenia groups, and comparable to OCD patients (see Contrast 1 above).

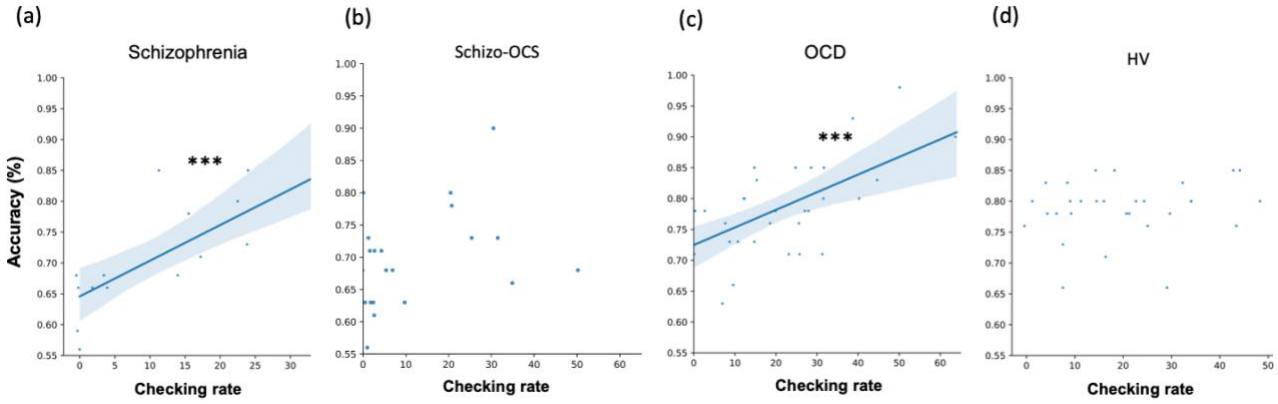


Figure.2.8 Relationship between checking and accuracy of answers in first block with high uncertainty and worse performance in (a) pure schizophrenia, (b) schizo-OCS, (c) OCD, and (d) healthy groups. (***) indicates a significance level below 0.001.

Correlations between checking on IVT and clinical measures

In OCD patients, checking in block 1, the first block with high uncertainty, was positively correlated with intolerance of uncertainty scores (r_s = 0.40, p = 0.02), but this was not the case in other groups. A Fisher's Z-test showed significant differences between these correlations between the OCD and healthy volunteers (z = 2.66, p = 0.004), and the OCD and schizo-OCS patients (z = 1.98, p = 0.02). This difference was not significant between the OCD and pure schizophrenia (z = 1.32, p = 0.09) groups for these correlations.

Two OCD patients were outliers, with checking rates of 27 and 28 times in block 3 which were more than 3 SD from the mean (mean + 3SD = 26). Both these patients were checkers according to their OCI-checking sub scores and after excluding them from the analysis, the YBOCS compulsion score in OCD patients (but not obsession score) was correlated with the amount of checking in block 3- the block with punishment for checking (r_s = 0.46, p = 0.009). Similarly, the schizo-OCS group's checking was positively related to their YBOCS compulsion score (r_s = 0.51, p = 0.01), although this was mainly caused by two outliers and checking was not normally distributed amongst schizo-OCS patients, the Spearman's correlation was still significant. Additionally, only in schizo-OCS group checking under punishment was associated with state anxiety (r_s = 0.60, p = 0.004). The difference between these correlations between the schizo-OCS group, and pure schizophrenia was significant (z = 2.19, p = 0.01), OCD (z = 2.02, p = 0.02), and healthy volunteers (z = 2.31, p = 0.01). There were no correlations between PANSS scores and checking rate in either schizophrenia groups. Lastly, after taking the number of cigarettes smoked per day into consideration, clozapine dose was still correlated with checking in schizo-OCS group (r_s = 0.428, p = 0.03), but not in pure schizophrenia (r_s = 0.14, p = 0.31). However, the difference between the two schizophrenia groups was not significant for these correlations (z = 0.84, p = 0.2). There were no correlations between treatment duration and checking on IVT in either schizophrenia group.

Correlations between checking on IVT and cognitive flexibility and working memory

Checking by schizophrenia group without OCS was correlated with digit span forward (r_s = 0.58, p = 0.02), whereas in OCD patients only accuracy was correlated with this measure (r = 0.39, p < 0.001). With regard to cognitive flexibility as measured by the IED task, the number of completed stages was only correlated with accuracy of answers (and not checking) in OCD across all blocks (r = 0.36, p < 0.001), healthy volunteers only in block 1 (r = 0.43, p = 0.02), and schizo-OCS group, only under punishment (r = 0.40, p < 0.001), but not in schizophrenia group without OCS and no correlations with checking. There were significant relationships between the number of errors made before the extradimensional shift and checking on the task in the pure schizophrenia group in final block and under high uncertainty (r_s = 0.67, p < 0.01), and accuracy of answers in healthy volunteers (r = - 0.46, p = 0.01). The errors made during the extradimensional shift on the IED task were positively correlated with checking in healthy volunteers across all blocks (r_s = 0.33, p < 0.001) and schizo-OCS in block 3 (r_s = 0.53, p = 0.01), negatively in pure schizophrenia only in 3rd block (r_s = - 0.56, p = 0.02). Although this relationship did not reach significance in OCD it, showed a negative pattern as well. Except for the healthy group, this relationship only was found for checking under punishment for the remaining groups. Additionally, in schizo-OCS group the extra-dimensional shift errors were negatively correlated with their accuracy of answers on high uncertainty blocks (r = - 0.45, p =0.03). In schizo-OCS group, spatial working memory between errors (SWMBE) were positively correlated with checking under

punishment in block 3 (r_s = 0.56 , p = 0.007), and negatively correlated with accuracy of answers in high uncertainty blocks, with a negative trend in block 1 (r_s = - 0.37, p < 0.1), and becoming significant in block 4 (r_s = - 0.59, p = 0.004). Although these correlations were not significant at all in schizophrenia group without OCS (p = 0.26 for checking and SWMBE, and p = 0.27 for accuracy and SWMBE), the differences between these correlations between the two schizophrenia groups were not significant (p > .05).

Summary: clinical measures, IED, verbal and spatial working memory

Table.2.2 shows all the clinical measures, IED, and working memory measures across groups. Severity of OCD symptoms as measured with YBOCS was higher in OCD than schizo-OCS group. The two OCD/schizo-OCS groups were more anxious (both state and trait anxiety), depressed, intolerant of uncertainty, and had higher OCI scores than the other two groups. However, the 'pure' schizophrenia patients were more intolerant of uncertainty and were more anxious and depressed than healthy volunteers. Clozapine dose, treatment duration, and abnormal involuntary movement scale scores were comparable between the two schizophrenia groups. However, the schizo-OCS group scored higher on positive and depressive symptoms than the schizophrenia group without OCS. Digit span forward was not different between participants, digit span backward was worse in two schizophrenia groups, and highest in OCD patients compared to all three groups. Cognitive flexibility and spatial working memory were worse in schizophrenia groups, OCD patients had worse cognitive flexibility than healthy volunteers but this did not reach significance. No correlations between YBOCS and clozapine dose, or YBOCS and schizophrenia symptoms were found in schizo-OCS group.

Summary: performance on IVT

All groups 1) checked less after receiving punishment for checking, 2) improved their accuracy over time, and 3) had less confidence after receiving feedback. However, OCD and healthy volunteers had higher accuracy (answering correctly whether the two images were the same or different at each trial: two sets of images) than the two schizophrenia groups, OCD patients also checked more than schizophrenia patients, especially in the beginning with higher uncertainty (i.e. no feedback block). After removing the punishment in block 4, schizo-OCS group continued to check less than previous blocks, and after removing the feedback, they also regained their confidence. The OCD and pure schizophrenia groups showed significant correlation between checking and accuracy (across all blocks in OCD, and only in uncertain blocks for schizophrenia patients), and had lower confidence than the other two groups.

Summary: checking and accuracy relationships with clinical and cognitive measures

Checking in the high uncertainty condition in OCD patients was correlated with their intolerance of uncertainty scores, while checking under punishment was related to the severity of their real life compulsions as measured by YBOCS. For the schizo-OCS group, checking under punishment was correlated with their state (but not trait) anxiety, general schizophrenia symptoms, and showed a positive trend with their positive symptoms. Checking under uncertainty however, displayed a positive relationship with their clozapine dose. None of this was found for the pure schizophrenia group. The spatial working memory performance measured with CANTAB SWM was comparable between the two schizophrenia groups, and worse than the other two groups. However, only the schizo-OCS group displayed a positive relationship between checking on the IVT task and the total number of times they revisited a box incorrectly in which a token had previously been found, which was also negatively correlated with their accuracy of answers.

2.2.3. Interim Discussion

Study 2 aimed to understand the psychological and behavioural characteristics of obsessive-compulsive symptoms in schizophrenia patients with OCS caused by clozapine, and OCD patients compared to healthy volunteers and schizophrenia patients on clozapine without OCS to understand what clinical and cognitive factors could underlie checking behaviour. We used a newly developed computerised checking paradigm to measure checking in an experimental context as we could not replicate the checking behaviour measured by Morein-Zamir et al (2018) in study 1.

Cognitive flexibility, working memory, and other clinical measures

As expected, both OCD and schizo-OCS groups showed higher anxiety (state and trait), depressive, obsessive compulsive symptoms, and were more intolerant of uncertainty than the other two groups. Clozapine dose, treatment duration, and abnormal involuntary movement scale scores were comparable between the two schizophrenia groups. However, the schizo-OCS group scored higher on positive and depressive symptoms than the schizophrenia group without OCS, which is in line with our previous findings in a sample of 231 clozapine treated patients (Biria et al., 2019). Cognitive flexibility, executive functioning, and spatial working memory are known deficits in schizophrenia patients (Bowie et al., 2006; Goldberg & Green, 2002; Leeson et al., 2009; Pantelis et al., 1999) and we also replicated these findings by measuring worse performance on IED across all measures, digit span backwards and spatial working memory of CANTAB in the two schizophrenia groups compared to OCD and healthy controls. We did not replicate the worse cognitive flexibility in OCD that have been previously reported (Chamberlain et al., (2007); Vaghi et al., 2017). However, consistent with the reported findings of a

recent meta-analysis (Chamberlain et al., 2021), our OCD group did show a trend towards making more mistakes during the extradimensional shift stage.

IVT performance differences

OCD and healthy volunteers performed better than schizophrenia groups on the IVT by having a higher number of correct answers on the perceptual decision making trials. To my surprise, OCD patients had both a good performance and showed functional checking by displaying a positive relationship between checking and accuracy of their answers. Despite having a worse performance, this positive relationship was also found in schizophrenia group without OCS. Healthy volunteers did not show this correlation but they were already performing optimally without the need to check. Our first hypothesis about finding excessive and dysfunctional checking, was only partly confirmed in schizophrenia patients with OCS, and not the OCD group. Interestingly, the schizo-OCS group showed dysfunctional checking by having both a poor performance, and not using checking to improve their answers. Neither the OCD nor the schizo-OCS groups showed excessive checking behaviour.

IVT checking and accuracy relationships with clinical and cognitive measures

Although excessive checking was not found in OCD nor schizo-OCS groups, their checking rate was positively correlated with severity of their compulsions as measured with YBOCS. This means the more severe their compulsions were, the less they could suppress checking despite being punished for it. This was worse in schizophrenia patients who also showed a relationship with state anxiety and checking. They were punished for checking, felt anxious, and yet the more compulsive checking they experienced in real life, the more they checked on IVT.

As hypothesized, intolerance of uncertainty seemed to play a role in checking for both OCD/schizo-OCS groups. Under high uncertainty, OCD patients showed a positive correlation between IOU scores and checking rate. Despite not replicating this finding in schizo-OCS group, they seemed to be sensitive to uncertainty as well. They showed a positive and significant correlation between clozapine dose (after correcting for smoking) and checking rate in high uncertainty blocks. The latter was not found in the schizophrenia group without OCS or healthy volunteers. The relationships between obsessive compulsive symptoms and clozapine dose &/or plasma levels in schizophrenia have been shown repeatedly (Biria et al., 2019; Fernandez-Egea et al., unpublished observations, personal communication; Gürcan et al., 2021; Kim et al., 2020; Lin et al., 2006; Mukhopadhaya et al. 2009; Reznik et al., 2004; Schirmbeck et al. 2011). However, in this study, we demonstrated the importance of uncertainty on dysfunctional checking behaviour induced by clozapine. However, no correlations between checking, positive and negative symptoms, and motor tics were found in schizophrenia patients. Despite having similar deficits on spatial working memory in both schizophrenia groups, only

the schizo-OCS group showed a positive correlation between checking, and spatial working memory performance (SWMBE: number of times they revisited a box incorrectly in which a token had previously been found). The SWMBE score was negatively correlated with accuracy of answers in schizo-OCS group. Again our hypothesis about working memory and checking relationship was partially confirmed. Spatial working memory did play a role on IVT checking in schizo-OCS group, but not in OCD. Thus the groups seem to have different underlying mechanisms driving their checking compulsions.

To summarise, we succeeded in measuring dysfunctional checking in the schizo-OCS group, despite using a slightly inferior task, and characterised their clinical, and cognitive differences compared to pure schizophrenia and OCD groups. Working memory deficits were related to checking in schizo-OCS patients but not in OCD and pure schizophrenia (despite both schizophrenia groups having comparable deficits in spatial working memory). The nature of the checking behaviour was different between groups as well, with OCD and schizophrenia patients without OCS showing goal-directed/functional checking, whereas schizo-OCS checking behaviour was dysfunctional. Both OCD and schizo-OCS groups are sensitive to intolerance of uncertainty which could be increasing or maintaining their checking behaviour. Clozapine dose was presumably causally related to checking in schizo-OCS patients, however, only under high uncertainty. Despite being punished for checking, the more severe OCD/schizo-OCS patients continued checking on IVT. Lastly, the IVT did not measure excessive checking in neither the OCD nor the schizo-OCS groups. This could be due to the task instructions placing an emphasis on both accuracy of answers and a time limit to finish the task. The speed/time limit requirement may have discouraged excessive checking. This finding however also indicates that OCD patients have the potential to show goal-directed behaviour, and instead of deficits in goal-directed system, they may rather experience a narrowing of the number of goals (Robbins et al., 2019). Although, despite the instructions encouraging all groups to show functional checking, schizo-OCS patients were the only group unable to do so, which may indicate the depth of their cognitive impairments.

In order to test the hypothesis that a time limit would discourage excessive checking, the task instructions needed to be changed to require only accurate answers without a time limit. This was also the instruction in a similar perceptual decision making task that did successfully measure checking in OCD (Rotge et al., 2008), although they did not replicate the results in another subsequent study (Rotge et al., 2015). Therefore, I decided to design a new version of the IVT checking paradigm, using new instructions, that would measure different aspects of cognition and decision making potentially influencing dysfunctional checking, rather than the functional checking measured in Study 2. Study 3 below will discuss the findings of this new IVT version in OCD and healthy controls.

2.3. Study 3 (IVT-version-2: OCD vs HV)

Study 2 demonstrated that uncertainty can play a role in checking behaviour in OCD patients and that under certain circumstances, OCD patients are able to show functional or goal directed checking as their checking improved their perceptual decision-making accuracy. However, despite being related to/predicting excessive real life checking in these patients, they did not show increased checking rates on the IVT compared to the healthy group. In Study 3, the task instructions were changed in order to encourage checking behaviour by removing the time limit and only focusing on accuracy of answers.

In Study 3 we address the following hypotheses:
H1 - By removing the time limit, OCD patients will show dysfunctional checking.
H2 – Dysfunctional checking in OCD is not goal-directed and rather driven by the habit system.

2.3.1. Method

2.3.1.1. Participants

This study included 23 healthy volunteers and 25 OCD patients who were fluent English speakers and were matched for age, gender and IQ. **Table.2.4** shows the demographic and clinical characterisation of both groups. This study was approved by the East of England - Cambridge South Research Ethics Committee (REC 16/EE/0465). All volunteers gave written informed consent before beginning the testing and received monetary compensation for taking part in the study. Patients met the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for OCD by a detailed interview with a clinician but no other Axis one mental disorders. All OCD patients had a primary diagnosis of OCD and anxiety and depressive symptom were present in all patients. OCD participants with comorbid major depressive disorder or GAD were not included in the study. OCD patients with a Yale- Brown Obsessive Compulsive Scale (YBOCS, Goodman et al., 1989) higher than 12 (Lewin et al., 2011) were enrolled in the study. Healthy controls had no current or past psychiatric disorders as determined by a screening interview including the Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1998) and the Montgomery-Asberg Depression Rating Scale (MADRS; Montgomery and Asberg, 1979). For all participants, excessive drug or alcohol use, neurological deficits or head injury were exclusion criteria. Six patients were unmedicated, and out of the 19 medicated patients, 1 was on beta blocker and clomipramine and the rest were on SSRIs. Lastly, all participants had normal or corrected-to normal vision and hearing.

2.3.1.2. Questionnaires and Neuropsychological battery

The following measures were collected for all participants:

- State/Trait Anxiety Questionnaire (Spielberger et al., 1983): standardized self-report measure of general anxiety
- Obsessive-Compulsive Inventory (Foa et al., 1998): standardized self-report measure of obsessive-compulsive symptoms
- Intolerance of Uncertainty Scale (Buhr and Dugas, 2002): standardized self-report measure on the unpleasantness of uncertainty
- National Adult Reading Test (Nelson & Willison, 1982) to provide an estimate of verbal intelligence
- Creature Of Habit Scale (COHS; Ersche et al., 2019), and Habitual Tendencies Questionnaire (HTQ; Ramakrishnan et al., 2022) to measure habitual tendencies. The three subscales of habitual compulsivity, preference for regularity and aversion for novelty were used in additional the total HTQ score (Ramakrishnan et al., 2022).

2.3.1.3. Stimuli and procedure

Cognitive flexibility and attention

The CANTAB Intra-Extra Dimensional Set Shift task (IED; Owen et al., 1991; Roberts et al., 1988) was used to assess cognitive flexibility, more specifically the set-shifting ability which tests rule acquisition and reversal learning. It was administered on a touch-screen tablet with a total duration of 7 minutes. The task features visual discrimination between colour-filled shapes and white lines, and shifting and flexibility of attention. The test is well validated in individuals experiencing OCD (Chamberlain et al., 2007; Vaghi et al., 2017) and is a computerised analogue of the Wisconsin Card Sorting test.

Checking behaviour

Checking behaviour was measured using the Image Verification Task described in Study 2, section 2.2.1.3. However, the instructions were different in the current version. In the first version of the task, the instructions were to “answer as accurately as possible in the limited time that you have”. As there were no group differences in the number of checking, I hypothesized that the instruction may have discouraged checking by setting a time limit. The instructions were changed to “Be as accurate as possible” and removing the time limitation. In the previous version, patients asked many questions during the task about whether it was measuring checking behaviour. Three patients shared their

unwillingness to check during the task because they mentioned that they knew that checking was measured, and were unhappy that we were trying to hide this information from them (although upon further inspection, these patients had the lowest YBOCS scores and this may have been the actual reason behind being able to suppress checking compulsions). In order to avoid this issue in the new version, instead of saying "You can go back and see the images again to decide if they are the same or different", the current version states "You can go back and check the images again to decide if they are the same or different". Everything else remained the same including the stimuli used within the task. **Figure.2.4** from Study 2 shows the different conditions of the task.

2.3.1.4. Statistical analysis

An independent sample t-test was used for descriptive data, clinical measures and task performance on IED task outcomes (more specifically, the errors made before and during the extradimensional set shift stage, and the number of total stages completed). In case the normality condition was not satisfied, Mann-Whitney U test was used instead. The average checking rates, accuracy of answers, and confidence ratings were used as dependent variables, repeated across blocks within subjects, and were also compared between groups using a mixed ANOVA in case of a normal distribution, and a robust mixed ANOVA if normality or homogeneity of variance were violated (Mair & Wilcox, 2021). In case of significant main effects, independent and paired sample t-tests were performed to understand where the differences laid. For the mixed ANOVA, if the sphericity condition for repeated measures was not satisfied, a Greenhouse-Geisser correction was applied. The relationship between primary outcome measures and clinical measures were compared using a Pearson's r or Spearman's r_s correlation depending on the distribution of the data. In the case of significant correlations, Fisher's Z-test was performed to compare the correlations across groups. R studio Version 1.2.5033, and package WRS2 were used to perform the robust mixed ANOVA, SPSS version 28 (SPSS IBM) was used for the rest of the analysis.

2.3.2. Results

2.3.2.1. Demographic and clinical measures

Table.2.4 depicts the descriptive group characteristics for both groups and the corresponding t and *p* values. Groups did not differ for age and verbal IQ and were matched for gender. However, healthy volunteers were slightly more educated than patients ($p = 0.04$). Patients scored significantly higher than healthy subjects ($p < .001$) on all clinical measures such as depression (MADRS), anxiety trait and state (STAI), intolerance of uncertainty (IOU), and obsessive-compulsive symptoms (OCI). They had higher habitual tendencies such as automaticity (COHS, $p = .01$), compulsivity (HTQ, $p < .001$), and aversion to novelty (HTQ, $p = .02$).

Table.2.4 Means and standard deviations of descriptive group characteristics.

Characteristics	Controls (n = 23) M (SD)	OCD (n = 25) M (SD)	t	*p*
Age (Years)	33.99 (14.13)	29.44 (9.78)	1.28 U	= .20
Gender (M:F)	10:13	11/14		
Verbal IQ (NART)	113.1 (8.2)	109.42 (6.23)	1.80	=.08
Education (Years)	17 (2.81)	15.48 (2.29)	2.10	= .04
YBOCS total score	NA	22.44 (5.58)		
Depression (MADRS)	5.7 (3.7)	17.88 (10.78)	- 5.31 U	< .001
State anxiety (STAI-S)	28.3 (7.2)	38.44 (9.81)	- 4.05	< .001
Trait anxiety (STAI-T)	38 (10.1)	55.68 (11.15)	- 5.74	< .001
IOU	53.65 (18.27)	83.72 (23.81)	- 4.87	< .001
OCI-R (total score)	7 (8.1)	59.28 (29.71)	- 8.15 U	< .001
OCI-Washing	0.87 (1.29)	9.48 (10)	- 4.25 U	< .001
OCI-Checking	1.61 (2)	12.8 (8.62)	- 6. 07 U	< .001
OCI-Doubting	0.39 (0.89)	6.12 (3.7)	- 7.22 U	< .001
OCI-Ordering	1.22 (1.47)	6.24 (4.81)	- 4.79 U	< .001
OCI-Neutralising	1.13 (1.74)	7.76 (5.75)	- 5.49 U	< .001
OCI- Hoarding	1 (1.88)	2.76 (2.97)	- 2.46 U	= .01
OCI-Obsessing	0.78 (1.5)	14.12 (7.38)	- 8.83 U	< .001
COHS-Routine	48.83 (10.38)	52.08 (10.44)	- 1.08	= .285
COHS-Automaticity	29.3 (8.51)	35.28 (8.04)	- 2.50	= .01
HTQ-total	92 (19.70)	118.76 (16)	- 5.18	< .001
HTQ-Compulsivity	13.57 (5.52)	24 (3.97)	- 7.56	< .001
HTQ-Preference for Regularity	16.48 (4.92)	18.72 (4.36)	- 1.67	= .10
HTQ-Aversion to novelty	6.78 (2.44)	8.76 (3.16)	- 2.40	= .02
IED completed stages	8.78 (0.60)	8.44 (0.917)	1.54	= .13
IED PRE-EDS errors	6.91 (3.19)	6.84 (2.56)	.088	= .93
IED EDS errors	5.57 (6.59)	10.84 (12.59)	- 1.83 U	= .07

NART: National Adult Reading Test, YBOCS: Yale-Brown Obsessive Compulsive Scale, MADRS: Montgomery-Asberg Depression Rating Scale (MADRS), STAI-S: State Trait Anxiety Inventory-State; STAI-T: State Trait Anxiety Inventory-Trait; IU: Intolerance Of Uncertainty; OCI: Obsessive Compulsive Inventory,, COHS: Creature Of Habits, HTQ: Habitual Tendencies Questionnaire, IED: Intra-Extra Dimensional Set Shift, ED: extradimensional shift, "U" stands for Mann-Withney U test in case of non-normal/inhomogeneous data.

2.3.2.2. Cognitive flexibility

With regard to cognitive flexibility there were no significant group differences for the number of stages completed on the CANTAB IED task, or the number of errors prior to the extra dimensional shift. Patients made more errors than healthy volunteers during the extra-dimensional shift stage. However, this difference was not significant (p = .07).

Table.2.5 IVT behavioural outcomes

Characteristics	Controls (n = 23) M (SD)	OCD (n = 24) M (SD)	t	*p*
Block 1 (high uncertainty):				
- Checking	24.48 (15.1)	46.17 (34.71)	2.34 U	= **.01** *
- Accuracy	0.78 (0.07)	0.80 (0.06)	- 0.86	= .39
- Confidence	3.57 (0.59)	3.33 (0.56)	1.48	= .07
Block 2 (optional feedback):				
- Checking	26.87 (19.36)	51.25 (37.55)	2.54 U	= **.01** *
- Accuracy	0.77 (0.07)	0.78 (0.07)	- 0.46	= .64
- Confidence	3.26 (0.54)	3.17 (0.637)	0.59	= .27
Block 3 (feedback + checking punished):				
- Checking	5.26 (5.92)	13.17 (16.37)	2.50 U	= **.01** *
- Accuracy	0.76 (0.08)	0.76 (0.08)	0.17	= .86
- Confidence	2.87 (0.54)	2.79 (0.65)	0.62	= .26
Block 4 (high uncertainty):				
- Checking	13.09 (11.95)	33.79 (29.39)	2.95 U	= **.003** **
- Accuracy	0.83 (0.08)	0.85 (0.15)	- 0.55	= .58
- Confidence	3.17 (0.65)	3.13 (0.61)	0.29	= .76

The task outcome measures were calculated as follows: checking is the total number of times someone checked the drawings, accuracy is percentage of the total correct answers, and confidence is the average confidence. These values were calculated per block.

2.3.2.3. Checking behaviour

One OCD patient was an outlier for checking by more than 3SD from the mean and was excluded from the correlation and ANOVA analyses with the number of checking on the task.

IVT outcome measures

After performing a robust mixed ANOVA for checking behaviour, the OCD group checked significantly more across all blocks than the healthy control group (F = 8.41, p = .008), and both groups checked less from block 1 and 2 to blocks 3 (**Figure.2.9.a**) where checking was punished (F = 20.06, p < .001). Additionally, there was an interaction between groups and blocks (F = 3.18, p = .04). The interaction was caused by the difference between groups from block 3 to 4. OCD patients checked significantly more from block 3 to 4 (after punishment was removed), whereas the healthy control group did not.

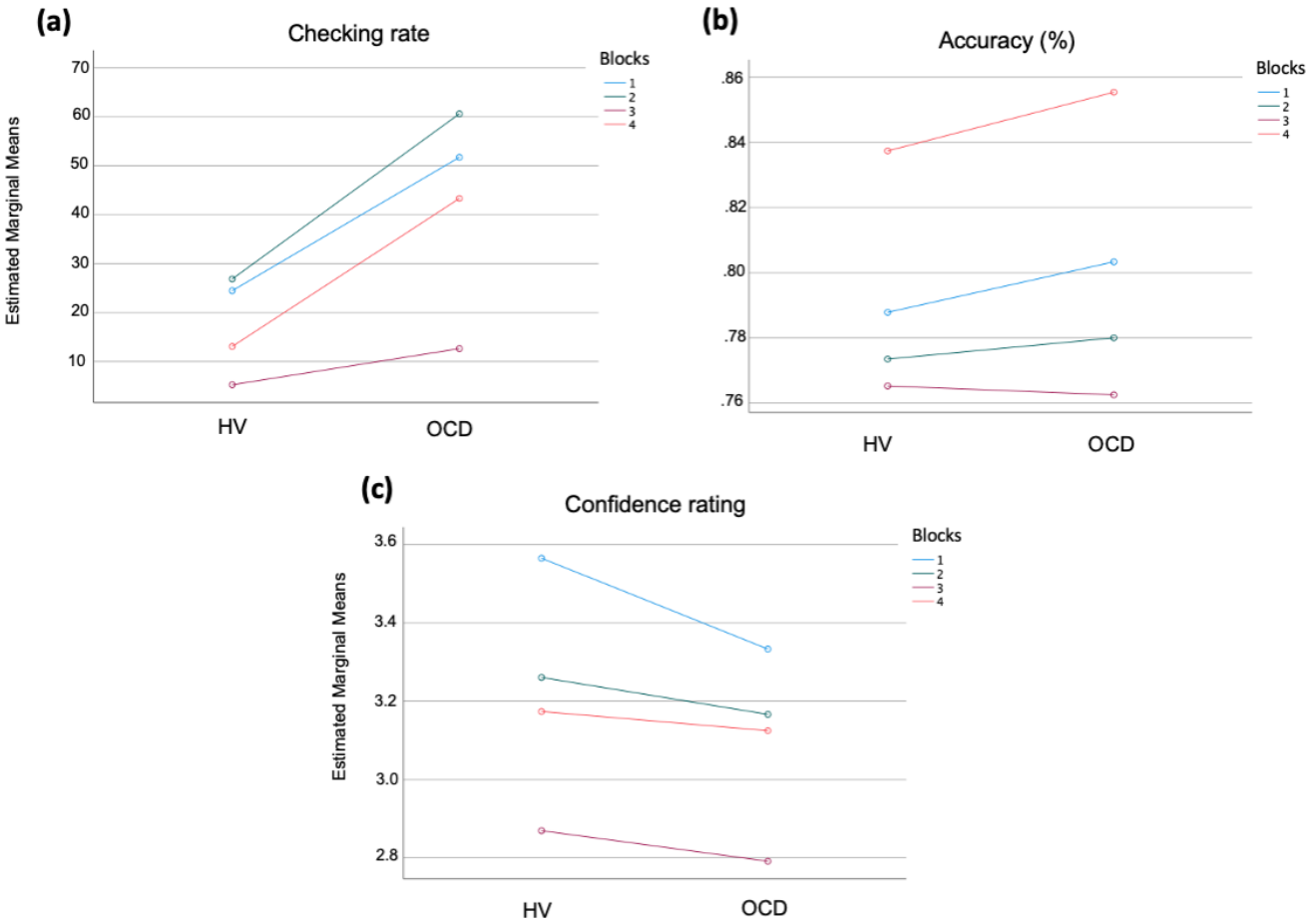


Figure.2.9 Estimated marginal means across 4 blocks and between two groups of OCD and healthy volunteers for **(a)** average number of checks **b)** percentage accuracy of answers, and **(c)** average confidence ratings.

Both groups became less confident (F = 18.63, $p < .001$) across blocks, with no significant group differences or interactions (**Figure.2.9.c**). **Table.2.5** shows all the IVT outcome measures across 4 blocks, and the corresponding t, and *p* values. The only significant differences were for checking rates across blocks, the biggest difference between groups was in the final block with OCD patients checking significantly more ($p < .005$ in block 4, as opposed to $p = .01$ in other blocks), despite having the best performance of all blocks, and even performing slightly better than controls. Lastly, there was a trend for lower confidence in patients in the first block with high uncertainty ($p = 0.07$).

Relationship between checking, accuracy and confidence on IVT

The relationship between checking and accuracy of answers in block 1 was investigated. As no feedback was provided about answers and participants were completing the task for the first time, checking could be used to improve performance and accuracy of answers. As opposed to Study 2, in Study 3, only healthy volunteers showed a positive relationship between checking and accuracy in the first block with high uncertainty ($r_s = .67$, $p < .001$), this was not the case in OCD group ($p > 0.05$). The difference between these correlations was significant ($z = 2.29$, $p < .05$). Next, the relationship between confidence and checking on first block with high uncertainty was investigated. OCD patients showed a negative correlation between checking (also a trend in other blocks) and confidence ratings ($r_s = - .50$, $p = .01$) on the first block. The Healthy group showed no correlations between checking and confidence, and the difference between these correlations between the two groups was significant ($z = - 1.99$, $p < .05$).

Relationship between checking on IVT, clinical measures and cognitive flexibility

There were no relationships between any of the IED measures and checking in either group. However, when combining both groups, OCI total score ($r = .38$, $p < .001$), OCI-checking ($r = .32$, $p < .005$), OCI-obsessing ($r = .32$, $p < .001$) and OCI-doubting ($r = .36$, $p < .001$) were positively related to checking on the task across all blocks, except the block with punishment for checking (block 3, $p > .05$). Again, when combining groups, automaticity (COHS; $r = .30$, $p < .001$) but not routine (COHS; $r = - 0.087$, $p = .65$), and habitual compulsivity (HTQ; $r = 31$, $p < .001$) in both groups were significantly and positively correlated with checking across all blocks. In OCD patients, state ($r = .37$, $p = .001$) and trait ($r = .41$, $r < .001$) anxiety showed a positive relationship with checking in blocks with high uncertainty (1 and 4), this correlation was not significant in healthy group and a Fisher's Z-test between these correlations yielded significant differences between groups ($z = 1.76$, $p < .05$; $z = 2.31$, $p = .01$; respectively for state and trait anxiety). No correlations between checking and YBOCS compulsions were found in OCD patients.

Summary: IVT performance

Both groups became more accurate over time, and less confident after receiving feedback about their answers. Checking was only functional in the healthy group as they showed a positive relationship between checking and accuracy of their answers in first block with high uncertainty. Whereas OCD patients' checking in the first block was negatively related to their confidence ratings. OCD patients checked significantly more than healthy group across all blocks. In the final block, despite improved accuracy, the OCD group showed the biggest difference in checking with the control group while their performance was even slightly superior to that of controls.

Summary: clinical findings and IVT checking correlations

As expected, OCD patients had significantly higher scores for obsessive compulsive symptoms, state and trait anxiety, depressive symptoms, intolerance of uncertainty, and habitual tendencies. Both groups displayed positive and significant correlations between checking on the IVT, and obsessing and doubting subscales of OCI, and the OCI total score, except when checking was punished. Additionally, both groups also showed positive relationships between IVT checking and automaticity and habitual compulsivity, across all blocks. However, only in OCD patients were state and trait anxiety significantly related with checking on high uncertainty blocks.

2.3.3. Interim Discussion

As hypothesised, when the time limit was removed and accuracy of answers was the only goal, OCD patients demonstrated excessive checking compared to the healthy group despite having a similar performance level as measured by the number of correct answers. Additionally, checking was dysfunctional in the OCD group as increased checking did not improve their accuracy, which was the case in the control group.

Under high uncertainty, confidence and checking were negatively related in patients but not in the control group. This could be related to how repeated checking can reduce confidence by reducing the vividness of memory (van den Hout et al., 2019). Moreover, the reduction in confidence was not related to their performance, they still performed comparably to the healthy group and even slightly better in the final block under high uncertainty. Thus, uncertainty seems to play a role in this relationship between checking and lower confidence, as the relationship disappears when uncertainty is removed. In addition to confidence, state and trait anxiety were also positively related to checking in OCD, only under high uncertainty conditions. This may rather reflect the impact that intolerance of uncertainty may have on anxiety feelings in OCD patients, as no direct impact of anxiety on checking compulsions has previously been shown in the literature, due to a third factor being required to induce anxiety in

OCD (e.g. harm avoidance or uncertainty). Additionally, excessive checking under uncertainty seems to be an OCD feature, as anxiety disorder patients are reported not to show increased checking under uncertainty (Toffolo et al., 2016).

Despite the superior performance of patients in the final block compared to the controls, OCD patients showed the biggest increase in checking compared with the healthy subjects at that stage. The final block had exactly same stimuli as the first block, and both groups' performance improved over time. However, regardless of their performance, of which they became aware in block 3 by receiving feedback, patients still showed excessive checking behaviour. However, uncertainty may not be sufficient to induce or maintain increased checking in OCD, as patients still showed excessive checking when uncertainty was removed in third block, and even when they were punished for checking. When combining the OCD and healthy groups in Study 3, there was also a moderate relationship between checking, and obsessive-compulsive symptoms (doubting, obsessing, and checking subscales of OCI), and compulsive habits, which confirms the sensitivity of the IVT to measure compulsive behaviour. The findings indicate dysfunctional checking in OCD, showing deficits in goal-directed behaviour, with checking rates being autonomous with respect to the goal (i.e. accuracy of responses, or avoiding punishment).

In Study 3, we showed that we can successfully measure excessive checking in laboratory. However, this behaviour does appear to depend on contextual features such as the task instructions. Therefore it would be very useful going forward to measure if there are any brain correlates of dysfunctional checking. It was not practically feasible to continue the neuroimaging in schizophrenia patients due to recruitment difficulties during the pandemic. Thus, the rest of the thesis will be focusing only on OCD and a healthy control group. In the next chapter, the relationship between excessive checking and its possible brain correlates will be discussed, using magnetic resonance spectroscopy at 7T in these same OCD patients and healthy controls as Study 3. The findings will also be interpreted by inspecting how patients and healthy volunteers relate their actions to the outcomes they achieve and whether there is a common neural underpinning between dysfunctional checking and deficits in action/outcome dissociations and judgements.

3. Chapter 3: Magnetic Resonance Spectroscopy in OCD

3.1. Literature review

3.1.1. General introduction

Much of OCD neuroimaging research depends on structural, functional and PET studies focusing on the role of the CSTC pathways. However, there is increasing evidence for change in other systems as well, such as the cerebellum (Zhang et al., 2011). In this chapter, I will introduce the use of magnetic resonance spectroscopy in OCD, which is a very effective complement to PET, by highlighting the possible neurochemical changes in the brain. It is especially relevant as MRS has been used more often than PET to measure neurochemical changes in the ACC, striatum, thalamus and frontal brain regions. Initial PET studies showing changes in glucose metabolism in OCD patients were mentioned briefly in the introduction to this thesis. PET has also been used neurochemically, although in a restricted way, to suggest changes in the dopamine and serotonin receptors in OCD, however, more research on this topic is required (Biria et al., 2021). There are many studies using the MRS in OCD, however, unfortunately most of the findings are inconsistent and inconclusive. This has been due to 1) a low signal-to-noise ratio translated into relatively low spatial resolution and neurochemical species discrimination due to low concentration of metabolites, as compared to water when magnetic fields lower than 3T are applied (Alger, 2010), 2) the small sample sizes not allowing for enough statistical power, and 3) not being able to control for other confounding variables such as medication. Below, I will summarise the neurochemical findings in OCD and attempt to synthesise the scattered literature on this topic. The relationship between treatment and severity of OCD on neurometabolite concentrations will also be discussed.

As mentioned in the first chapter of this thesis, the cortico-striato-thalamo-cortical circuitry is consistently found to be abnormal in structure, neurochemistry and function in patients with OCD and provides the prevailing neural model of the disorder (Robbins et al., 2019; Saxena & Rauch, 2000). The main circuits involve glutamatergic (excitatory) projections from medial prefrontal regions, such as orbitofrontal and cingulate cortex, to ventral and dorsal striatal nuclei, which in turn send GABA-ergic (inhibitory) projections to the globus pallidus. Further, the GABA-ergic projections are sent to the thalamus, which then conveys glutamatergic projections back to the prefrontal cortex, thus closing the 'loops'. These circuits have been functionally divided into three broad networks engaging different domains of processing, the dorsal cognitive circuit (dlPFC - dorsal caudate - thalamus), the limbic circuit (ventral striatal - thalamus - prefrontal cortex) and the sensorimotor circuit (OFC/ supplementary motor area (SMA)/ dorsal ACC (dACC) - putamen- thalamus) loops (Milad & Rauch, 2012). Patterns of neural activity within these circuits are dynamic and change constantly to adapt to new cognitive and

motor demands. These different behavioural states are mainly regulated by glutamatergic neurons, which trigger neural communication, and by the GABAergic neurons, which in turn inhibit communication by decreasing the likelihood that the neurons will fire. Therefore perturbations in glutamate or GABA neurotransmission may translate into neuronal hyper- or hypoactivity.

The prefrontal–subcortical circuits mediate different behavioural functions, and imbalanced glutamate and GABA levels within this widespread network might translate into symptomatic behavioural expression observed in OCD. Striatal hypoactivation during inhibition and interference tasks and prefrontal hypoactivation in the anterior cingulate, medial frontal and dorsolateral prefrontal cortices during switching tasks (Eng et al., 2015), as well as hypoactivation of the caudate and prefrontal areas alongside with hyperactivation of the subthalamic nuclei and putamen during symptom provocation in patients with OCD (Banca et al., 2015) might suggest abnormal glutamate and GABA expression. However, such complex behavioural deficits cannot be explained simply by glutamate\GABA perturbation. Modulatory systems, and particularly the serotonergic and dopaminergic systems, are also tuning the information flow through these circuits, and may thus contribute to dysregulation of behaviours like response inhibition, cognitive flexibility or working memory in OCD (Robbins et al., 2019). Animal models reveal modulatory effects of dopaminergic neurons on postsynaptic cortical and thalamic glutamatergic release and synaptic plasticity on GABAergic medium spiny inhibitory neurons of the striatum; glutamate signalling is enhanced through dopamine D1 receptors in striato-nigral neurons, while reduced through dopamine D2 receptors in striato-pallidal neurons (Surmeier et al., 2007). In humans, functional neuroimaging combined with pharmacological manipulation reveal increased functional connectivity between caudate nuclei and thalamic nuclei and ventral midbrain following treatment with the dopaminergic drug sulpiride (Honey et al., 2003).

In addition, modulation of glutamate transmission by prefrontal serotonergic system (5-HT) has been described. In animal models, activation of prefrontal glutamate NMDA receptors inhibits local glutamate release and stimulates subcortical striatal glutamate release through both pre- and post-synaptic mechanisms, namely 5-HT_{1A} and 5-HT_{2A} receptors (Ciranna, 2006). Serotonin can also stimulate receptors in GABA interneurons to modulate inhibitory inputs onto pyramidal excitatory neurons (Celada et al., 2013). In humans, serotonin has been shown to modulate cognitive behaviours, such as enhancement of cognitive flexibility (Kehagia et al., 2010) and response inhibition (Cools et al., 2008). These findings suggest that fine regulation of excitation and inhibition are modulated by monoamine systems that might be involved in the abnormal functioning of CSTC circuits in patients with OCD (Saxena & Rauch, 2000).

More than two decades ago, Saxena et al. (1998) hypothesized that hyperactivity in orbitofrontal-subcortical circuits underlies the symptoms of OCD (Saxena et al., 1998). Since then, a large number of structural and functional neuroimaging data have substantiated and extended this hypothesis. Dysfunction in the orbitofrontal-striatal circuits and connected structures, such as the ACC, NAc and thalamus, have been found to contribute to the pathology of OCD (Robbins et al., 2019) and abnormal neurochemical changes have been hypothesised to modulate the structural and functional abnormalities within these circuits (Graat et al., 2017).

Although structural and functional studies have illuminated the neurobiological underpinnings of OCD, the literature on neurochemical changes is more sparse. Glutamate (Glu) and gamma-amino-butyric acid (GABA) are evidently two important neurotransmitters for the normal functioning of the fronto-striatal-thalamic circuits. Hypothetically, a loss of brain volume and abnormal function could be explained by reduced functioning of GABAergic interneurons that results in disinhibition of pyramidal cells and hence increased glutamatergic neurotransmission within the main CSTC pathways. This glutamatergic overactivity may lead to excitotoxic brain tissue damage, reduced neuropil (i.e. the dense mesh of dendrites, axon terminals and glia) and synaptic loss (Hardingham & Do, 2016), which ultimately results in cognitive and behavioural impairments underpinning OCD symptoms. Although such hypotheses have been explicitly investigated in disorders such as schizophrenia, similar studies have been lacking in OCD.

3.1.2. Magnetic resonance spectroscopy (MRS)

Magnetic Resonance Spectroscopy is a non-invasive neuroimaging technique that allows the *in vivo* quantification of different brain metabolites such as choline, creatine, N-acetylaspartate (NAA, a marker of neuronal health), glutamate (the main excitatory neurotransmitter), and GABA (the main inhibitory neurotransmitter) and thus provides direct evidence of biochemical levels and tissue integrity across cortical and subcortical in both healthy and clinical population. MRS measures the magnetic resonance signal amplitudes generated by atomic nuclei in living tissues. This technique is particularly well suited for studying OCD, as it is capable of quantifying concentrations of glutamate, and GABA in localised regions of the human brain. Proton Magnetic Resonance Spectroscopy (1H-MRS) is the most widely used MRS technique. Using the magnetic field and a brief radio-frequency pulse, 1H-MRS generates resonance signals with peaks unique to each brain metabolite (see **Figure 3.1**). The scanner generates a signal from the protons of the hydrogen atoms of the molecules and the strength of this signal indicates the molecular concentration of the specific neurochemical in the brain (Brennan et al., 2013). The signal sensed by the scanner usually contains considerable field variability. To control for this, researchers usually obtain a reference signal which can be internal or external.

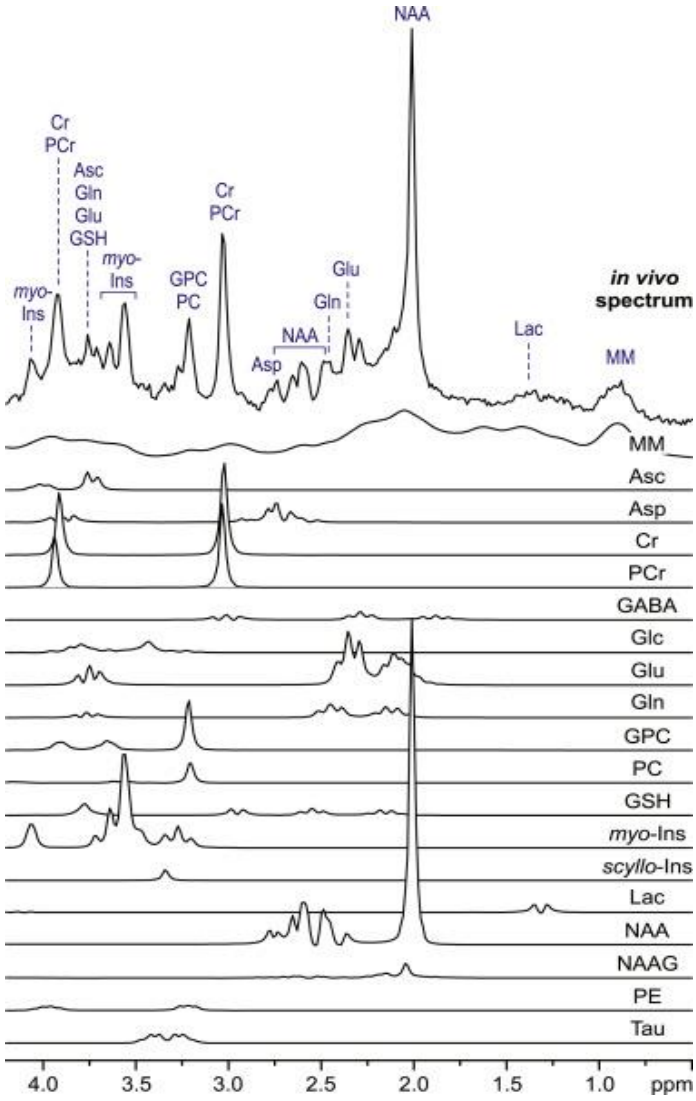


Figure 3.1. shows the LCModel analysis of an *in vivo* ^{1}H MR spectrum acquired from a human brain at 3T (semi-LASER, TE = 28 ms, TR = 5 s, NT = 160, VOI = 8 ml, grey-matter-rich occipital cortex). Reprinted from Xin and Tkáč, (2017), with permission from Elsevier

An internal reference is generated in the brain such as a neurometabolite or water, whereas an external reference signal is generated outside the brain, using a phantom. The metabolites are then expressed in terms of their ratios to these references. See Box 1 for further technical details regarding MRS methodology.

BOX 1 Technical aspects of MRS

Water suppression

In the healthy human brain, grey and white matter are respectively made up of 83% and 70% water (Turner, 1986). In contrast with water, concentrations of brain metabolites are 103-104 times smaller and much harder to detect. The commonly used solution to this problem is to directly suppress the water signal from the MR spectra by exciting the water resonance using a frequency-selective radio frequency pulse. For this purpose, differences in chemical shifts of water and other neurometabolites are used to suppress water signals, while the signals of other brain neurochemicals remain unaffected. This is called the CHEmical Shift Selective (CHESS) method for water suppression (Haase et al., 1986) and is the most commonly used solution to detect metabolites in the brain that would otherwise be camouflaged by the high concentrations of water.

Magnetic Field Homogeneity and Shimming

When the magnetic field does not vary a lot within a target voxel, a single frequency or peak will be observed. For example, see the simulated NAA peak in **Figure.3.1** and how narrow it is. However, when the magnetic field does vary a lot, instead of a single narrow peak, a distribution of frequencies will be measured. The broadening of the spectral peaks is a sign of inhomogeneity of the magnetic field and the loss of spectral sensitivity and resolution. Various radio frequency shimming methods have been developed to correct for the inhomogeneity of the magnetic field while acquiring data (Gruetter, 1993; Juchem et al., 2010; Juchem et al., 2011; Shen et al., 1999). Unfortunately, this cannot be corrected for once the data is acquired. Therefore it is important to make sure the magnetic field is homogeneous while acquiring the data. Poor shimming is one common cause of bad data quality, especially one that cannot be corrected for once the data is acquired.

Poor shimming or inhomogeneity of magnetic field can also be numerically detected by looking at the full width at half maximum (FWHM) parameter, which is the spectral width at the half amplitude of the signal. If the shimming is poor, the peaks will be broad and the FWHM will be larger. Smaller FWHM indicates better shimming. If the shimming is found to be poor, indicating inhomogeneity of magnetic field, this can sometimes be improved by changing the shim currents or by repositioning of the voxel to be farther from factors leading to this inhomogeneity. The latter will of course be at the cost of less spatial consistency across subjects or between scans in case of longitudinal studies. Overall, this technique requires careful assessment of the settings at the right time, considering the specific measurements being investigated, in order to optimise the quality of the collected data.

Referencing to standard values

In MRS there are two ways to report metabolite ratios, i.e. the ratio of the neurochemical species of interest to a standard metabolite. The first method, more commonly found in OCD studies, is internal referencing using a neurometabolite with a stable concentration. Creatine (Cr) is often used as an internal reference, as it is assumed to have a constant concentration in both the healthy and clinical populations and also under different physiological conditions (Bagory et al., 2007). However, one must be aware that this implicit assumption of constant Cr concentrations is often not substantiated, and using creatine as reference has its own disadvantages: 1) the fact that the Cr measurements can be contaminated by other neurochemicals (e.g. choline) or by noise, and 2) the Cr concentration is less uniform throughout the brain than once thought (Alger, 2010). The second approach to reporting metabolite values, less commonly found in the OCD studies reviewed, is to report absolute concentrations. This terminology can be confusing when incorrectly interpreted as true values rather than ratios. Absolute concentration simply refers to a comparison with either brain water concentration (internal reference) or other metabolites in a phantom (external reference) for which the concentration is already known (Bagory et al., 2007). Using water as an internal reference has the advantage that it can be obtained very quickly, with a very strong signal. Nevertheless, this determination usually requires an additional step as the water concentration must be recorded separately from other neurochemicals and without water suppression. However, this technique also has its disadvantages as the water signal may change due to disease, depending on region of the brain, and hydration levels (Alger, 2010). Each signal referencing technique has its own pros and cons, and careful thought must go into choosing a sufficiently optimal reference according to the specific needs of the study, while taking into account different factors such as the sample size, study design and other possibilities such as recording the water signal separately. When choosing LCModel (Provencher, 1993) for data analysis, all metabolites will automatically be referenced to creatine and phosphocreatine (this is the software used for this thesis).

Single voxel versus multi-voxel MRS

Based on the number of voxels or the volume of interest (VOI) studied, MRS can be divided into single-Voxel Spectroscopy and Magnetic Resonance Spectroscopic Imaging (MRSI). Single voxel spectroscopy is widely used for assessing *in vivo* brain metabolite changes, as it is usually easier to perform in a shorter time period and tends to have a higher signal-to-noise ratio. However, this technique only evaluates the metabolite profiles within *a priori* selected areas/voxels of the brain, with a high risk of missing the target region where disorder-related differences in neurochemicals might be observed. As such, strong *a priori* hypotheses about which regions are most relevant for investigation are essential. In contrast, MRSI simultaneously measures metabolite concentrations in many voxels covering more areas of the brain, thus having a higher spatial coverage (a top-down approach). However, longer acquisition times, more complex processing and a lower signal-to-noise ratio are important considerations (Boer & Klomp, 2014). In MRSI, neighbouring voxels can contribute to the spectrum and the larger volumes make shimming and water suppression more difficult (Kraguljac et al., 2012).

3.1.3. MRS in OCD

The most commonly studied neurometabolites using the 1H-MRS in OCD are choline (Cho), creatine (Cr), GABA, Glu, glutamine (Gln), myo-Inostitol (Ins), and N-acetylaspartate (NAA). However, due to the smaller concentrations or peaks of some neurometabolites and the difficulty of measuring them reliably, sometimes depending on their relationship and specific hypotheses, they may be combined (see below). All these neurometabolites have important implications for healthy brain functioning and therefore have been used in exploratory MRS studies in OCD. Nevertheless, GABA and Glu are probably more relevant to study, given their implications for neuropathology within the fronto-striatal-thalamic circuits, which will be discussed in more detail in the next sections.

Choline is involved in membrane synthesis and degradation and is thought to be a marker of cell membrane turnover (Miller, 1991). *Creatine* and *phosphocreatine* are important for cerebral intracellular energy production or cerebral bioenergetics (Govindaraju et al., 2000). *GABA* and *Glu* are the main inhibitory and excitatory neurotransmitters. GABA mediates inhibitory modulation, generally via local interneurons (Hardingham & Do, 2016). Whereas Glu, in addition to playing a central metabolic role in the brain (Krebs, 1935), is involved in processes such as fast synaptic transmission and plasticity (Hardingham & Do, 2016). In addition to being an intermediate in neurotransmitter metabolism, *Gln* is the main precursor of Glu and GABA (Hertz & Zielke, 2004), although the *de novo* synthesis of Glu through tricarboxylic acid (TCA) cycle is also possible (de Graaf et al., 2011). In the glutamate-glutamine cycle, the synthesis of Glu is dependent on Gln and vice versa (Ramadan et al., 2013). Additionally, the structure of Gln is very similar to Glu and at a magnetic field below 3T, their resonance overlaps, thus preventing independent measurement. This is also true to some extent for GABA. Traditionally Glu and Gln have been measured together as a combined peak termed ‘Glx’. However, Glx may not be a precise measure of neurochemicals of interest as Glu and Gln changes might be in opposite directions and the Glx may still remain unchanged. For example, a meta-analysis in schizophrenia showed no Glx changes but reduced glutamate ratios (Marsman et al., 2013). Thus a separate quantification of these neurochemicals is very important and the more recently developed 7T ultra-high magnetic field does enable this (Choi et al., 2010; Yang et al., 2008). To date (and to my knowledge), no ultra-high field studies have been published so far in OCD patients. *Myo-Inositol (Ins)* is suggested to be a marker of glial cell proliferation, a precursor of intracellular second messengers and a major osmolyte in the central nervous system, important for the integrity of cells (Fisher et al., 2002). Lastly, *NAA* appears to be a marker of neuronal density (Ebisu et al., 1994).

Since the CSCT circuitry is strongly implicated in the pathophysiology of OCD, MRS studies to date have focused on regions of interest within the cognitive, affective or motor loops mentioned above. I will therefore review first the specific anatomical regions within the CSTC circuits that have been

investigated in OCD using MRS. These include the rostral and dorsal anterior cingulate cortex (rACC and dACC), dlPFC, OFC and medial pre-frontal cortex (mPFC), striatum (putamen and caudate), lenticular nuclei (putamen and globus pallidus), thalamus, posterior cingulate cortex (PCC), and frontal and parietal white matter. Next, the neurometabolite associations with symptom severity and treatments effect, including the outcomes of CBT and medication with SSRIs will be reported.

It is important to note that the majority of these studies were based on small sample sizes (fewer than 15 participants per group), many failed to control for the effects of medication and those that did were lacking a large sample size, reducing the reliability of their findings. In addition, they acquired the MRS data at lower magnetic field strengths (1.5 to 3 Tesla), which prevented accurate separation of Glu from Gln, accurate measurement of GABA or even regional separation of the caudate nucleus from the putamen. These limitations may well contribute to the inconsistent findings that will be reviewed below.

3.1.4. MRS and OCD: Regional findings

Neurometabolite concentrations in the rostral anterior cingulate cortex

One of the functions of the rACC is to integrate action values in control networks (Rushworth et al., 2004). It has extensive connections with OFC, vmPFC, as well as the amygdala (Haber & Behrens, 2014) and has been identified as an important region for the development of OCD symptomatology. The rostral ACC, as defined in the articles reviewed for this chapter, refers to Volumes of Interest (VOI) in the perigenual and subgenual ACC, and also its border with mOFC and mPFC (**Figure 3.2.A**). This region, together with the OFC, has an important role in mediating contingency learning, relevant for goal-directed behaviour (Jackson et al., 2016) which as mentioned previously, has been shown to be compromised in patients with OCD (Gillan et al., 2014) and may therefore account for their rigid behaviour. The rACC is a good example of a region that lacks anatomical consistency within the MRS literature, with most of its investigations also including some parts of the mPFC or OFC when defining their target rACC voxel. Therefore, some findings concerning the rACC reported below must be considered with caution.

With the exception of one study by Zhu et al. (2015) that tested unmedicated patients, and reported lower Glu levels in the rostral ACC of OCD patients as compared to that of healthy volunteers (Zhu et al., 2015), most studies used a mixed sample of medicated and unmedicated patients, and reported no differences in Glu or Gln concentrations within this region (Brennan et al., 2016; Li et al., 2019; O'Neill et al., 2016; Wang et al., 2018; Zheng et al., 2020; Zurowski et al., 2012). Findings on Glx are still more inconsistent. Despite most studies reporting no between group differences in the combined measures of

Glu and Gln (Glx) (Brennan et al., 2016; Parmar et al., 2019; Simpson et al., 2012; Zhang et al., 2016; Zheng et al., 2020; Zhu et al., 2015), two studies found higher Glx levels in OCD patients compared with healthy controls (de Salles Andrade et al., 2019; O'Neill et al., 2016) whereas one study (Yücel et al., 2008) found lower Glx concentrations in OCD patients. All these studies were carried out using a 3T scanner, and the differences do not seem to be related to medication type or sample size. Only three studies have measured GABA levels in the rACC of OCD patients. Results are also erratic, with one study showing higher GABA and GABA/Glu ratios in the rACC of patients with OCD (Li et al., 2019) and two others reporting lower GABA levels in OCD, as compared with the healthy control group (Simpson et al., 2012; Zhang et al., 2016).

Inconsistent results have also been found with regards to concentrations of the total NAA (tNAA) in the rACC. Three studies reported no between-group differences (de Salles Andrade et al., 2019; Parmar et al., 2019; Zheng et al., 2020), while two other studies found lower tNAA concentrations in OCD patients (O'Neill et al., 2013; Zhang et al., 2016). Despite the inconsistency of findings for tNAA, there might be a trend for lower NAA levels in unmedicated patients as the study by Zhang et al., (2016) had the largest sample size of patients with OCD (N=78) of all the studies reviewed in this chapter (Zhang et al., 2016). Consistent results have been found in the rACC only for metabolites such as Cho, Ins and Cr, with no abnormalities reported by nine studies in adults with moderate to severe OCD symptoms (de Salles Andrade et al., 2019; O'Neill et al., 2016; Parmar et al., 2019; Yücel et al., 2007; Yücel et al., 2008; Zheng et al., 2020; Zhu et al., 2015; Zurowski et al., 2012). Only one study reported higher Cho levels in rACC, which may be a less reliable finding since the investigators used a multivoxel MRSI technique (Hatchondo et al., 2017).

To summarise, MRS in OCD does not demonstrate abnormalities in Cho, Ins or Cr within the rACC. Despite mixed findings for Glx, GABA and tNAA, the majority of studies do not observe significant differences in the concentration levels of the main neurometabolites within the rACC between adult OCD and healthy controls, particularly for Glu and Gln.

Neurometabolite concentrations in the dorsal anterior cingulate cortex

The dACC (**Figure 3.2.B**) is important for error monitoring as shown by electrophysiological studies and tends to be hyperactive in OCD patients during resting state functional neuroimaging (McGovern & Sheth, 2017). As reported previously, this hyperactivity is also associated with increased error-related negativity following making an incorrect response, measured in electrophysiological studies using tasks provoking conflict (Mathews et al., 2012).

With the exception of a single study in female patients with OCD reporting lower Glx concentrations in the dACC (Yücel et al., 2008), no abnormalities in Glu (Fan et al., 2017; O'Neill et al., 2016; Starck et al., 2008), Gln (Ebert et al., 1997; Fan et al., 2017; Starck et al., 2008) or Glx (Bédard & Chantal, 2011; O'Neill et al., 2016; Starck et al., 2008; Yücel et al., 2007; Zhang et al., 2016) concentrations have been described in OCD. Findings in GABA measurements of the dACC of OCD patients are less consistent. While Zhang et al. (2016) reported lower GABA levels in the dACC of OCD patients (Zhang et al., 2016), Starck et al. (2008) did not find any GABA abnormalities.

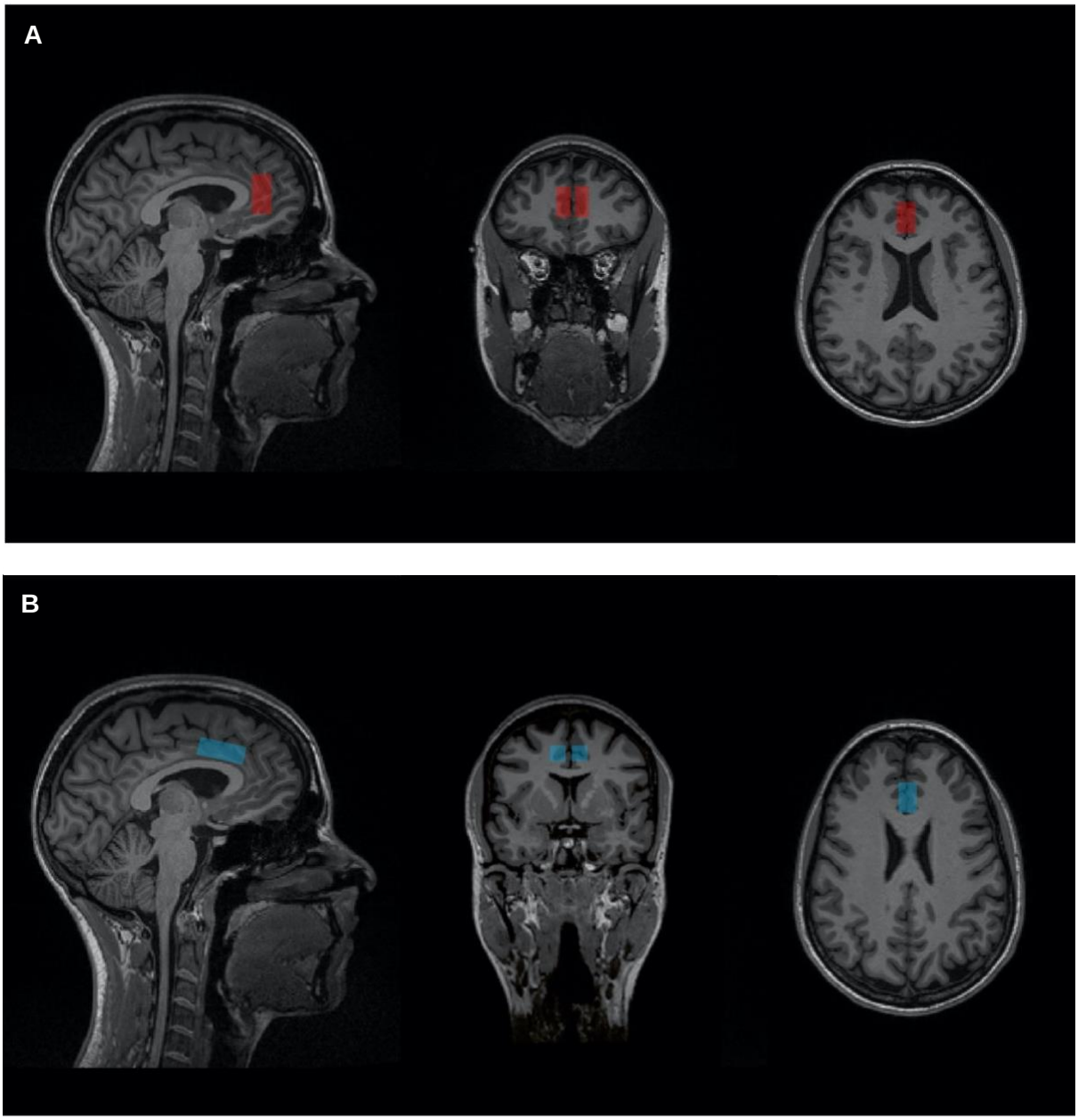


Figure 3.2 highlights the areas of the Anterior Cingulate Cortex (ACC) coverage in Magnetic Resonance Spectroscopy (MRS) studies reviewed in this chapter. Figure.3.2.A depicts the location for the rostral ACC (rACC) voxels, and Figure.3.2.B shows the location for dorsal ACC voxels (dACC) in sagittal, coronal and horizontal planes.

Less contradictory findings are reported for NAA concentrations in the dACC, with only two studies reporting no differences in NAA concentrations between OCD and healthy volunteers (Bédard & Chantal, 2011; Starck et al., 2008) while two other studies in medicated patients found lower NAA or tNAA concentrations in OCD patients (Ebert et al., 1997; M. Yücel et al., 2007). The lower NAA concentrations were also reported in treatment naïve (Jang et al., 2006) and unmedicated OCD patients (Tükel et al., 2015; Tükel et al., 2014). Similarly to the rACC, no abnormalities were found for Cho, Ins (Bédard & Chantal, 2011; Ebert et al., 1997; Fan et al., 2017; Kitamura et al., 2006; O'Neill et al., 2016; Starck et al., 2008; Tükel et al., 2014; M. Yücel et al., 2007; Yücel et al., 2008; Zhang et al., 2016), and Cr (Fan et al., 2017; Kitamura et al., 2006; O'Neill et al., 2016; Starck et al., 2008; M. Yücel et al., 2007; Yücel et al., 2008; Zhang et al., 2016) in the dACC.

To summarise, no altered concentrations of Glu, Gln or Glx have been found in the dorsal ACC of OCD patients. Nevertheless, this region may be characterised by lower NAA concentrations in both medicated and unmedicated patients with OCD. As for the rostral ACC, no clear abnormalities have been found in Cho, Ins and Cr within the dACC. Possible GABA changes in this region have not yet been investigated by many studies.

Neurometabolite concentrations in the basal ganglia

The basal ganglia (BG) are a crucial component of the cortico-striatal circuitry that underlies the pathophysiology of OCD. For this reason, many studies have focused on investigating its neurochemical basis in these structures. The striatum, comprising the caudate nucleus and the putamen, represents the main input region of the BG, whereas the globus pallidus represents the main output region of this constellation of nuclei. Several meta-analyses found increased volumes in different components of these deep structures, most consistently in putamen and globus pallidus (de Wit et al., 2014; Kong et al., 2020). Further, abnormal functional connectivity has been reported during resting state fMRI, with increased connectivity between the ventral striatum and the orbitofrontal cortex that predicted overall symptom severity (Harrison et al., 2013) and reduced ventrolateral PFC-caudate and dl-PFC-putamen functional connectivity respectively during attentional set-shifting and planning (Vaghi et al., 2017). Neurochemical investigations have therefore been conducted on the dorsal and ventral components of the striatum, given their different functions and distinct circuitries, as well as lenticular nuclei (putamen and globus pallidus).

Neurochemical concentrations in the ventral striatum have been investigated in two studies using both multi- (Simpson et al., 2015) and single-voxel approaches (Zurowski et al., 2012). The results were similar in showing no neurochemical concentration abnormality. Simpson et al. (2015) investigated

specifically Glu levels in unmedicated adults with OCD and reported no Glu differences and no volumetric differences as compared to healthy controls (Simpson et al., 2015). Additionally, Zurowski et al. (2012) investigated the NAA and myo-inositol levels in the right ventral striatum, in unmedicated OCD patients compared to healthy controls and reported no diagnosis effects on neurometabolite concentrations (Zurowski et al., 2012). Less consistent are MRS data for the dorsal striatum. Ebert et la. (1997) showed decreased concentrations of NAA in the left striatum of unmedicated patients with OCD, which correlated with illness severity. On the other hand, Hatchondo et al. (2017) reported increased Cho and NAA ratio. Within the dorsal striatum, several authors have argued they could technically isolate the caudate and putamen and assess their respective neurochemistry. However, following our aforementioned explanations about the difficulties in separating caudate and putamen reliably using lower spatial resolution scanners, I recommend that the findings reported below are interpreted with caution. Three studies have found reduced NAA concentrations and NAA/Cr ratios in the caudate nuclei of unmedicated patients with moderate to severe OCD symptoms (Bartha et al., 1998; Chen et al., 2017; Tükel et al., 2015). Tükel et al. (2015) found reduced caudate NAA/Cr ratio in the OCD group (Tükel et al., 2015). However, other studies have not observed such changes in NAA (Simpson et al., 2015; Tükel et al., 2014; Whiteside et al., 2012b). In fact, they reported no differences, not only in the concentration of NAA, but also in the levels of Glu, Glx and Cr concentrations between healthy controls and medicated and unmedicated adult patients with moderate obsessive and compulsive symptoms.

Only 3 studies have investigated specifically the neurochemistry of the putamen in patients with OCD, two using MRSI and one using MRS. All reported no significant differences in either absolute values of Glu levels (Simpson et al., 2015) or the NAA, Cho and myo-inositol ratios between patients and controls (Simpson et al., 2015; Tükel et al., 2015; Tükel et al., 2014). One study has, however, focused on the lenticular nuclei (putamen and globus pallidus) but revealed no abnormalities in the NAA and Cho concentrations in medicated patients with severe symptoms (Ohara et al., 1999). Two studies reported no differences in the concentration of the metabolites NAA, Cho and Cr between medicated patients with moderate OCD symptoms and healthy individuals in the basal ganglia (Kitamura et al., 2006; Sumitani et al., 2007). Nevertheless, the results should be considered carefully as they might have been influenced by selection of the ROI. In the study of Kitamura et al. (2006) the basal ganglia were defined as putamen and globus pallidus accompanied by the adjacent white matter (Kitamura et al., 2006), while in the study of Sumitani et al. (2007) the basal ganglia were measured across both the caudate and putamen.

Altogether, no robust and consistent neurochemical abnormalities in the basal ganglia of patients with OCD have emerged.

Neurometabolite concentrations in the thalamus

The thalamus is another important element of the CSTC circuitry, serving as a hub between the striatum and the prefrontal cortex. The thalamic nuclei are heavily interconnected with the OFC and are known to be involved in cognitive flexibility and goal-directed actions, as measured behaviourally by reversal learning and action-outcome contingency learning tasks (Alcaraz et al., 2018; Parnaudeau et al., 2015; Pergola et al., 2018). Furthermore, a meta-analysis of structural alterations (Rotge et al., 2009) has reported increased bilateral thalamic volumes in OCD patients that correlated with the severity of obsessive-compulsive symptoms.

Proton MRS studies investigating the neurochemical underpinnings of thalamus in OCD have also revealed inconsistent findings. Zhu et al. (2015) observed lower levels of Glu concentrations in the right thalamus of unmedicated adult patients with moderate OCD symptoms. These authors also found increased thalamus Cho bilaterally in patients (Zhu et al., 2015). More recently, Parmar et al. (2019) also showed increased total choline (tCho), Ins, and Glx concentrations in the medial thalamus of unmedicated patients with an illness duration of less than 5 years (Parmar et al., 2019). However, increased levels of thalamic Cho/tCho were confirmed by one study in unmedicated patients with moderate OCD symptoms (Chen et al., 2017) and two studies on with medicated patients with severe and moderate OCD symptoms (Fan et al., 2017; Hatchondo et al., 2017). Nevertheless, four other studies have not found any reliable between group differences in the concentrations of Cho, Glu, Glx, NAA, tNAA, myo-inositol and Cr between medicated patients with OCD and healthy volunteers (Bédard & Chantal, 2011; Gnanavel et al., 2014; Kitamura et al., 2006; O'Neill et al., 2016).

In conclusion, MRS investigations of the neurochemistry of the thalamus in OCD converge to suggest that enhanced thalamic cholinergic concentrations might be a marker of abnormality in adult and paediatric populations diagnosed with OCD. Nevertheless, it would perhaps be wise to consider these promising results as preliminary hypotheses that require future investigation.

Neurometabolite concentrations in other brain regions

Fewer studies have investigated neurochemical imbalances related to OCD in other brain regions. Below we briefly describe a few findings in the following areas: DLPFC, mPFC, OFC, PCC and frontal and parietal WM.

The DLPFC is part of the CSTC circuit and plays a role in executive functioning and attention, both being impaired in patients with OCD (Vahabzadeh & McDougle, 2014). Additionally, DLPFC is reported to be hypoactive in OCD which may underlie the deficits in executive functioning observed in this patient group (McGovern & Sheth, 2017; Vaghi et al., 2017). A positive correlation has been found

between the grey matter volume of the right DLPFC (and right OFC) and the severity of sexual and religious obsessions (Alvarenga et al., 2012). Furthermore, this region has been successfully used as a target region for treating OCD using rTMS (Sachdev et al., 2007; Sachdev et al., 2001), which additionally supports its role in the pathophysiology of this disorder. Sachdev et al. (2001), highlighted the DLPFC in the right hemisphere as being a more effective target for treating OCD symptoms, as compared to the same region in the left hemisphere. This is probably the reason why the three MRS studies examining metabolite imbalance in this area in OCD chose their VOIs in the right hemisphere (Moon & Jeong, 2018; Park et al., 2017; Simpson et al., 2012). The first study found no differences in GABA and Glx measures between unmedicated OCD patients and healthy volunteers (Simpson et al., 2012). The second study, however, found reduced GABA and increased Ins in medicated patients with OCD compared with healthy controls (Park et al., 2017). A third study, also in medicated patients, found increased GABA and reduced NAA and Cho levels in DLPFC of patients with OCD (Moon & Jeong, 2018). However, half of the patients in the latter study were taking psychedelic drugs in addition to their antidepressant medication, which may well have contributed to the differences observed.

The PFC is crucial for human cognitive performance and has been proved to be dysfunctional in OCD. As mentioned in the introduction to this thesis, its lateral and medial connections to the striatum were found to be functionally autonomous and context-dependent in OCD (Robbins et al., 2019). Hypoactivation in the lateral portions of the OFC and hyperactivation of the vmPFC including medial OFC in OCD patients were found at rest, during reversal learning paradigms (Apergis-Schoute et al., 2018; Remijnse et al., 2006) and in first-degree unaffected relatives (Chamberlain et al., 2008). However, this pattern of activation is intriguingly apparently reversed when patients are exposed to symptom provocation paradigms (Banca et al., 2015; Morgiève et al., 2014). Studies investigating the potential neurochemical abnormalities in OCD within the OFC are very scarce, due to the close proximity of this brain region to the sinuses, and hence to bone and air. This can in turn lead to increased inhomogeneity of the magnetic field which makes this region a very difficult target for spectroscopy. One study that tested medicated patients found no between group differences in NAA, Glx, Ins and Cho concentrations in the OFC (Bédard & Chantal, 2011). However, another that tested unmedicated OCD patients reported higher NAA concentrations in the OCD group, as compared to healthy controls, and no differences in Cho levels (Chen et al., 2017). Finally, Zurowski et al. (2012) found no abnormalities in the OFC Ins levels in OCD patients. None of these 3 studies investigated GABA in the OFC, which could be due to the weaker magnets they were using in their scanner and thus the lower power to detect GABA. Within the PFC (including the mPFC), one study that tested unmedicated patients found lower Glu concentrations in patients with OCD but no differences in the levels of Glx, NAA, tNAA, Cho and Ins (Zhu et al., 2015). However, another study that tested medicated OCD patients found lower NAA levels in prefrontal cortex (Jang et al., 2006).

A few studies also explored metabolite ratios in posterior cingulate cortex and hippocampus, brain areas less commonly explored in OCD. Two studies investigated the neurochemical concentrations in the PCC and found no abnormalities in Glu, Gln, GABA, Cho, Ins and NAA concentration levels in patients with OCD and no correlations with the OCD symptoms (Brennan et al., 2016; Jang et al., 2006). Measurements in the hippocampus have shown that reduced levels of choline normalised NAA (NAA/Cho) co-occurred with smaller volumes of the hippocampus (Atmaca et al., 2009). However, this finding should be interpreted with caution as this was an MRSI study at 1.5T with a higher noise in the signal, thus making this result less reliable.

Finally, potential abnormalities in the white matter were also investigated in the frontal and parietal lobes of the OCD brain. While two studies found no abnormalities in tNAA and Cho concentration levels in the prefrontal WM of medicated adolescent and adult OCD patients (Kitamura et al., 2006; Sumitani et al., 2007), another study investigating the same region in paediatric OCD patients reported higher tNAA and Cho levels in patients (Weber et al., 2014). Age and medication status of the patients are likely driving these inconsistent findings. Finally, lower Cr and NAA levels in the right orbital white matter (Whiteside et al., 2012b) and high concentrations of Cho in the parietal white matter were also reported in adult OCD patients (Kitamura et al., 2006).

To summarise, no neurochemical abnormalities were found in the PCC of OCD patients. Similarly, no significant differences between OCD and healthy volunteers were found for most of the neurometabolites within the frontal regions of the brain such as PFC and OFC, with the exception of possibly higher NAA concentrations in the OFC and lower glutamate in the PFC. Contradictory results were, however, found in the DLPFC and the frontal and parietal white matter.

3.1.4.1. Associations between neurometabolite concentrations and clinical symptoms

Studies using MRS could additionally inform us about how differences in neurometabolite concentrations could relate with different clinical symptoms. The MRS studies conducted so far using 1.5-3T scanners, report mainly correlations between neurometabolite changes in some frontal areas and depressive and anxiety symptoms. These findings will be discussed in more detail below.

OCD symptom severity

Symptom severity as measured with the Yale Brown Obsessive Compulsive Scale (Goodman et al., 1989) was found to be correlated positively with concentrations of the following neurochemicals: 1) Cho in the parietal WM (Kitamura et al., 2006); 2) Glx in the rostral and dorsal ACC but only in female

OCD patients (Yücel et al., 2008); 3) NAA ratios in dACC with the obsessions subscale (Yücel et al., 2008). Several studies, however, reported negative associations between the YBOCS scores and 1) the NAA in dACC (Ebert et al., 1997; Tükel et al., 2014) and caudate nucleus (Tükel et al., 2015); 2) Cr and Cho ratios of the rostral and dorsal ACC (O'Neill et al., 2016) ; 3) GABA and tNAA in the OFC including the rACC (Zhang et al., 2016); 4) Ins concentrations in the left OFC (Bédard & Chantal, 2011); and 5) tCho levels in medial thalamus (Parmar et al., 2019) and Glx ratios in thalamus, specifically the compulsion subscore of the YBOCS (Zhu et al., 2015).There are also studies reporting no significant correlations between YBOCS scores and the different neurometabolites in dACC (Starck et al., 2008), posterior cingulate cortex (Brennan et al., 2016), rACC (Andrade et al., 2019; Li et al., 2019), striatum and thalamus (Hatchondo et al., 2017), dorsal caudate, dorsal putamen and ventral striatum (Simpson et al., 2012), MPFC (Zhu et al., 2015) or DLPFC (Simpson et al., 2015).

To conclude, there are no clear trends towards specific associations between the neurometabolite concentrations and symptom severity as measured with YBOCS. The findings do not seem to be robust and thus more studies are needed to provide more reliable evidence.

Anxiety and depression scales in OCD

In adult patients with OCD, thalamic Glu levels were positively correlated with anxiety scores (Fan et al., 2017). In another study, Whiteside et al. (2006) found decreased caudate myo-inositol ratio, which were linked to anxiety traits (measured with STAI-T) but after correcting for anxiety, the differences observed became insignificant (Whiteside et al., 2006). Anxiety symptoms were additionally reported to be negatively correlated with 1) NAA and tNAA levels in the left OFC, dACC (Bédard & Chantal, 2011), and rACC (Zhang et al., 2016) and 2) Glu concentration in rACC (Zheng et al., 2020). No correlations were found between concentrations of NAA, Cho, Ins in the dACC and anxiety scores (Tükel et al., 2014). In addition, severity of depression was negatively correlated with Cho and Cr levels in rACC and dACC (O'Neill et al., 2016). The OFC concentrations of NAA were also negatively associated with depressive symptoms (Zhang et al., 2016). There were no correlations reported between concentrations of GABA and Glu, Glx, tNAA or Cho in the rACC and clinical variables such as anxiety, depression (Andrade et al., 2019; Li et al., 2019). Furthermore, no associations were observed between concentrations of NAA, Cho, Ins in the dACC and depression (Tükel et al., 2014) .
In summary, the most robust finding seems to be the negative association between the rACC, dACC and OFC concentrations of NAA and tNAA and comorbid anxiety symptoms. In other words, the higher the NAA level in the OFC, which included in most studies a large part of rACC, the lower the anxiety symptoms in OCD patients. Additionally, the higher the Cho and Cr levels in the anterior cingulate and the higher the NAA levels in the OFC, the lower the severity of depression symptoms seem to be in

OCD patients. However, more studies are needed to confirm these findings, especially with a more precise voxel placement to avoid including the OFC in an ACC voxel.

3.1.4.2. Treatment effects on neurometabolite concentrations in OCD

Cognitive behavioural therapy

Cognitive behavioural therapy, ameliorates OCD symptoms in about 60% of patients (Rufer et al., 2006), and has also been shown to alter brain function (Olatunji et al., 2014) . Several studies have shown decreased resting state glucose metabolism or blood flow in the right caudate of OCD patients with a successful treatment response (Linden, 2006). Therefore, it is plausible to hypothesise that CBT may also induce changes in the concentration of neurometabolites in areas of brain of relevance for OCD.

Lower NAA levels in the rACC were found at baseline in a group of OCD patients, which were then elevated after 20 sessions of successful CBT treatment (90 minutes per session) as measured by reductions in their YBOCS scores (O'Neill et al., 2013). Additionally, baseline levels of NAA in the rACC could predict treatment outcome, with higher NAA levels at baseline correlating with better response to CBT (O'Neill et al., 2013). Importantly, these findings should be interpreted with caution, as the latter study had a sample of 10 OCD patients only and used a 1.5T scanner and a MRSI method. However, Zurowski et al. (2012) found no differences in NAA and Ins concentrations in rACC, OFC and right ventral striatum, neither at baseline between OCD patients and healthy controls nor after 24 sessions of CBT (40 minutes per session) within patients, while the Ins levels in the OFC were negatively correlated with treatment outcome as measured with the YBOCS (Zurowski et al., 2012). Although these findings are in contradiction to the findings by O'Neill et al. (2013), Zurowski et al (2012) had a slightly larger sample of unmedicated OCD patients (N=16) and used a single voxel imaging technique on a 3T scanner. One other study looking at caudate NAA observed a significant increase in patients with OCD after 16 successful CBT sessions leading to a decrease in OCD and anxiety symptoms (Whiteside et al., 2012b). Although it is important to note that the latter study included mixed medicated and unmedicated patients, a small sample size, and employed a 1.5T MRS scanner. Nevertheless, we the larger peak of the NAA may still be detected even with weaker techniques.

Whiteside et al. (2012) reported decreased Glx in the right caudate after the 16 sessions of successful behavioural treatment and lower levels of NAA and Cr at baseline in non-medicated patients compared to medicated patients and controls (Whiteside et al., 2012a), while Benazon et al. (2003) reported no change in metabolite concentrations after 12 sessions of behavioural therapy that led to improvements of OCD, depressive, and anxiety symptoms (Benazon et al., 2003). In the PCC, Glu levels at baseline

were predictive of better treatment outcome, given its associations with a reduction in the YBOCS score (O'Neill et al., 2017).

In summary, no clear conclusion can be drawn based on the studies reviewed above. There might be a trend of reduced Glu and Glx after CBT treatment in respectively rACC and caudate, and elevated NAA levels in rACC. However, better MRS studies are required to confirm these results, especially by reliably detecting Glu, and Gln separately.

SSRI medication

It would be expected that SSRI would also influence the neurometabolite ratios within the different areas of the brain. There are two ways to study these medication effects: 1) with a within-group longitudinal design and measuring the neurometabolite concentrations before and after the SSRI treatment or 2) by having a between group, cross sectional design and comparing medicated and unmedicated patients with one another.

Three longitudinal studies have focused on the effect of SSRI treatment on the neurometabolite concentrations of the ACC (dorsal and rostral). Two of them showed lower NAA levels in dACC at baseline, with an increase after 12 weeks of successful sertraline (Tükel et al., 2015) and citalopram (Jang et al., 2006) treatment. It is noteworthy that the latter studies had samples respectively of 19 and 13 unmedicated (mostly drug naïve) OCD patients using a 1.5T scanner. A third 3T study using a slightly larger sample (N=21) found no neurochemical differences between OCD and healthy volunteers at baseline and no changes after 12 weeks of escitalopram treatment in OCD patients (Parmar et al., 2019). Although it is important to note that only half of the patients in the latter study responded to their SSRI treatment, whereas the other half showed no improvement in their OCD symptoms after 12 weeks of escitalopram treatment. However, they did find reduced tCho and Glx in medial thalamus after the escitalopram treatment, which were higher at baseline. In the study by Tükel et al. (2015), the lower caudate NAA levels remained unchanged after 12 weeks of successful treatment with sertraline (Tükel et al., 2015).

Cross-sectional studies comparing medicated and unmedicated patients have largely failed to observe significant differences in Glu and Gln in the rACC (Brennan et al., 2016), Cr, Cho, NAA, Glx and Ins in the dorsal and rostral ACC (Yücel et al., 2008), and Glx, mI, NAA Cho and Cr in the dACC (Yücel et al., 2007). Although the latter study found a trend for lower Glx levels in unmedicated patients, it did not reach statistical significance (M. Yücel et al., 2007). Only one study has found lower Glu, Glx, Cr,

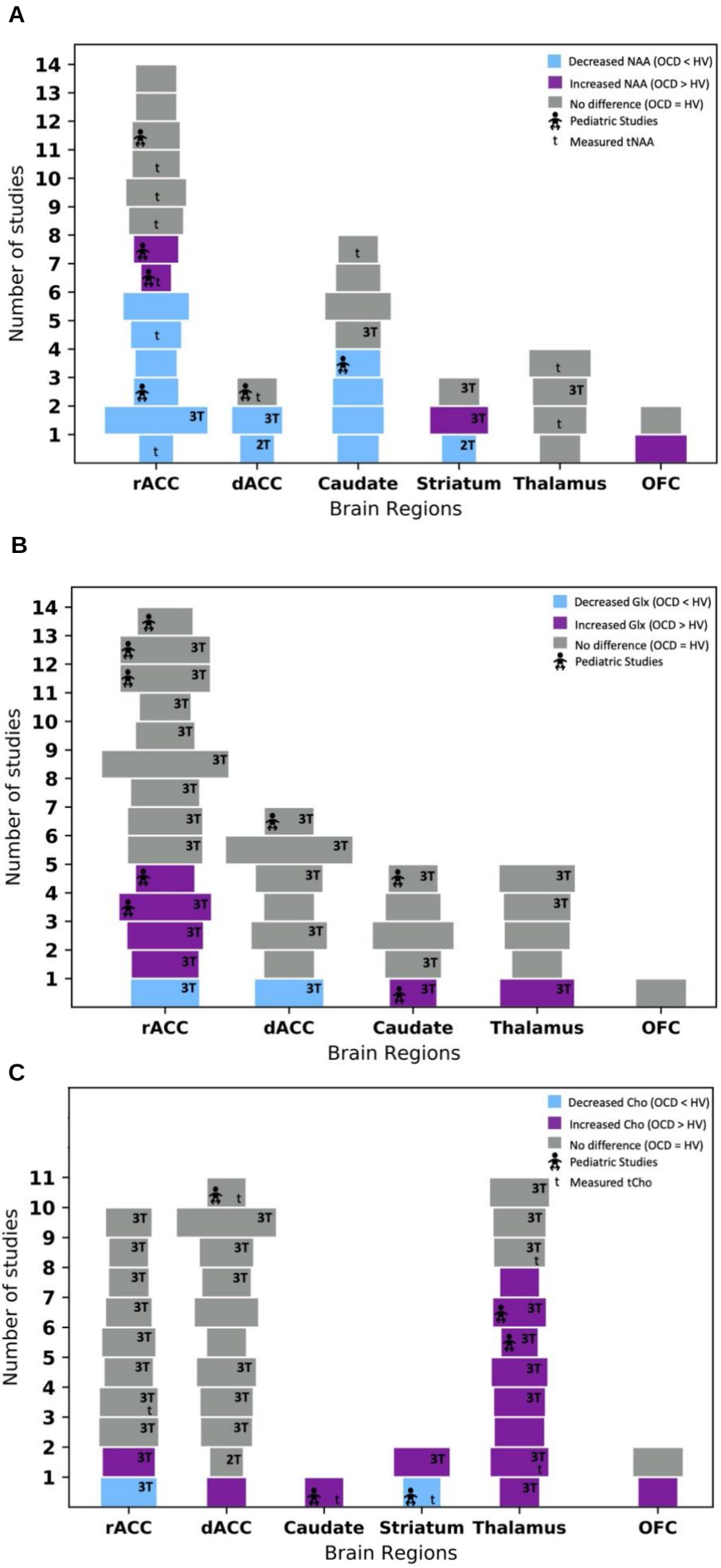
Cho and Ins concentrations in the dACC of medicated patients when compared to an unmedicated group (O'Neill et al., 2016). In general, the cross-sectional studies did not identify clear differences in neurochemical levels between the medicated and unmedicated OCD groups.

In summary, besides the probability of increased NAA in improving the OCD symptoms, no clear conclusions can be drawn based on the reviewed studies. It is important to note that the treatment studies had an average sample of 10 patients only. The absence of changes in neurochemical concentrations could be either a true absence or a false negative finding due to various limitations in methodology and design to be discussed below.

3.1.5. Summary literature review

Figure 3.3 shows the summary of all findings for NAA, tNAA, Cho, tCho, and Glx in different brain regions. Following an exhaustive review of the literature, the majority of available MRS studies appear to have failed to find convincing evidence of group differences between OCD and healthy subjects for Glu, Gln, and GABA in several of the key brain regions. This null conclusion could mean that the differences were simply not detected due to limitations of the methods used rather than a true absence. There is also no evidence of abnormality in Cr and Ins levels in OCD patients. There are however, a few findings that show some degree of consistency, such as lower NAA levels in dorsal (and to a lesser extent rostral) ACC of OCD patients. The lower NAA level seems to increase after successful treatment with CBT and SSRI's and is correlated with improvement of OCD symptoms. Since NAA is a marker of neuronal density, it would be relevant to further investigate possible correlations with structural data in OCD and their implications to the pathophysiology of this disease. The NAA concentration seems to be sensitive to pathological brain processes, and thus appears to be potentially reversible following successful treatment by either CBT or SSRI's. Another, rather more consistent finding, is a higher Cho concentration in the thalamus. Since Cho is a marker of cell membrane turnover, involved in membrane synthesis and degradation, a misadjusted concentration of this metabolite could lead to dysfunctional communication between neurons and ultimately unbalanced neurotransmission, which could contribute to OCD deficits. Further studies are warranted to enable further conclusions. Nevertheless, the question remains whether the Cho and NAA are the only neurochemicals impacted by OCD, or whether they can just be better detected due to their higher peaks compared to other neurochemicals. Studies using a higher magnetic field can potentially answer this intriguing question.

Figure.3.3 depicts a summary of the studies which used 1H-MRS to measure the brain neurochemicals of patients with Obsessive Compulsive Disorder (OCD) and healthy volunteers (HV). Findings are summarised by brain region: rostral anterior cingulate cortex (rACC), dorsal anterior cingulate cortex (dACC), caudate, thalamus, striatum (which includes both nucleus and putamen) and orbitofrontal cortex (OFC). Figure.3.3.A shows the findings for N-acetylaspartate (NAA) and total NAA (tNAA); Figure.3.3.B shows the findings for Glx (glutamate + glutamine); and Figure.3.3.C shows the findings for Choline (Cho) and total Cho (tCho). The letter "t" indicates studies measuring the total NAA in Figure.3.3.A or total Choline in Figure.3.3.B. The baby icon indicates studies in pediatric population. The power of the magnetic field used in the studies is also indicated: 3T, 2T and if none, it is 1.5T. The width of each block corresponds to square root of the total sample size (OCD + HV) of each study.



3.1.6. Limitations

The absence of differences, or mixed findings, could be due to the differences in the scanner field strengths, using mostly scanners at 1.5T or 3T, but also the limited overlap in voxel placements. With this technique, voxels are placed manually and this can cause variability between voxel placement in different scan sessions but also different studies. A third major problem with almost all the studies reviewed in this chapter is the small sample sizes used, with the majority of the studies recruiting fewer than 20 patients. For example, to detect a 10% change in NAA (at 1.5 T), which has the largest peak, with 80% power, a sample size larger than 39 has been suggested per group (Steen et al., 2005). Only 4 studies reviewed met this criterion. Using lower magnetic fields, most studies had low spatial resolution that would not allow satisfactory separation of some relevant regions such as the caudate from putamen. Another potentially confounding variable is the difference in the sequences employed by different studies. Each sequence is optimised for a different purpose to either optimise the detection of some neurochemicals over others, or to be sensitive to the size of voxel selected. All these factors could lead to variable data quality and accuracy of detecting specific neurometabolites in different regions. Another confounding variable could be the difference in reference signals used to normalise the data, although the majority of studies have used creatine as their reference. Moreover, only a few studies performed brain tissue content corrections. The lack of consistency of the set-up parameters and the type of MRS sequences used by the different studies provide yet further sources of confound. There are some parameters that can be set manually before or during data collection such as calculating the best transmitter voltage and flip angle for water saturation pulse.

3.1.7. Conclusions and future directions

Despite the aforementioned difficulties, the MRS studies so far conducted provide preliminary findings that are quite promising. They have been helpful in establishing current issues causing reduced data reliability. Nevertheless, they indicate that the methodology is promising and could be much more valuable if better controlled study designs could be implemented, preferably using stronger magnetic fields (e.g. 7T), and by including sufficiently large samples. Once these issues are overcome, MRS studies are well placed to inform the molecular underpinning of cognitive problems at the basis of OCD. A future way forward could involve using MRS at 7T 1) to detect changes in the concentrations of Glu, GABA, Gln, and NAA and 2) understand the interaction between the different neurotransmitters and their link to the behavioural and clinical symptoms in OCD. In order to overcome the limitations described above, the next two studies were conducted in OCD patients and healthy volunteers using MRS at 7T, and a larger sample size to study neurochemical differences important for OCD and their link to behavioural and clinical measures.

3.2. Study 4: MRS in ACC, SMA, & Occipital lobes (OCD vs HV)

To overcome the limitations of the above literature review, we employed magnetic resonance spectroscopy at 7T, recruited a larger sample of patients with OCD than most studies reviewed (although as will be discussed at the end of this section, we aim to continue data collection to reach our desired power), while carefully selecting clear landmarks to increase overlap between the voxel locations across subjects, or avoid overlap between some voxels within subjects. Based on the above literature, and the General Introduction, one of the regions highly relevant for OCD is anterior cingulate cortex. The intersection of all ACC findings were used to choose the location of our ACC voxel, which included mostly the grey matter volume of anterior cingulate cortex. To do so, the coordinates of all these studies were taken into account and converted into the same space. Additionally, due to implication of SMA, as part of the PFC in sensorimotor circuit (Milad & Rauch, 2012), and the efficacy of SMA/pre-SMA as targets for brain stimulation in improving OCD symptoms (D'Urso et al., 2016; Gowda et al., 2019; Hazari et al., 2016; Mukherjee et al., 2021), a second box was placed where most of the OCD SMA/pre-SMA findings were reported, including mostly grey matter volume of the SMA or pre-SMA regions. Lastly, similar to Murley et al., (2020), an additional box was placed in occipital lobe, under the assumption that this area is less influenced by a psychiatric diagnosis and could be used as a control region, correcting for physiological changes within subjects. Again, the occipital voxel included mostly grey matter volume from the occipital lobes.

Prior to this study, I also performed 27 pilot scans to detect the best location and size for our voxels, and to finally arrive to the voxel size and locations we used in this study providing us with the best shimming and the highest signal to noise ratio. To take advantage of the higher resolution of a 7T scanner, the neurometabolites that were previously difficult to quantify, such as GABA, Glu, Gln were quantified separately as well in combinations such as Glx, and GABA/Glu and compared across groups. Lastly, to test whether the findings are due to neuronal integrity rather than an OCD diagnosis, and to confirm the previously reported NAA findings in ACC (Ebert et al., 1997; Jang et al., 2006; Tükel et al., 2014; Yücel et al., 2007), the NAA was also one of our neurometabolites of interest. Additionally, the correlations between ACC structural data and the NAA levels measured in this voxel were investigated.

Study 4 aimed to:

- Characterise the neurochemical abnormalities in OCD patients in ACC and SMA, while using the occipital lobe metabolite ratios to correct for physiological changes.

We aimed to test the following hypotheses:

H1: Patients with OCD display increased glutamate in ACC and SMA that would explain the increased activity in these areas.

H2: Patients with OCD display reduced GABA in ACC and SMA that would explain the increased activity in these areas.

3.2.1. Methods

3.2.1.1. Participants, questionnaires, inclusion criteria

There were 23 healthy volunteers and 25 patients with OCD included in this study. However, a smaller subset was selected for some metabolites (**Table.3.2**) depending on the spectroscopy exclusion criteria described below. The participants and questionnaires used in this study were the very same used in Study 3 (please see sections 2.3.1.1 and 2.3.1.2), and thus the ethics number, and inclusion/exclusion criteria was the same as well, with the exception of one additional exclusion criteria which was the MRI safety for both groups. As a general rule, out of every 5 interested participants, only 1 was suitable for MRS at 7T.

3.2.1.2. MRS data acquisition

The proton magnetic resonance spectroscopy (^{1}H MRS) took place at the Wolfson Brain Imaging Centre, University of Cambridge (United Kingdom). Participants underwent whole-brain T1-weighted MR and single-voxel proton MRS scans using a 7T Siemens Magnetom-Terra scanner. The scanner was equipped with a Nova single-channel transmit, and 32-channel array head coil for signal reception (Nova Medical). T1-weighted MP2RAGE (Marques et al., 2010) images were acquired to guide voxel placement and used in the analysis to perform tissue corrections (see below). The following specifications were used: echo time = 4300 ms, repetition time = 1.99 ms, inversion times (1/2)= 840/2370 ms, flip angles = 5/6°, acceleration factor (A ≫ P) = 3, bandwidth = 250 Hz/px, voxel size = 0.75 mm. To increase the SNR and the amount of GM in each voxel, the spectra were measured bilaterally from one 12 x 20 x 33 mm^3 voxel at the ACC (Figure 3.1.a), and two 20 x 20 x 20 mm^3 voxels at the SMA, and occipital cortex (Figure 3.1 b and c).

All the voxels were located manually by the same researcher (M.B). Clear landmarks were used while placing the voxels to increase the reliability of the voxel placements across subjects. A sagittal view was used for this purpose, first connecting the anterior and posterior commissure via a horizontal line, and then drawing a vertical and perpendicular line through the anterior commissure. The ACC voxel was placed above the corpus callosum, and starting after the vertical line going through the anterior commissure, while the SMA was placed right above the pons, and staying behind the line going through the anterior commissure. This also helped avoiding an overlap between the SMA and ACC voxels in participants with a thinner cortex. Lastly, the occipital voxel was placed in the outermost corner, while avoiding any region that could introduce noise in the data (such as the skull and sinuses) by going through all brain slices. Then the left side of the square shaped voxel, was parallel with the line right

above cerebellum. See **Figure.3.1** for a visual representation of all three voxel positions. After acquiring the MP2RAGE image, and placing the voxels, a short-echo semi-LASER (Deelchand et al., 2015; Öz & Tkáč, 2011) sequence was used to acquire the spectra, collecting 64 repetitions and time/echo time of 5000/26 ms. For each voxel, the FASTESTMAP (Gruetter & Tkáč, 2000) sequence for shimming, and variable radio frequency pulses with optimised relaxation delay (VAPOR) for water suppression calibration (Tkáč et al., 1999) were used. Lastly, for each subject, the 64 individual spectral repetitions were saved separately, later to be combined after corrections (see below).

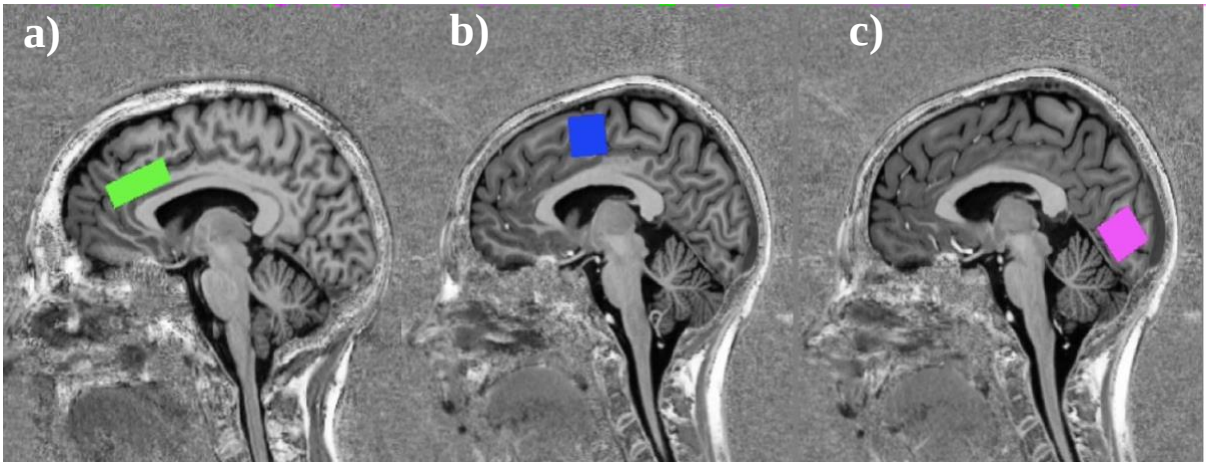


Figure 3.1 showing the voxel positions for **a)** Anterior Cingulate Cortex in green, **b)** Supplementary Motor Area in blue, and **c)** Occipital cortex in purple.

3.2.1.3. MRS data preparation and analysis

Data preparation

Within subjects, the 64 individual spectra files were corrected for effects of eddy currents, frequency, and phase shifts using MRspa (Dinesh Deelchand, University of Minnesota, www.cmrr.umn.edu/downloads/mrspa) and converted to one single averaged file. Next, LCModel (Provencher, 1993) version 6.2-3 was used with an automated fitting routine, to generate model spectra for 20 different neurometabolites (including the GABA, Glutamate, Glutamine and NAA) between 0.5 and 4.2 parts per million (ppm), and relative to Creatine and phosphocreatine. The metabolites were water scaled using 8 unsuppressed water spectra acquired before and after the 64 spectral repetitions (automatically detected by MRspa), and using a simulated basis set that included experimentally acquired macromolecule spectra. Each LCModel output generates all 20 neurometabolites without having the option to specify an a-priori number of metabolites. The generated model spectra were for Minimum alveolar concentration (Mac), Alanine (Ala), Aspartate (Asp), Ascorbate/vitamin C (Asc), Creatine (Cr), γ-amino-butyric acid (GABA), Glucose (Glc), Glutamine (Gln), Glutamate (Glu),

Glycerophosphocholine (GPC), Glutathione (GSH), myo-inositol (Ins), scyllo-inositol (sIns), Lactate (Lac), Phosphocreatine (PCr), Phosphocholine (PCho), Phosphoethanolamine (PE), N acetylaspartate (NAA), Nacetylaspartylglutamate (NAAG), and Taurine (Tau).

Once the metabolite concentrations were acquired using LCModel, a segmentation analysis was performed using SPM12 and the MP2RAGE images to extract tissue fractions for each subject for Grey Matter (GM), White Matter (WM) and Cerebrospinal fluid (CSF). Then a mask was created using the specific voxel location for each subject (the corresponding sLaser-LW file), to extract the GM, WM, and CSF fractions within each individual voxel, for each subject. These values were then used for partial volume corrections.

Due to the differential availability of GABA in different tissues (e.g. CSF less relevant for GABA), the partial correction for GABA was performed separately following Harris et al., (2015). For the rest of the metabolites we followed the LCModel manual (Provencher, 2021). Lastly, in order to avoid exclusion of values that are disorder/group specific and can provide insight into the nature of a disorder, a straight cut-off score (which is still being used by many of studies and are highlighted by most softwares as noisy, including the LCModel which uses a threshold of 15 %) is not recommended (Kreis, 2016). Instead, per metabolite and per group, the average and standard deviation were calculated for Cramér-Rao Lower Bound (CRLB) of each metabolite, and individual metabolite concentrations. Next, values larger than 2SD from the mean of each group were excluded for both measures. This means, both of these criteria needed to be respected for the data to be included. This led to exclusion of quite a few metabolite per voxel, however, this is comparable to other similar studies using the same exclusion criteria (Frangou et al., 2019) and helps avoiding introduction of noise in the data and loss of informative but unusual data. For ACC, in OCD patients, 2 GABA, 1 Gln, 2 Gln, and 1 NAA, and in healthy group, 2 GABA, 1 Glu, and 1 NAA values were excluded. For SMA, in OCD group, 1 GABA, 2 Gln, 1 Glu, and 3 NAA, and in healthy participants, 2 GABA, 3 Gln, 1 Glu were excluded. Lastly, for occipital lobe, for patients, 2 GABA, 3 Glu, and 1 NAA, and in healthy subjects, 2 GABA, 1 Gln, 2 Glu, and 1 NAA data were excluded. The occipital lobe was selected to collect metabolite levels within subjects, and to use the information to correct for physiological changes. However, as opposed to our assumption, since groups differed on occipital lobe metabolite concentrations, this correction was not performed, and the group differences for occipital lobe will also be reported below in the results section.

Data analysis

Independent sample t-tests were performed to compare concentration of metabolites between groups for all voxels. More specifically, we were interested in ratios of GABA, Glu, Gln, GABA/Glu, Glx (Glu/Gln), and NAA (as a measure of neuronal integrity). As groups did not differ for age, gender, and IQ, the spectroscopy data could be used without any further corrections (see section 2.3.2.1 for demographic data). To rule out additional confounding variables such as the influence of noise introduced by poor shimming, or other unrelated factors, differences between FWHM, and signal to noise ratio (SNR), and CRLB were also compared between groups. To test the relationship between NAA and brain structure, and to provide additional sanity check for the NAA findings in ACC, and in accordance to the role of NAA in providing neuronal integrity, the GM and WM fractions within the ACC voxel (which had a constant size across all subjects) were also compared between groups. Independent t-test was used for data with a normal distribution, and in case the normality assumption was violated, the Mann-Whitney U test was performed. Depending on distribution of the data, a Pearson or Spearman correlation analysis was conducted to more directly investigate the relationship between NAA and GM and WM fraction in the ACC voxel. In case of significant correlations, a Fisher's Z test was performed to compare these correlations between groups. Python version 3.7.6 was to analyse the data.

3.2.2. Results

Table 3.1 shows the technical quality of the scanning measures, and the brain tissue compositions for both groups separately. For none of the voxels, the signal to noise, FWHM, and metabolite CRLB differed significantly between the two groups, except for the CRLB of Gln in ACC (U = 171, $p = 0.03$) which was slightly larger in the healthy group, indicating a larger variance or noise in the data in healthy subjects. **Table.3.2** shows all the metabolite concentrations for both groups, including the sample sizes used for each comparison. The groups showed similar concentration levels for all SMA neurometabolites studied (GABA, Glu, Gln, GABA/Glu, Glx, and NAA). For V1, Glutamate was higher in OCD patients (t = 2.23, $p = 0.03$), and there was a trend for a higher Glx in patients compared with the healthy group (t = 1.90, $p = 0.06$). The voxel that showed the biggest difference between groups was the ACC, with lower GABA/Glu (t = - 3.91, $p = 0.0003$), higher Glu (t = 2.26, $p = 0.02$), and Gln (t = 2.27, $p = 0.02$), and a trend for lower GABA (t = - 1.97, $p = 0.05$) concentrations in patients. **Figure 3.2** illustrates the main group differences in ACC. Additionally, there were no differences between groups for GM and WM percentage fractions within the ACC voxel between OCD, and healthy volunteers (see **Table.3.1**). Lastly, no correlations were found between anterior cingulate NAA levels and ACC voxel GM fractions in OCD (r = - 0.23, $p = 0.25$), nor healthy volunteers (r = - 0.25, p

= 0.26). Similarly, there were also no correlations between WM and NAA in ACC for OCD (r = - 0.3, p = 0.13), nor healthy subjects (r = - 0.11, p = 0.62).

Table.3.1 Measures of magnetic resonance spectroscopy quality and tissue composition (% of MRS voxel)

Measures	Controls (n = 23) M (SD)	OCD (n = 25) M (SD)	t / U	p
ACC				
SNR	57 (6.64)	61.38 (6.81)	367 U	= 0.053
FWHM	0.024 (0.002)	0.023 (0.004)	-0.99	= 0.32
GM	0.83 (0.029)	0.84 (0.036)	0.76	= 0.44
WM	0.10 (0.021)	0.096 (0.027)	200 U	= 0.11
CRLB (GABA)	9.30 (1.26)	9.17 (1.12)	274 U	= 0.42
CRLB (Glu)	1.87 (0.34)	1.79 (0.41)	243 U	= 0.74
CRLB (Gln)	7.00 (0.953)	6.25 (1.11)	171 U	= 0.03 *
CRLB (NAA)	1.00 (0.0)	1.00 (0.0)	NA	NA
SMA				
SNR	58.48 (10.72)	55.72 (14.56)	206 U	= 0.74
FWHM	0.026 (0.004)	0.027 (0.008)	-0.48	= 0.63
GM	0.71 (0.06)	0.72 (0.052)	0.55	= 0.58
WM	0.17 (0.036)	0.16 (0.039)	-1.03	= 0.30
CRLB (GABA)	9.87 (2.78)	9.56 (1.98)	264 U	= 0.78
CRLB (Glu)	2.04 (0.21)	2.24 (0.83)	275 U	= 0.69
CRLB (Gln)	13.35 (7.32)	11.87 (5.57)	232 U	= 0.77
CRLB (NAA)	1.09 (0.41)	1.08 (0.27)	-0.41	= 0.67
Occipital cortex				
SNR	81.61 (11.36)	77.04 (20.44)	261 U	= 0.78
FWHM	0.03 (0.003)	0.03 (0.003)	0.31	= 0.75
GM	0.80 (0.03)	0.82 (0.04)	379 U	= 0.06
WM	0.16 (0.031)	0.14 (0.033)	-1.6	= 0.11
CRLB (GABA)	9.26 (2.09)	13.72 (15.96)	202 U	= 0.35
CRLB (Glu)	1.87 (0.34)	2.0 (0.64)	222 U	= 0.74
CRLB (Gln)	4.48 (1.27)	5.40 (2.19)	357 U	= 0.056
CRLB (NAA)	1.09 (0.29)	1.16 (0.37)	284 U	= 0.46

ACC = anterior cingulate cortex, SMA = supplementary motor area, SNR: Signal-to-Noise Ratio, FWHM = Full Width at Half Maximum, CRLB: Cramer-Rao Lower Bounds, GABA = Gamma-aminobutyric acid, Glu = Glutamate, Gln = Glutamine, NAA = N-acetylaspartate, GM = Grey Matter, WM = White Matter, t = independent sample t-test, U = Mann-Whitney U test, * = $p < 0.05$, NA = not applicable, test could not be performed as values for all subject for both groups were exactly the same.

Table.3.2 MRS metabolites

Metabolites (ppm)	OCD *N*	HV *N*	Controls M (SD)	OCD M (SD)	t / U	*p*
ACC	**24**	**23**				
GABA	23	21	2.88 (0.29)	2.67 (0.41)	-1.97	= 0.05
Glu	23	22	12.14 (0.71)	12.61 (0.70)	2.26	= 0.02 *
Gln	24	23	3.08 (0.43)	3.37 (0.43)	2.27	= 0.02 *
Glx	22	22	3.96 (0.50)	3.75 (0.46)	-1.44	= 0.15
GABA/Glu	22	20	0.24 (0.017)	0.21 (0.26)	-3.91	= 0.0003***
NAA	24	22	12.49 (0.76)	12.70 (0.89)	0.85	= 0.39
SMA	**25**	**23**				
GABA	24	21	3.21 (0.81)	3.32 (1.04)	0.39	= 0.69
Glu	24	22	12.28 (1.28)	12.60 (0.92)	0.98	= 0.32
Gln	22	20	2.39 (0.71)	2.59 (0.98)	0.74	= 0.46
Glx	22	19	5.55 (1.94)	5.42 (1.43)	0.23	= 0.81
GABA/Glu	23	20	0.25 (0.06)	0.26 (0.06)	-0.59	= 0.55
NAA	22	23	15.34 (1.28)	15.13 (1.53)	0.49	= 0.62
Occipital cortex	**25**	**23**				
GABA	22	22	2.41 (0.45)	2.60 (0.72)	1.04	= 0.30
Glu	22	21	9.92 (0.62)	10.34 (0.60)	2.23	= 0.03*
Gln	25	22	4.09 (0.48)	3.84 (0.67)	-1.41	= 0.16
Glx	22	20	2.47 (0.27)	2.73 (0.55)	1.90	= 0.06
GABA/Glu	22	22	1.38 (0.54)	1.52 (0.61)	297 U	= 0.20
NAA	22	22	14.68 (0.94)	14.46 (1.05)	0.73	= 0.46

ACC = anterior cingulate cortex, SMA = supplementary motor area, ppm = parts per milion, GABA = Gamma-aminobutyric acid, Glu = Glutamate, Gln = Glutamine, Glx = Glu/Gln, NAA = N-acetylaspartate, GM = Grey Matter, WM = White Matter, CSF = Cerebrospinal fluid, N stands for total number of participants that were used in the analysis after exclusion of CRLB and metabolite concentration of bigger than 2 standard deviations from the group averages, M = mean, SD = standard deviation, OCD = obsessive compulsive disorder, HV = healthy volunteers, t = independent sample t-test, U = Mann-Whitney U test, * = $p < 0.05$, *** = $p < 0.0005$.

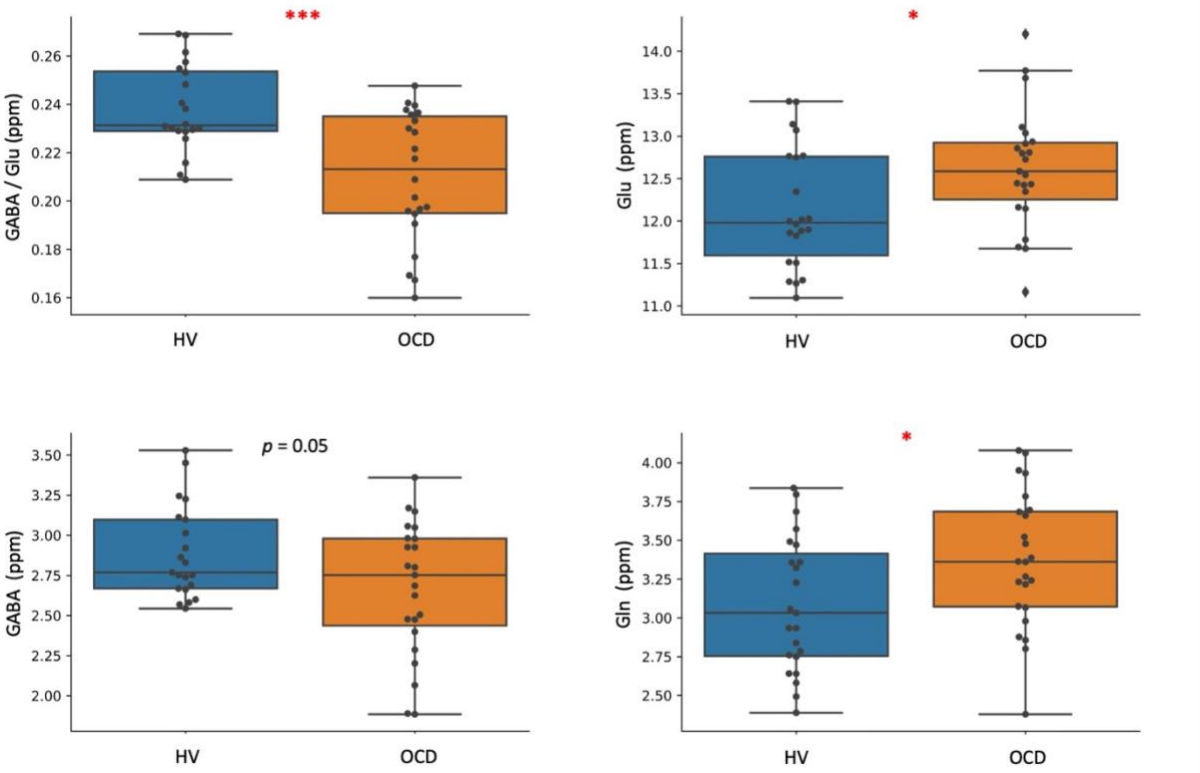


Figure 3.2 depicts the ACC **a**) GABA/Glu, **b**) Glutamate, **c**) GABA, and **d**) Glutamine concentrations in ppm (parts per million) in OCD (orange) and healthy (blue) groups. * = $p < 0.05$, *** = $p < 0.0005$.

3.2.3. Interim Discussion

As hypothesised, we found increased Gln and Glu, and lower GABA in anterior cingulate cortex of patients with OCD, leading to a strong significant decrease in GABA/Glu ratios in OCD group. Additionally, the Glu concentration levels in OCD happens to show similar increasing pattern in both ACC and occipital cortex compared with healthy subjects, however, this was not the case for GABA, and Gln. There were no differences in anterior cingulate NAA levels in OCD patients.

These are novel findings which have not been reported before in the literature and presumably must relate in some sense either to patient selection, or more likely, the use of the 7T MRS scanner. Of course, we must explore various possible artefact or limitations of the data, in view of the fact that the data do not agree with the published literature. One such finding in particular is the similar NAA levels between both groups, as opposed to the reductions reported in the literature, discussed above (Jang et al., 2006; Tükel et al., 2015). To gain more information about the reliability of the latter finding, the structural

information (fractions of GM and WM) were compared between groups, and also in association with NAA levels. Importantly, the results agreed with the structural imaging, finding no differences between GM and WM fractions in ACC voxel between groups, and no correlations with ACC NAA concentrations. Having had mostly medicated patients in our sample could have increased the NAA levels to 'normal', as NAA reduction was observed mostly at baseline before treatment with SSRI's, and increased after the treatment (Jang et al., 2006; Tükel et al., 2015). Another possible factor could be the fact that our patients were quite functional. The majority of our sample was educated (having a university degree), 6 of which were medical or PhD students in Cambridge, and 92% were working full time. There were only two patients that were severe and could not work at the time of the study, with the rest of the patients showing moderate symptoms. These factors could explain either 1) normal baseline NAA levels in our patients, or 2) an increase to normal NAA levels after the SSRI treatment. However, considering that there was no degeneration or loss of GM within the selected ACC voxel of our patients (which had a constant size across all subjects), the first explanation seems more plausible.

With regard to the GABA, Glu, Gln, and GABA/Glu findings in anterior cingulate, only one study reporting decreased GABA in ACC of patients with OCD (Zhang et al., 2016) is in line with our finding, and the literature has been very scarce and inconsistent with not many studies reporting Glu, GABA and Gln results individually. This is mostly due to the lower strengths of the magnetic field that was employed by these studies and the higher spatial resolution that is required to detect these neurometabolites reliably. Although the use of 7T scanner, which to this date, and to my knowledge has not previously been used in OCD, enabled us to have an advantage compared to the previous studies, there were nevertheless two important limitations that will be discussed below.

Limitations and future directions

One important shortcoming of most studies was their small sample size, and although our sample size was larger than most MRS studies discussed above, it was still below our desired number of 30 in each group. In order to decide the ideal sample size for our study, as there were no previous 7T MRS studies in OCD, and as NAA findings seemed to be the most consistent OCD finding, a 3T study by Yücel et al. (2007) reporting NAA reductions was used to perform a power analysis. A sample size of larger than 29 participants per group was required (at 3T) to achieve power of 80% to reject the null hypothesis, with an alpha of 1% (one-tailed t-test). Although we still aim to reach this sample size (30 participants in each group), at the time of writing, due to the slowness of the recruitment during the pandemic, and the thesis submission deadline, I have had to present the data with a slightly smaller sample size (23 healthy and 25 OCD participants).

Although it therefore may be premature to place too much emphasis on these findings until the intended numbers are achieved, considering that the power of 80% could be achieved by a sample of 39 using 1.5T scanners (Steen et al., 2005), and it comes down to 29 subjects per group using a 3T scanner (Yücel et al., 2007), we could hypothesize that the 7T scanner would in general require a smaller number of subjects to achieve a comparable power (if this reduction were to be linear, it could mean about 18-19 subjects would be enough to reach the same power using a 7T magnet). Nevertheless, further scanning is conducted in accordance with the initially targeted sample size, to reach 30 participants in each group.

The second limitation in most studies, was the inability to control for the effects of medication on neurometabolite ratios, especially relevant as most studies had mixed medicated and unmedicated patients and did not have a large enough sample size to control for medication effects. We were in a similar position. Although studies have not provided any evidence to conclude our findings could be caused by the SSRI medication, the absence of findings should be interpreted with caution since their lower magnetic field would not have allowed the detection of smaller metabolite reliably, and thus could be the reason they could not replicate our ACC findings using a 7T scanner. As we could not rely on the literature on SSRI and GABA, Glu, Gln ratios in ACC, and since we could not take the effect of medication into account, we intended to gain greater insight into the possible significance of these differences by relating them to behavioural and clinical measures, which will be discussed next in the final study of this thesis, Study 5.

3.3. Study 5: Behavioural correlates of brain metabolites (OCD vs HV)

Study 3 successfully demonstrated excessive and dysfunctional checking in patients with OCD in a lab setting. Significantly higher scores were also measured in OCD patients compared to healthy subjects for OCD symptom severity, anxiety, depression, intolerance of uncertainty, and habitual tendencies. Study 4 measured differences in Glu, GABA, Gln, and GABA/Glu levels within the anterior cingulate cortex between the OCD and healthy groups.

In the final study of this thesis, I aimed to merge the previous two studies by investigating the relationships between the measurements in Study 3 and 4 to learn more about the neural basis of behavioural and clinical symptoms in OCD patients. Knowing more about this relationship could also validate the neurometabolite findings in Study 4, especially relevant due to the inability to control for the possible effects of medication on neurometabolite levels in OCD. As compulsive checking may also be associated with deficits in goal-directed behaviour or increased habitual tendencies, performance on a contingency degradation task (explained briefly in the General Introduction of this thesis) was also compared between groups, in addition to its correlations with neurometabolite levels in ACC and SMA.

In Study 5 we aimed to:

- Study the relationships between checking, habit versus goal directed behaviours and neurometabolite levels in ACC and SMA in both OCD and healthy subjects.
- Study the relationships between anxiety, depression, OCD symptom severity, habitual tendencies, intolerance of uncertainty, and neurometabolite levels in ACC and SMA.

3.3.1. Methods

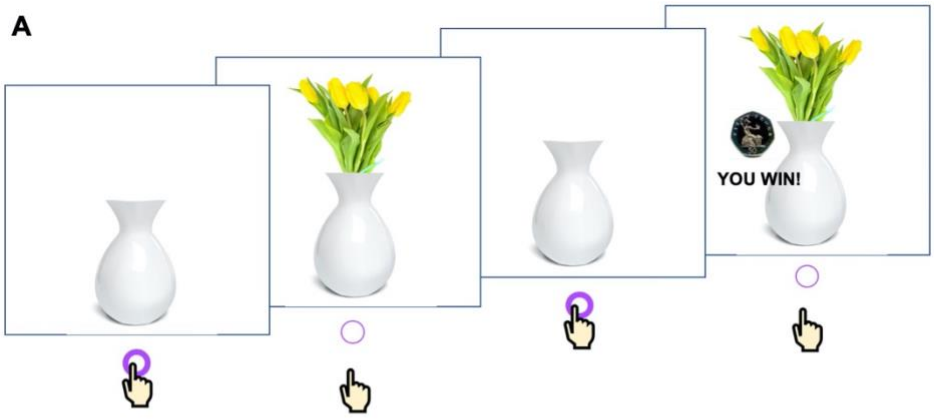
3.3.1.1. Participants and clinical measures

There were 23 healthy volunteers and 25 patients with OCD included in this study. The participants and questionnaires used in this study were the very same used in Study 3 (see sections 2.3.1.1 and 2.3.1.2), and thus the ethics number, and inclusion criteria were the same as well.

3.3.1.2. Behavioural measures

The behavioural data for Image Verification Task reported in Study 3, were used in this study. For a description of the IVT please refer to section 2.2.1.3. The second behavioural task administered in this study was a contingency degradation task used by Ersche et al. (2021), which consisted of 8 blocks of 120 trials, lasting 1 second each. **Figure 3.3.A** depicts the stimuli used in this task.

A



B

P (O I A)	**0.60**	**0.60**	**0.60**
P (O I ~A)	**0.00**	**0.30**	**0.60**
DELTA P	**0.60**	**0.30**	**0.00**
Programmed contingencies:	**non** degraded	**partially** degraded	**fully** degraded

Figure.3.3 (A) Depicts the stimuli from the contingency degradation task. Participants are shown a white empty vase on the screen, which fills with flowers when participants press the space bar, and which in 60% of the times is associated with winning 20 pence. Depending on the experimental condition, not pressing the space bar can also be associated with winning 20 pence. **(B)** shows the

experimental design of the varying degradation. This image was reconstructed from Ersche et al., (2021).

Participants were presented with a white vase on the screen which could be filled with flowers every time the space bar key was pressed. In 60% of the trials, the key press was associated with a financial reward of 20 pence and the message "YOU WIN" on the screen for 500 ms (see **Figure.3.3.A**). In the first 3 blocks, the association between the action of pressing the key, and winning 20 pence was established (non-degraded action-outcome contingency). This duration has been shown to be enough to induce habits in humans (De Wit et al., 2018). In block 4, in addition to the original probability of 60%, participants also received a free reward with a 30% probability (partially degraded condition). In block 5, the chance of receiving the free reward was also 60%, which was equal to the probability of reward after pressing the key/performing an action (fully degraded condition). In the final 3 blocks, the initial contingencies were reinstated (non-degraded conditions), followed by a partially degraded condition in block 7, and a fully degraded condition in block 8. **Table 3.3** depicts an overview of all blocks and conditions. In addition to the space bar key presses measured in all blocks, the causality judgements were also collected, that is rating on a scale of 0 (never) to 100 (always), how likely their actions were associated with the reward they received.

Table.3.3 Depicts an overview of the contingency degradation experimental design

Block Number	Condition	*P* (20p received following action)	*P* (20p received without action)	ΔP: programmed contingency
1	Non-degraded	0.60	0.00	0.60
2	Non-degraded	0.60	0.00	0.60
3	Non-degraded	0.60	0.00	0.60
4	Partially-degraded	0.60	0.30	0.30
5	Fully-degraded	0.60	0.60	0.00
6	Non-degraded	0.60	0.00	0.60
7	Partially-degraded	0.60	0.30	0.30
8	Fully-degraded	0.60	0.60	0.00

3.3.1.3. Statistical analysis

The IVT behavioural measures and clinical data were described in Study 3. In Study 5, the contingency degradation findings were compared between groups. First, the response rates, and causality judgements were calculated and averaged per condition: 1) the non-degraded (blocks 1,2,3), 2) partially degraded (blocks 4 and 7), 3), and fully degraded (blocks 5 and 8) conditions. Next, the difference (Delta) between [non- minus - partially degraded], and [non - minus - fully degraded] conditions were calculated for both Response Rates (RR) and Causality Judgements (CJ).

A mixed analysis of variance (ANOVA) was performed to study the effects of condition within subjects and between groups for the three conditions (non, partially, and fully degraded), and the two Delta's described above for RR, and CJ. In case the homogeneity or normality assumptions were not met for the mixed ANOVA, a robust ANOVA (Mair & Wilcox, 2021) was used instead. In case of significant main effects, independent and paired sample t-tests were performed to understand where the differences resided. For the mixed ANOVA, if the sphericity condition for repeated measures was not satisfied, a Greenhouse-Geisser correction was applied. Next, Pearson correlation coefficients, r, were calculated for each group separately, and in case of non-normally distributed data a Spearman correlation coefficient r_S was used to test the relationships between the Delta's of RR with OCI total score, and anxiety, as the latter were related to checking rates in the same OCD patients from Study 3. In case of significant correlations, a Fisher's Z-test was performed to study the differences between group for these correlations. R studio Version 1.2.5033, and package WRS2 were used to perform a robust mixed ANOVA in case of inhomogeneous data, and SPSS version 28 (SPSS IBM) for the mixed ANOVA in case the normality and homogeneity assumptions were not violated.

In a second analysis, the relationships between relevant 1) behavioural measures on the IVT from Study 3 (checking, and accuracy of answers under high uncertainty), 2) contingency degradation (Delta's of response rates- see **Figure.3.5**, and 3) relevant clinical measures described in Study 3 (anxiety state and trait, depression, intolerance of uncertainty, OCD symptoms as measured with OCI, and YBOCS total scores, and habitual tendencies such as COHS automaticity, and HTQ Compulsivity, and preference for regularity), with neurometabolite concentrations in ACC and SMA such as GABA, Glu, and GABA/Glu from Study 4 were investigated in both OCD and healthy volunteers. Pearson's r correlation coefficient was used for normally distributed data, and in case of non-normality, Spearman's correlation coefficient r_s was calculated. In case of significant correlations, a Fisher's Z test was used to compare the correlations between groups. For this part of the analysis Python version 3.7.6 was used.

3.3.2. Results

The demographic, clinical and neurometabolite differences between OCD and healthy groups were described in Studies 3 and 4. In this section, the results of the contingency degradation task performance, and the correlations between neurometabolite levels in ACC, SMA, and behavioural and clinical measures are described for both OCD and healthy volunteers.

Contingency degradation

Both groups responded less after degradation ($F = 24.60$, $p < 0.001$), with smaller causality judgements ($F = 47.13$, $p < 0.001$) from non to fully degraded conditions. Despite patients responding more than healthy volunteers in the partially and fully degraded conditions (**Figure.3.4.A),** and having higher causality judgements in these conditions **(Figure.3.4.B),** these differences did not reach significance ($p > 0.05$). Nor was there a significant interaction of group with degradation condition ($p > 0.05$)

For the Delta-RR, and Delta-CJ between [non – partial] and [non – full] degradation, there were significant within group differences, with both groups responding less from Delta [non – partial] to Delta [non – full] degradations ($F = 25.78$, $p < 0.001$), and similarly, having lower CJ from Delta [non – partial] to Delta [non – full] degradations ($F = 40.08$, $p < 0.001$). Although the data show that patients responded more than healthy volunteers (**Figure.3.5.A**), and had higher causality judgement scores (**Figure.3.5.B**), especially for Delta [non – partial] degraded conditions, these differences were not significant, and nor was there an interaction of group with degradation condition ($p > 0.05$). There were no significant correlations between Delta's of RR with OCI total score, anxiety state, nor trait in either groups ($p > 0.05$).

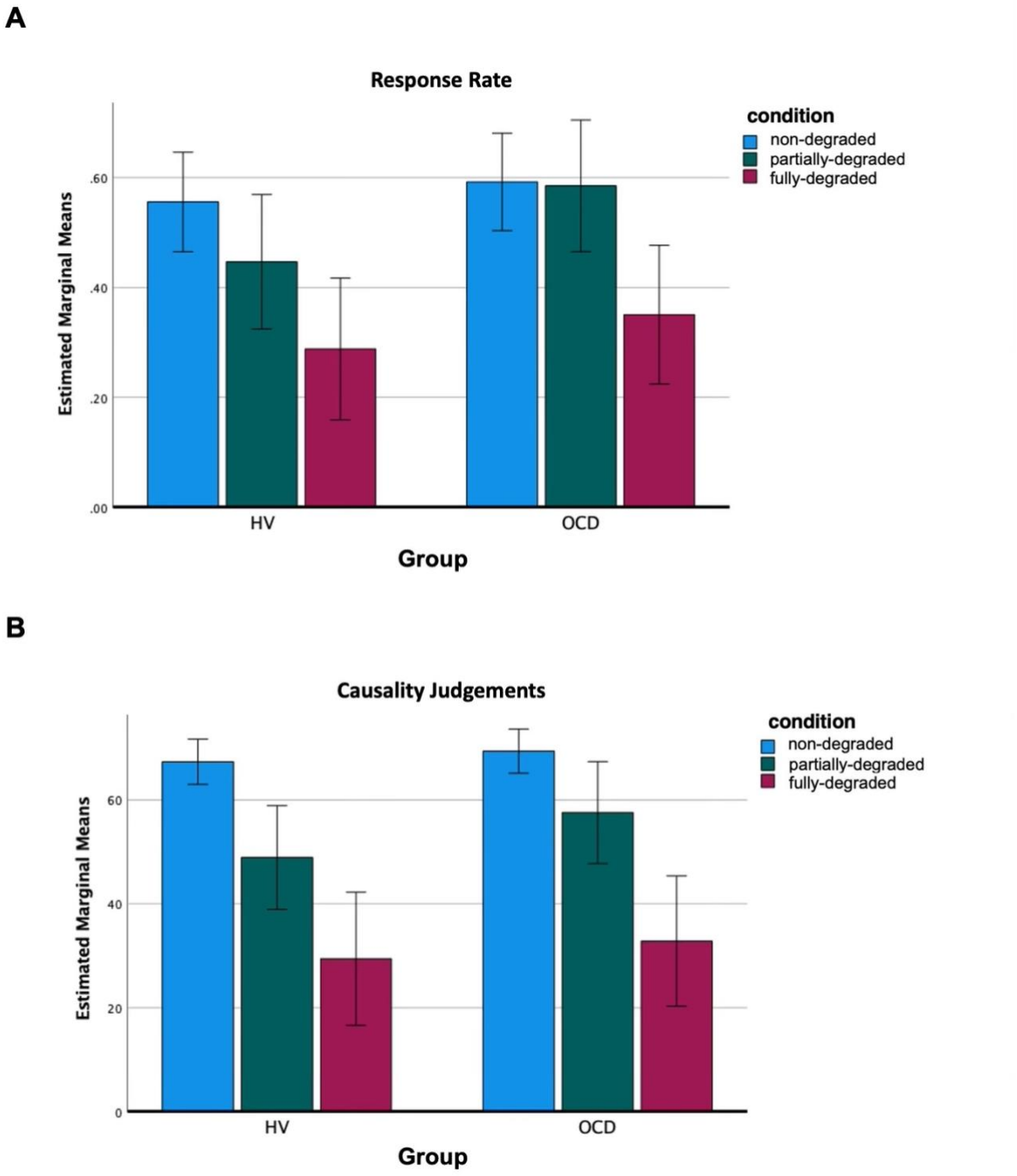
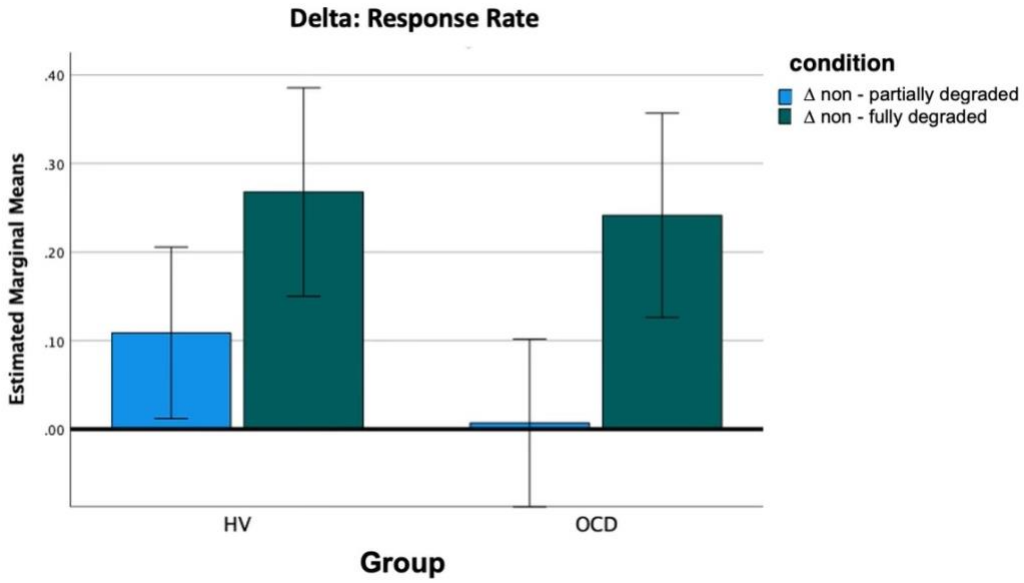


Figure 3.4 (A) depicts the Response Rates in OCD and health volunteers, in all three conditions of non-degraded (blue), partially-degraded (green), and fully degraded (red) conditions. **(B)** shows the Causality Judgements in OCD and health volunteers, in all three conditions of non-degraded (blue), partially-degraded (green), and fully degraded (red) conditions. Error bars represent ± 2 standard errors.

A



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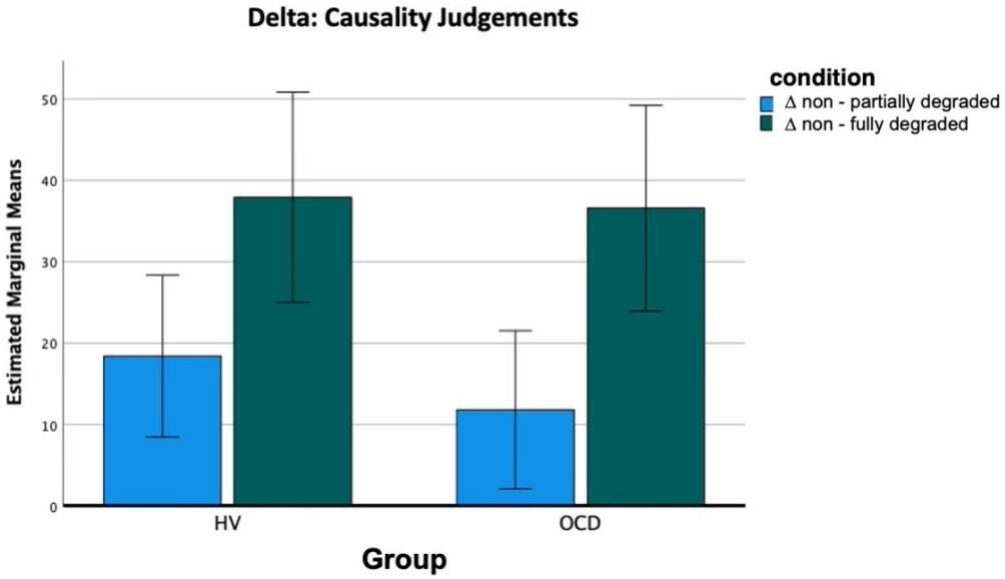

Figure.3.5 (A) Depicts the Delta [non minus partially degraded] in blue, and Delta [non minus fully degraded] in green, for Response Rates of OCD and healthy volunteers. **(B)** Shows the Delta [non minus partially degraded] in blue, and Delta [non minus fully degraded] in green, for Causality Judgements of OCD and healthy volunteers. The larger the Delta values for Response Rates, the better subjects detected the degradation and responded accordingly, and the higher the Delta of Causality Judgements, the better subjects could associate their actions with reward. The Δ symbol stands for Delta. Error bars represent ± 2 standard errors.

Correlations between neurometabolites and IVT checking/accuracy

Table.3.4.A shows the correlations between neurometabolite ratios and IVT checking under high uncertainty, and accuracy of answers for both groups. OCD patients showed positive and significant correlations between the GABA/Glu levels in ACC, with both checking (r = 0.59, p = 0.003) and accuracy of answers (r = 0.45, p = 0.03) under the high uncertainty condition. These correlations were significantly different between OCD patients and controls (z = -1.78, p = 0.03; z = -2.43, *p* = 0.007, respectively for checking and accuracy correlations). OCD patients also showed a positive trend for the correlation between the GABA levels in ACC and checking (r = 0.41, *p* = 0.05). In healthy volunteers there was a negative relationship between checking under high uncertainty and Glu levels in SMA (r = - 0.45, *p* = 0.03). The difference between these correlations in OCD patients and controls was highly significant (z = -2.72, *p* = 0.003). There were no other significant correlations for either groups (*p* > 0.05).

Correlations between neurometabolites and contingency degradation

Table.3.4.B presents the correlations between neurometabolite levels and contingency degradation for both groups. Interestingly, for contingency degradation, the two groups showed correlations at different stages of the task, and with metabolites in different regions. OCD patients showed positive correlations between Delta of response rates for [non – full] degradation and GABA (r = 0.43, *p* = 0.04), and GABA/Glu (r = 0.48, *p* = 0.02) levels in ACC. There were only trends for the Fisher's Z-test measuring the difference between these correlations between both groups (*p* = 0.08 and *p* = 0.06, respectively). By contrast, healthy volunteers showed a positive correlation between the GABA levels measured within the SMA voxel and the Delta of response rates between [non – partial] degradations (r = 0.69, *p* = 0.0005). The latter correlations were significantly different between groups (z = 3.33, *p* = 0.001). There were no other significant correlations/trends for either group (*p* > 0.10).

Correlations between neurometabolites and clinical measures

As anxiety and depression scores in OCD patients were highly correlated with one another (r = 0.62, *p* = 0.001), we used only the anxiety scores for this part of the analysis as they were more relevant for our hypotheses related to OCD symptoms, as discussed in the General Introduction. Similarly, the OCI total scores were correlated with the total YBOCS scores (0.61, *p* = 0.001), however, since YBOCS was only available in patients and OCI was available in both groups, we used both these measures to compare severity of obsessive compulsive symptoms within the patient group, but also between OCD and healthy subjects. In OCD patients, there was a trend for a correlation between anxiety trait and GABA/Glu ratio in ACC (r = 0.40, *p* = 0.06). In Study 3, a similar (but significant) positive relationship between anxiety and checking was reported under high uncertainty. There was also a trend for a positive

correlation between COHS automaticity, and Glu levels in ACC of OCD patients (r = 0.41, p = 0.05). The difference between these correlations however was not significant between groups (p > 0.05).

Table.3.4 MRS metabolite correlations with behavioural and clinical measures

Metabolites	Clinical/behavioural measures	OCD			HV		
		N	r	*p*	*N*	r	*p*
A							
GABA/Glu (ACC)	Checking (IVT)	22	0.59 $_S$	0.003**	20	0.08	0.71
GABA (ACC)	Checking (IVT)	23	0.41 $_S$	0.05	21	- 0.08	0.72
GABA/Glu (ACC)	Accuracy (IVT)	22	0.44 $_S$	0.04*	20	0.09	0.71
Glu (SMA)	Checking (IVT)	24	0.36 $_S$	0.09	22	- 0.45	0.03*
B							
GABA/Glu (ACC)	Delta-RR[non-full]	22	0.48	0.02*	20	0.007	0.97
GABA (ACC)	Delta-RR[non-full]	23	0.42	0.04*	21	- 0.10	0.64
GABA (SMA)	Delta-RR[non-partial]	24	- 0.19	0.38	21	0.69	0.0005***
C							
GABA/Glu (ACC)	Anxiety Trait (STAI-T)	22	0.40	0.06	20	- 0.004	0.98
Glu (ACC)	Automaticity (COHS)	23	0.40	0.05	22	0.08	0.70
Glu (SMA)	YBOCS-total	24	0.58	0.003**		NA	
Glu (SMA)	OCI-total	23	0.57	0.004**	22	0.39 $_S$	0.07
GABA/Glu (SMA)	OCI-total	23	- 0.19	0.40	20	- 0.56 $_S$	0.01*
GABA/Glu (SMA)	Regularity-preference(HTQ)	23	0.49	0.02**	20	-0.06	0.80

(A) shows the correlations between neurometabolite ratios and IVT checking under high uncertainty and accuracy of answers, **(B)** describes the relationships between neurometabolite ratios and contingency degradation, and **(C)** shows the correlations between neurometabolite ratios and clinical measures. All the latter are presented for the two groups separately.

Abbreviations: ACC = anterior cingulate cortex, SMA = supplementary motor area, GABA = Gamma-aminobutyric acid, Glu = Glutamate, N = sample size, OCD = obsessive compulsive disorder, HV = healthy volunteers, IVT = Image Verification Task, Delta-RR [non-full] = difference between response rates of non minus full degraded conditions, Delta-RR [non-partial] = difference between response rates of non minus partial degraded conditions, STAI-T = State Trait Anxiety Inventory-Trait, YBOCS = Yale-Brown Obsessive Compulsive Scale, OCI = Obsessive Compulsive Inventory, HTQ = Habitual Tendencies Questionnaire, r = Pearson correlation, S = Spearman correlation used in case of non-normal data, * = p < 0.05, ** = p < 0.005, *** = p < 0.001.

Despite the previous findings in OCD patients being all related to ACC neurometabolites, the OCI and YBOCS total scores were positively and significantly correlated with the Glu levels in SMA (r = 0.57, p = 0.004; r = 0.58, p = 0.003, respectively). Healthy volunteers also showed a positive similar trend for the correlation between the OCI scores and SMA Glu concentration (r_s = 0.39, p = 0.07).

There was a negative correlation in healthy volunteers between the GABA/Glu levels in SMA and OCI total score (r_s = - 0.56, p = 0.01). This correlation was not significant in patients, however, they also showed a negative correlation (r= -0.19, p = 0.40) and a Fisher's Z-test was non-significant comparing the correlations of the two groups (p > 0.05). There were no other significant correlations or trends. **Table.3.4.C** shows a summary of these correlations for both groups.

3.3.3. Interim Discussion

Study 5 aimed to understand the relationship between neurometabolite findings of Study 4, with behavioural and clinical results from Study 3. The performance on a contingency degradation task in OCD and healthy volunteers is reported below in addition to its relationship with neurometabolite levels in ACC and SMA from Study 4.

Contingency degradation and metabolites in ACC and SMA

Although the significant findings by Vaghi et al., (2019) for contingency degradation using a more elaborate test paradigm were not replicated in our OCD sample, their performance still showed a similar trend. OCD patients showed a bias towards excessive, presumed habitual, behaviour (away from a goal-directed tendency, expressed as large Deltas) despite being relatively similar to healthy control subjects in understanding the relationship between their actions and their consequences.

Healthy volunteers were more sensitive to the degradation at its earliest stage as reflected by larger Delta-RR [non – partial degradations], and its positive correlation with increased GABA levels in SMA. OCD patients showed a similar relationship between GABA and GABA/Glu levels and Delta-RR, however, in the ACC and for a later stage of contingency degradation [non – full degradations]. These differences between groups suggest that the SMA, although apparently normal in terms of GABA/GLu concentrations, is not undertaking its normal function in OCD patients, and possibly the ACC has taken over and behaves in a dysfunctional manner, leading to excessive habitual behaviour. (The correlation of YBOCS scores with neurometabolites in the SMA also supports this interpretation of functional abnormality in the SMA of OCD patients, see below).

Checking behaviour and metabolites in ACC and SMA

Similar to the contingency degradation findings, SMA was more relevant in healthy volunteers whereas in OCD patients ACC was related to their IVT performance, such as checking under high uncertainty and accuracy of answers. In patients, GABA/Glu levels in ACC were *positively* related to both rates of checking and accuracy of answers, indicating the possibility that ACC may have contributed to

functional checking by improving the answers and increasing the checking rates, and yet not being completely effective at accomplishing this goal. In healthy subjects, a negative correlation between glutamate levels in SMA and checking under high uncertainty was found, meaning that the lower the Glu levels in SMA the more they checked and vice versa. The discrepancy between the engagement of SMA and ACC between groups shows again that SMA is impaired in patients and ACC may have "taken over" a compensatory role but in a dysfunctional manner. It is important to note that the IVT checking measured in the lab, despite not being equivalent to compulsive checking, is functional in some sense by optimising decisional choice, and yet excessive in nature. Thus, this may be how compulsive checking is initially induced by anxiety or intolerance of uncertainty feelings, and eventually becoming compulsive in real life over time and by repetition. Thus, in Study 3 and by using the IVT task in a laboratory setting, we may be capturing the initial phase of excessive checking behaviour in OCD patients, before the habitual/compulsive component develops.

Correlations between metabolites in ACC and SMA and clinical measures

There was a positive trend for the relationship between anxiety trait and GABA/Glu ratios in ACC of OCD patients. A similar correlation was found between checking under high uncertainty and anxiety trait in the same participants in Study 3. Although causality cannot be concluded based on these correlations, there is the possibility that dysfunctional checking has an underlying affective component induced by increased anxiety and intolerance of uncertainty. Interestingly, despite the lack of 7T GABA studies in ACC related to anxiety, a dynamic activity pattern for ACC has been reported during anticipatory anxiety using a functional MRI study, with increased activity during moderate, but reduced activity during strong feelings of threat (Straube et al., 2008), which is in line with the current findings of increased GABA relationship with a more pronounced anxiety trait of our patients. Moreover, reduced ACC activity has been reported in patients with different anxiety disorders during emotion regulation (Blair et al., 2012).

Next, a positive correlation between the total OCI scores and the glutamate levels in SMA of both OCD and healthy volunteers was found. In addition to this finding, the total YBOCS scores were also positively related to the Glu concentration in SMA of OCD patients. Healthy volunteers also showed a negative correlation between the GABA/Glu levels in SMA and OCI total scores, whereas OCD patients showed a positive correlation with the preference for regularity and GABA/Glu in SMA. Yet again, therefore, there is evidence for an involvement of SMA in healthy volunteers in compulsivity, but this time, OCD symptom severity is also associated with SMA neurometabolite levels in both groups, indicating the relevance of SMA in OCD as well.

Conclusion

SMA is apparently not functioning as normal in OCD patients and thus ACC may be playing a somewhat unsuccessful compensatory role. While in OCD patients symptom severity and clinical symptoms were correlated with SMA neurometabolite levels such as Glu and GABA/Glu, the ACC metabolites such as GABA/Glu levels, were related to more subtle behavioural measures such as dysfunctional checking, deficits in goal directed behaviour and excessive habitual responding. However, checking and habitual responding possibly have different underlying mechanisms, as excessive checking was related to affective components such as anxiety trait and intolerance of uncertainty, whereas excessive habitual responding was not. Nevertheless, both IVT checking, and habitual responding during contingency degradation were related to the same neurometabolite ratios in ACC, though in opposite directions. One interpretation of this pattern of findings is that my IVT task is capturing an initial tendency towards excessive checking, perhaps related to affective factors such as uncertainty and anxiety, and that repetition (e.g. in everyday life) causes compulsive checking through an underlying bias to habitual control, mediated by changes in the anterior cingulate cortex.

Limitations and future directions

The desired sample size of 30 participants in each group was not achieved by the time of writing this thesis due to the delay caused by the pandemic and the thesis submission deadline. Additionally, there may have been other, more appropriate, multivariate techniques to analyse the relationships between different measures in this study, such as a multiple regression analysis or structural equation modelling, especially relevant to study the moderating role of anxiety on the correlation between checking and ACC neurometabolites. Using a single method, as opposed to multiple comparisons, would reduce the probability of making Type I errors (false positive discoveries). However, due to the sample size limitations, a simple correlational analysis was more appropriate at this stage. Data collection is still ongoing after submission of this thesis until the desired sample size is achieved and a more appropriate method will then be used to re-analyse the data presented in Study 5.

Chapter 4: General Discussion

In this thesis I focused on a clinical condition of OCS in schizophrenia patients, likely precipitated by treatment with second generation antipsychotics, notably clozapine. I showed that it was possible to measure a plausible form of compulsive behaviour, specifically dysfunctional checking, in a laboratory task and to compare it carefully not only with patients with schizophrenia but also patients with OCD. Thus, this behaviour presents a unique form of compulsive behaviour which most likely depends upon an interaction of clozapine dose with affective state, based on correlations with anxiety and enhanced checking behaviour under conditions of uncertainty, and treatment at higher clozapine dosage.

I have also added to the literature on OCD itself, specifically by demonstrating how contextual factors may affect the expression of compulsive checking in the laboratory. Moreover, I have investigated neural and neurochemical mechanisms in OCD, including not only checking but also another laboratory task which examines the balance between goal-directed behaviour and habitual control. I have shown important inter-relationships among these variables, as well as some aspects of affective functioning in OCD. Overall, this analysis contributes importantly to understanding the roles of anterior frontal cortex including SMA and ACC in OCD symptoms and in laboratory models of these symptoms. The following discussion will initially consider specifically the phenomenon of clozapine induced obsessive compulsive symptoms in schizophrenia, followed by a more detailed interpretation of the neural basis of cognitive change in OCD and clinical and theoretical implications thereof.

3.3.4. Clozapine induced OCS

One of the aims of this thesis was to understand and characterise the cognitive and clinical features of clozapine induced OCS in schizophrenia, compared to schizophrenia without OCS, OCD patients and healthy controls. More specifically, I sought to understand the nature of checking behaviour which is one of the most common obsessive compulsive symptoms reported in both schizo-OCS (Grover et al., 2015) and OCD patients (Strauss et al., 2020). The results of this study in schizo-OCS patients are discussed below in comparison to patients with schizophrenia and OCD.

3.3.4.1. Schizo-OCS compared to schizophrenia without OCS

In this thesis, I compared the clinical characteristics of patients on clozapine who developed OCS, with the patients who were on the same medication but did not develop OCS. The schizo-OCS patients showed higher positive, and depressive symptoms, which was also shown in my previous study in a larger sample of 231 patients (Biria et al., 2019). Additionally, in the current cohort of patients, increased anxiety and intolerance of uncertainty were measured when comparing them to the 'pure '

schizophrenia group (and healthy controls). Both schizophrenia groups were on similar doses of clozapine, had comparable treatment duration, and showed similar levels of deficits in spatial working memory, although schizo-OCS patients had worse cognitive flexibility, this deficit was not related to their OCS. Deficits in executive functioning such as cognitive flexibility and spatial working memory have been shown previously in schizophrenia patients (Bowie et al., 2006; Goldberg & Green, 2002; Leeson et al., 2009; Pantelis et al., 1999).

Checking compulsions have been one of the most commonly reported symptoms in schizo-OCS patients (Grover et al., 2015; Fernandez-Egea et al., 2018), and yet to this date, this behaviour has never been measured using objective tests in the lab. Thus, I designed and developed a new checking paradigm, the Image Verification Task, to measure this behaviour in the laboratory. The need for a new task arose because I could not replicate the previously reported findings by Morein-Zamir et al (2018) in the experimental context that we used the task, perhaps because it required the induction of anxiety by ambiguous task instructions, but also because this task did not rely on assessment of externally derived information. The IVT, however, is based on perceptual decision making involving response selection after the gathering of sensory information. In fact, although there has been considerable inconsistency in previous attempts to measure checking in the laboratory, those tasks requiring perceptual decision making (Arntz et al., 2007; Jafari et al., 2013; Rodge et al., 2008; Toffolo et al., 2016) as opposed to reasoning within a task before making a decision have proved most reliable in inducing checking behaviour in OCD patients (Strauss et al., 2020).

As hypothesized, only the schizo-OCS group demonstrated dysfunctional checking which was excessive and not goal-directed (i.e. operationally speaking, did not lead to improvement of their poor performance). Despite both schizophrenia groups being on comparable doses of clozapine, and experiencing similar spatial working memory deficits, only the schizo-OCS patients showed correlations between checking with clozapine dose and spatial working memory performance, thus confirming our hypothesis of a positive relationship between clozapine dose and checking. However, the latter correlations were only significant when checking occurred under high uncertainty conditions, indicating the role of uncertainty intolerance in schizo-OCS patients. The previous findings of a positive correlation between the clozapine treatment duration and OCI checking subscale (Fernandez-Egea et al., 2018) were not replicated in our patient groups, possibly due to our smaller sample size (leading to a smaller range for the number of years on clozapine) compared to the 118 patients in the latter study. On the contrary, the relationship between OCS and clozapine dose/plasma levels in schizophrenia have been shown repeatedly (Biria et al., 2019; Fernandez-Egea et al., unpublished observations, personal communication; Gürcan et al., 2021; Kim et al., 2020; Lin et al., 2006; Mukhopadhaya et al. 2009; Reznik et al., 2004; Schirmbeck et al. 2011), although to my knowledge this is the first time that the OCS symptoms, such as checking, have been isolated behaviourally in a laboratory context.

The question still remains whether schizo-OCS patients are more severe to begin with, as evident from their higher positive and depressive symptoms, and worse cognitive flexibility, or whether their response to clozapine has not been as effective as the pure schizophrenia patients to reduce their schizophrenia symptoms to a comparable level as for the schizophrenia patients without OCS. Although we did not find a relationship between positive symptoms and checking rates in our study, and after psychosis remission checking compulsions seem to be directly related to clozapine dose and plasma levels in schizo-OCS patients (Fernandez-Egea et al., unpublished observations, personal communication), psychosis may have an indirect effect on checking, such as through induction of obsessions. The latter has been found in one of our unpublished studies in schizophrenia patients, together with genetic moderation of the serotonergic (HTR 2A/C and SCL6A4) and glutamatergic transmission (GRIN2B) in schizo-OCS patients (Fernandez-Egea et al., unpublished observations, personal communication). However, there are probably more factors involved than increased clozapine dose or positive symptoms. Affective state is another relevant construct that seems necessary to induce checking in schizo-OCS patients. In addition to increased anxiety in these patients, they showed a positive relationship between checking and state anxiety when punished for checking, and the positive relationships between clozapine dose, spatial working memory were found only under high uncertainty conditions. Although the possible role of anxiety is not yet completely clear is in these patients - and the causes of these stronger feelings of anxiety, it is possible that clozapine alters their affective state through reductions of serotonin function, although the precise role of serotonin in anxiety is controversial (Stahl 2000). Although clozapine is mainly a 5-HT receptor antagonist (especially at 5-HT2A, 5-HT1A and 5-HT2C- receptors) as well as having dopamine receptor antagonism (especially at D2 receptors; Meltzer et al., 2008), the highly complex binding properties of clozapine (Stahl, 2000), makes it difficult to test this hypothesis.

3.3.4.2. Schizo-OCS compared to OCD patients

Although the schizo-OCS patients in our sample had a primary diagnosis of schizophrenia, their verbal IQ levels were closer to those of OCD and healthy participants than the 'pure' schizophrenia group. Thus, the deficits discussed below could not be linked to lower IQ levels.

Both OCD and schizo-OCS patients showed higher anxiety, intolerance of uncertainty, and obsessive compulsive symptoms compared to the pure schizophrenia and healthy controls. However, with regard to cognitive deficits, schizo-OCS patients performed more poorly than OCD patients, more specifically they had worse spatial working memory and cognitive flexibility. As mentioned earlier in this thesis, cognitive flexibility impairments are not only present in schizophrenia (Bowie et al., 2006; Goldberg

& Green, 2002; Leeson et al., 2009; Pantelis et al., 1999), but also in OCD (Chamberlain et al., 2007; Vaghi et al., 2017). The OCD group in our sample displayed cognitive flexibility deficits expressed as greater errors during extradimensional set-shifting, in line with a recent meta-analysis (Chamberlain et al., 2021), although they were significantly less impaired than the schizo-OCS group.

At a behavioural level, our initial hypothesis of excessive dysfunctional checking was only partly confirmed in schizo-OCS patients. Neither schizo-OCS nor OCD patients showed increased checking rates compared to the other two groups, however, in schizo-OCS, their checking behaviour was not used in a goal-directed manner to improve their poor performance, and in this sense it was excessive and dysfunctional. Similarly, our second hypothesis of finding no relationship between working memory and checking was only confirmed in the OCD group, as checking under high uncertainty in schizo-OCS patients was positively correlated with their spatial working memory performance. The latter finding also suggests a role of uncertainty and checking in schizo-OCS, which they also had in common with OCD patients. Thus, our hypothesis of the role of uncertainty intolerance on checking behaviour was supported by these findings in both groups.

The absence of excessive checking in OCD and schizo-OCS patients in our study could be due to the nature of the task instructions that discouraged checking behaviour, and as will be discussed below, was reversed in OCD patients by changing the task instructions. Nevertheless, schizo-OCS patients were unable to perform functional checking even when the task instructions required them to and discouraged excessive checking by placing an emphasis on both accuracy of answers but also speed, indicating the severity of impairment in these patients compared to the OCD group. What both these patient groups had in common however, was an association of affective state with checking behaviour. OCD patients showed a positive association between intolerance of uncertainty scores and checking under high uncertainty. However, the possible importance of intolerance of uncertainty in schizo-OCS patients was not evident from any significant correlation with the intolerance of uncertainty scale, but from significant relationships found for these patient of correlations for clozapine dosage and spatial working memory with checking rates that were present only under the high uncertainty conditions of the IVT. Additionally, the schizo-OCS group also showed a positive relationship between checking under punishment and anxiety state. In both groups, the more severe patients had difficulty suppressing checking despite being punished for it, consistent with our hypothesis.

3.3.5. Conclusion

Schizo-OCS patients displayed obsessive compulsive symptoms after treatment with clozapine, such as dysfunctional checking measured successfully in a laboratory context. Although clozapine dose was positively related to checking rate in these patients, affective state such as anxiety and uncertainty

intolerance, and executive functioning deficits such as spatial working memory impairments were likely moderators of this relationship. Despite the absence of excessive and dysfunctional checking in OCD patients, both OCD and schizo-OCS patients exhibited a common role for uncertainty conditions in eliciting checking. It remains unclear how clozapine exactly induces these checking symptoms and what differentiates patients with and without OCS from one another, although, a biological or genetic predisposition related to 5HT2 receptor abnormalities seems plausible. Clozapine may make some individuals more susceptible to anxiety or intolerance of uncertainty, and thus leads to the induction of OCS initially, similar to the possible induction of symptoms in OCD patients. Since both OCD and schizo-OCS patients present excessive checking behaviour in their daily lives, and yet the IVT did not capture this excessive behaviour, the task instructions were changed in order to capture this excessive behaviour by removing the time/speed limit. However, due to the difficulties in recruiting another sample of schizophrenia patients in the circumstances of the pandemic, the use of the new IVT task and the planned comparison of the neural and neurochemical basis of the two patient groups with controls was restricted instead to comparing OCD patients with healthy volunteers.

3.3.6. Behavioural, neural and neurochemical correlates of OCD

In this section, the behavioural, structural and neurochemical findings underlying obsessive compulsive disorder will be discussed. As mentioned previously, due to feasibility issues, the latter studies could not be conducted in schizophrenia patients and were continued in OCD and healthy control subjects.

3.3.6.1. Checking behaviour

As mentioned repeatedly throughout this thesis, checking is one of the most commonly reported compulsions in OCD patients (Fontenelle et al., 2006). Yet, this behaviour and its underlying mechanisms are not fully understood. Studies have tried to measure the influence of uncertainty (Toffolo et al., 2013, 2016), memory confidence (Dar, 2004; Hermans et al., 2003; Tolin et al., 2001), and increased responsibility feelings (Arntz et al., 2007, Mantz et al., 2019) on checking behaviour. However, none of these studies tried investigating these questions in the same participants using different task conditions to understand the cognitive and affective aspects underlying checking in OCD. Most of the latter studies used subjective measures such as self-reporting questionnaires to measure checking behaviour. One common theme amongst studies reporting excessive checking in OCD has been the need to gather information prior to decision making, more specifically perceptual as opposed to reasoning for decision making seems to induce checking behaviour in OCD (Strauss et al., 2020). Several researchers have used perceptual decision making tasks to study checking in OCD (Jaafari et al., 2013; Rotge et al., 2008), however, with some failures to replicate the findings (Rotge et al., 2015). In general, patients with OCD (Banca et al., 2015a) and high compulsive individuals without an OCD

diagnosis (Hauser et al. 2017c) have been shown to require more information before making a decision and have a higher decision threshold.

In order to measure checking and its underlying cognitive aspects more reliably in a lab setting, I designed a new checking paradigm relying on perceptual decision making, under various conditions of 1) high versus low uncertainty, 2) absence or presence of punishment, and 3) encouragement of goal-directed responding in the task instruction. The first version of the task failed to measure excessive checking in OCD patients (Study 2), despite the presence of this behaviour in their daily lives. One hypothesis I formulated was that the task instructions may have previously discouraged checking by placing not only an emphasis on both accuracy of answers but also a time/speed limit, and OCD patients were able to show goal-directed checking by being aware of the consequences of their checking behaviour. In my second attempt, the task instructions were modified by removing the time limit, and omitting the explicit explanation that checking more will lead to staying in the lab for longer. In the second version of the task, OCD patients checked significantly more than healthy subjects despite having comparable performance, and thus showing dysfunctional checking. I will discuss below why this excessive checking behaviour could still be seen as a failed attempt to performing a functional checking behaviour, when discussing the findings of the brain correlates of checking performance.

Although in Study 3, both OCD and healthy controls showed a relationship between checking and obsessive compulsive symptoms (measured with OCI; Foa et al., 1998), automaticity (COHS; Ersche et al., 2019), and habitual compulsivity (HTQ; Ramakrishnan et al., 2022), only anxiety (state and trait; Spielberger et al., 1983) was positively correlated with checking within the OCD group. In addition to anxiety, checking rates were the highest under high uncertainty conditions in OCD patients. However, as opposed to Study 2, there was no correlation between OCD symptom severity as measured with YBOCS and checking under punishment. In other words, all OCD patients checked more than healthy controls under punishment, and not only the most severe patients did so (which had been the case in Study 2). Although the relationship between intolerance of uncertainty, anxiety and checking seems plausible in helping to explain what could be causing checking, this relationship alone does not seem to be sufficient. Patients with anxiety disorder do not seem to show excessive checking under high uncertainty conditions (Toffolo et al., 2016), thus, there must be other factors at play moderating the relationship between anxiety, uncertainty and checking in OCD. Although OCD patients in Study 3 also showed a negative relationship between confidence and checking, low memory confidence has also been reported in anxiety disorder patients (Ouellet-Courtois et al., 2018), and may not be the main drive for checking behaviour in these patients. Thus, it is likely that all these cognitive and affective components are inter-related and somehow contribute to the induction or maintenance of checking behaviour in OCD. However, it is unlikely that they are the main mechanisms underlying checking compulsions on their own. Although excessive checking was successfully measured in our OCD

patients in the lab, this behaviour may resemble the initial stage of checking induced by anxiety and uncertainty intolerance, as opposed to the clinical checking compulsions which are acquired by performing this repetitive behaviour over time, leading to compulsive and possibly habitual checking behaviour. Since excessive checking could be seen as a failure to perform goal-directed behaviour, a contingency degradation task was additionally administered to the same patient cohort in order to investigate possible relationships between checking and the balance between goal-directed and habitual behaviour - as well as the neural and neurochemical basis of these behaviours.

3.3.6.2. Goal-directed versus habitual behaviour

Patients with OCD are reported to show deficits in goal-directed behaviour, in favour of increased habitual tendencies (Gillan et al., 2011). As checking compulsions could be seen as excessive and not goal-directed, and habitual over time, a contingency degradation task was administered to learn more about the neural mechanisms underlying both checking and habitual control. This task was a simplified version of the task by Vaghi et al., (2019), and is the equivalent of the task used by Ersche et al. (2021).

In the contingency degradation task, the link between an action and its outcome is tested by modifying the contingencies. Subjects are instructed to optimise reward, and rate how much their actions were predictive of the outcome. In some conditions actions can predict the outcome/reward with a high probability, whereas in other conditions, reward/outcome occurs without the need to perform an action. By learning this contingency, the outcome/reward can be achieved without the need to perform an action and thus perform a goal-directed behaviour. However, if an instrumental behaviour, trained in a previous phase, is still continued after degrading the contingency between the action and outcome, this behaviour is believed to be driven by the habitual system (Balleine and O'Doherty, 2010). OCD patients tend to keep responding even after degrading the relationship between action and outcome, and having learnt the contingencies, showing habitual responding (Vaghi et al., 2019).

At a behavioural level, although the significant findings previously reported in OCD (Vaghi et al., 2019) using a rather different procedure were not replicated in our OCD patients, they did numerically show a similar increased habitual responding after the degradation phase. When comparing the Delta's of response rates (calculated by subtracting the response rate of either partial or full degradation, from the non-degradation conditions), OCD patients showed a smaller Delta than healthy controls, suggesting they did not respond in a goal directed manner. This was especially the case for the Delta of [non – full degradation], whereas the healthy control subjects were sensitive to a change in degradation at its earliest stage [non – partial degradation]. This is despite the fact that in OCD patients, causality judgements were close to those of healthy controls, similar to the findings reported by Vaghi et al.,

(2019). This means that despite learning the association between actions and outcomes, OCD patients still responded excessively or habitually, even after the degradation of this association.

3.3.6.3. Neurochemical and neural changes in OCD

Even following an extensive literature review, the neurochemical underpinning of OCD were still unclear, mostly due to the use of conventional MRS scanners at a strength below 3 Tesla, small sample sizes (majority of studies had a sample size below 20), and not controlling for confounding variables such as medication effect. In this study, an MRS scanner at 7T, and a sample size of 23 healthy and 24 OCD subjects were employed to study the neural and neurochemical underpinnings of OCD. As the majority of our patients were medicated, the metabolite ratios were studied in relation to behavioural and clinical data to rule out accidental findings related to medication, rather than OCD symptoms.

Three voxels were placed at the ACC, SMA and occipital lobes, bilaterally, and including mostly grey matter volumes. Anterior cingulate cortex is one of the highly relevant regions to study in OCD due to its involvement in error monitoring (Carrasco et al., 2013; Endrass et al., 2008) and reward prediction errors (Hauser et al., 2017a; Murray et al., 2019). Additionally, ACC is implicated in both checking and goal-directed behaviour. Inactivation and hyperactivation of ACC or reduced excitatory input from ACC to caudate, all caused deficits in goal directed behaviour in marmoset monkeys (Duan et al., 2021). Moreover, OCD patients showed increased ACC activity in response to provoking checking compulsions by seeing pictures of objects related to checking, such as a stove, purse or door (Mataix-Cols et al., 2004). A second voxel was placed in the Supplementary Motor Area (SMA) which participates in a sensorimotor circuit with the putamen (Milad & Rauch, 2012) and is an effective target for brain stimulation and improvement of OCD symptoms (D'Urso et al., 2016; Gowda et al., 2019; Hazari et al., 2016; Mukherjee et al., 2021). A third voxel was placed in a region within the occipital lobes according to Murley et al., (2020), to control for physiological changes, as this region is assumed not to be influenced by a psychiatric diagnosis, however, as will be discussed below, the latter was not the case in our OCD patients. The individual data for each metabolite, each voxel, per individual subjects were corrected for the GM, WM, and CSF ratios to make sure the findings were not due to brain structure differences. The GM, WM, CSF fractions and metabolite ratios within each selected voxel were then compared between OCD and healthy control subjects.

There were no structural differences in GM, WM, and CSF fractions in any of the voxels. As hypothesised, OCD patients showed increased Gln and Glu, and lower GABA, and GABA/Glu ratios in the ACC. These findings were not previously reported in the literature, except for the decreased GABA levels in one study (Zhang et al., 2016).

In general, as discussed in survey in Chapter 3, the literature has been somewhat scarce and inconsistent, with not many studies reporting Glu, GABA and Gln results individually, mostly due to the higher spatial resolution required to detect these metabolites reliably, and the lower strengths of the magnetic field employed in those studies. As opposed to the reduced NAA findings in the literature (Jang et al., 2006; Tükel et al., 2015), the NAA levels in our patients were comparable to those in healthy controls, indicating that our findings were not due to neuronal integrity changes. As an additional control, the association between the NAA levels and structural data were investigated, with no significant relationship found. As discussed in the Interim Discussion of Study 4, there are two possibilities that could explain the similar NAA levels between groups: 1) medication could increase the NAA levels to 'normal' (Jang et al., 2006; Tükel et al., 2015) and most of our patients were medicated, or 2) our patients were more functional compared to the ones reported in other studies, with majority of our patients having either university degrees or working full time- there were only two severe unemployed patients in our sample. However, considering there were no structural changes that would point towards neuronal degeneration, the second factor seems more plausible.

There were no differences found between neurometabolite ratios in the SMA voxel, nevertheless, I will discuss below how functioning of the SMA may nevertheless be impaired in patients. Additionally, Glu concentration levels were increased in occipital cortex compared with healthy volunteers, showing a similar pattern to ACC Glu levels. Thus, occipital lobe metabolite levels could not be used to correct for physiological changes within subjects. Although we found no functional deficits in relation to this change, patients with OCD are known to have deficits in sensory processing and it has been hypothesized that this deficit together with cognitive impairments may underlie OCD symptomatology (Szalai, 2019). These sensory phenomena may in fact be what OCD and schizophrenia patients have in common with one another, except for the lack of delusions in OCD patients (Szalai, 2019). Compulsive behaviours in OCD could be seen as compensatory acts to reduce the impact of the sensory (including visual) prediction errors, as a result of a mismatch between the expected and actual outcomes (Gentsch et al., 2012). Thus, the increased Glu in the occipital lobes may possibly be related to aberrant sensory processing. However, it would be of perhaps greater interest to have found such changes in somatosensory regions related to sensorimotor feedback; this could be a future target for MRS studies of OCD.

All the above findings are novel and not previously reported in OCD patients. These novel results could be either due to the process of our patient selection, or the use of a 7T scanner. The latter, seems more likely considering that our neurometabolites of interest required very high spatial resolution to be detected reliably. Nevertheless, this study still had one shortcoming similar to the ones reported in the literature, such as the medication being a confounding variable. In order to study the effects found in

OCD patients more carefully, and be able to rule out the role of medication on the neurometabolite concentrations, the relationship between clinical, behavioural measures and neurochemical changes in the brain were investigated in both OCD patients and healthy controls.

3.3.6.4. Correlations between brain neurochemistry, checking and contingency degradation

There is ample evidence suggesting the relevance of GABA/Glu levels in ACC for goal-directed (higher GABA/Glu) versus habitual responding (lower GABA/Glu). As mentioned earlier, the GABA/Glu levels in ACC were significantly lower in patients, and this ratio was also *positively* correlated with superior performance on both contingency degradation and image verification tasks. In other words, the more goal-directed a patient was, the closer their ACC GABA/Glu ratios were to normal levels in healthy controls. Goal-directed responding was measured using contingency degradation directly (a larger Delta-RR), and IVT by inference (a higher accuracy of answers, as it was the main goal of the task). Additionally, there was a *positive* relationship between Glu levels in ACC and automaticity (COHS) measures in patients. Automatic behaviours do not serve any specific goals and are triggered by the context (Ersche et al., 2019). In line with the latter findings, Ersche et a. (2021) also reported a negative relationship between automaticity and goal-directed responding on the contingency degradation task in patients with cocaine use disorder (CUD). In other words, the higher the automaticity scores, the more habitual as opposed to goal-directed responding someone with CUD displayed on the contingency degradation task (Ersche et al., 2021).

Surprisingly, in addition to the accuracy of answers on the IVT, the GABA/Glu levels in ACC were also correlated *positively* with excessive checking rates, under high uncertainty, in OCD patients. Although at first sight these findings may seem contradictory (how can GABA/Glu levels be related to both goal-directed behaviour and excessive/dysfunctional checking at the same time?), there may be an explanation for these puzzling results. IVT checking rates were related to the affective state in OCD patients (both anxiety state, and trait), and patients checked the most under the high uncertainty condition despite very good accuracy. The anterior cingulate cortex seems to have a dynamic activity profile in relation to anxiety state, with increased activity during moderate, but reduced activity during strong feelings of threat (Straube et al., 2008). Additionally, reduced ACC activity has been reported in anxiety disorder patients during emotion regulation (Blair et al., 2012). Additionally, GABA/Glu ratios in ACC were *positively* correlated with the anxiety trait in our OCD patients. Although correlation does not imply causality, these relationships suggest that excessive checking in OCD may have an underlying affective component induced by increased anxiety and intolerance of uncertainty, influenced in some way by the GABA/Glu ratios in ACC.

With regard to contingency degradation performance, OCD patients showed a bias towards excessive, presumed habitual, behaviour (expressed as larger Deltas of RR). As mentioned briefly above, they showed a *positive* relationship between Delta of RR [non – full degradation] and GABA/Glu ratios in ACC. Although this correlation seems similar to the one described above for checking, the direction of the relationship is in fact opposite. In other words, the larger the Delta of RR, the better the performance, and the higher the GABA/Glu ratio (whereas, for excessive checking a negative relationship would have been predicted). There are again two possible explanations for this opposite relationship: 1) The ACC may have been recruited to resolve uncertainty and hence elicit checking, thus improving accuracy; in that sense, checking could be viewed as somewhat functional, 2) Alternatively, checking and response to contingency degradation may be unrelated, as contingency degradation showed no associations with affective factors such as anxiety state nor trait, whereas checking rates under high uncertainty did so. In general, ACC is implicated in many different processes such as error detection, conflict monitoring, integration of outcome uncertainty and values of actions before making a decision, reward prediction error, choice difficulty (Rushworth et al., 2004; Vassena et al., 2017), and exploration of alternative choices (Kolling et al., 2016) and so it is possible that different ACC circuits are implicated in these two forms of behaviours. Structurally, ACC can also be divided into different sub-regions, our ACC voxel including both the ventral and dorsal ACC regions described by Rushworth et al. (2004) as regions 24a, 24b, 24a′, 24b′. Thus, it is perhaps not surprising to find ACC serving two different functions in our patients, such as excessive checking and a goal-directed response to contingency degradation. Besides the structural and functional diversity within the ACC, its activation level is another variable relevant for goal-directed control. Duan et al., (2021) found deficits in goal-directed behaviour in marmoset monkeys after both inactivation and overactivation of the ACC (specifically, area 24), so an optimal level of ACC activity is required for effective goal-directed behaviour.

Our second voxel SMA seemed initially more relevant in healthy controls than OCD patients, with a *negative* correlation between SMA Glu levels and checking rates on the IVT under high uncertainty, and a *positive* correlation between GABA levels in SMA and Delta-RR [non – partial degradations]. The latter findings in healthy subjects shows their higher sensitivity to degradation as this correlation occurred at a more subtle stage of action-outcome degradation than OCD patients. However, these findings imply that SMA, despite exhibiting normal metabolite levels, is in fact impaired in OCD patients, and this deficit may have led to ACC providing a compensatory, though ineffective, dysfunctional role. Indeed, there is supporting evidence for the latter hypothesis in the form of the significant *positive* relationships between OCD symptoms (both self-reported OCI and clinician rated YBOCS) and the SMA glutamate levels in my study. This implies that the higher Glu levels in SMA, the greater the severity of symptoms in OCD patients. Hyperactivity of pre-SMA (a region also included in our SMA voxel, according to the reported x-y-z coordinate of this study) has been suggested to be a

neurocognitive endophenotype of OCD, possibly related to inefficient neural processing, as measured during a response inhibition task (de Wit et al., 2012). Additionally, the authors reported two negative correlations between left SMA and Stop Signal Reaction Time (ms), and right SMA and OCD symptom severity as measured with YBOCS (de Wit et al., 2012). Although intuitively, higher Glu levels in SMA could possibly lead to increased activity in SMA, and under this assumption our results appear to be the opposite to the latter fMRI findings, one needs to bear in mind that higher Glu levels do not necessarily equate to increased BOLD fMRI activity. Resting state functional connectivity studies are required to understand if there are indeed baseline differences between OCD patients and controls that constrain interpretation of fMRI BOLD results- and may also indicate what and where is the impact of elevated Glu levels in the pre-SMA/SMA, implying greater output, as well as greater input sensitivity of this structure.

According to the findings in this study, frontal regions within the CSTC circuits such as ACC and SMA seem to be somehow functionally interconnected, due to their involvement in both perceptual decision making and the response to changes in action-outcome contingencies. Although there have been controversies about the role of sub-regions of PFC, choice difficulty effects (resulting from conflict between several choice options), have been found from more dorsal regions such as ACC towards the pre-SMA- a region included in our SMA voxel (Kolling et al., 2016). There is more evidence indicating the involvement of the fronto-striatal pathways in goal-directed control, such as the inactivation and hyperactivation of ACC, and reduced excitatory input from ACC to caudate, all causing deficits in goal directed behaviour in marmoset monkeys (Duan et al., 2021).

In addition to the ACC and SMA, OFC is another relevant region for OCD within the CSTC circuits, having extensive connections with ACC (Haber and Behrens, 2014). However, due to poor shimming in this region using a 7T MRS scanner, this region was not studied in our OCD sample. While the ACC seems to be involved in instrumental learning (Schweimer and Hauber, 2005), the anterior OFC is suggested to contribute to ACC function by mediating Pavlovian-to-Instrumental transfer (PIT; Cartoni et al., 2016). Inactivation and hyperactivation of anterior OFC led to enhancement and impairments in goal-directed behaviour in marmoset monkeys respectively, and thus, OFC may be competing with instrumental responding of ACC by expressing Pavlovian control (Duan et al., 2021). According to this hypothesis, the stimuli relevant to OCD patients may first be provoking activity in OFC (Rauch et al., 1994) and thereby affecting the ACC neurometabolites or activation. Consequently, deficits in neurometabolite projections from OFC to ACC, may mediate the instrumental influence of ACC on goal-directed behaviour. However, resting state connectivity studies, together with 7T MRS study of OFC neurometabolites would be required to test this hypothesis.

Conclusion

OCD patients showed excessive checking, with comparable accuracy/performance to healthy subjects, on the IVT, and excessive or presumably habitual responding on the contingency degradation task as hypothesized. The GABA/Glu levels in ACC were related *positively* to a good (or goal-directed) performance on both IVT and contingency degradation tasks in the OCD group. Strikingly, these ratios also showed a similar positive relationship with excessive checking under high uncertainty in patients. Additionally, anxiety trait was related to both GABA/Glu levels in ACC of patients with OCD, but also to excessive checking under high uncertainty. However, contingency degradation performance on the other hand displayed no relationship with anxiety. The latter findings indicate two separate functions within the ACC for goal-directed versus habitual responding on one hand, and checking in relation to an affective state on the other hand. As the intermediate stage at which compulsive behaviours are acquired require anxiety for the behaviour to become compulsive over time, we may have been measuring this initial stage of the development of a compulsive behaviour in the lab. Overall, ACC seemed to be more implicated in behavioural measures of patients, whereas, the SMA appeared more relevant in healthy subjects. This could indicate that SMA may not be functioning normally in OCD patients and is related to more explicit symptom severity, and ACC may be showing a suboptimal compensatory role, as measured more subtly at a behavioural level. Additional support for the possible impairment of SMA in OCD was provided by a positive relationship between SMA Glu levels and OCD symptom severity in both groups. A larger sample size, better control over possible medication effects, and additional multimodal approaches involving functional and connectivity studies would be required to provide more definitive answers about the neural and neurochemical deficits in OCD and the explicit role of each region.

3.3.7. Methodological limitations and future directions

There are several methodological limitations in this study that need to be considered.

3.3.7.1. Image Verification Task

There were three issues that would be addressed if I had to modify the IVT for a third time. The blocks were not counterbalanced and thus some effects such as the high uncertainty influence on checking rates for example, could be somewhat due to performing the task for the first time. However, similar findings, with smaller magnitudes, were also found for Block 4 with high uncertainty. Additionally, contrasting the first and last 20 trials did not show significant differences in checking rates for the first high uncertainty block. Nevertheless, counterbalancing the blocks would still abolish possible confounding effects. The next issue is the inability to take choice difficulty into account, especially considering the role of ACC and SMA in this cognitive decision making. As there were only 40 trials in each block, and there were 4 difficulty levels ranging from 'easy', 'medium easy', 'medium difficult', 'very difficult', and diverse types of objects being used across trials, choice difficulty using computational modelling could not be measured. To modify the task, I would increase the number of trials, and reduce the different types of objects used. Lastly, in order to compensate for the additional number of trials that would be added in accordance to the previous two suggestions, I would remove Block 2 of IVT. Especially because the results were less clear as they resembled somewhat the results from both Block 1 and 3, depending on whether subjects asked for feedback.

3.3.7.2. YBOCS cut-off point

There were differences in the YBOCS cut-off scores used between Study 2 and Study 3, 4 and 5. In Study 2 as it has been challenging to recruit schizophrenia patients, we chose a lower cut-off score such as a mild score of higher than 6 for both schizo-OCS and OCD groups. Whereas, a cut-off score of higher than 12 according to Lewin et al. (2011) was used to select patients for the three final studies, to ensure I could capture neurometabolite changes associated with OCD. Although this discrepancy may have led to different findings in the task performance on the two IVT versions, this seems unlikely considering that the average YBOCS values in the two OCD samples are comparable.

3.3.7.3. 7 MRS study

Although this is the first study using a 7T MRS scanner in OCD patients, enabling us to have additional advantage in measuring GABA, Glu, and Gln separately, there were nevertheless a few limitations in this study. First, due to the exclusion of milder patients with a YBOCS score below 12, and the participation of only two severe patients in the study, the range of symptom severity in our patients was

quite small. Thus, lack of correlations between different measures cannot necessarily be interpreted as a true absence, as we could not cover a wide range of OCD symptom severity in our patient cohort. Nevertheless, lack of more severe patients may have led to intact neuronal integrity in our patients (as measured by normal NAA levels), and the greater likelihood that the differences found may have been due to an OCD diagnosis rather than grey matter degeneration. Despite having tried to interpret the metabolite findings in conjunction with their associations with clinical and behavioural measures, medication effects could still have impacted our results. However, medication could not be both associated with lower GABA/Glu we find in ACC, and the excessive checking rates in our patients. Additionally, cross-sectional studies comparing medicated and unmedicated patients have either failed to find any differences between groups in ACC metabolites (Brennan et al., 2016; Yücel et al., 2007; Yücel et al., 2008), or found lower Glu levels (O'Neill et al., 2016) of OCD patients which is opposite to our findings, and thus could not explain the increased Glu in our study. Nevertheless, these studies have used lower magnetic fields and also failed to measure the metabolite differences found in our study. Thus, a larger sample size and a cross sectional study with medicated and unmedicated patients would resolve possible confounding effects of medication on metabolite ratios measured in our study.

Due to the delay caused in our study by the pandemic, and the deadline for my PhD thesis submission, smaller sample sizes (N = 23, and 24 for HV and OCD groups) were used in this study than initially planned (30 subjects per group). However, the original sample size estimation was based on the available NAA data using 3T studies, rather than the Glu and GABA levels using a 7T scanner.
For NAA, a sample size larger than 29 subjects per group was required at 3T to achieve power of 80% to reject the null hypothesis, whereas, this number was 39 using a 1.5T scanner. If this relationship were to be linear, a sample size of 18-19 would be enough to achieve a power of 80%. Nevertheless, these estimations were for NAA and to measure GABA, and Glu we may need larger sample sizes to achieve the same power, using a 7T scanner. Thus, the study recruitment is still ongoing at the time of writing this thesis to reach our desired sample size of 30 participants per group.

Lastly, one methodological shortcoming in Study 5 was the use of several correlation analyses that could increase the false discovery rates, although the values for the correlations were often high ($p < 0.01$). However, due to the relatively small sample sizes, multiple regression methods, or Structural Equation Modelling, which would be more appropriate for detailed hypothesis testing, were not feasible at this stage. However, after completion of our data collection, a more suitable method will be used according to the collected number of data points to compare the relationships using a single analysis.

3.3.8. Theoretical and clinical implications

Clozapine induced OCS

A few clinical and theoretical implications should be considered. Although there may be different avenues for the development of OCS in schizophrenia, the schizo-OCS patients in Study 2 showed these symptoms only after the start of their clozapine treatment. This was ensured not only during screening, but also by consultation with their psychiatrist (Dr Fernandez-Egea). For example, patients would reveal/disclose that they have started checking only recently, and did not understand why but they felt compelled to check despite knowing it to be senseless, thus, dissociating the OCS from their delusions. According to the findings in Study 2, such as the positive relationship between clozapine dose and dysfunctional checking, it is apparent that clozapine may not be suitable for treating certain groups of schizophrenia patients. Although it is not yet clear how clozapine works due to its complex binding mechanisms (Stahl, 2000), only some schizophrenia patients on clozapine developed OCS, despite being on a similar dosage and having a comparable treatment duration. Moreover, schizo-OCS patients showed more severe clinical and cognitive profiles such as higher positive, depressive, and anxiety symptoms, and a worse executive functional such as higher cognitive flexibility deficits than patients on clozapine without OCS. All the latter findings may be an indication that a biological or genetic factor could be causing a different response in different groups of patients. Indeed, genetic moderations of the serotonergic (HTR 2A/C and SCL6A4) and glutamatergic transmission (GRIN2B) have been observed in schizo-OCS patients (Fernandez-Egea et al., unpublished observations, personal communication).

Lastly, one of my personal observations was that many of schizo-OCS patients are not aware of their symptoms. Even when they sensed an abnormal behaviour, such as sudden increase in checking, they assumed it is related to their schizophrenia symptoms (despite having insight into the irrationality of their behaviour) and would be ashamed to share it with their clinician or family members. For example, one patient disclosed to me that after performing excessive checking behaviour she thought “I am just being weird again”; another patient was embarrassed to share her experience with checking compulsions with anyone and as a consequence only found out about this side effect of clozapine after 12 years of treatment. Therefore, making patients more widely aware of this possible adverse side-effect of clozapine is clearly indicated.

Contingency degradation

SMA function is apparently impaired in patients with OCD and this is reflected in a possible compensatory role of the ACC. However, ACC itself also appears to be malfunctioning and promoting a maladaptive tendency to overrespond in the degradation task. Nevertheless, this overresponding does not appear to be a simple disinhibition of responding, as patients are not showing a similar relationship

for the IVT, since the relationships between checking and ACC metabolites were opposite to that of contingency degradation. More specifically, the increased GABA/Glu levels in ACC were related to increased checking, as opposed to the increased GABA/Glu ratios being related to less responding after the degradation. It may however be related to how actions are processed in relation to their outcomes and is not related to an affective change, and probably distinct from anxiety trait. The question still remains why OCD symptom severity was only correlated with SMA Glu levels, and not those of ACC. Assuming that the deficits in the SMA appear before the hypothetical compensatory involvement of ACC, deficits in ACC may be related to more subtle behavioural changes rather than more explicit symptom severity. There are already studies showing improvements in OCD symptom severity after stimulating the SMA using Repetitive Transcranial Magnetic Stimulation (rTMS; Rostami et al., 2020) or Transcranial direct current stimulation (tDCS; D'Urso et al., 2016; Gowda et al., 2019; Hazari et al., 2016; Mukherjee et al., 2021). Nevertheless, our study provides additional neurochemical evidence that SMA is indeed a good target to alleviate the obsessive compulsive symptoms. Additionally, detecting the obsessive compulsive symptoms early on in OCD development, and stimulating the SMA activity as early as possible may also reduce the ACC impairments later on, in case ACC has indeed taken over a compensatory role to make up for SMA's aberrant functioning. There is evidence showing cognitive inflexibility is present in adult but not adolescent OCD, suggesting that the adult disorder itself may contribute to this (Marzuki et al.,2021). Thus, similarly, a longitudinal study might be useful to test the inter-relationship between ACC and SMA at different time points and whether deficits in ACC only appear after initial SMA impairments and then worsen over time. Another current methodological issue is the lack of clarity on what frequency should best be used to stimulate the SMA for effective treatment (Liang et al., 2021).

Checking behaviour

The IVT was especially sensitive to excessive checking in patients for whom it was their main compulsion, as two patients with OCD with IVT checking rates higher than 3 standard deviations from the mean, showed mostly checking compulsions in real life according to their OCI sub-scores. However, one clear clinical implication of the findings in Study 3 is that despite some clinicians' beliefs concerning different subtypes of OCD (e.g. Mataix-Cols et al., 2004), the heterogenous sample of OCD patients in our study, showed excessive checking behaviour compared to healthy controls, suggesting that checking is a general propensity in OCD patients, although other compulsions (such as washing) may also be their preferred behaviour. In general, this argues for a more unitary account of OCD nosology, but it leaves unanswered the precise aetiology of specific compulsions.

Additionally, as discussed previously, it is important to note that what is being measured using the IVT in the laboratory, is not the clinical symptom checking. What is measured is a task based behaviour, which may be important for gauging processes underlying the acquisition of checking. As suggested by van den Heuvel et al., (2016) (as well as by Robbins et al., 2019), a goal-directed behaviour could become habitual over time in response either to reward or anxiety. If OCD patients experience anxiety, they could check more under conditions of high uncertainty. Thus, the checking behaviour measured in the lab, using the IVT, could provide substrates for the development of checking behaviour, which has not yet become fully compulsive. In order for compulsive checking to develop, repetition of the behaviour over a long period of time may be required for habitual control to occur (as hypothesised for avoidance behaviour in general; see chapter by Gillan et al 2016). According to this account, despite the importance of anxiety during acquisition of avoidance of doubt (i.e. checking), once compulsive behaviour has developed, such anxiety relief may become less relevant to induce compulsions which become autonomous of the goal, due to their habitual nature. How then can one study the aetiology of OCD symptoms in an experimental setting if they only develop over long time periods? Of course, the classic provocation studies are relevant, but they are nonetheless artificial in nature; seeing provocative pictures in the scanner is not the same as performing the behaviour themselves in response to specific environmental contexts. Thus, measuring checking behaviour on a task such as the IVT may be the closest to capturing initial stages in the development of compulsive behaviour.

To conclude, despite gaining a considerable amount of insight into the nature and neural basis of OCD symptomatology, the 5 studies discussed in this thesis were insufficient to provide a fully integrated causal account of this fascinating puzzle. Future multimodal studies using functional, connectivity, and spectroscopy scans in the same well-diagnosed, unmedicated individuals will be required to understand OCD, with theoretically motivated tasks subject to computational analyses, and over a longitudinal time-frame, for example, to learn more about the inter-relationship of changes in SMA and ACC over time.

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