EFFECTS OF MCPP ON COMPULSIVE CHECKING BEHAVIOUR

MCPP MODULATES COMPULSIVE CHECKING BEHAVIOUR IN RATS: NEUROBIOLOGICAL AND BEHAVIOURAL CORRELATES OF A POTENTIAL ROLE FOR SEROTONERGIC STIMULATION IN THE QUINPIROLE SENSITIZATION MODEL OF OBSESSIVE-COMPULSIVE DISORDER (OCD)

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ABSTRACT

The 5-HT agonist drug mCPP contributed to a 5-HT hypothesis of obsessive-compulsive disorder (OCD), but the effects of the drug in human and animal studies have been inconsistent. The objective of this thesis was to shed light on the behavioural and neurobiological effects of mCPP using the quinpirole sensitization rat model of OCD and in a reciprocal manner, to use the drug to further reveal behavioural and neurobiological components of the animal model. The utility in using the quinpirole model is that the process of analysis by experimentation can be employed to observe effects of the drug on three separate behavioural components identified to underlie the model compulsive behaviour: vigor, focus and satiety.

Four original studies were designed to address this objective, and the findings yielded novel contributions to the literature. We suggest that mCPP attenuates compulsive checking by attenuating the exacerbated vigor and satiety characteristic of compulsive behavior, but this effect may not have been captured in previous clinical studies because OCD was measured as a unitary phenomenon across different symptom subtypes. We also reveal that separate systems underlie the development and performance of compulsive behaviour in the animal model, and mCPP reduces its performance but not its development. Hence, the animal model findings suggest that mCPP can attenuate performance of OCD behavior but the drug does not reverse the pathology of OCD or arrest the pathogenesis of OCD. Neurobiologically, we hypothesize that the underlying mechanism mediating the response to mCPP is mediated downstream

of the nucleus accumbens core (NAc), at the substantia nigra pars reticulata, based on the finding that the effects of mCPP on vigor and satiety are present in NAc lesioned animals. Finally, although findings of this thesis indicate that 5-HT2A/C receptors do not mediate the response to mCPP, an oppositional role for DA and 5-HT on the model of compulsive behaviour is proposed, consistent with a security motivation theory of OCD. Overall, this thesis shed new light on the effects of mCPP on OCD, and reveals novel neurobiological and behavioural correlates of the quinpirole model.

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This journey would not be possible without the help and support of a number of important individuals. Foremost, my development as a researcher would not be possible without the kind mentorship provided by my supervisor, Dr. Henry Szechtman. Henry, your knowledge, patience, encouragement, and rigour as a supervisor have helped my development immensely, and I am thankful you welcomed me into your laboratory. To my supervisory committee members, Dr. Fahnestock and Dr. Sakic, your thoughtful care towards my work and development encouraged me to strive for the next level. There are numerous members of the Szechtman Laboratory who contributed their own time towards the studies in this thesis. I know that on many occasions you thanked me for giving you laboratory experience, but in return, you all gave me the chance to hone my supervisory skills while you collected that data. One member of the Szechtman Laboratory in particular, Dawn Graham, deserves a special acknowledgement: Dawn, you truly taught me how to manage what can otherwise become a chaotic environment! To all the past and present members of the MiNDS Program, especially Josh Pinto and Allison Fenney, thank-you for your fellowship and encouragement along the way. To all the support staff, Marianne Hannon, Sandra Murphy, and Bert Visheau, thanks for just being there to help me out when I needed it. I also wish to acknowledge Dr. Joe Gabrielle; when the days grew long and I needed a smile, I knew that Dr. Joe would be just around the corner to make that happen. Finally, thanks to all my friends at the Sunnybrook Research Institute for staying late before a long weekend for my practice defense.

DEDICATIONS

This thesis has been a significant journey for myself, and for those closest to me. To my family and friends, you were always interested to know 'how it was going', and my answers changed like the weather – from frustration to excitement and elation. I know you didn't always understand what I was doing – and sometimes neither did I – but I know that you deeply cared. To my wife Nicola, you have been at my side throughout this entire process. I am grateful for your patience, enthusiasm, and encouragement. I could not imagine a better person to come home to after a day in the lab. To my Mom and Dad, you encouraged me to go to school and achieve all that I can. I remember my days at Dr. Thornton struggling through grade 7. I could never imagine I would be here now. You pushed me to overcome obstacles and reminded me that things happen for a reason – and they did. Mike and Shelley, you gave me your daughter, but it was a while before we could go into the world alone. Thanks for your support and accommodations during my lengthy training period. I am grateful for the care and consideration you showed towards my work. Shannon, thanks for moving out to make room for me and reviewing my lay summaries – there's always sacrifice in the name of science. Finally, to my grandparents, aunts, uncles, cousins, and friends – some who are no longer here – I am glad to have shared this journey with you. Even though we didn't see each other every day, I know that you cared about my progress and were excited to see me finish. I will continue to be there to answer your medical questions. As this journey comes to a close, I thank God for this experience.

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LIST OF ALL ABBREVIATIONS

5-HIAA…….5-hydroxyindoleacetic acid
5-HT…….serotonin
8-OH-DPAT…….8-hydroxy-2-(di-*n*-propylamino)tetralin
ACC…….anterior cingulate cortex
APA…….American Psychiatric Association
CBGTC loops…….cortical-basal ganglia-thalamic-cortical loops
CBT…….cognitive behaviour therapy
CNS…….central nervous system
COMT…….catechol-O-methyltransferase
CSF…….cerebrospinal fluid
DA…….dopamine
DAT…….dopamine transporter
DBS……deep-brain stimulation
DPAT..... 8-hydroxy-2-(di-*n*-propylamino)tetralin
DSM-5…….Diagnostics and Statistics Manual, Fifth Edition
DSM-IV-TR…….Diagnostics and Statistics Manual, Fourth Edition, Text Revision
ERP…….exposure and response prevention
GABA…….*gamma*-Aminobutyric acid
HVA…….homovanilic acid
MAO-A…….monoamine oxidase-A
mCPP…….meta-chlorophenylpiperazine hydrochloride
MDMA.....3,4-methylenedioxy-*N*-methylamphetamine
MK-212.....6-chloro-2-(I-piperazinyl)pyrazine
NA.....nucleus accumbens
NAc…….nucleus accumbens core
NMDA.....N-methyl-D-aspartate
OCD…….obsessive-compulsive disorder
OFC…….orbitofrontal cortex
PBS.....phosphate buffered saline
PANDAS…….paediatric auto-immune neuropsychiatric disorder
PFC…….prefrontal cortex
ROI.....region of interest
RSA…….respiratory sinus arrhythmia
SERT…….serotonin transporter
SMS.....security motivation system
SNr…….substantia nigra pars reticulata
SRI…….serotonin reuptake inhibitor
SSRI…….selective serotonin reuptake inhibitor
YBOCS.....Yale-Brown Obsessive-Compulsive Scale
VTA…….ventral tegmental area

DECLARATION OF ACADEMIC ACHIEVEMENT

I, Mark C. Tucci, with the advice of my supervisor, was the primary contributor to the contents of this thesis. The reader will note that for each of the publications and manuscripts contained in the thesis, I am listed as the primary author. This achievement reflects my role in designing, performing, interpreting, and communicating each of the studies. This work would not be possible, however, without the excellent assistance of contributing co-authors. The detailed contributions by me and each co-author are indicated in the preface to each publication and manuscript.

STRUCTURE OF THESIS

The structure of this thesis takes the form of a sandwich thesis. Chapter 1 is a general introduction providing background information and context for the publications and manuscripts comprising the subsequent Chapters (Chapters 2-5). Here, the objectives, hypotheses and predictions for the forthcoming studies are indicated. An overarching hypothesis tying the studies together is also stated. It should be noted that the rationale for some studies stem from the findings of a previous one. Hence, I try to indicate in the introduction how the results of one study led to new questions that seemed appropriate to address in a subsequent study.

The studies comprising chapters 2-5 are at various stages in the publication process; from published to being submitted for publication. The reader will note that for each of the manuscripts I am the first author reflecting the significance of my contributions to the studies.

Chapter 6 is a general discussion on how the results of the preceding studies addressed the objectives and hypotheses set forth in the introduction. Implications for the findings are discussed, followed by an analysis of the outcome for the overarching hypothesis. The thesis is concluded with a final comment reiterating the contributions of the novel findings to the literature.

CHAPTER 1: INTRODUCTION

Obsessive-compulsive disorder (OCD) is a psychiatric affliction that negatively affects the quality of life for patients suffering with the disorder (Hou, Yen, Huang, Wang, & Yeh, 2010), family members, and caregivers (Grover & Dutt, 2011). Living with OCD is also associated with impairments in social, work, and regular activities (Torres et al., 2006). The DSM-IV-TR describes obsessions as recurrent, relatively persistent thoughts, images, or impulses; compulsions are described as repetitive behaviours or mental acts usually performed as a response to an obsession, or as a set of rigid rules (*Diagnostic and statistical manual of mental disorders: DSM-IV*, 1994). Patients with OCD are usually aware that their obsessions or compulsions are excessive and unreasonable (*Diagnostic and statistical manual of mental disorders: DSM-IV*, 1994). Until recently, OCD was categorized as an Axis 1 anxiety disorder (*Diagnostic and statistical manual of mental disorders: DSM-IV*, 1994). However, the revised edition of the DSM – the DSM-5 (*Diagnostic and statistical manual of mental disorders: DSM-5*, 2013), altered the traditional categorization for OCD and related disorders into a new, independent chapter (American Psychiatric Association., 2013). The American Psychiatric Association (APA) indicates that this revision reflects a growing body of evidence that OCD and related disorders are relatively different from other anxiety disorders (American Psychiatric Association., 2013).

Recent epidemiological studies estimate a lifetime prevalence for OCD of 2-3% (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012; Ruscio, Stein, Chiu, &

Kessler, 2010). OCD afflicts both males and females and can strike during child or adult years (Graybiel & Rauch, 2000; Maia, Cooney, & Peterson, 2008), albeit somewhat differently. In children, there is a greater prevalence of OCD in males, while in adults, the higher prevalence shifts to females (Graybiel & Rauch, 2000; Ruscio et al., 2010). The overall mean age of onset for OCD is approximately 19.5 years (Ruscio et al., 2010). The prevalence of comorbid conditions diagnosed in patients with OCD is also strikingly high. Approximately 65% are diagnosed with another Axis 1 disorder; most frequently, with major depressive disorder (Hasler et al., 2005; Tukel, Polat, Ozdemir, Aksut, & Turksoy, 2002).

The content of obsessions and compulsions can vary dramatically – from concerns regarding orderliness or religious thoughts, to the exaggerated performance of checking, washing, or hoarding behaviour to name just a few (Heyman, Mataix-Cols, & Fineberg, 2006; Stein, 2002). Studies indicate that compulsive checking behaviour is the most prevalent subtype of OCD (Ball, Baer, & Otto, 1996; Rasmussen & Eisen, 1992). The following excerpt provides a good description of the behavioural performance of an individual with a checking compulsion as they describe turning on the television:

> Before I start to turn it on, I have to wash and dry my hands. Then I go and touch the corner curtain followed by touching the side of the TV two times. Then I have to go back and wash my hands. When I am finished with that I will look behind the lamp 2 times, go back and wash my hands, come back, move the lamp to the left and look behind it, move the lamp two times to the right and look behind it, go back, wash my hands, and then look in back of the TV on the left 4 times, washing my hands in between each one. Then I look in back of the TV on the right 8 times, wash my hands, and put the TV on channel 6. Then I turn the knob from channel 6 to 7, 4 times, and from channel 6 to channel 8, 4 times. Then finally I turn it on. The whole thing probably takes around half an hour (Rasmussen & Eisen, 1991, p. 28)

As can be gleaned from this excerpt, the performance of compulsive checking appears relatively organized in terms of spatial-temporal structure, and is characterized by distinct motor routines or rituals. Given the heterogeneity of compulsive symptoms, some suggest that studying the spatial-temporal *form* of OCD behaviour versus the *content* may provide a better understanding of the mechanisms underlying the disorder (Reed, 1985). As will be discussed later, measuring the form of compulsive behaviour has been employed in an animal model to study the mechanisms of OCD-like behaviour.

Theories of OCD

There are a number of theories that attempt to account for the development and maintenance of OCD. Here, the most prominent theories are surveyed. Of the cognitive-behavioural theories, some suggest that OCD is a result of 'erroneous cognitions' (Franklin & Foa, 2011). That is, normal appraisal of the danger and outcome of objects or situations is negatively exaggerated. For example, touching a doorknob will result in the spread of disease to the individual or those they in turn may touch (Franklin & Foa, 2011). Along this line of reasoning, lack of evidence for safety is perceived to indicate danger for individuals with OCD, while individuals without the affliction view the absence of evidence for danger to indicate that something is safe (Franklin & Foa, 2011). For example, an individual with a contamination obsession may avoid eating at a restaurant because they cannot readily observe the cleanliness of the kitchen (Franklin & Foa, 2011). Others suggest that obsessions provoke negative automatic thoughts, particularly thoughts of self-blame and an exaggerated sense of responsibility (Franklin &

Foa, 2011). As a result of these negative thoughts, individuals with OCD attempt to 'neutralize' them by performing mental or behavioural compulsions (Franklin & Foa, 2011). Using the above example of the supposed individual who is obsessed with the idea of spreading a disease acquired by touching a doorknob, they may wash their hands repetitively, and hence in a compulsive manner, to neutralize the sense of responsibility.

OCD has also been conceptualized in a motivational framework (Boyer & Lienard, 2006; Szechtman & Woody, 2004). OCD behaviour is often directed toward stimuli related to safety, security, or well-being of the species (Reed, 1985). As such, behaviour directed towards such stimuli may reflect a *special motivation* (Szechtman & Woody, 2004). Special motivations are biologically derived motivated behaviour directed towards stimuli related to well-being or survival of the species; for example: hunger, thirst, and sex (Hebb, 1966). According to Szechtman and Woody (2004), security-related behaviours may then reflect a special kind of motivated behaviour directed at preservation of self or the species. A 'security motivation system' (SMS) is thus tuned to activate such behaviours in response to potential threat, that is, a potential danger that is not readily detectable. In considering what shuts down the activated security motivation, the existence of environmental cues signalling the absence of danger may not necessarily be present, and thus, cannot be relied upon. For example, a predator may not be visible to its prey because it is hiding behind an object waiting to pounce. Hence, it is suggested that something else shuts down the SMS and corresponding behaviour. According to the authors, the signal that shuts down security motivation is an internally generated 'feeling of knowing'. This feeling of knowing, also termed 'satiety',

shuts down the species typical behaviours. However, in the absence of generating a feeling of knowing, the performance of behaviour continues unabated, giving rise to OCD behaviour. Hence, OCD behaviour is considered to reflect a 'stopping' problem rather than a 'starting' problem with respect to its behavioural performance. This motivational framework for OCD is known as the 'security motivation theory' (Szechtman & Woody, 2004).

Recently, a study by Hinds and colleagues (2012) asked whether the performance of OCD behaviour could be explained by an abnormally high activation of performance reflective of a starting problem, or a dysfunction in the shut down of performance reflective of a stopping problem, and hence, consistent with a security motivation theory for OCD. Findings showed that individuals with a washing compulsion did not differ from controls on the initial activation of symptoms following exposure to a potential threat, however, differed from controls on their ability to shut down the activated behaviour (Hinds, Woody, Van Ameringen, Schmidt, & Szechtman, 2012). Such observations are consistent with a security motivation theory of OCD, lending empirical support for this explanation of OCD behaviour.

Neurobiology of OCD

Neurobiological correlates of OCD have been reported using a number of different study designs and techniques in both human and animal subjects. A great deal of evidence and agreement amongst researchers suggests that OCD probably involves hyper-activity in a series of cascading, partially segregated parallel 'loops' running

through the basal ganglia (Aouizerate et al., 2004; Baxter, 1992; Graybiel & Rauch, 2000; Huey et al., 2008; Insel & Winslow, 1992; Modell, Mountz, Curtis, & Greden, 1989; Saxena, Bota, & Brody, 2001; Stein, 2002; Szechtman & Woody, 2004; Vermeire et al., 2012; Wise & Rapoport, 1989). Several loops have been identified; however a general schema may be used to account for the basic circuitry of each loop (Alexander, DeLong, & Strick, 1986): information from separate cortical regions is sent to partially overlapping areas in the striatum. This information becomes further integrated as it is relayed to the pallidum/substantia nigra (pars reticulata), and finally to the thalamus. Partially closing the circuit, the information is sent back to one of the cortical areas it originated from. Hence, such loops may be referred to as cortical-basal ganglia-thalamic-cortical loops, or CBGTC loops (Alexander et al., 1986; Maia et al., 2008).

Several separate clusters of nuclei make up the structures of the basal ganglia: the striatum, (comprised of the caudate, putamen, and nucleus accumbens), globus pallidus, substantia nigra, and subthalamic nucleus (Parent & Hazrati, 1995). The basal ganglia have been implicated in a number of behavioural functions ranging from emotion to motor control (Saint-Cyr, Taylor, & Nicholson, 1995). There is some agreement that the basal ganglia selects which of several possible behavioural actions to release at a given time (Chakravarthy, Joseph, & Bapi, 2010; Stocco, Lebiere, & Anderson, 2010).

Neuroanatomical correlates of OCD

In general, imaging studies performed on patients with OCD have revealed several neuroanatomical structures that are inherent components of CBGTC loops which show altered activity during rest, symptom provocation, and normalization following

therapeutic intervention. In particular, these regions are: orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), and the basal ganglia (Graybiel & Rauch, 2000; Maia et al., 2008). The OFC plays a role in decision making and planning, while the ACC seems to be involved in affective and motivated behaviour (Graybiel & Rauch, 2000). Other studies reported alterations in the volume of structures comprising these circuits. Overall, results seem to indicate a reduction in volume of the OFC, an increase in volume of thalamus, and inconsistent findings with respect to volume of the striatum (Maia et al., 2008). Another study analysing reward processing in patients with OCD reported decreased activity in the nucleus accumbens of patients with OCD which was correlated with deficits in reward anticipation (Narayanaswamy, Jose, Kalmady, Venkatasubramanian, & Reddy, 2013). The nucleus accumbens is suggested to play a role in reward processing (Knutson, Adams, Fong, & Hommer, 2001), a function identified to be dysfunctional in some patients with OCD (Figee et al., 2011). It should be noted though, that dysfunctions in brain regions not necessarily a part of CBGTC loops have been identified; for example in the amygdala and hippocampus (Maia et al., 2008). However, such regions may modulate the function of inherent CBGTC structures. For example, the amygdala projects to the mediodorsal nucleus of the thalamus (Maia et al., 2008).

Studies using animals to model compulsive-like behaviour (herein termed compulsive behaviour for simplicity) also report brain regions associated with CBGTC circuitry to mediate the model compulsive behaviour. For example, it has been reported that lesions to the OFC in rats produce an increase in compulsive lever-pressing

behaviour (Schilman, Klavir, Winter, Sohr, & Joel, 2010). Dvorkin et al. (2010) report that lesions to the lateral OFC of rats alters attention or *focus* on the task of checking such that animals are more likely to stay away from objects/places that elicit checking behaviour. In the same study, the authors showed that lesions to the nucleus accumbens core subregion (NAc) produced an activating or energizing effect on the motor performance of checking which appeared to reflect an increase in the *vigor* of checking performance. Other studies using rats where high-frequency deep brain stimulation (DBS) was performed on the nucleus accumbens (core or shell subregions) report decreases in the performance of compulsive checking behaviour (Winter et al., 2008). For DBS procedures, electrical stimulation is directed at a given neuronal target (de Koning, Figee, van den Munckhof, Schuurman, & Denys, 2011). Using a mouse model of excessive grooming to study compulsive behaviour, it was found that repeated hyperactivation of OFC-ventralmedial striatal stimulation increased the model compulsive behaviour (Ahmari et al., 2013). Studies in dogs with canine compulsive disorder (characterised by excessive grooming, pacing, predatory behaviour) revealed alterations in the volume of brain structures similar to those implicated in the human disorder: lower dorsal ACC and right anterior insula volumes, and an increase in overall gray matter volume (Ogata et al., 2013). Overall, brain regions identified in animal models of compulsive behaviour thought to be homologous in structure and function to humans also appear to mediate OCD behaviour.

Neurochemical correlates of OCD

Serotonin (5-HT) is probably one of the most studied neurochemicals related to OCD. 5-HT is a neurotransmitter in the central nervous system (CNS) and peripheral nervous system (Dutton & Barnes, 2008). Almost all central 5-HT producing neurons are situated in the raphe nuclei, located in the brainstem (Dutton & Barnes, 2008). These neurons project extensively throughout the brain such that almost all neural regions receive 5-HT innervation (Dutton & Barnes, 2008). Within the mammalian CNS, there are currently 14 different 5-HT receptor subtypes, divided into 7 families, of which the majority are G-protein coupled (Dutton & Barnes, 2008). 5-HT is implicated in many processes including: mood, sleep, aggression, cognition, memory, feeding, and in psychiatric disorders such as: depression, schizophrenia, and OCD (Dutton & Barnes, 2008).

Early challenge studies using 5-HT agents such as meta-chlorophenylpiperazine hydrochloride (mCPP) performed on patients with OCD contributed to a 5-HT hypothesis of OCD (Murphy et al., 1989). mCPP is a non-selective 5-HT agonist drug that was first reported to exacerbate symptoms in patients with OCD (Zohar, Mueller, Insel, Zohar-Kadouch, & Murphy, 1987). However, further studies using this drug yielded relatively equivocal results, with mCPP exacerbating symptoms in some studies (Broocks et al., 1998; Gross-Isseroff, Cohen, Sasson, Voet, & Zohar, 2004; Hollander et al., 1991; Pigott et al., 1993), while failing to alter symptoms in others (Charney et al., 1988; de Leeuw & Westenberg, 2008; Goodman et al., 1995; Ho Pian, Westenberg, den Boer, de Bruin, & van Rijk, 1998; Khanna, John, & Reddy, 2001; Pigott et al., 1993). Further contributing

to a 5-HT hypothesis of OCD were studies demonstrating that drugs with potent 5-HT reuptake inhibition properties as opposed to inhibition of other transmitters systems (e.g. norepinephrine) produce a therapeutic effect in patients with OCD (Micallef & Blin, 2001).

However, studies performed on patients with OCD are less clear on the nature of 5-HT dysfunction. For example, levels of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of 5-HT, are reported to be higher in the cerebrospinal fluid (CSF) of un-medicated patients with OCD compared to healthy controls, however, other studies report no difference (Westenberg, Fineberg, & Denys, 2007). Additionally, peripheral markers of 5-HT function have been studied. Blood and platelet levels of 5-HT are relatively normal in patients with OCD (Westenberg et al., 2007).

A greater understanding for the role of specific 5-HT receptors in OCD may be gleaned from pharmacological studies performed on patients with OCD. Activation of the 5-HT1A receptor does not seem to alter OCD symptoms (Blier & Bergeron, 1996; Lesch, Hoh, Schulte, Osterheider, & Muller, 1991). Reports from the literature indicate that stimulation of the 5-HT1D receptor produces inconsistent results; some report that stimulation exacerbates OCD symptoms (Koran, Pallanti, & Quercioli, 2001), while others report that stimulation had no effect (Pian, Westenberg, van Megen, & den Boer, 1998), or decreased OCD symptoms (Zohar, Kennedy, Hollander, & Koran, 2004). One study reported that in some patients 5-HT1D receptor stimulation exacerbated symptoms while in other patients it decreased symptoms (Stein et al., 1999). Concerning 5-HT2 receptors, some have reported a therapeutic effect on OCD symptoms from stimulation of

5-HT2 receptors (Leonard & Rapoport, 1987; Moreno & Delgado, 1997). However, others have suggested that selective antagonism of the 5-HT2A receptor as an adjunct to selective serotonin reuptake inhibitor (SSRI) treatment yields an enhancement to the therapeutic effects of SSRIs alone (Marek, Carpenter, McDougle, & Price, 2003). Hence, there may be a role for 5-HT2 receptors, but it remains unclear. Finally, the literature indicates that blockade of the 5-HT3 receptor subtype may yield an enhancement to the therapeutic effects of SSRI treatment (Askari et al., 2012; Pallanti, Bernardi, Antonini, Singh, & Hollander, 2014). Overall, it seems as though various 5-HT receptor subtypes modulate OCD in humans, but to some extent the effects remain unclear.

Other studies performed using animals are consistent with a 5-HT hypothesis of OCD. For example, in a model of compulsive checking behaviour, treatment with the serotonin reuptake inhibitor clomipramine attenuated compulsive checking behaviour (Szechtman, Sulis, & Eilam, 1998). Other studies report a role for some of the same specific 5-HT receptors in model compulsive behaviour as those implicated in the human disorder. Using the reinforced spatial alternation model of OCD, 5-HT2C receptor antagonism reduced directional persistence induced by the non-selective 5-HT agonist drug mCPP (Papakosta et al., 2013). In this model, directional persistence is meant to reflect exaggerated behavioural performance related to compulsions in the human disorder (Tsaltas et al., 2005).

A role for dopamine (DA) in OCD has relatively recently emerged and is gaining more support based on the reports of a number of studies. DA is a neurotransmitter which may bind to one or more of several DA receptor subtypes (DA1-5) which are G-

protein coupled (Beaulieu & Gainetdinov, 2011). There are a number of DA pathways in the mammalian brain (Beaulieu & Gainetdinov, 2011; Bjorklund & Dunnett, 2007), however two prominent pathways are the nigrostriatal pathway which originates in the substantia nigra pars compacta and projects to the striatum, and the mesolimbic and mesocortical pathways which project from the ventral tegmental area (VTA) to limbic structures and the cerebral cortex, respectively. DA modulates processes ranging from movement to reward, and psychiatric afflictions such as Parkinson's disease, Huntington's disease, Tourette's syndrome (Beaulieu & Gainetdinov, 2011) and OCD (Fineberg, Brown, Reghunandanan, & Pampaloni, 2012; Szechtman, Culver, & Eilam, 1999; Westenberg et al., 2007).

A putative dysfunction in DA signalling in OCD stems from observations that patients with neurological disorders such as Parkinson's disease and Tourette's syndrome who displayed OCD-like behaviour benefited from pharmacotherapy with DA blocking agents (Micallef & Blin, 2001; Zohar, Chopra, Sasson, Amiaz, & Amital, 2000). Augmentation of SSRI pharmacotherapy with DA blocking drugs produces a therapeutic effect in some patients who do not respond to SSRI treatment alone (Fineberg et al., 2012). For the most part, atypical antipsychotic drugs are used as an augmentation strategy (Fineberg et al., 2012).

In a similar manner to the findings of neurochemical studies of markers of 5-HT function in patients with OCD, studies of DA markers do not yield a clear dysfunction in DA signalling. For example, levels of homovanilic acid (HVA), which is the major metabolite of DA, are not found to be different in the CSF of patients with OCD after

drug treatment, or, in un-medicated patients with OCD compared with healthy controls (Westenberg et al., 2007).

Challenge studies using DA agents, however, led to the suggestion that an increase in DA activity may be related to OCD. Cocaine challenge induces OCD-like symptoms and exacerbates symptoms in patients with OCD (Westenberg et al., 2007). Cocaine acts as a DA blocker, resulting in an increase in DA activity and the density of the DA transporter (DAT) (Little et al., 1999). Other drugs which increase DA activity such as amphetamine and methylphenidate both increase and decrease symptoms in patients with OCD, respectively (Westenberg et al., 2007). It must be noted though that in addition to DA signalling, all of these drugs alter activity of the 5-HT system, so the nature of the role of DA in the pathophysiology of OCD is not entirely clear (Westenberg et al., 2007). However, studies using radioligand binding and imaging techniques generally show that the density of the DAT is higher, and, that there is a down-regulation of the DA D2 receptor in the basal ganglia of patients with OCD (Westenberg et al., 2007). This alteration may reflect in the basal ganglia an increase in the concentration of DA, and hence, is in line with the suggestion that DA activity is higher in OCD (Westenberg et al., 2007).

Animal studies that model compulsive behaviour also implicate a role for DA in OCD. In rats, compulsive checking behaviour is induced by chronic administration of the DA D2/3 receptor agonist drug quinpirole and this preparation has been proposed as 'the quinpirole sensitization model of OCD' (Szechtman et al., 1998). As will be discussed shortly, the quinpirole model is a relatively good model to probe the neurobiological and

behavioural components of OCD. Quinpirole treatment induces compulsive behaviour in another rat model of OCD. In this model, rewarded alternation in a T-maze is used to measure directional persistence in quinpirole and saline treated animals. Quinpirole treated animals tend to show a reduction in alternation and increase in directional persistence, a behaviour considered to be exaggerated as it relates to compulsive behaviour (Kontis et al., 2008). The results of these studies further suggest a role for increased DA activity in OCD.

Given evidence suggesting that both 5-HT and DA neurotransmitter systems may be implicated in OCD, the possibility exists that an interaction between these systems may underlie the disorder (Goodman et al., 1990; Westenberg et al., 2007; Zohar et al., 2000). It has been shown that stimulation of either 5-HT neurons or DA neurons can modulate the release of transmitters of the opposite system. For example, stimulation of 5-HT2A receptors localized in the prefrontal cortex (PFC) of rats produces an increase in the release of DA in the prefrontal cortex (PFC) and the VTA (Bortolozzi, Diaz-Mataix, Scorza, Celada, & Artigas, 2005). Furthermore, atypical antipsychotics can alter in addition to dopamine signalling, 5-HT signalling (Westenberg et al., 2007). A putative mechanism for how antipsychotic drugs may reduce OCD symptoms through a DA-5-HT interaction has been suggested. For example, Westenberg and colleagues (2007) indicate that hyperactivity in DA circuits projecting to the cortex and subcortical areas may produce symptoms of OCD. In addition to effects at DA D1/2 receptors, blockade of 5-HT2A and stimulation of 5-HT1A receptors at the prefrontal cortex by antipsychotics may dampen cortical output to subcortical areas, yielding a reduction in OCD symptoms.

Animal models, for the most part, have not assessed a putative 5-HT-DA interaction in OCD behaviour. Some studies show that the model compulsive behaviour is sensitive to both 5-HT and DA manipulations. The quinpirole sensitization model, where compulsive checking behaviour is induced by stimulation of DA D2/3 receptors, reports a transient attenuation of the model compulsive behaviour following stimulation with the serotonin reuptake inhibitor (SRI) clomipramine (Szechtman et al., 1998). However, it is unclear whether this effect is mediated by 5-HT receptors or receptors of another system given that SRIs are not selective for 5-HT (Micallef & Blin, 2001).

Other neurobiological correlates of OCD

Other neurobiological substrates have been studied for their potential role in OCD as well. Although less extensive than studies of 5-HT and DA systems, such reports warrant mention. A growing amount of evidence is shedding light on a putative dysfunction in glutamate neurotransmission. Glutamate is the main excitatory neurotransmitter in the brain, and is also found within CBGTC circuitry (Pittenger, Bloch, & Williams, 2011). A number of studies using various techniques seem to show an increase in glutamate neurotransmission in patients with OCD (Pittenger et al., 2011). However, an understanding of how glutamate dysregulation may mediate OCD is still relatively unknown.

The immune system is also implicated in OCD. Such findings have come largely from reports identifying children who developed acute OCD symptoms following streptococcal infection, an occurrence referred to as paediatric auto-immune neuropsychiatric disorder associated with streptococcal infection (PANDAS) (Graybiel &

Rauch, 2000; Stein, 2002). Although the immune mechanism(s) responsible are not well known, some studies report an increase in the B lymphocyte antigen D8/17 in patients with PANDAS (Stein, 2002).

OCD is also identified to run in families, indicating the possibility of a genetic component. Genetic studies of OCD, however, are still relatively early and have yielded inconsistent findings. Nevertheless, there seems to be some indication that functional genetic polymorphisms may contribute to the development of OCD. Putative sexually dimorphic associations related to low activity in catechol-O-methyltransferase (COMT) are reported (Stein, 2002). COMT is an enzyme which is involved in the degradation of catecholamines such as the neurotransmitter DA. Additionally, sexually dimorphic associations are reported with an allele of the monoamine oxidase-A (MAO-A) gene (Stein, 2002). MAO-A is involved in the degradation of amine neurotransmitters such as 5-HT and DA. However, further work needs to be done to shed light on the nature of gender differences.

Treatment of OCD

There are a number of different treatment strategies for OCD, but two interventions are most commonly employed in clinical practice (Bjorgvinsson, Hart, & Heffelfinger, 2007): exposure and response prevention (ERP) and cognitive behavioural therapy (CBT). In ERP therapy, the patient creates a 'fear hierarchy' of stimuli that provoke OCD symptoms. The patient, with the help of a therapist, gradually engages in provocation of OCD symptoms listed in the hierarchy (e.g. turning off a tap at the sink),

and is then prevented from performing their typical response (e.g. checking that the tap is off). At first, the patient experiences an increase in anxiety, however, the goal of the therapy is for the patient to realize that the anxiety will decrease on its own without performance of ritualistic behaviour. In accordance with cognitive theories of OCD where pathological behaviour results from erroneous beliefs or appraisals of stimuli or situations, patients receiving CBT learn to correct such erroneous beliefs or appraisals of stimuli that provoke OCD. By correcting erroneous beliefs or appraisals, pathological behaviour usually performed to neutralize such thoughts (e.g. compulsions) is also corrected.

First-line pharmacotherapy for OCD is generally with selective serotonin reuptake inhibitors (SSRIs) (Fineberg et al., 2012). SSRIs are drugs that block the serotonin transporter (SERT), increasing levels of 5-HT at the synaptic cleft to bind to receptors (Schloss & Williams, 1998). Drugs with potent SSRI properties such as fluvoxamine, sertraline, fluoxetine, paroxetine, and citalopram, have been reported to reduce OCD symptoms in patients (Fineberg et al., 2012; Marazziti & Consoli, 2010).

For patients who do not experience a reduction in OCD symptoms following a course of pharmacotherapy with SSRI's, drugs of the antipsychotic class such as risperidone and haloperidol may be used as an augmentation strategy (Fineberg et al., 2012). These drugs have potent DA blocking effects. Drugs modulating other transmitter systems such as the glutamatergic system, and, direct serotonin agonists and antagonists are also being studied for their potential in treating OCD, however their effects are not entirely clear (Fineberg et al., 2012).

Reserved for more severe cases of OCD where pharmacotherapy fails to yield a therapeutic response, deep brain stimulation (DBS) and psychosurgery may be performed, however, these procedures are still considered to be relatively experimental. As discussed above, for DBS procedures, electrical stimulation is directed at targeted areas within the brain; targets are generally chosen based on the type of OCD symptoms the patient experiences (de Koning et al., 2011). Some typical targets for DBS that produce a therapeutic effect are the anterior limb of the internal capsule and nucleus accumbens (de Koning et al., 2011). For psychosurgery procedures, the neuronal connections between brain regions believed to mediate OCD are severed (Micallef & Blin, 2001). Procedures such as cingulotomy and leucotomy show promising results, but the potential for severe adverse effects exists (Marazziti & Consoli, 2010).

Animal models of OCD

Studies performed on patients with OCD in the past have provided important insights into the disorder, and such studies will continue to do so in the future. However, there are certain limitations inherent to human clinical studies, for example, invasively manipulating brain regions thought to mediate the disorder. To this end, we may turn to using animal studies. Animal models may be used to study the underlying neurobiology of a disorder, or as screening tools for potential therapeutics. Accordingly, a number of animal preparations have been proposed to model OCD. A comprehensive review of current animal models of OCD has been reported by Albelda and Joel (2012) and Joel (2006). The following is a brief survey of some of these animal models.

There are several genetic mouse models of OCD: Hoxb8 mutant mice (Greer & Capecchi, 2002); 5-HT2C knockout mice (Chou-Green, Holscher, Dallman, & Akana, 2003), and; *Sapap3*-mutant mice (Welch et al., 2007). Overall, genetic models are not necessarily based on a genetic component of OCD that has been identified in the human, but rather, mice with these genetic manipulations express behaviour considered to reflect human OCD behaviour (Albelda & Joel, 2012). For example, Hoxb8 mutant mice and *Sapap-3* mutant mice display excessive grooming considered to reflect the excessive grooming by patients with trichotillomania related to OCD (Albelda & Joel, 2012; Greer & Capecchi, 2002; Joel, 2006a; Welch et al., 2007). However, some genetic models such as the Hoxb8 and 5-HT2C knockout mouse models have been criticized for the presence of behaviours not specific to, or unrelated to, OCD (Albelda & Joel, 2012; Joel, 2006a).

Other models of OCD are based on naturally occurring repetitive or stereotypic behaviours, innate motor behaviours, and displacement behaviours (Albelda & Joel, 2012; Joel, 2006a). For example, excessive lever pressing displayed by rats in the 'signal attenuation model' is suggested to be a result of a deficit in feedback from normal goal-directed behaviour (Joel, 2006b). According to the author (Joel, 2006b), excessive or 'compulsive' lever pressing is suggested to reflect the excessive and unreasonable nature of human compulsions. Strengths of this model are suggested to be good construct validity given that a deficient feedback mechanism is suggested to underlie OCD, and good predictive validity given that the model compulsive behaviour responds to SSRIs but not to tricyclic antidepressants (Albelda & Joel, 2012; Joel, 2006a; Joel, 2006b). In another behavioural model, the spontaneous stereotypic performance of vertical jumping,

backward somersaulting and patterned running by deer mice is suggested to reflect the stereotypic performance of OCD behaviour (Korff, Stein, & Harvey, 2008). Good predictive and construct validity of the model is indicated by a reduction in stereotypic behaviour by SSRI treatment but not by treatment with a tricyclic antidepressant, and demonstrated roles for DA and 5-HT systems in the model compulsive behaviour, respectively (Albelda & Joel, 2012).

In yet other models of OCD, the model compulsive behaviour is induced by pharmacological manipulations (Albelda & Joel, 2012; Joel, 2006a). For example, Tsaltas and colleagues (2005) have suggested that the directional persistence induced by mCPP in a reinforced alternation task reflects an exaggeration of normal behaviour and hence, 'compulsive' behaviour. A strength of this model is suggested to be good predictive validity given that the model compulsive behaviour responds to SSRI treatment but not to treatment with a tricyclic antidepressant or anxiolytic (Albelda & Joel, 2012). However, the effect of mCPP on directional persistence appears to be transient given that it tolerates after several treatments (Kontis et al., 2008; Tsaltas et al., 2005). In another pharmacological model employing mCPP, it has been reported that the drug induces ritualistic chewing behaviour which is suggested to reflect the ritualistic performance of behaviour by individuals with OCD (Kreiss et al., 2013). This model is relatively new and not much else is known about it. In yet another pharmacological model of OCD, compulsive 'checking' is induced in rats by chronic sensitization with the DA D2/D3 receptor agonist drug quinpirole and exposure to a novel environment (Dvorkin,

Perreault, & Szechtman, 2006; Szechtman et al., 1998). This model is employed in the present thesis, and the following is a detailed account of the model.

Quinpirole sensitization rat model of OCD

A disorder such as OCD with such a rich heterogeneity of symptoms, cognitive components, and extensive neurobiology poses a significant challenge to the study of the disorder using an animal model. Each of the animal models discussed above possess their own strengths and weaknesses of which a detailed analysis is beyond the scope of this thesis. However, one common observation across most of the models is that the behaviour considered to reflect OCD does not possess much face validity. Consider for example the excessive lever pressing or stereotypic backwards somersaults displayed by animals in other models. These behaviours were suggested to reflect compulsive behaviour because their performance is excessive and unnecessary. These qualities, that is, unnecessary and unreasonable behavioural performance, could conceivably be attributed to almost any type of behaviour, and not necessarily behaviour that more accurately reflects OCD. An animal preparation that models a specific subtype of OCD may be useful for cross-species comparisons, and hence strengthen the generalization and implications for findings.

The suggestion that the *form* of compulsions constitute analysis of compulsive behaviour (Reed, 1985) allows for the empirical study of such behaviour across species (Szechtman et al., 1998). One such model which measures the compulsive-like behaviour of rats by analyzing the spatial-temporal structure of behavioural performance and

reflects a specific subtype of OCD, that is, compulsive checking, is the 'quinpirole sensitization rat model of OCD' (Dvorkin et al., 2006; Szechtman et al., 1998). The induction of compulsive checking behaviour in the quinpirole model requires two components: one is exposure to a novel environment (a large open field), and the second is chronic, bi-weekly, treatment with the DA D2/3 receptor agonist drug quinpirole immediately before exposure to the novel environment for the development of behavioural sensitization (Szechtman et al., 1998).

The following describes the quinpirole model developed by Szechtman and colleagues (1998), and how it is a useful animal model to study compulsive checking behaviour. In the rat, compulsive checking behaviour is characterized by a preoccupation with, and reluctance to leave one locale in the environment to which the animal returns to repeatedly. This locale, termed the 'key locale' or 'key place' (these terms may be used interchangeably) is almost always the location where the animal spends the longest cumulative duration of time. For the rat, this key place is referred to as its 'home base'. When exposed to a novel environment, the rat will form one, or sometimes two, home bases (Eilam & Golani, 1989). The spatial-temporal structure of locomotor performance in the environment is organized around the home base, and further, grooming, rearing, and crouching behaviours are often highest at the home base (Eilam & Golani, 1989). As such, the home base may be viewed as a familiar locale or place of security. This view is consistent with reports that grooming and crouching behaviour indicate emotional relief in the rat (van Erp, Kruk, Meelis, & Willekens-Bramer, 1994). Interestingly, quinpirole-

treated animals do not show grooming or crouching behaviours at the home base, suggestive of the absence of emotional relief.

Given the salient nature of the home base to the rat, it seems reasonable that the performance of checking behaviour in the rat would be organized around its home base. As such, a visit to this key place is referred to as a 'check' or 'checking'. In order to empirically measure the performance of compulsive checking behaviour in rats, a set of four criteria measures were derived based on the spatial-temporal features of human compulsive behaviour; that is, a preoccupation with and reluctance to leave the object or place being checked, and, a ritual-like quality to motor performance related to checking. Hence, the following four parameters of checking are measured in the animal model: 1) frequency of checking (total number of visits to the key place; 2) length of check: total duration of stay at the key place divided by the total frequency of visits (shorter durations of stay in the key place are also an indirect index of ritual-like behaviour as quinpirole-treatment is associated with the display of motor rituals; 3) recurrence time of checking: mean duration of return time to the key place (return time is the interval between departure and arrival to the key place), and; 4) number of stops before returning to check: mean number of other places visited before returning to the key place. For animals to be considered showing 'compulsive' checking behaviour, quinpirole-treated animals must differ significantly from saline-treated control animals on all four measures. As a result, the measures are referred to as 'criteria measures' for compulsive checking. Quinpirole-treated animals typically show a significantly increased frequency of checking, and

decreased length of check, recurrence time of checking, and number of stops before returning to check compared to saline-treated control animals.

The criteria measures for compulsive checking behaviour have been empirically dissociated into a set of constitutive 'functional components' providing a rich set of parameters by which compulsive checking behaviour can be studied in the rat (Dvorkin et al., 2010). According to Dvorkin et al. (2010) at least three functional components are observed. The first is an increase in the *vigor* by which compulsive checking is performed (as indexed by an increase in the 'frequency of checking' and decrease in 'length of check'). Lesion to the nucleus accumbens core (NAc) appears to increase this component. As discussed previously, the nucleus accumbens is reported to mediate the value of reward, which may produce an activating effect on motivated behaviour (Robbins & Everitt, 2007). In the case of compulsive checking, this may be related to the checked place/object. Hence, animals appear to display an increase in the vigor of checking performance. The second is a heightened *focus* on the task of checking (as indexed by a decrease in the 'recurrence time of checking' and 'number of stops before returning to check'). Lesion to the OFC appears to decrease this component. As discussed previously, the OFC is reported to be involved in decision making, and in a related manner, to compute the value of a potential future reward (Wallis, 2007). As it relates to checking, the OFC mediated response may be to focus behavioural performance on obtaining the reward; i.e. checking the place/object. In order to be considered showing either of these components, both measures related to the components must be exaggerated compared with controls. The third functional component is a reduced amount of rest, or

satiety following a bout of checking (as indexed by the amount of 'time to next checking bout'). This component is decreased by NAc lesion and quinpirole treatment, and may reflect the inability of the animal to achieve a sense of task completion or satiety signal. This functional component is related to the security motivation theory of OCD discussed previously (Szechtman & Woody, 2004). In a recent study, each of the separate functional components were 're-synthesized' simultaneously by non-quinpirole neurobiological manipulation, yielding the re-constitution of compulsive checking in rats (Tucci, Dvorkin-Gheva, Johnson et al., 2014). More specifically, NAc lesion exaggerated vigor and satiety, while the addition of systemic treatment with the selective 5-HT1A agonist drug DPAT exaggerated the focus component, yielding full-blown compulsive behaviour. This finding strengthens the suggestion that compulsive checking displayed in the animal model consists of three underlying behavioural processes, each greatly exaggerated. Altogether, analysing the functional components of compulsive checking behaviour in response to neurobiological manipulations provides for a richer understanding of the behavioural response to experimentation, and furthermore, the associated neurobiology. This approach is different from other animal models which index compulsive behaviour as a unitary phenomenon, and highlights the utility of the quinpirole model.

Overall, several lines of evidence support the validity of the quinpirole sensitization model as a useful model to study OCD behaviour. First, the spatial-temporal structure of behavioural performance on the open field during chronic quinpirole treatment is similar to the salient features of OCD compulsions; that is, a preoccupation

with stimuli of concern that is greatly exaggerated, motor performance that appears ritual-like, and behaviour that is environmentally dependent (Szechtman et al., 1998). These qualities indicate strong face validity for the model; a claim endorsed by others (Albelda & Joel, 2012; Joel, 2006a; Robbins & Everitt, 2007). Second, compulsive checking induced by quinpirole and checking performed by humans appears to be motivationally driven towards stimuli related to safety and security (in the case of the rat, this is its home base), and is a greatly exaggerated form of normal checking of such stimuli (Boyer & Lienard, 2006; Szechtman et al., 1998; Szechtman & Woody, 2004). Third, OCD compulsions and compulsive behaviour induced by quinpirole may both be modulated by external stimuli and can be temporarily suppressed (Ben-Pazi, Szechtman, & Eilam, 2001; Szechtman et al., 2001; Zadicario, Ronen, & Eilam, 2007). Finally, treatments that are therapeutic for humans with OCD are also beneficial on compulsive behaviour induced by quinpirole; for example: clomipramine (Foa et al., 2005; Szechtman et al., 1998), nicotine (Lundberg, Carlsson, Norfeldt, & Carlsson, 2004; Salin-Pascual & Basanez-Villa, 2003; Tizabi et al., 2002), and deep brain stimulation (Djodari-Irani et al., 2011; Greenberg et al., 2006; Mundt et al., 2009; Winter et al., 2008). The latter two examples suggest relatively good predictive validity for the model. Overall, the quinpirole model is a relatively useful model to study the neurobiological and behavioural correlates of OCD.

Objectives of the present thesis

As can be gleaned thus far, OCD is a very heterogeneous affliction – a phenomenon agreed upon by others (Bjorgvinsson et al., 2007; Fineberg et al., 2012; Leckman, Bloch, & King, 2009; Stein et al., 2010). To some extent, this may contribute to inconsistent effects reported for certain pharmacological agents tested in both human and animals. One such pharmacological agent which has been observed to produce inconsistent results in OCD research that has not been empirically explained is the non-selective 5-HT agonist drug meta-chlorophenylpiperazine, otherwise known as mCPP. mCPP was one of the first direct pharmacological agents used to test a putative role for the 5-HT system in psychiatric afflictions (Kahn & Wetzler, 1991). mCPP crosses the blood-brain barrier (Hoyer & Neijt, 1988; Rurak & Melzacka, 1983), and binds to the 5-HT1A/1B/1C/1D/2/3 receptor subtypes (Fiorella, Rabin, & Winter, 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick, Jones, & Tyers, 1987). Changes in the classification schema of 5-HT receptors have altered the binding profile such that the 5-HT1C receptor was re-classified as the 5-HT2C receptor, and stimulation at the 5-HT2 receptor is considered to be at the 5-HT2A receptor subtype (1994 receptor and ion channel nomenclature, 1994). The drug also has affinity for alpha-1/2 and beta adrenergic receptors, and to a lesser extent dopamine and cholinergic receptors (Hamik & Peroutka, 1989; Smith & Suckow, 1985). The drug binds with highest affinity to 5-HT2C and 5-HT3 receptor subtypes where it acts as an agonist and antagonist, respectively (Kahn & Wetzler, 1991).

In humans, mCPP produces a number of physiological and behavioural effects including: nausea, sweating, weakness, an increase in anxiety and in some studies participants report the occurrence of panic attacks (Kahn & Wetzler, 1991). Other psychiatric afflictions reported to be potentiated by mCPP treatment include: Alzheimer's disease, schizophrenia, and alcohol craving (Kahn & Wetzler, 1991). In rats, mCPP produces a number of physiological and behavioural effects including: reduced food intake, decreased locomotor activity, lower body temperature, increased heart rate, and decreased social interaction suggestive of an anxiogenic effect (Kahn & Wetzler, 1991).

mCPP was also one of the earliest pharmacological agents that contributed to the 5-HT hypothesis of OCD (Murphy et al., 1989). However, the drug has produced inconsistent effects when administered to patients with OCD. In some studies it exacerbated OCD symptoms (Broocks et al., 1998; Gross-Isseroff et al., 2004; Hollander et al., 1991; Pigott et al., 1993; Zohar et al., 1987), while in other studies no effect of the drug was reported (Charney et al., 1988; de Leeuw & Westenberg, 2008; Goodman et al., 1995; Ho Pian et al., 1998; Khanna et al., 2001; Pigott et al., 1993).

mCPP has also been tested in animal models of OCD behaviour. In deer mice, the drug has been reported to decrease spontaneous stereotypic behaviour suggested to model compulsive behaviour (Korff et al., 2008). Using the reinforced alternation task as a model of compulsive behaviour in rats, mCPP was observed to produce a transient increase in the model compulsive behaviour that tolerated after repeated treatments (Kontis et al., 2008; Tsaltas et al., 2005). Others report that mCPP induces ritualistic chewing behaviour considered to model OCD (Kreiss et al., 2013).

Given the relatively inconsistent effects observed for mCPP treatment on OCD behaviour, the objective of this thesis was to identify the behavioural and neurobiological effects of mCPP using the quinpirole sensitization rat model of OCD, and in a reciprocal manner, to use the drug to dissect and further understand behavioural and neurobiological components of compulsive checking in the quinpirole model. By implication, outcomes may explain in part the inconsistencies reported in the literature.

One way to approach this objective, and what separates this line of research versus previous studies, is to leverage the tools of neuroscience by way of the complementary methods of analysis and synthesis to reduce and isolate behavioural phenomena to their constitutive components (Teitelbaum, 2012; Teitelbaum & Pellis, 1992). Analysis contributes to this approach by breaking down a seemingly unitary behavioural phenomenon, while synthesis attempts to re-build it from its constitutive components. Both of these methods are achieved by manipulating the nervous system, which reveals neurobiological mechanisms underlying behavioural features. Overall, this method provides the opportunity to observe functional neurobiological and behavioural components that may otherwise remain unidentified when a phenomenon is studied as being a unitary entity. The advantage of using the quinpirole model to address this objective is that the processes of analysis and synthesis contributed to the observation that performance of compulsive checking by rats is not a unitary phenomenon, but rather, comprised of at least three greatly exaggerated behavioral processes: vigor, focus and satiety (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014). The present thesis employed these known behavioural components and the process of analysis in

addressing how mCPP alters compulsive checking behaviour in rats, illuminating in greater detail the effects of the drug and underlying neurobiology.

For the present thesis, four separate studies were designed to address specific questions related to this objective. Below, the rationale, objective and hypothesis for each of these studies are discussed in detail. This section is ended with the statement of an overarching hypothesis for the thesis. Figure 1 below provides an overview for the plan of study.

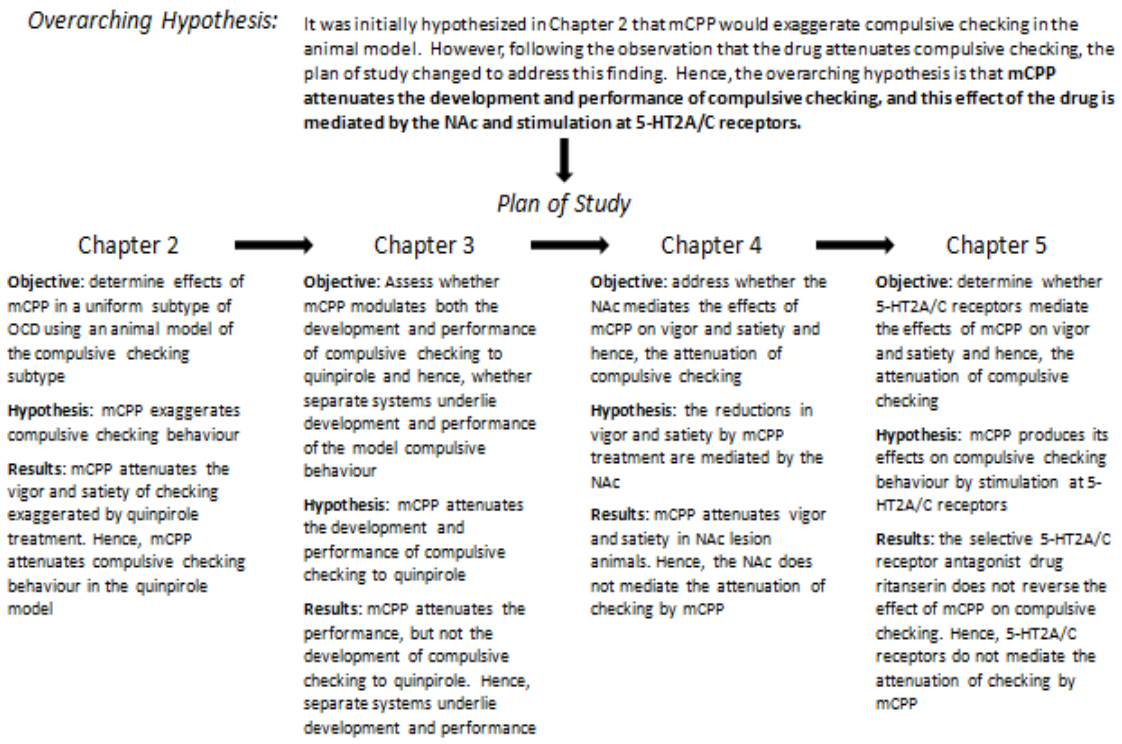

Figure 1. Plan of study for thesis.

Chapter 2: Effects of the serotonergic agonist mCPP on male rats in the quinpirole sensitization model of obsessive-compulsive disorder (OCD); (Tucci et al., 2013)

A number of human studies have administered mCPP to patients with OCD, and report inconsistent effects of the drug. For example, some studies report that mCPP exacerbates OCD symptoms (Broocks et al., 1998; Gross-Isseroff et al., 2004; Hollander et al., 1991; Pigott et al., 1993; Zohar et al., 1987), while others report that mCPP does not alter OCD symptoms in the patient group (Charney et al., 1988; de Leeuw & Westenberg, 2008; Goodman et al., 1995; Ho Pian et al., 1998; Khanna et al., 2001; Pigott et al., 1993). A number of different suggestions have been put forth in an attempt to explain these inconsistent findings. For example, it has been suggested that the dose of the drug administered, route of its administration, assessment of patients, or quality of the compound may have contributed to the discrepant outcomes (Goodman et al., 1995). Some of these possibilities have been empirically tested. For example, in an attempt to test whether the route of administration may account for inconsistent results, Goodman and colleagues (1995) attempted to replicate the earlier findings that oral administration of mCPP exacerbates OCD symptoms in patients. In a double-blind, placebo-controlled study, mCPP was administered orally or intravenously to OCD patients, and behavioral outcomes were measured. The authors did not replicate earlier findings. Such an outcome was interpreted to suggest that the route of mCPP administration does not account for the discrepant effects of the drug reported in the literature.

Animal models of OCD have also been used to test the response of the model compulsive behaviour to mCPP. For example, in deer mice, mCPP was reported to

reduce the performance of compulsive behaviour (Korff et al., 2008). However, in the reinforced spatial alternation model of OCD, the effects of mCPP were reported to exacerbate the model compulsive behaviour (Kontis et al., 2008; Tsaltas et al., 2005). Hence, even the outcomes from animal studies are somewhat discrepant.

Interestingly, some suggest that the seemingly inconsistent findings may be a result of the relative heterogeneous subtypes of patient OCD symptoms in the clinical studies (Goodman et al., 1995; Khanna et al., 2001). No study employed a group of patients reporting a relatively uniform symptom subtype. Although these authors (Goodman et al., 1995; Khanna et al., 2001) do not explicitly suggest why this possibility may have contributed to this outcome, it is known that some drugs produce different effects depending on the subtype of OCD being treated. For example, individuals with the hoarding subtype respond less favourably to SSRIs, while in a similar manner the presence of sexual obsessions predicts the absence of a therapeutic response to SSRIs (Leckman et al., 2009). It is possible then, that the effect of mCPP on OCD symptoms depends, at least in part, on the subtype of OCD the patient experiences. If, as in the previous studies the subtype was not uniform or controlled for, the results may be inconsistent. Therefore, in order to more clearly understand the effects of the drug, it would be beneficial to test the drug's effects on a homogeneous subtype of OCD behaviour.

The objective of Chapter 2 was to test the effects of mCPP treatment on a uniform subtype of OCD behaviour, that is, the compulsive checking subtype using the quinpirole sensitization model. The advantage of using the animal model comes from the fact that it

is a well-validated model of a specific symptom subtype, and does not merely model indecision or some other general behavioural characteristic associated with OCD. Further, by reducing analysis to effects of the drug on separate behavioural features of checking, that is, the vigor of checking performance, focus on the task of checking, and satiety following a bout of checking (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014), we may reveal whether the drug differently alters behavioural processes related to checking, and whether such an observation may contribute to an explanation of the inconsistent findings from the human clinical studies. Clinically, OCD is often measured as a unitary phenomenon. For example, the Yale-Brown Obsessive Compulsive Scale (YBOCS) (Goodman et al., 1989) is often used to measure OCD symptoms in humans, and was used in some of the clinical studies administering mCPP. This measure indexes the individual's subjective experience of OCD and is a valuable tool in assessing severity of symptoms. However, the YBOCS measures OCD as a relatively uniform phenomenon, without being designed to shed light on the processes that may underlie OCD behaviour.

The present study tested the hypothesis that mCPP exaggerates compulsive checking behaviour. Accordingly, it was predicted that mCPP would exaggerate one or more of the functional components underlying compulsive checking in quinpirole-treated animals. To address this, rats were chronically treated with quinpirole to induce compulsive checking behaviour, followed by an acute treatment with mCPP. A second experiment was utilised to test the effects of the drug alone on naive animals. The

response of the constitutive functional components vigor, focus, and satiety were measured and compared across treatment conditions.

Chapter 3: Separate mechanisms for development and performance of compulsive checking in the quinpirole sensitization rat model of obsessive-compulsive disorder (OCD); (Tucci, Dvorkin-Gheva, Sharma et al., 2014)

Results of Chapter 2 revealed that acute mCPP treatment reduced the vigor of checking performance and normalized satiety following a bout of checking in animals showing quinpirole-induced compulsive checking behaviour. Such a pattern of results was suggested to indicate that mCPP had ameliorative effects on the model compulsive behaviour. Given mCPP is a non-selective agonist at 5-HT receptors (Fiorella et al., 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick et al., 1987), it was further suggested that the ameliorative effects of mCPP on compulsive checking was by activity at 5-HT receptors. This finding led us to change our plan of study to further address this effect. Hence, we first ask whether a similar process would modulate the performance of checking during the induction or 'development' of compulsive checking by quinpirole.

For some behavioural phenomena, separate mechanisms exist for the development of a response and the performance of it. For example, psychostimulant drugs have been shown to induce locomotor sensitization by acting in one brain region, while the maintenance of this response appears to be mediated by another brain region (Carlezon & Nestler, 2002; Vezina & Stewart, 1990). Addressing this question goes beyond merely identifying whether mCPP also inhibits the development of OCD behaviour. Arguably

more revealing is the ability to shed light on whether the development of compulsive checking and performance of it are mediated by separate neurobiological processes, or whether a unitary mechanism is involved. Accordingly, the objective for Chapter 3 was to use the process of analysis by experimentation to address whether separate mechanisms underlie the development and performance of the model OCD behaviour.

The value in addressing this using mCPP comes from the fact that it is a non-selective 5-HT agonist drug (Fiorella et al., 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick et al., 1987), and hence not of the same neurochemical class as the drug used to induce the model compulsive behaviour; that is, DA D2/3 receptor stimulation by quinpirole. Any drug that may block DA D2/3 activity during the induction phase would merely inhibit the development and therefore performance of compulsive checking, and it would not be possible to address whether separate systems exist because of the complete absence of any checking behaviour. However, mCPP is known to alter the performance of compulsive checking, probably by 5-HT stimulation (see Chapter 2), and this eliminates the possibility of blocking induction of checking by quinpirole stimulation of DA D2/3. Hence, it is possible to test whether development of compulsive checking by quinpirole is open to the same modification as performance by mCPP.

Therefore, the extent to which mechanisms mediating development versus performance of compulsive checking overlap can be addressed by asking two related questions: first, whether mCPP co-treatment during the induction of compulsive checking by quinpirole attenuates its development; and second, whether the attenuation in checking

by mCPP co-treatment during the induction to quinpirole phase reduces the development and performance of full-blown compulsive checking as tested by a quinpirole-only challenge following the induction phase. Given evidence from Chapter 2 that mCPP reduces checking, and that sensitization of checking by quinpirole during the induction phase is necessary for its full-blown development and performance (Szechtman et al., 1998), **it was hypothesized that mCPP attenuates the development and performance of compulsive checking to quinpirole.** Accordingly, it was predicted that mCPP would attenuate the induction of compulsive checking by quinpirole and hence, its development. As a function of the reduction in the development of checking, it was accordingly predicted that we would observe a decrease in performance of compulsive checking on the quinpirole-only challenge. By implication, such an outcome would be consistent with a unitary mechanism mediating development and performance of the model OCD behaviour.

Chapter 4: The nucleus accumbens core does not mediate the attenuation of compulsive checking produced by mCPP treatment in a rat model of obsessive-compulsive disorder (OCD); Tucci, Dvorkin-Gheva, Johnson, Cheon et al. (submitted to *The European Journal of Neuroscience* on 2014-07-18)

Localising brain correlates for a particular behavioural response is possible by reducing and isolating nervous system components using the method of analysis by experimentation to reveal functional correlates. Many studies have identified a role for the nucleus accumbens in modulating OCD behavior by manipulating its function. For

example, bilateral DBS to the nucleus accumbens of treatment-resistant patients with OCD was reported to decrease the severity of OCD symptoms. Works from animal models have suggested a similar effect for DBS on the model OCD behaviour. Using the quinpirole sensitization rat model, high-frequency stimulation of both the shell and core subregions of the nucleus accumbens decreased compulsive checking behaviour (Mundt et al., 2009). Work from our laboratory has suggested that lesions to the nucleus accumbens core subregion (NAc) exaggerates the performance of some, but not all constitutive behavioural components related to compulsive checking behaviour (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014).

Chapters 2 and 3 reported that mCPP treatment attenuated the exaggerated vigor of checking performance and satiety following a bout of checking in quinpirole-treated animals. This outcome was suggested to reflect a reduction of the model OCD behaviour possibly by mCPP stimulation at 5-HT receptors. However, given that in both of these studies mCPP was administered systemically to intact rats, it is unknown where in the brain the drug may be producing its effects. Therefore, the objective for Chapter 4 was to attempt to localise where in the brain mCPP reduces the exaggerated vigor and satiety to yield an attenuation of compulsive checking using the process of analysis by reducing and isolating brain function to identify functional correlates.

Lesions to the NAc exaggerate the vigor of checking performance and satiety following a bout of checking, but do not appreciably alter the amount of focus on the task of checking (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014). Hence, the lesion alters 2 of the 3 constitutive functional components related to compulsive

checking behaviour. The authors (Dvorkin et al., 2010) suggest that the NAc may produce inhibitory control on vigor and satiety, and NAc lesion disinhibits this yielding OCD behaviour. Given evidence from Chapter 2 and 3 that mCPP also inhibits the exaggerated vigor and satiety produced by quinpirole treatment, it is possible that mCPP produces these effects by stimulation at the NAc. It is also possible that the drug produces these effects by acting elsewhere in the brain. Therefore to test which of these possibilities is true, we used an animal preparation consisting of NAc lesion rats to show exaggerations in vigor and satiety, and treated them with mCPP. **It was hypothesized that the reductions in vigor and satiety by mCPP treatment are mediated by the NAc.** Hence, it was predicted that if the NAc mediates reductions in vigor and satiety by mCPP stimulation, animals with NAc lesion would not show such a response to mCPP treatment. However, if another neural region mediates the response to mCPP, NAc lesion animals would continue to display reductions in vigor and satiety by mCPP treatment.

Chapter 5: 5-HT2A/C receptors do not mediate the attenuation of compulsive checking by mCPP in the quinpirole sensitization rat model of obsessive-compulsive disorder (OCD); Tucci, Dvorkin-Gheva, Johnson, Wong et al. (accepted in *Behavioural Brain Research*)

There is a growing body of evidence that a DA-5-HT interaction may underlie OCD behaviour. For example, it has been suggested that DA hyperactivity in circuits that project to the cortex and subcortical areas may yield obsessive-compulsive symptoms, and this DA hyperactivity may be attenuated by the blockade or stimulation of 5-HT2A and 5-HT1A receptors, respectively, located in the prefrontal cortex (Westenberg et al.,

2007). According to the authors, the net effect would be a reduction in cortical activity projecting back to subcortical areas. Westenberg and colleagues (2007) further suggest that such a putative mechanism may explain why antipsychotic drugs which block DA D1/D2 receptors and block and stimulate 5-HT2A and 5-HT1A receptors, respectively, may have a calming effect on OCD symptoms. However, studies demonstrating a DA-5-HT interaction modulating OCD behaviour through experimentation are lacking.

Chapters 2, 3 and 4 have each suggested the possibility for a DA-5-HT interaction underlying the modulation of OCD behaviour by mCPP as revealed through experimentation. It is reasonable to suggest that mCPP modulates compulsive checking in the animal model through activity at 5-HT receptors given the wide use of the drug as a probe of 5-HT function (Kahn & Wetzler, 1991), and in considering that mCPP contributed to the '5-HT hypothesis' of OCD (Murphy et al., 1989). However, mCPP is a non-selective 5-HT drug, with affinity for a number of 5-HT and some non-5-HT receptors. For example, as discussed above, the drug binds to the 5-HT1A/1B/1D /2A/2C/3 receptor subtypes (Fiorella et al., 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick et al., 1987). The drug also binds at non-5-HT receptor subtypes including: alpha-1/2 and beta adrenergic receptors, and to a lesser extent dopamine and cholinergic receptors (Hamik & Peroutka, 1989; Smith & Suckow, 1985). The drug binds with highest affinity to 5-HT2C and 5-HT3 receptor subtypes where it acts as an agonist and antagonist, respectively (Kahn & Wetzler, 1991), and some have referred to the drug as a 'preferential' 5-HT2C agonist (Blier & de Montigny, 1998). Nevertheless, it remains unknown which of these receptor subtypes mediates the effects of mCPP on compulsive

checking. Demonstrating a role for specific 5-HT receptors would strengthen the argument that mCPP produces its effects on compulsive checking by activity at 5-HT receptors, and the presence of a DA-5-HT interaction.

Therefore, the objective for Chapter 5 was to use the process of analysis to identify which 5-HT receptor subtypes mediate the effects of mCPP, and a possible DA-5-HT interaction in the animal model. Given reports that mCPP has high affinity for 5-HT2A/C receptor subtypes, and suggestions from the literature that stimulation of these same receptors may underlie the therapeutic effect produced by SSRIs on OCD symptoms (Blier & de Montigny, 1998; El Mansari & Blier, 2006), **we hypothesized that mCPP produces its effects on compulsive checking behaviour in the rat model by stimulation at 5-HT2A/C receptor subtypes.** Accordingly, we predicted that blockade of 5-HT2A/C receptors by the selective 5-HT2A/C receptor antagonist drug ritanserin would inhibit the attenuating effects of mCPP on vigor and satiety exaggerated by quinpirole treatment.

Overarching hypothesis

It was initially hypothesized in Chapter 2 that mCPP would exaggerate compulsive checking in the animal model. However, following the observation that the drug attenuates compulsive checking, the plan of study changed to address this finding. Hence, the overarching hypothesis is that **mCPP attenuates the development and performance of compulsive checking, and this effect of the drug is mediated by the NAc and stimulation at 5-HT2A/C receptors.**

CHAPTER 2: EFFECTS OF THE SEROTONERGIC AGONIST MCPP ON MALE RATS IN THE QUINPIROLE SENSITIZATION MODEL OF OBSESSIVE-COMPULSIVE DISORDER (OCD)

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Contributions by authors:

Mark C. Tucci - PhD candidate. With the assistance and advice of Dr. Henry Szechtman, designed the study, collected data, analyzed results and prepared the manuscript for submission.

Dr. Henry Szechtman - PhD candidate's supervisor. Provided assistance and advice to Mark C. Tucci on all aspects of the study, and contributed in particular to the statistical analysis and manuscript preparation.

Dr. Anna Dvorkin-Gheva - Collaborator. Performed computational analysis on the obtained co-ordinates of the animal on the open field to generate the spatial-temporal form of activity on the open field.

Dawn Graham, Sean Amodeo, Paul Cheon, Ashley Kirk, John Peel and Leena Taji - Laboratory members. Performed a portion of the data collection and edited the manuscript.

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ORIGINAL INVESTIGATION

Effects of the serotonergic agonist mCPP on male rats in the quinpirole sensitization model of obsessive–compulsive disorder (OCD)

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Abstract
Rationale The serotonergic agonist, *meta*-chlorophenylpiperazine (mCPP), produces inconsistent effects on obsessive–compulsive disorder (OCD) symptoms, perhaps because clinical studies have not utilized a homogenous OCD subgroup of patients.
Objectives This study aimed to evaluate mCPP effects on functional components of compulsive checking, using the quinpirole sensitization rat model of OCD.
Methods In study 1, the effects of mCPP were evaluated in quinpirole rats with compulsive checking. Two experimental groups were co-injected with quinpirole (0.125 mg/kg) and mCPP (0.625 or 1.25 mg/kg), while one control group was co-injected with quinpirole (0.125 mg/kg) and saline and the other control group received co-injections of saline. In study 2, mCPP (0, 0.3125, 0.625, and 1.25 mg/kg) was administered repeatedly to naïve rats and induction of compulsive checking evaluated.
Results mCPP significantly attenuated quinpirole-induced compulsive checking behavior by reducing vigor of checking (indexed by *frequency of checking* and *length of check*) and increasing rest after a bout of checking (indexed by *time to the next checking bout*), but it did not affect focus on the task of checking (indexed by *recurrence time of checking* and *number of stops before returning to check*). In naïve rats, mCPP did not induce compulsive behavior, but the highest dose reduced vigor of checking performance compared to saline controls.
Conclusions mCPP did not exacerbate or induce compulsive checking behavior. Instead, it ameliorated compulsive checking by reducing vigor of checking and increasing post-checking satiety, without affecting focus on checking. Ameliorative effects of mCPP may involve $5HT_{2A/2C}$ receptors in substantia nigra pars reticulata that inhibit expression of motor vigor.

Keywords Obsessive–compulsive disorder · Rat · Animal model · mCPP · Quinpirole · Serotonin · Behavioral sensitization

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Introduction

The serotonergic agent, *meta*-chlorophenylpiperazine (mCPP), is one of the first direct 5-HT receptor agonists used widely in psychiatry to examine the 5-HT receptor system (Kahn and Wetzler 1991). Early results from such studies contributed to the "serotonergic hypothesis" of obsessive–compulsive disorder (OCD) (Murphy et al. 1989) by reporting that mCPP exacerbated obsessive–compulsive symptoms in patients with OCD (Broocks et al. 1998; Gross-Isseroff et al. 2004; Hollander et al. 1991; Pigott et al. 1993; Zohar et al. 1987). However, other reports did not find an increase in obsessive–compulsive symptoms (Charney et al. 1988; de Leeuw and Westenberg 2008; Goodman et al. 1995; Ho Pian et al. 1998; Khanna et al. 2001; Pigott et al. 1993).

mCPP also was used in several animal models to examine the serotonergic modulation of compulsive behavior. Using deer mice, (Korff et al. 2008) found that mCPP reduced

compulsive behaviors. However, in another model—the reinforced spatial alternation model of OCD (Kontis et al. 2008; Tsaltas et al. 2005), the effects of mCPP were biphasic: early mCPP treatment exacerbated the model compulsive behavior, while chronic treatment with mCPP attenuated it.

It has been suggested (Goodman et al. 1995; Khanna et al. 2001) that a critical variable that may account for discrepant findings with mCPP is the heterogeneity of OCD. Clinical diagnosis of OCD encompasses several subtypes of the disorder (Mataix-Cols et al. 2005; van den Heuvel et al. 2009), and conceivably, mCPP is effective for particular subgroups only, but this was not considered in the patients tested. Importance of OCD phenotype for effectiveness of mCPP can be evaluated systematically using animal models reflecting a different homogenous subtype of OCD. One prevalent OCD subtype is characterized by "compulsive checking" (Lind and Boschen 2009; Matsunaga et al. 2001) and the purpose of the present study was to test the effects of mCPP on the compulsive "checking" behavior of rats using the quinpirole sensitization model of OCD (Szechtman et al. 1998). For this purpose, we coadministered mCPP acutely to rats expressing quinpirole-induced compulsive checking behavior. Because the literature suggested that mCPP may exacerbate OCD symptoms, we chose to use in the present study a lower dose of quinpirole (0.125 mg/kg) than is typically administered in our standard protocol (0.5 mg/kg) to minimize the possibility of a ceiling effect on compulsive checking. Moreover, in a separate study, we examined whether mCPP alone can induce compulsive checking behavior in naïve rats.

In the quinpirole sensitization rat model of OCD (Szechtman and Eilam 2005; Szechtman et al. 1998), repeated treatment with the D2/D3 dopamine agonist quinpirole induces compulsive checking behavior. In the rat, compulsive checking behavior is characterized by exaggerated preoccupation with one location in the environment to which the animal returns repeatedly. Such behavior in the rat is similar to OCD checking in the human, according to four lines of evidence (reviewed in Eilam and Szechtman 2005; Hoffman 2011; Joel 2006; Korff and Harvey 2006; Man et al. 2004; Westenberg et al. 2007). First, the temporal structure of quinpirole-induced compulsive checking and its organization in the environment satisfies performance criteria that define the salient features of an OCD compulsion (Szechtman et al. 1998), namely, an exaggerated preoccupation with the item(s) of concern, a ritual-like quality in motor performance and environmental dependence for display of the behavior. Second, the motivational basis of quinpirole-induced compulsive checking and OCD checking appear similar (Boyer and Lienard 2006; Feygin et al. 2006; Szechtman et al. 1998; Szechtman and Woody 2004; Whishaw et al. 2006; Woody and Szechtman 2005), in that both represent an exaggerated form of normal checking of stimuli related to safety and security (the "home base" in the case of the rat model). Third, quinpirole-induced compulsive checking is subject to similar modulation as OCD compulsions in that the performance of each is modified by external stimuli and can be suppressed temporarily (Ben Pazi et al. 2001; Szechtman et al. 2001; Zadicario et al. 2007). Finally, treatments that are therapeutically useful for OCD are also effective in attenuating quinpirole-induced compulsive checking, e.g., clomipramine (Foa et al. 2005; Szechtman et al. 1998), nicotine (Lundberg et al. 2004; Salin-Pascual and Basanez-Villa 2003; Tizabi et al. 2002), and deep brain stimulation (Djodari-Irani et al. 2011; Greenberg et al. 2006; Mundt et al. 2009; Winter et al. 2008).

Materials and methods

Animals

Subjects were 112 experimentally naïve adult male Long-Evans rats (Charles River, Quebec, Canada) weighing approximately 250–300 g at the onset of the experiments. Animals were individually housed in a climate-controlled rat colony room, with a 12-h light/dark cycle (6 AM lights on, 6 PM lights off), with food and water freely available. Upon arrival, rats were allowed to habituate to the animal facility for 7 days and were then handled for approximately 2–5 min each day for 5 days before the start of the experiment. Testing was conducted during the light phase. Animals were housed and tested as approved by the Animal Research Ethics Board, McMaster University in compliance with the Canadian Council on Animal Care guidelines.

Drugs

All drugs were obtained from Sigma-Aldrich, St Louis, MO, USA. 1-(3-Chlorophenyl)-piperazine hydrochloride (mCPP) was administered to rats at doses of 0.625 or 1.25 mg/kg. These doses of mCPP were chosen because they produce compulsive-like behavior in a rat model (Kontis et al. 2008). (−)-Quinpirole hydrochloride was administered at a dose of 0.125 mg/kg (rather than 0.5 mg/kg; Dvorkin et al. 2006; Szechtman et al. 1998) to minimize the possibility that a full dose of quinpirole produces a ceiling effect on compulsive checking that would mask a potential exacerbation with mCPP cotreatment. All drugs were dissolved in 0.9 % physiological saline and administered at a volume of 1 ml/kg through a subcutaneous injection under the nape of the neck. Control animals received a similar volume of 0.9 % physiological saline in a similar manner.

Apparatus

Rats were tested on large open field (160×160 cm blue table without walls) located in a noncolony room, as described

previously (Dvorkin et al. 2006, 2010). The table was divided virtually into a grid of 25 rectangular places (locales), but no actual lines were marked on the table surface. Four small Plexiglas/glass boxes (approximately 8×8×7.5 cm) were present throughout the study at the same fixed location of the open field: two at corners and two at places near the center of the open field. Objects were wiped clean with a diluted solution of an antibacterial cleaner (Lysol) after each rat was tested. Behavior was videotaped continuously by a camera affixed to the ceiling (providing a stationary top view of the entire open field and the rat in it). Videotapes were converted to MPEG files (Canopus MPEGPro EMR Realtime MPEG-1 MPEG-2 Encoder) and these digitized videos were used to automatically track the trajectories of locomotion using EthoVision 3.1 (Noldus Information Technology, Wageningen, The Netherlands) software (Noldus et al. 2001; Spink et al. 2001).

Data analysis

EthoVision 3.1 software was used to extract the time series of the x, y coordinates of the rat from digitized video recordings (Dvorkin et al. 2006). Digitized tracking data were preprocessed to remove noise (by applying appropriate filters to smooth the x, y coordinates) (Hen et al. 2004), and the obtained coordinates were divided into episodes of forward locomotion (called progression) and episodes of small movements or immobility (called lingering), as described previously (Drai et al. 2000; Drai and Golani 2001; Golani et al. 1993). The coordinate system was mapped onto 25 open field locales (places) (Szechtman et al. 1998), and the frequency of visits and duration of stops in each locale were computed (the terms "visit" and "stop" are equivalent and are used interchangeably). Checking behavior was defined with reference to the most visited locale (labeled "key place" or "key locale"; these terms are equivalent), which was in most instances also the locale with the longest total duration of stops (Eilam and Golani 1989; Szechtman et al. 1998). A visit to the key place is also referred to as a "check" or "checking," and the following group of four criteria measures of checking behavior were computed: (1) Frequency of checking: total number of visits to the key locale. (2) Length of check: total duration of stay at the key locale divided by the frequency of visits there; this measure is also an indirect index of ritual-like behavior as a very short duration of stay in the key locale is associated with the appearance of motor rituals in quinpirole-treated rats (Ben Pazi et al. 2001; Szechtman et al. 1998). (3) Recurrence time of checking: mean duration of return times to the key locale ("return time" is the interval from departure to next arrival at the locale). (4) Stops before returning to check: mean number of places visited between returns to the key locale. Compulsive checking behavior is identified by the presence of a significant difference between quinpirole-treated and saline-treated rats—all four measures need to differ from controls for the claim of compulsive checking (Szechtman et al. 1998), and hence, the group of these four measures is termed "criteria measures" for compulsive checking.

The criteria measures for compulsive checking were dissociated empirically in a recent lesion study (Dvorkin et al. 2010). Specifically, nucleus accumbens core (NAc) lesions affected measures indicative of the amount of checking behavior (frequency of checking and length of check), whereas orbitofrontal cortex (OFC) lesions affected indices of staying away from checking the key locale (time to return to check and number of stops before returning to check). This pattern of results suggested that the functional roles of the NAc and OFC in checking behavior are to control the vigor of motor performance and focus on goal-directed activity, respectively. Accordingly, we consider vigor and focus as two relatively independent components of checking behavior, indexed by the first two and the last two criteria measures for compulsive checking, respectively.

In addition to these four criteria measures, we also evaluate "time to next checking bout" (Dvorkin et al. 2006). This measure is greatly reduced in quinpirole-sensitized rats and has been proposed to index the third constitutive component of compulsive checking behavior—"satiety" or rest after checking (Dvorkin et al. 2010). It was reasoned (Dvorkin et al. 2010) that, in the animal model, the foreshortened "satiety" or "rest" after a bout of checking corresponds to notions that OCD reflects failure to achieve "sense of task completion" (Pitman 1989) or "feeling of knowing" (Szechtman and Woody 2004).

The computation of checking bouts is detailed in Dvorkin et al. (2006). Briefly, the method follows the logic used to identify the clustering of a bout of eating behavior into a "meal" and the time between meals into a period of post-ingestion satiety (Tolkamp et al. 1998; Tolkamp and Kyriazakis 1999). According to those authors, a bout of behavior is defined on the basis of the distribution of time intervals between behavioral events (inter-event intervals). This distribution is examined to locate and extract a timepoint that will produce a natural split between clusters of inter-event intervals. Specifically, the identified timepoint will separate the time intervals into a class of (relatively long) intervals that are between the bouts of behavior (inter-bout intervals) and a class of (relatively shorter) intervals that belong within a bout of behavior (intra-bout intervals) (Tolkamp et al. 1998; Tolkamp and Kyriazakis 1999). This principle was employed in an algorithm developed to identify bouts of checking behavior (Dvorkin et al. 2006) and extract "time to next checking bout." In the present study, we introduce a slight modification in the computation of "time to next checking bout." In particular, in the present study, the value for this variable contributed by an individual rat for statistical analysis

is the interval from the end of the first bout to the start of the second bout, rather than the mean of inter-bout intervals if the rat had more than two bouts of checking in the session. The advantage of this approach lies in utilizing the same parameter across subjects as only a subset of subjects complete two or more bouts of checking in a test session. It should be noted that a rat may complete a bout of checking but not start the next bout during the session, and hence, the number of rats for "time to next checking bout" is generally smaller than for the four criteria measures for compulsive checking. Saline-treated rats generally have one to two bouts of checking behavior in a session, while quinpirole-treated rats usually perform two or more bouts (Dvorkin et al. 2010).

Design and procedure

Two independent experiments were performed: experiment 1 examined the effects of mCPP on quinpirole-induced compulsive checking, while experiment 2 evaluated the effects of mCPP on the induction of compulsive checking in naïve rats. For experiment 1, four independent groups (N=14/group) were used: two experimental groups, each co-injected with quinpirole and mCPP (either 0.625 or 1.25 mg/kg of mCPP), and two control groups, one of which was co-injected with quinpirole and saline, while the other one received injections of saline. The experiment had two phases. Phase 1 involved the induction of compulsive checking. The two experimental groups and the quinpirole control group received 10 injections of quinpirole in the open field to induce compulsive checking (the saline control group received 10 injections of saline). Phase 2 was the test for the effects of mCPP on compulsive checking. Here, on injection 11, the groups received the appropriate additional injection of mCPP or saline, and checking behavior was monitored as before.

The design of experiment 2 was similar to phase 1 of experiment 1, except for treatment with mCPP instead of quinpirole. Four independent groups (N=14/group) were used: three experimental groups were injected with mCPP (0.3125, 0.625, or 1.25 mg/kg of mCPP), while the control group received injections of saline. Rats received nine injections of mCPP or saline in the open field and their behavior assessed for presence of compulsive checking. Results for analysis of injections 1, 2, and 9 are shown in Table 1 as findings were not different for the in-between injections.

Rats were allocated into groups based on body weight at the start of the experiment. For all runs in phase 1 and phase 2, the same procedure was followed: Animals were weighed, transported in their home cage to an adjoining noncolony testing room, and administered the appropriate injection(s). Immediately afterwards, the rat was placed into the open field for 55 min and its behavior videotaped for offline analysis. Each rat had two open field tests per week, at approximately the same time of day, and was allocated to the same experimenter for the duration of the study.

Statistical analysis

A one-way analysis of variance (ANOVA) was computed for each of the dependent variables, followed by Duncan's new multiple range test. Chosen level of significance was $p<0.05$. Calculations were performed using SPSS 20 for Windows.

Results

Figure 1 shows the performance of different treatment groups on a test for compulsive checking. As is evident, animals treated with quinpirole (0.125 mg/kg) displayed compulsive checking, in that, compared to saline controls, their frequency of checking was significantly greater, the length of check and the recurrence time of checking were significantly shorter, and the number of stops before returning to check was significantly smaller. In addition to meeting these criteria for compulsive checking, the quinpirole-treated group had a significantly shorter time to the next checking bout, as found for animals treated with a higher dose of the drug (Dvorkin et al. 2006). Thus, as expected, quinpirole treatment enhanced all three components of compulsive checking: vigor of performance (indexed by *frequency of checking* and *length of check*), focus on the task of checking (indexed by *recurrence time of checking* and *number of stops before returning to check*), and rest after task completion (indexed by *time to the next checking bout*).

As shown in Fig. 1, the effects of mCPP on the criteria measures for compulsive checking were selective—mCPP altered the quinpirole response for the vigor and rest components, but not for the focus on the task of checking component. Specifically, for measures of vigor (frequency of checking and length of check), both doses of mCPP produced a significant decrease in the frequency of checking compared to the quinpirole group; for the other measure of vigor, length of check, it was elevated compared to the quinpirole group, as evidenced by the mCPP-treated groups not being statistically different from either the saline or quinpirole groups. For measures of focus, both mCPP doses had no effect on recurrence time of checking and number of stops before returning to check, compared to the quinpirole group. Finally, there was a dose-dependent effect for mCPP on rest after task completion, in that the high-dose mCPP group was statistically not different from either the saline or quinpirole groups for time to next bout, indicating a return of rest towards normal controls and away from the very brief period of rest under quinpirole.

Table 1 Performance on criteria measures for compulsive checking after 1, 2 or 9 injections of three doses of mCPP

Injection no.	Compulsive checking measure	Saline		mCPP (0.312 mg/kg)		mCPP (0.625 mg/kg)		mCPP (1.25 mg/kg)		One-way ANOVA
		Mean±SEM	*N*	Mean±SEM	*N*	Mean±SEM	*N*	Mean±SEM	*N*	
1	Frequency of checking	17.8±1.0	14	13.3±1.7	14	14.4±2.3	13	8.1±0.8*	12	$F(3,49)=6.3$, $p=0.001$
	Length of check (s)	50.9±6.5	14	128.0±37.9	14	199.8±63.5*	13	261.5±57.0*	12	$F(3,49)=4.0$, $p=0.013$
	Recurrence time of checking (s)	97.1±11.7	14	88.9±15.3	14	72.2±18.2	13	56.3±19.5	12	$F(3,49)=1.2$, $p=0.3$
	No. of stops before returning to check	5.1±0.4	14	4.7±0.9	14	3.2±0.3*	13	3.0±0.4*	12	$F(3,49)=3.35$, $p=0.026$
2	Frequency of checking	10.6±0.7	13	7.7±0.4*	12	8.6±0.5	10	8.0±1.3*	6	$F(3,37)=4.1$, $p=0.013$
	Length of check (s)	69.3±9.9	13	198.1±50.1	12	201.3±35.2	10	326.7±85.1*	6	$F(3,37)=5.4$, $p=0.003$
	Recurrence time of checking (s)	153.1±20.8	13	86.9±28.5	12	103.0±20.5	10	109.0±64.8	6	$F(3,37)=1.1$, $p=0.36$
	No. of stops before returning to check	4.1±0.4	13	3.4±0.5	12	3.2±0.2	10	3.7±1.2	6	$F(3,49)=0.58$, $p=0.6$
9	Frequency of checking	12.4±1.7	13	8.1±1.1	11	8.4±1.5	10	5.3±0.5*	6	$F(3,36)=3.5$, $p=0.024$
	Length of check (s)	129.4±31.5	13	174.9±45.0	11	171.9±67.5	10	343.3±78.4*	6	$F(3,36)=2.4$, $p=0.085$
	Recurrence time of checking (s)	101.2±13.0	13	85.6±25.0	11	128.0±42.0	10	102.4±36.7	6	$F(3,36)=0.4$, $p=0.76$
	No. of stops before returning to check	4.5±0.4	13	4.0±0.9	11	3.2±0.5	10	4.6±0.8	6	$F(3,36)=0.9$, $p=0.45$

*$p<0.05$ compared to saline group, Duncan multiple range test

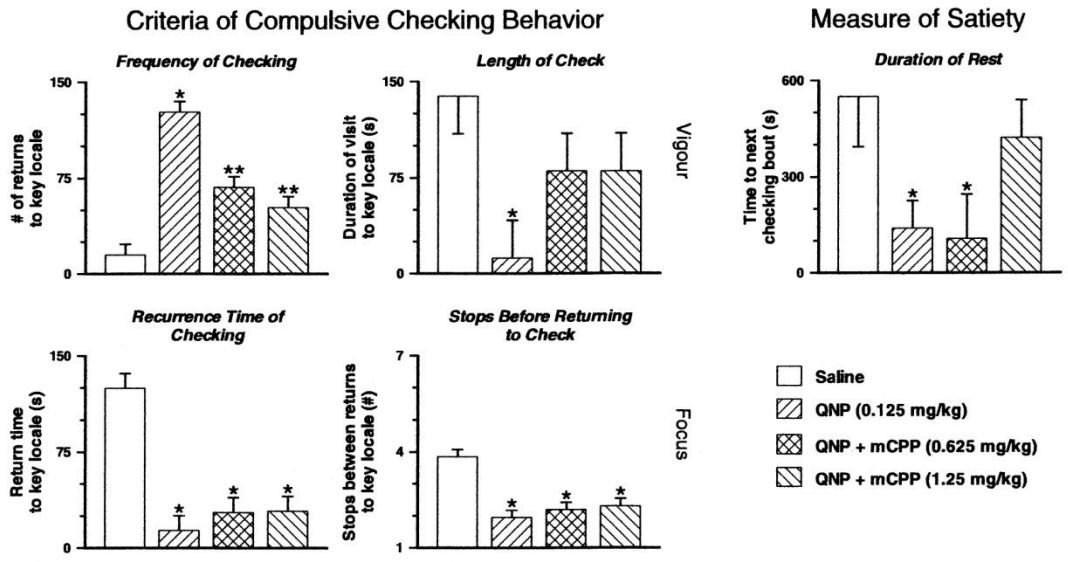

Fig. 1 Effects of mCPP coadministration on functional components of compulsive checking. Rats are said to show compulsive checking behavior when their performance is significantly different from saline controls on all four criteria measures (*left panel*): frequency of checking (no. of stops in key locale); length of check (mean duration in seconds of stay in key locale); recurrence time of checking (mean duration in seconds of return times to key place); and no. of stops before returning to check (mean number of places visited between returns to key locale). The first two measures index the vigor of checking and the last two index the focus on the task of checking. The third component of compulsive checking is rest (satiety) after task performance (*right panel*) and is indexed by time in seconds to the next bout of checking. *Bars* are marginal means and standard error of the mean (SEM). *$p<0.05$ vs saline controls, **$p<0.05$ vs saline and quinpirole control groups, Duncan multiple range test, following a significant effect of group for frequency of checking [$F(3,56)=30.323$, $p<0.001$]; length of check [$F(3,56)=3.088$, $p=0.035$]; recurrence time of checking [$F(3, 56)=19.347$, $p<0.001$]; no. of stops before returning to check [$F(3,56)=14.184$, $p<0.001$]; and time to next bout [$F(3,29)=2.839$, $p=0.058$]

Thus, both doses of mCPP reduce the motor vigor with which checking is performed, without any effects on the attention or concentration on the task of checking itself; moreover, mCPP dose-dependently increases the rest, or satiety, following a bout of checking. Hence, mCPP has selective effects on the components of compulsive checking.

Table 1 shows in naïve rats the effects of mCPP treatment alone on performance of checking behavior. As is evident, chronic administration of mCPP over a range of doses did not induce "compulsive" checking behavior, as even nine injections of mCPP did not lead to a significant difference from the saline controls on all four criteria measures. However, the highest dose of mCPP (1.25 mg/kg) did produce a consistent effect on two measures: it reduced significantly the frequency of checking and increased the mean length of checks compared to saline controls. Thus, mCPP attenuates the vigor of checking performance also in saline-treated rats, without altering the measures of focus. The effects of mCPP on "time to next checking bout" could not be assessed, as too few animals treated with mCPP alone initiated a second bout of checking.

Discussion

The present study showed that stimulation of 5HT receptors with mCPP does not produce an exacerbation of compulsive checking behavior. It is unlikely that this lack of exacerbation was due to a ceiling effect because we used a lower dose of quinpirole to induce compulsive checking. Moreover, even in naïve rats, mCPP treatment failed to induce compulsive checking. These findings are consistent with those reports in the clinical literature which did not find that mCPP exacerbates or induces OCD symptoms (Charney et al. 1988; de Leeuw and Westenberg 2008; Goodman et al. 1995; Ho Pian et al. 1998; Khanna et al. 2001; Pigott et al. 1993). Hence, the present results suggest that mCPP is not pro-compulsive, at least for the "checking" subtype of OCD.

Unexpectedly, rather than exacerbate, mCPP attenuated two components of compulsive checking: It reduced the vigor of checking (as indexed by *frequency of checking* and *length of check*) and it increased the post-checking "satiety" (as indexed by *time to next checking bout*). mCPP did not alter the focus on the task of checking (as indexed by *recurrence time of checking* and *number of stops before returning to check*). Nevertheless, even those limited effects of mCPP were sufficient to render the quinpirole-treated animals no longer meeting criteria for "compulsive" checking behavior. Therefore, rather than exacerbating compulsive checking, mCPP appears to have an ameliorative effect on this compulsive behavior.

Prior studies with OCD patients did not report such an ameliorative effect of mCPP on OCD symptoms. However, those studies did not have a homogeneous sample of compulsive "checkers" only. As discussed earlier, mCPP may produce different effects (or none at all) in different OCD subtypes and our findings may indicate that the therapeutic effectiveness of mCPP applies only to certain compulsions, including compulsive checking behavior. Furthermore, it is important to note that, in our experimental paradigm, we examined, in essence, the effects of mCPP in an animal that is currently engaged in compulsive behavior (evoked by quinpirole). In other words, we examined the effects of mCPP on the active "compulsive" state, as opposed to using mCPP to provoke OCD symptoms in a resting individual with the OCD trait. Thus, it may be the case that the ameliorative mCPP effects are evident only in the presence of florid symptoms, but in human studies, special care was used to ensure that OCD symptoms are not provoked spontaneously (de Leeuw and Westenberg 2008; Goodman et al. 1995; Ho Pian et al. 1998). Unfortunately, there are no published clinical studies that examined the effects of mCPP in actively symptomatic OCD patients to ascertain symptom reduction expected from the quinpirole model. Thus, mCPP may have ameliorative effects on OCD symptoms, depending on the OCD subtype and/or the state of behavioral engagement in compulsions and obsessions.

In this regard, two animal studies report a reduction of OCD-related behavior following mCPP treatment. In particular, Korff et al. (2008) reported that mCPP attenuated the spontaneous high levels of compulsive-like behavior in deer mice. Similarly, in the reinforced spatial alternation model of OCD, mCPP attenuated the expression of compulsive-like behavior in rats (Kontis et al. 2008; Tsaltas et al. 2005). Conceivably, reduction of compulsive-like behavior in these animal models also involves an attenuation of the vigor with which those behaviors are performed.

Present findings indicate that mCPP has normalizing effects in quinpirole-treated rats on two components of compulsive checking behavior (vigor and satiety) and that, in naive animals, it likewise has an attenuating effect on checking performance, reducing the vigor of checking below normal levels. This reduction in vigor with mCPP is consistent with reports in the human literature of a similar decline with mCPP in OCD patients and healthy controls. In particular, human subjects administered mCPP report altered mood states as rated by reduced "energy" and increased "drowsiness" (Charney et al. 1988).

Given agonist properties of mCPP at $5HT_{2A/2C}$ receptors (Rajkumar et al. 2009), the ameliorating effects of mCPP on compulsive checking are consistent with suggestions that the mechanism for the therapeutic effects of selective serotonin reuptake inhibitors on OCD may involve stimulation of $5HT_{2A/2C}$ receptors (de Leeuw and Westenberg 2008; El Mansari and Blier 2006). However, further studies are necessary to ascertain whether the effects observed in the

present study involve stimulation of $5HT_{2A}$ or $5HT_{2C}$ or both of these serotonergic receptors.

Regardless of receptor subtype involved, the finding that a 5HT agonist drug ameliorated the quinpirole effect on compulsive checking suggests an oppositional dopamine–serotonin interaction in mediating OCD behavior. One possible mechanism could involve the blockade of the quinpirole-induced dopaminergic increase in checking vigor through mCPP stimulation of $5HT_{2A/2C}$ receptors in the substantia nigra pars reticulata (SNr). This hypothesis stems from findings suggesting that NAc normally exerts inhibitory control on checking vigor and quinpirole inhibits this suppression (Dvorkin et al. 2010); that NAc innervates the SNr (Berendse et al. 1992; Heimer et al. 1991); and that $5HT_{2A/2C}$ receptors in SNr (Eberle-Wang et al. 1997; Hamada et al. 1998) may inhibit nigrothalamic output (Deniau et al. 1994). Hence, we propose that upon reaching the SNr, the quinpirole-disinhibited NAc output mediating increased vigor is inhibited by activation of $5HT_{2A/2C}$ receptors in the SNr. Accordingly, mCPP ameliorates compulsive checking by blocking SNr-mediated expression of motor vigor. Studies that address this hypothesis are currently underway.

In summary, mCPP serotonergic stimulation attenuates measures of the vigor of checking and enhances the rest period after a bout of checking, but does not alter measures of the focus on the task of checking in quinpirole-treated animals. Changes in these components were sufficient to render the animals no longer meeting the criteria for "compulsive" checking behavior. Further, mCPP alone does not induce compulsive checking behavior in naïve rats but does attenuate measures of the vigor of checking performance. Therefore, mCPP has selective effects on components of compulsive checking behavior. The effects of mCPP on compulsive checking behavior may be due to stimulation of $5HT_{2A/2C}$ receptors, possibly within the SNr to inhibit the expression of compulsive checking behavior.

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Conflict of interest The authors declare that there is no conflict of interest.

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CHAPTER 3: SEPARATE MECHANISMS FOR DEVELOPMENT AND PERFORMANCE OF COMPULSIVE CHECKING IN THE QUINPIROLE SENSITIZATION RAT MODEL OF OBSESSIVE-COMPULSIVE DISORDER (OCD).

Tucci, M. C., Dvorkin-Gheva, A., Sharma, R., Taji, L., Cheon, P., Peel, J., Kirk, A., & Szechtman, H. (2014). Separate mechanisms for development and performance of compulsive checking in the quinpirole sensitization rat model of obsessive-compulsive disorder (OCD). *Psychopharmacology*, doi:10.1007/s00213-014-3505-6

Contributions by authors:

Mark C. Tucci - PhD candidate. With the assistance and advice of Dr. Henry Szechtman, designed the study, collected data, analyzed results and prepared the manuscript for submission.

Dr. Henry Szechtman - PhD candidate's supervisor. Provided assistance and advice to Mark C. Tucci on all aspects of the study, and contributed in particular to the statistical analysis and manuscript preparation.

Dr. Anna Dvorkin-Gheva - Collaborator. Performed computational analysis on the obtained co-ordinates of the animal on the open field to generate the spatial-temporal form of activity on the open field.

Renee Sharma, Sean Amodeo, Leena Taji, Paul Cheon, John Peel, and Ashley Kirk - Laboratory members. Performed a portion of the data collection and edited the manuscript.

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ORIGINAL INVESTIGATION

Separate mechanisms for development and performance of compulsive checking in the quinpirole sensitization rat model of obsessive-compulsive disorder (OCD)

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Abstract
Rationale Acute administration of serotonergic agonist, meta-chlorophenylpiperazine (mCPP), attenuates performance of compulsive checking in an animal model of obsessive-compulsive disorder (OCD). It is not known whether mCPP has a similar effect on development of compulsive checking.
Objectives The objective of the study was to examine whether similar mechanisms mediate the development versus the performance of compulsive checking in the rat model.
Methods Four groups of male rats (N=14/group) were tested: two experimental groups co-injected with D2/D3 dopamine agonist quinpirole (0.25 mg/kg) and mCPP (0.625 mg/kg or 1.25 mg/kg), and two control groups, one co-injected with quinpirole and saline, the other receiving injections of saline. The time course of development of compulsive checking across injections 1 to 10 in quinpirole-treated rats was compared to rats co-injected with quinpirole and mCPP.
Results Results showed that during the course of chronic treatment, mCPP (1.25 mg/kg) significantly attenuated performance of checking behavior. However, when these rats were retested for expression of compulsive checking (that is, with an injection of quinpirole only), their profile of compulsive checking was no different from the control rats treated throughout with quinpirole only.
Conclusions Findings show that mCPP inhibits performance of compulsive checking but does not block quinpirole from inducing the neural substrate underlying this compulsive behavior. Hence, a separate mechanism underlies the induction of compulsive checking and the performance of it. It is suggested that development of the OCD endophenotype reflects neuroplastic changes produced by repeated dopamine D2/D3 receptor stimulation, while stimulation of serotonergic receptors mediates a negative feedback signal that shuts down the motor performance of checking.

Keywords Compulsive checking behavior · Dopamine-serotonin interaction · Security motivation · mCPP · Quinpirole

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Introduction

The serotonin hypothesis for the neurobiology of obsessive-compulsive disorder (OCD) arose from the clinical experience of therapeutic benefit with serotonin reuptake inhibitors (Aouizerate et al. 2005; Barr et al. 1993; Denys 2006; Insel et al. 1985; Murphy et al. 1989). The success of this pharmacotherapy is often enhanced through coadministration of various dopamine blockers (Bloch et al. 2006; Denys 2006; Dougherty et al. 2004; Fineberg et al. 2006), raising the current hypothesis of a dopamine-serotonin interaction in OCD (Goodman et al. 1990; Westenberg et al. 2007; Zohar et al. 2000). Research using animal models of OCD supports the current hypothesis in that experimental manipulations of serotonin (Alkhatib et al. 2013; Andersen et al. 2010; Bos et al. 1997; Flaisher-Grinberg et al. 2008; Greene-Schloesser et al. 2011; Kontis et al. 2008; Korff et al. 2008; Martin et al. 1998; Papakosta et al. 2013; Schilman et al. 2010; Shanahan et al. 2011; Yadin et al. 1991) or dopamine neurotransmission (Amato et al. 2008; Campbell et al. 1999; Einat and Szechtman 1995; Hoffman and Rueda Morales 2012; Jimenez-Gomez et al. 2011; Joel et al. 2001; Kurylo 2004;

Sesia et al. 2013; Szechtman et al. 1998) affects the model OCD compulsive behavior. Further support for the dopamine-serotonin hypothesis comes from animal studies in which both dopamine and serotonin neurotransmission were targeted directly, with evidence of an oppositional effect on the model compulsive behavior (e.g., Joel 2006; Schepisi et al. 2013; Tucci et al. 2013).

Even though the expression of OCD symptoms involves dopamine-serotonin interaction, it is not known whether the development of this psychopathology does as well, given that a separation often exists between mechanisms for the acquisition of a response and the expression of it. For instance, some regions of the brain are activated during learning of a new sequence of actions but not during performance of the learned sequence (Hikosaka et al. 2002). Likewise, psychostimulant drugs induce locomotor sensitization by acting on specific brain regions but evoke the sensitized response by acting elsewhere (e.g., Carlezon and Nestler 2002; Vezina and Stewart 1990). Hence, the pathogenesis of OCD and the functional manifestation of OCD pathophysiology could involve non-overlapping neural circuits. The present study investigates such a possibility by examining whether a serotonergic agonist that attenuates compulsive checking also blocks the development of this compulsive behavior, as would be expected if the same mechanism mediated both the expression and the development of OCD symptoms.

The design of the present study takes advantage of our recent findings that in the quinpirole sensitization rat model of OCD (Eilam and Szechtman 2005; Szechtman and Eilam 2005), an acute injection of the serotonergic (5-HT) agonist drug, 1-(3-chlorophenyl)-piperazine (mCPP), ameliorated compulsive checking induced by chronic treatment with the D2/D3 agonist quinpirole (Tucci et al. 2013). This result was surprising, given that mCPP exacerbates obsessive-compulsive symptoms in patients with OCD (Broocks et al. 1998; Gross-Isseroff et al. 2004; Hollander et al. 1991; Pigott et al. 1993; Zohar et al. 1987), although this is not a uniform finding (Charney et al. 1988; de Leeuw and Westenberg 2008; Goodman et al. 1995; Ho Pian et al. 1998; Khanna et al. 2001; Pigott et al. 1993). In considering a plausible interpretation of mCPP results in the quinpirole sensitization model, we took account of prior findings indicating that compulsive checking is not a unitary phenomenon but is constituted of three relatively independent components, all greatly exaggerated by quinpirole, namely: (1) vigor of checking; (2) the focus on checking; and (3) rest or "satiety" after a bout of checking (Dvorkin et al. 2010). Our analysis (Tucci et al. 2013) showed that mCPP normalized two of these components: the 5HT agonist counteracted strongly the energizing effects of quinpirole on vigor of checking and, to a lesser extent, it counteracted the quinpirole truncated post-checking satiety; however, mCPP had no effect on quinpirole-induced hyperattention to check. Hence, we suggested that mCPP, possibly by stimulating 5HT receptors, inhibits the expression of compulsive motor vigor (Tucci et al. 2013).

The above mCPP findings (Tucci et al. 2013) produced two intertwined empirical questions. First, is mCPP inhibition also evident during the period of induction of compulsive checking by repeated injections of quinpirole? Second, does the coadministration of mCPP protect the rat from acquiring a full-blown compulsive checking response to quinpirole? Answers to these questions would indicate whether inhibition of motor vigor produced by mCPP is sufficient to prevent the development of compulsive checking and by implication whether the same mechanisms underlie the development of compulsive checking and the performance of it. Results showed that coadministration of mCPP did indeed attenuate checking performance during the induction period but, surprisingly, even such diminution of motor activity did not prevent quinpirole-induced pathogenesis of compulsive behavior.

Materials and methods

Animals

Subjects were 56 experimentally naïve adult male Long-Evans rats (Charles River, Quebec) that weighed between 250 and 300 g at the onset of the experiment. Animals were housed individually in a climate-controlled colony room and exposed to a 12-h light/dark cycle (6 AM lights on, 6 PM lights off). Food and water were freely available. Upon arrival, rats were allowed to habituate to the animal facility for 7 days and were then handled for approximately 2-5 min each day for 5 days before start of experiment. Testing occurred during the light phase. Animals were housed and tested as approved by the Animal Research Ethics Board, McMaster University in compliance with the Canadian Council on Animal Care guidelines.

Drugs

All drugs were obtained from Sigma-Aldrich, USA. 1-(3-Chlorophenyl)-piperazine hydrochloride (mCPP) was administered to rats at doses of 0.625 or 1.25 mg/kg. These doses of mCPP were chosen because they were shown to attenuate compulsive checking behavior when administered acutely to rats showing quinpirole-induced compulsive checking behavior (Tucci et al. 2013). Quinpirole was administered at a dose of 0.25 mg/kg. The dose of quinpirole used in the present study was higher than the dose used in our previous study (0.125 mg/kg) (Tucci et al. 2013). This particular dose of quinpirole was selected because the drug reaches a maximum behavioral effect at a dose of about 0.2–0.5 mg/kg (Dvorkin et al. 2006; Perreault et al. 2005; Szechtman et al. 1994a; Szechtman et al. 1994b; Szumlinski et al. 1997).

All drugs were dissolved in 0.9 % physiological saline and administered at a volume of 1 ml/kg through a subcutaneous injection under the nape of the neck. Control animals were similarly injected with 1 ml/kg of 0.9 % physiological saline.

Apparatus

Animals were tested on a large open field (160×160 cm and 60-cm-high table without walls) that was located in a non-colony experiment room, as described previously (Dvorkin et al. 2006; Dvorkin et al. 2010). The table was divided virtually into a grid of 25 rectangular places (locales), but no actual lines were marked on the table surface. Four small Plexiglas/glass boxes (approximately 8×8×7.5 cm) were located at the same fixed location on the open field throughout the study: two at corners and two at places near the center of the open field. After each rat was tested, the table and objects were wiped clean with a diluted solution of an antibacterial cleaner (Lysol). Behavior on the open field was videotaped continuously by a camera affixed to the ceiling (providing a stationary top view of the entire open field and the rat in it). Videotapes were converted to MPEG files (Canopus MPEGPro EMR realtime MPEG-1 MPEG-2 encoder), and these digitized videos were used to automatically track the trajectories of locomotion using EthoVision 3.1 (Noldus Information Technology, Netherlands) software (Noldus et al. 2001; Spink et al. 2001).

Data analysis

EthoVision 3.1 software was used to extract the time series of x, y coordinates of the rat from digitized video recordings (Dvorkin et al. 2006). To remove noise, digitized tracking data were preprocessed (by applying appropriate filters to smooth the x, y coordinates) (Hen et al. 2004), and the obtained coordinates were divided into episodes of forward locomotion (called progression) and episodes of small movements or immobility (called lingering), as described previously (Drai et al. 2000; Drai and Golani 2001; Golani et al. 1993). The coordinate system was mapped onto the 25 open field locales (places) (Szechtman et al. 1998), and the frequency of visits and duration of stops in each locale were computed (the terms "visit" and "stop" are equivalent and are used interchangeably). Checking behavior was defined with reference to the most visited locale (labeled "key place" or "key locale"; these terms are equivalent), which in most instances is also the locale with the longest total duration of stops (Eilam and Golani 1989; Szechtman et al. 1998). A visit to the key place is also referred to as a "check" or "checking," and the following set of four measures of checking behavior were computed. (1) Frequency of checking: total number of visits to the key locale. (2) Length of check: total duration of stay at the key locale divided by the frequency of visits there; this measure is also an indirect index of ritual-like behavior as the appearance of motor rituals in quinpirole-treated rats is associated with a very short duration of stay in the key locale (Ben Pazi et al. 2001; Szechtman et al. 1998). (3) Recurrence time of checking: mean duration of return times to the key locale ("return time" is the interval from departure to next arrival at the locale). (4) Stops before returning to check: mean number of places visited between returns to the key locale. Compulsive checking behavior is identified by the presence of a significant difference between quinpirole- and saline-treated rats—all four measures need to differ from controls for the claim of "compulsive" checking (Szechtman et al. 1998), and hence the group of these four measures is termed "criteria measures" for compulsive checking.

The criteria measures for compulsive checking were dissociated empirically in a lesion study (Dvorkin et al. 2010). Specifically, a lesion to the nucleus accumbens core (NAc) altered the amount of checking behavior (as indexed by frequency of checking and length of check) whereas a lesion to the orbitofrontal cortex (OFC) affected the delay between checks of the key locale (as indexed by time to return to check and number of stops before returning to check. This pattern of results suggested that the functional roles of the NAc and OFC in checking behavior are to control the vigor of motor performance and the focus on goal-directed activity, respectively (Dvorkin et al. 2010). Accordingly, we consider vigor and focus as two relatively independent components of checking behavior, with the vigor of checking indexed by frequency of checking and length of check, and the focus on checking indexed by time to return to check and number of stops before returning to check.

In addition to the above criteria measures, we also evaluate "time to next checking bout" (Dvorkin et al. 2006). This measure is greatly reduced in quinpirole-sensitized rats and has been proposed to index the third constitutive component of compulsive checking behavior—satiety or rest after checking (Dvorkin et al. 2010). It was reasoned (Dvorkin et al. 2010) that in the animal model, the reduced satiety or "rest" after a bout of checking corresponds to notions that OCD reflects failure to achieve "sense of task completion"(Pitman 1989) or "feeling of knowing" (Szechtman and Woody 2004).

The computation of checking bouts is detailed in Dvorkin et al. (2006). Briefly, the method follows the logic used to identify the clustering of a bout of eating behavior into a "meal" and the time between meals into a period of post-ingestion satiety (Tolkamp et al. 1998; Tolkamp and Kyriazakis 1999). A bout of behavior, according to those authors, is defined on the basis of the distribution of time intervals between behavioral events (inter-event intervals). This distribution is examined to locate and extract a time-point that will produce a natural split between clusters of inter-event intervals. Specifically, the identified time-point will separate the time intervals into a class of (relatively long)

intervals that are between the bouts of behavior (inter-bout intervals) and a class of (relatively shorter) intervals that belong within a bout of behavior (intra-bout intervals) (Tolkamp et al. 1998; Tolkamp and Kyriazakis 1999). This principle was employed in an algorithm developed to identify bouts of checking behavior (Dvorkin et al. 2006) and extract "time to next checking bout." A rat may complete a bout of checking but not start the next bout during the session and hence the number of rats used for "time to next checking bout" is generally smaller than for the criteria measures for compulsive checking. Generally, saline-treated rats have one to two bouts of checking behavior in a session while quinpirole-treated rats usually perform 2 or more bouts (Dvorkin et al. 2010). When a rat did complete more than one bout of checking, only the first "time to next checking bout" was used for statistical analysis (Tucci et al. 2013).

Design and procedure

The experiment addressed two research questions: (1) does mCPP attenuate the performance of checking behavior during the period of induction of compulsive checking with quinpirole; and (2) does mCPP prevent the full-blown induction of compulsive checking to quinpirole? For question 1, the time course of development of compulsive checking across injections 1 to 10 in quinpirole-treated rats was compared to rats co-injected with quinpirole and mCPP. Four independent groups of rats (N=14/group) were tested: two experimental groups, each co-injected with quinpirole (0.25 mg/kg) and mCPP (either 0.625 mg/kg or 1.25 mg/kg of mCPP), and two control groups, one of which was co-injected with quinpirole and saline, while the other one received injections of saline. For question 2, same groups received two additional trials (trial 11 and trial 12), and the performance of mCPP co-treated rats was compared to their performance with quinpirole only. To control for possible order effects, half of the animals in each experimental group continued with their usual treatment on trial 11, whereas on trial 12, they received quinpirole, and saline was substituted for mCPP. This sequence of treatments was reversed for the other half of the rats in the two experimental groups. The two control groups continued with their usual treatments on trials 11 and 12; however, for statistical analysis, each control group was randomly subdivided for trials 11 and 12 into a "usual treatment-no mCPP" vs "no mCPP-usual treatment" sequence order to correspond to the subdivision of the experimental groups. For analysis of results, the subgroups were realigned such that trial 11 refers to the test with "usual treatment" while trial 12 refers to the test where no mCPP was administered.

Rats were allocated into groups based on body weight at the start of the experiment. For all trials, the same procedure was followed: Animals were weighed, transported in their home cage to an adjoining non-colony experimental testing room, and was administered with the appropriate injections. Immediately afterwards, the rat was placed into the open field for 55 min and its behavior was videotaped for offline analysis. Each rat had two open field tests per week, and hence, the experiment was 6 weeks in total duration. Each rat was run on the same day of the week (Mon/Thu or Tue/Fri), at approximately the same time of day, and was allocated to the same experimenter for the duration of the study.

Statistical analysis

To assess the effects of mCPP on performance of checking behavior across injections 1 to 10 (research question 1), regression estimates for each dependent variable of the criteria measures were analyzed using a one-way analysis of variance (ANOVA), followed by between-group comparisons using Duncan multiple range test. A regression line was fitted for each individual rat and the obtained slopes and intercepts served as input for the one-way ANOVAs above (Table 1). A regression analysis was employed for group comparison across injections, rather than repeated measures ANOVA, because regression analysis is less sensitive to missing data points due to sporadic absence of compulsive checking or random technical malfunction. For analysis of the variable time to next checking bout, multilevel regression (MLM) was used to estimate group slope and intercept and for between-group comparisons, because of higher incidence of missing data for this variable given that rats do not necessarily continue to a second bout of checking. To correct for skew in the data, the variables duration of visit to key locale and time to next checking bout were log transformed for statistical analysis.

To compare performance with and without mCPP (research question 2), a 4×2 ANOVA was used with chronic drug treatment group (saline vs. quinpirole vs. quinpirole+mCPP low dose vs. quinpirole+mCPP high dose) as the between-group factor and trial (with mCPP vs. without mCPP) as a within-group factor (preliminary analysis showed that trial order was not significant and hence is not included in data presentation); where appropriate, simple effects were evaluated by comparing the relevant marginal means and 95 % confidence intervals. For analysis of the variable time to next checking bout, a separate one-way ANOVA was performed for each trial to maximize the number of rats providing data for the analysis. The chosen level of significance was P less than 0.05. Calculations were carried out using IBM SPSS Statistics 20.0

Results

Profile of induction of compulsive checking by quinpirole

To appreciate the effects of mCPP on the induction of compulsive checking by quinpirole, we first highlight the change

Table 1 Parameters of regression lines fitted to measures of compulsive checking shown in Fig. 1

Compulsive checking measures	Regression parameter	Group				ANOVA	
		Saline	QNP	QNP + $mCPP_1$	QNP + $mCPP_2$	$F(3,52)$	$P\leq$
Frequency of checking	Slope	−0.22±0.66	**7.46**±0.66[a]	**7.94**±0.66[a]	**4.88**±0.66[abc]	31.68	0.001
	Intercept	**18.63**±4.28	**27.53**±4.28	**15.39**±4.28	6.97±4.28[b]	3.95	0.013
Length of check (log s)	Slope	**0.04**±0.01	**−0.10**±0.01[a]	**−0.12**±0.01[a]	**−0.10**±0.01[a]	34.12	0.001
	Intercept	**1.49**±0.11	**1.89**±0.11	**2.17**±0.11[a]	**2.33**±0.11[ab]	10.88	0.001
Recurrence time of checking (s)	Slope	1.69±1.59	−0.86±1.59	**−3.70**±1.59[a]	**−5.41**±1.59[a]	3.88	0.014
	Intercept	**78.25**±11.94	**36.25**±11.94[a]	**54.15**±11.94	**79.07**±11.94[b]	3.00	0.039
# of stops before returning to check	Slope	**−0.09**±0.04	0.02±0.04	0.01±0.04	−0.02±0.04	1.75	0.169
	Intercept	**4.77**±0.29	**2.56**±0.29[a]	**2.36**±0.29[a]	**2.45**±0.29[a]	16.54	0.001
Time to next checking bout (log s)	Slope	0.03±0.02	**−0.06**±0.02[a]	**−0.10**±0.02[ab]	**−0.07**±0.02[a]		
	Intercept	**2.68**±0.10	**2.74**±0.10	**3.12**±0.88[ab]	**3.03**±0.15		

Estimates of group slope and intercept are means (and SEM) of individual rat regression parameters fitted to the dependent variable data across injections 1 to 10. Values in bold are significantly different from 0. Saline refers to control group injected chronically with saline + saline; QNP group refers to rats injected chronically with quinpirole (0.25 mg/kg) + saline; QNP + $mCPP_1$ group received quinpirole (0.25 mg/kg)+mCPP (0.625 mg/kg); and QNP + $mCPP_2$ group was treated with quinpirole (0.25 mg/kg)+mCPP (1.25 mg/kg). Groups were compared using a one-way analysis of variance and the obtained F values and statistical significance are indicated. ANOVAs are based on 14 rats per group that provided valid data for analysis. Duncan multiple range test was used for post hoc comparisons, and significant differences ($p<0.05$) are indicated by a letter superscript: [a] vs. Saline, [b] vs. QNP, [c] vs. QNP+$mCPP_1$. For *time to next checking bout,* multilevel regression (MLM) was used to estimate group slope and intercept and for between-group comparisons. The actual number of rats in a group that provided valid data for analysis of this variable was 11, 14, 14, and 12 for the *saline + saline*, *QNP*, *QNP + $mCPP_1$*, and *QNP + $mCPP_2$* groups, respectively

in measures of compulsive checking as a function of repeated treatment with quinpirole. Figure 1 (left panel) displays means for the criteria measures of compulsive checking—frequency of checking, length of check, recurrence time of checking, and number of stops before returning to check—shown by each group during injections 1 to 10; open and solid circles represent saline and quinpirole rats, respectively, and the solid and dashed thin lines indicate the calculated regression lines for saline and quinpirole groups. Inspection of these regression lines suggests that during the course of repeated treatment, quinpirole induced one pattern of change for frequency of checking and length of check (Fig. 1, left panel, top row), and a different pattern of change for recurrence time of checking and stops before returning to check (Fig. 1, left panel, bottom row), compared to the saline group. For the former measures, repeated injections of quinpirole altered the slope but not the intercept of the regression line while for the latter measures, quinpirole produced a shift in the intercept of the regression line without effect on the slope. Indeed, as shown in Table 1, these observations are supported by statistical analysis.

As noted in the "Materials and methods," frequency of checking and length of check are variables that index the vigor in the motor performance of checking while recurrence time of checking and stops before returning to check are variables related to the focus of the task checking. Accordingly, the statistically significant effects on the slope of the regression line indicate that only measures of vigor sensitize with repeated injections of quinpirole. In contrast, the statistically significant effects on the intercept of the regression line suggest that quinpirole affects measures of focus acutely, and this acute effect persists unabated throughout the course of chronic quinpirole treatment.

Figure 1 (right panel) displays a similar type of analysis for the measure of time to the next checking bout. This measure is suggested to constitute a component of compulsive checking related to satiety or negative feedback engendered by completion of the task of checking (Dvorkin et al. 2006; Dvorkin et al. 2010). Inspection of Fig. 1, and results of statistical analysis presented in Table 1, show that the change in this variable across injections is similar to the change observed with length of check, namely, a decline across injections in its duration, compared to the slight increase in duration across saline injections (Table 1).

In all, the present findings with quinpirole replicate the pattern of results across injections obtained earlier using a somewhat different method of analysis (Dvorkin et al. 2006).

Effects of mCPP on quinpirole induction profile

Higher dose of mCPP (1.25 mg/kg)

As shown in Fig. 1 (left panel) and Table 1, the higher dose of mCPP altered markedly the quinpirole profile for the vigor of compulsive checking (frequency of checking and length of check). In contrast, mCPP had only a small effect on the quinpirole profile for focus on checking, in that there was only a transient influence on one of the measures (recurrence

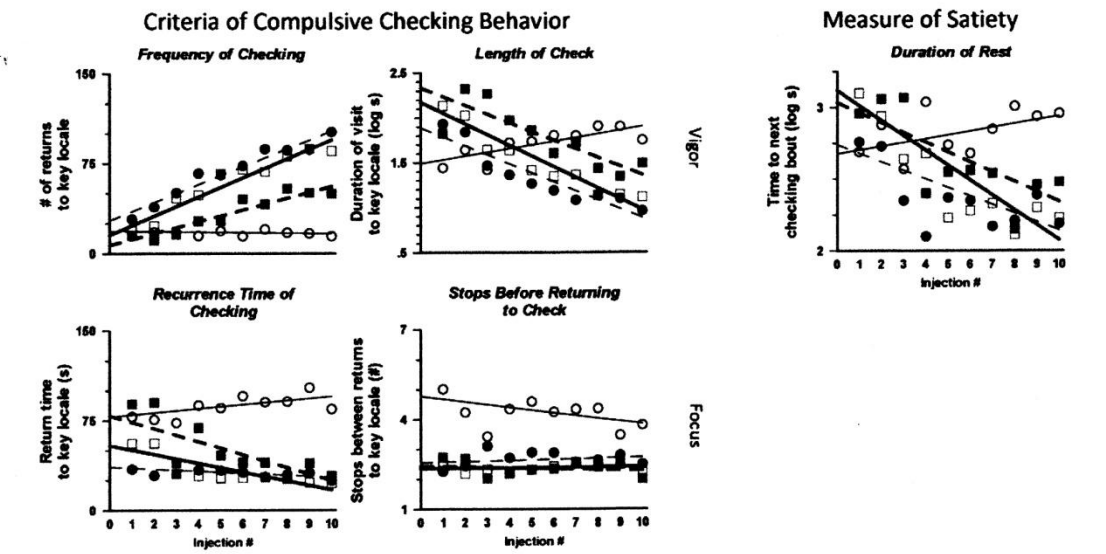

Fig. 1 Effects of mCPP on the criteria measures for compulsive checking and on the measure of post-checking satiety during the course of chronic treatment with quinpirole. See Table 1 for parameters of the indicated regression lines. Each *symbol* is the mean value for the indicated dependent variable at the indicated injection obtained from all rats showing a value. For the saline control group, the number of values at each injection for the criteria measures ranged from 9 to 14 while for the satiety graph they ranged from 3 to 7. For the QNP control group, the corresponding numbers ranged from 9 to 14 and from 4 to 11; for the QNP+mCPP$_1$ group, they were 8 to14 and 5 to 10. For the QNP+mCPP$_2$ group, the numbers for the criteria measures ranged from 7 to 14 while for the satiety graph the numbers for injections 1 through 4 were 1, 2, 3, and 4, respectively, and for injections 5 to 10, they ranged from 5 to 10. Note that the parameters of the indicated regression lines were not obtained using these actual means but were computed from the regression parameters of each individual rat as described in the "Materials and methods." *Open circles*, Saline control group injected chronically with saline+saline; *solid circles*, QNP control group injected chronically with quinpirole (0.25 mg/kg)+saline; *open squares*, QNP+mCPP$_1$ group injected chronically with quinpirole (0.25 mg/kg)+mCPP (0.625 mg/kg); and, *solid squares*, QNP+mCPP$_2$ group injected chronically with quinpirole (0.25 mg/kg)+mCPP (1.25 mg/kg). The corresponding regression lines are: *thin solid line*, Saline control group; *thin dashed line*, QNP control group; *thick solid line*, QNP+mCPP$_1$ group; *thick dashed line*, QNP+mCPP$_2$ group

time of checking) and no influence at all on the other measure of focus (number of stops before returning to check). Specifically, for vigor, co-treatment with mCPP yielded a significantly smaller slope and lower intercept for frequency of checking, and a significantly different intercept for length of check, compared to treatment with quinpirole alone (Table 1). The asymmetry of findings for the two measures of vigor suggests differential mCPP effects. The slope findings suggest a lowering effect of mCPP on the quinpirole sensitization rate in the amount of checking, without affecting the sensitization rate in the duration of checks. In contrast, the intercept findings suggest that mCPP has acute effects on both measures of vigor. Interestingly, these acute mCPP effects do not prevent the development of quinpirole sensitization (Fig. 1, left panel, top row).

The higher dose of mCPP also had an effect on one measure of focus (recurrence time of checking) producing a significantly higher intercept compared to quinpirole alone (Table 1). However, by injections 8 to 10, the mCPP co-treated group had a similar return time to key locale as the quinpirole alone group (Fig. 1, left panel, bottom left row). This suggests that the acute mCPP effect on recurrence time of checking may tolerate with repeated injections. Hence, in the absence of mCPP modulating the quinpirole effect on stops before returning to check, it appears that the higher dose of the drug does not alter the quinpirole profile for the focus on checking.

Finally, as shown in Fig. 1 (right panel), mCPP produced a significant increase in the intercept of the regression line for time to the next checking bout, compared to quinpirole alone (Table 1). As was observed for length of check, this suggests an acute mCPP effect on the quinpirole profile for satiety, without affecting the rate of sensitization to quinpirole.

Lower dose of mCPP (0.625 mg/kg)

As shown in Fig. 1 (left panel) and Table 1, the lower dose of mCPP did not alter the quinpirole profile for the vigor or focus of compulsive checking. However, this dose of mCPP did have significant effects on the slope and the intercept for time to the next checking bout, compared to quinpirole alone (Table 1). As noted above, the higher dose of mCPP altered the intercept but not the slope of the regression line for time to the next checking bout (Table 1). Hence, it may be the case

that the lower, but not the higher dose of mCPP, potentiates the quinpirole sensitization of satiety. Accordingly, there may be two, seemingly opposite, effects of low-dose mCPP co-treatment on the "rest" following a bout of checking behavior: an acute effect that prolongs this interval and a repeated treatment effect that quickens the rate of negative feedback foreshortening with repeated exposure to quinpirole.

Long-term effects of chronic mCPP co-treatment on quinpirole-induced compulsive checking

Figure 2 compares performance of the mCPP co-treated rats on a test with mCPP (left bar cluster of each graph) with a test on which mCPP was omitted (right bar cluster of each graph). The test with mCPP (trial 11) is in essence a continuation for every group of the same treatment as they received for injections 1 to 10; the test without mCPP (trial 12) is a further continuation of the same treatment for the saline and quinpirole groups but an omission of mCPP for the two quinpirole+mCPP co-treatment groups. As is evident, the test with mCPP confirms the results shown in Fig. 1, namely, a significant attenuation in the vigor of checking with the higher dose of mCPP but no effect on the focus of checking or on the measure of satiety. The novel findings are the results without mCPP: strikingly, the attenuation of vigor is no longer present in that every group injected with quinpirole performs similarly, regardless of mCPP history. Hence, the attenuation of vigor with mCPP co-treatment during induction of compulsive checking (Fig. 1) does not impact development of full-blown compulsive checking, as evident by the challenge test with quinpirole alone in the absence of mCPP.

Discussion

A challenge injection of the serotonergic agonist mCPP to rats with quinpirole-induced compulsive checking inhibits their vigor of checking and such animals no longer meet the criteria for compulsive checking behavior (Tucci et al. 2013). In the present study, mCPP was coadministered with quinpirole to naïve rats and the development of compulsive checking was monitored. Here, too, mCPP diminished motor vigor during the course of chronic treatment with quinpirole, seemingly reducing the development of compulsive checking. And yet, despite reduced performance during the induction phase, these rats showed fully developed compulsive checking upon challenge with quinpirole alone. It appears therefore that even though mCPP inhibits the performance under quinpirole of compulsive checking, it does not block the induction by quinpirole of the compulsive checking phenotype. Thus, in the quinpirole model, the neural circuit necessary to acquire compulsive checking must be distinct in some part from the circuit mediating performance of this compulsive behavior. Similarly, these animal model findings suggest that for the human condition, pathogenesis of OCD and functional manifestation of OCD pathophysiology probably involve partially non-overlapping neural circuits, with only the latter subject to modulation by mCPP.

Present findings of diminished performance during the induction phase suggest further that development of compulsive checking behavior is not a direct function of motor practice. Instead, the induced compulsive behavior may reflect plastic changes underlying sensitization to quinpirole. A number of brain changes had been found in quinpirole-sensitized rats. For instance, using the 2-deoxyglucose technique to measure local cerebral glucose utilization in quinpirole-sensitized animals (Carpenter et al. 2003; Richards et al. 2005), alterations had been found in cortical (the cingulate cortex area 1, frontal cortex area 3, lateral orbital cortex, medial/ventral orbital cortex, and parietal cortex) and subcortical areas (ventral pallidum and nucleus accumbens). Others report increased high-affinity states of dopamine D2 receptors ($D2^{High}$) in quinpirole-sensitized animals (Culver et al. 2008; Perreault et al. 2007; Seeman et al. 2006) and decreased dopamine levels in the left prefrontal cortex (Sullivan et al. 1998). However, which of those changes, if any, are necessary to produce compulsive checking requires further research.

The differential effects of mCPP coadministration on the expression and the induction of compulsive checking with quinpirole implicate a dopamine-serotonin interaction in the expression of compulsive checking and suggest minimal serotonergic modulation in the induction of this compulsive behavior. Such an interpretation is consistent with the theoretical framework that OCD is a manifestation of a disturbance in security motivation (Szechtman and Woody 2004; Woody and Szechtman 2005; 2011), as elaborated below.

The security motivation system (SMS) evolved to handle potential threats to survival, such as the possibility of contamination or predation. The output of SMS is a precautionary behavior such as washing or checking and performance of those security-related behaviors generates a negative feedback satiety signal which deactivates security motivation. However, if the negative feedback signal is not generated due to a dysfunction, the motivation will not be deactivated. Consequently, the persistent motivational state will continue to drive performance of security-related behaviors for a prolonged period of time, yielding OCD compulsions such as compulsive washing or compulsive checking.

The pathophysiology of OCD (for review, see Szechtman et al. 2013) probably involves overly persistent and uncontrolled activity in a neural circuit composed of a series of cascading cortico-striatal-thalamic-cortical loops (Aouizerate et al. 2004; Baxter 1992; Graybiel and Rauch 2000; Huey et al. 2008; Insel 1992; Modell et al. 1989; Saxena et al. 2001; Stein 2002; Szechtman and Woody 2004; Vermeire et al. 2012; Wise and Rapoport 1989). This same neural circuit is

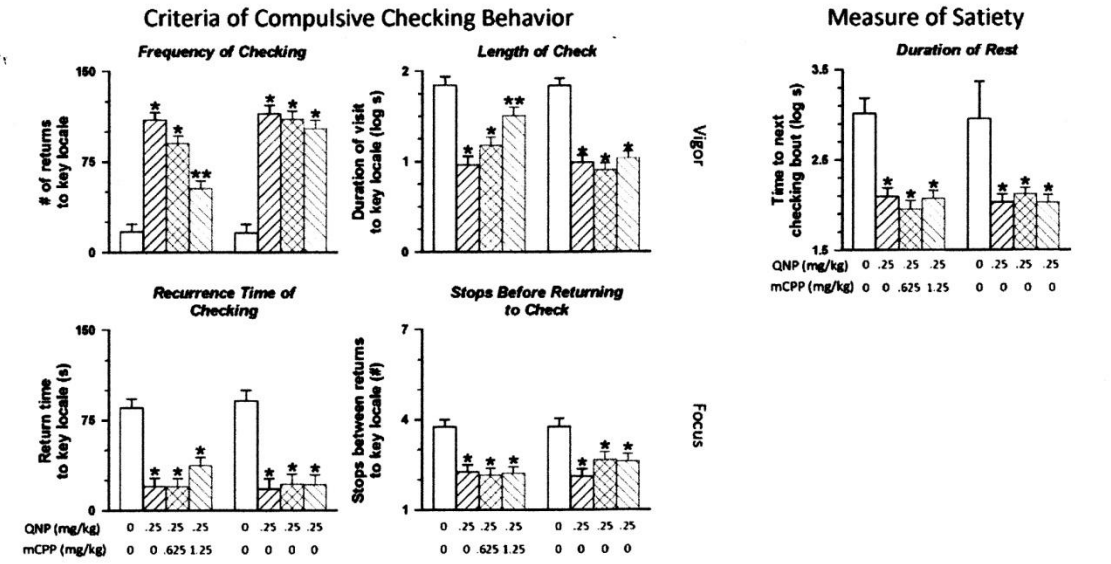

Fig. 2 Test for compulsive checking with and without co-injection of mCPP. The *left* cluster of bars of each graph is the trial with co-injection of quinpirole and mCPP, and the *right* cluster of bars is the trial with injection of quinpirole only. There was a significant interaction between chronic drug treatment group and trial, for frequency of checking ($F(3,51)=8.98$, $P<0.001$) and for length of check ($F(3,51)=8.44$, $P<0.001$), and a significant main effect of chronic drug treatment group for recurrence time for checking ($F(3,51)=25.59$, $P<0.001$) and stops before returning to check ($F(3,51)=11.37$, $P<0.001$); simple effects were evaluated within each trial only. For the satiety graph, there was a significant effect of group for the test with mCPP ($F(3,28)=13.69$, $P<0.001$) and for the test without mCPP ($F(3,30)=5.99$, $P=0.003$); the actual number of rats providing valid data for statistical analysis for the test with mCPP was 5, 13, 9, and 5 for the *saline+saline*, *QNP*, *QNP+mCPP*$_1$, and *QNP+mCPP*$_2$ groups, respectively; the corresponding numbers for the test without mCPP were 2, 13, 8, and 11; between-group differences in a test were evaluated using Duncan multiple range test. * $p<0.05$ *vs* saline controls; ** $p<0.05$ *vs* all other groups

considered also the neuroanatomical substrate of SMS (Szechtman and Woody 2004; Woody and Szechtman 2011) and hence, the pathophysiology of OCD is equivalent to prolonged activity in the SMS circuit. According to the authors (Szechtman and Woody 2004; Woody and Szechtman 2011), activation of SMS by a potential threat induces a motivational drive mediated by dopaminergic inputs from the ventral tegmental area and the substantia nigra. The ensuing performance of security-related behaviors generates a negative feedback signal that in turn deactivates security motivation; this feedback satiety signal to shut down security motivation is carried by serotonergic pathways from the brainstem to the limbic striatum and the medial and orbital frontal cortex. Thus, the OCD phenotype may be produced by an excessive dopaminergic motivational drive, an insufficient serotonergic negative feedback or both.

In the quinpirole sensitization model, the open field environment presents a potential threat that activates the dopaminergic motivational drive of the security motivation circuit. Quinpirole induces compulsive checking behavior by driving continually the dopamine receptors of the circuit and overpowering any negative feedback to terminate the behavior. Compulsive checking in the open field is induced not only by quinpirole but also by the 5-HT$_{1A}$ agonist 8-OH-DPAT (Alkhatib et al. 2013). However, the mechanism by which 8-OH-DPAT induces compulsive checking is not excessive stimulation of the motivational drive but probably involves insufficient serotonergic negative feedback. Specifically, it has been suggested that because 8-OH-DPAT has inhibitory effects on serotonin activity (Albert and Le François 2010; Alex and Pehek 2007; Barnes and Sharp 1999), this would make the serotonergic negative feedback signal from security-related behaviors functionally ineffective and so fail to shut down the dopaminergic motivational state induced by the potential danger of the open field. Such a proposed mechanism is consistent with findings that 8-OH-DPAT can perpetuate an excitatory effect on dopaminergic activity via its combined action on 5-HT$_{1A}$ autoreceptors and heteroreceptors (Barnes and Sharp 1999; Fink and Göthert 2007; Hayes and Greenshaw 2011), and evidence for a dysfunctional negative feedback signal in OCD patients (Hinds et al. 2012).

Considering that mCPP acts as an agonist at several 5-HT receptor subtypes (for review, see Gatch 2003), further research is needed to identify which receptor subtypes mediate the mCPP negative feedback effects on quinpirole-induced compulsive checking. Although mCPP has high affinity for 5-HT$_{2c}$ receptors (Rajkumar et al. 2009), the relevance of 5-HT$_{2c}$ stimulation for the mCPP effect may be questioned given that

in other animal models, inhibitory effects on compulsive behavior are produced by relatively specific antagonist drugs of 5-HT_{2c} receptors (Boulougouris et al. 2008; Flaisher-Grinberg et al. 2008; Papakosta et al. 2013; Schepisi et al. 2013).

In contrast to the above mCPP findings suggesting dopamine-serotonin interaction on checking vigor, the observed absence of an effect with mCPP on the induction of compulsive checking points to a different interaction between these neurotransmitter systems in the development of this compulsive phenotype. Specifically, it suggests that the particular serotonergic negative feedback signal stimulated by mCPP—though sufficient to inhibit the performance of even compulsive checking (Tucci et al. 2013)—is inadequate to block the cascade of changes produced by repeated injections of quinpirole, and consequently, that those quinpirole-induced changes underlie the compulsive checking endophenotype (and by implication, the OCD endophenotype in the human). As noted before, induction of compulsive checking is associated with development of sensitization to quinpirole, and thus the elucidation of mechanism(s) by which quinpirole produces behavioral sensitization should illuminate the neurobiology of the OCD endophenotype.

Because mCPP treatment was effective in blocking the motor output of checking but did not arrest the acquisition for its enhanced performance, it is likely that the serotonergic negative feedback signal which deactivates an activated security motivation includes more than just the pathway or receptors stimulated by mCPP. In a previous study, the acute administration of mCPP had normalizing effects on measures of post-checking satiety and vigor of checking (Tucci et al. 2013). Together, these results suggested that the drug reduced compulsive performance and appeared to restore in full the normal negative feedback signal to deactivate security motivation. However, in the present study, a normalizing effect of mCPP on post-checking satiety was acute and by end of chronic treatment, this measure was similar to quinpirole controls. This absence of an effect on post-checking satiety suggests that chronic mCPP could not fully restore the normal negative feedback signal to deactivate security motivation. Conceivably, the negative feedback signal is composed of a component that is sensitive to mCPP and can inhibit motor vigor, and a component that has only acute sensitivity to mCPP and can terminate motivational drive. Such a hypothesis can be tested experimentally by assessing the extent to which the neurobiological effects of chronic quinpirole are reversed or blocked by treatments with mCPP.

In all, the present results indicate that stimulation of 5HT receptors inhibits performance of compulsive checking but does not prevent the development of compulsive checking in the quinpirole model. These findings strongly suggest that 5HT receptors play a role in a serotonergic negative feedback signal that shuts down the motor output of an activated dopaminergic security motivation drive, but it is still undetermined whether the receptors stimulated by mCPP play a role in the satiety signal which deactivates the actual motivation to engage in checking behavior. However, the pathogenesis of compulsive checking is not modulated by receptors stimulated by mCPP. Instead, the development of compulsive checking probably reflects the neuroplastic changes produced by stimulation of D2/D3 receptors with quinpirole. Those changes in the animal model may be the neurobiological substrate of the OCD endophenotype.

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CHAPTER 4: THE NUCLEUS ACCUMBENS CORE DOES NOT MEDIATE THE ATTENUATION OF COMPULSIVE CHECKING PRODUCED BY MCPP TREATMENT IN A RAT MODEL OF OBSESSIVE-COMPULSIVE DISORDER (OCD)

Tucci, M. C., Dvorkin-Gheva, A., Johnson, E., Cheon, P., Taji, L., Agarwal, A., Foster, J. A., & Szechtman, H. The nucleus accumbens core does not mediate the attenuation of compulsive checking produced by mCPP treatment in a rat model of obsessive-compulsive disorder (OCD). Submitted to *The European Journal of Neuroscience* on 2014-07-18.

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Mark C. Tucci - PhD candidate. With the assistance and advice of Dr. Henry Szechtman, designed the study, collected data, analyzed results and prepared the manuscript for submission.

Dr. Henry Szechtman - PhD candidate's supervisor. Provided assistance and advice to Mark C. Tucci on all aspects of the study, and contributed in particular to the statistical analysis and manuscript preparation.

Dr. Anna Dvorkin-Gheva - Collaborator. Performed computational analysis on the obtained co-ordinates of the animal on the open field to generate the spatial-temporal form of activity on the open field.

Dr. Jane Foster - Collaborator. Provided laboratory resources and guidance for the histology process, and edited the manuscript.

Eric Johnson, Paul Cheon, Leena Taji, and Arnav Agarwal - Laboratory members. Performed a portion of the data collection and edited the manuscript.

Abstract

The 5-HT agonist drug mCPP reduces two of the three functional components related to compulsive checking exaggerated by quinpirole treatment in rats: vigor and satiety, but not focus. However, it is unknown where the mCPP effect is mediated in the brain. Given suggestions that the NAc mediates inhibitory control on vigor and satiety, the present study asked whether the NAc mediates the mCPP effect on vigor and satiety, or whether another brain region mediates this mCPP effect. To address this question, the experimental approach was to exaggerate vigor and satiety with bilateral nucleus accumbens core (NAc) lesion (lesion there has been shown to exaggerate these two functional components, with no appreciable effect on the focus component) and treat lesion and sham-lesion groups with mCPP (0.625 mg/kg) and monitor checking behavior in a large open field. Results showed that as expected NAc lesion produced an increase in the vigor of checking and decreased satiety. Treatment with mCPP reduced the vigor and the satiety exaggerated by NAc lesion. These results suggest that the NAc does not mediate the effects of mCPP on vigor and satiety. Accordingly, a site downstream of the NAc, possibly the substantia nigra pars reticulata, is suggested to mediate the response to mCPP.

Key Words

Obsessive-compulsive disorder (OCD); animal model; compulsive checking behavior; nucleus accumbens core lesion; substantia nigra pars reticulata

Introduction

Obsessive-compulsive disorder (OCD) is a psychiatric affliction that probably involves persistent activity in a series of cascading cortico-striatal-thalamic-cortical loops (CBGTC loops) (Aouizerate et al., 2004; Baxter, 1992; Graybiel & Rauch, 2000; Huey et al., 2008; Insel & Winslow, 1992; Modell, Mountz, Curtis, & Greden, 1989; Saxena, Bota, & Brody, 2001; Stein, 2002; Szechtman & Woody, 2004; Vermeire et al., 2012; Wise & Rapoport, 1989). The nucleus accumbens (NA) is located in the ventral portion of the striatum, and has been reported to modulate OCD symptoms in clinical studies and OCD-like behavior in animal models of the disorder. For example, one study of treatment-resistant OCD patients found that bilateral stimulation of the NA significantly reduced the severity of symptoms and had no adverse effects (Denys & Mantione, 2010). However, a recent review indicates that longer term follow-up is required before concluding on the effectiveness of this treatment (Blomstedt, Sjoberg, Hansson, Bodlund, & Hariz, 2012). Studies from the animal literature also reveal a role for the NA on model compulsive behavior. For example, using the quinpirole sensitization rat model of OCD, it was reported that high-frequency stimulation of the NA core and shell subregions decreased the model compulsive behavior (Mundt et al., 2009). Others have shown that bilateral excitotoxic lesions to the NA core subregion results in the exaggeration of some, but not all, behavioural features related to the performance of checking behavior (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014).

Recently, it was reported that acute treatment with the 5-HT agonist drug mCPP attenuates the exaggerated vigor and satiety displayed by quinpirole-treated animals

showing compulsive checking behavior, rendering the animals no longer meeting criteria for 'compulsive' checking (Tucci et al., 2013). Given that mCPP is a non-selective 5-HT agonist drug (Fiorella, Rabin, & Winter, 1995; Hamik & Peroutka, 1989; Kilpatrick, Jones, & Tyers, 1987), it was suggested by the authors that the ameliorative effects produced by mCPP on the model compulsive behavior were mediated by stimulation of 5-HT receptors (Tucci et al., 2013). However, it is unknown where in the brain the receptors stimulated by mCPP are localised.

Work from our laboratory has shown that in saline-treated rats bilateral excitotoxic lesions of the nucleus accumbens core subregion (NAc) exaggerates two constitutive behavioral components related to compulsive checking: the vigor of checking performance and the amount of satiety following a bout of checking (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014). The lesion does not appreciably alter the third constitutive component, that is, focus on the task of checking. Such findings were interpreted to suggest that the NAc is inhibitory over vigor and satiety. Given that the NAc appears to exert inhibitory control over vigor and satiety (Dvorkin et al., 2010), and that mCPP inhibits these same constitutive behavioral components (Tucci et al., 2013), it is possible that the effects produced by the drug are mediated by the NAc. Alternatively, the response to mCPP may be mediated by receptors elsewhere in the brain. The present study tested which of these two possibilities is correct. To address this, we employed an animal preparation with bilateral NAc lesion to exaggerate the vigor of checking performance and the satiety following a bout of checking (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014). If mCPP produces its effects outside of the NAc,

then it follows that lesion animals injected with mCPP would show a reduction in vigor and satiety. However, if mCPP produces its effects at the NAc, then no decrease in vigor and satiety in lesion animals injected with mCPP should be evident.

Materials and Methods

Animals

To minimize the number of animals used, this experiment was conducted simultaneously with another that tested the effects of a different drug (8-OHDPAT) on NAc lesion animals and has since been published (Tucci, Dvorkin-Gheva, Johnson et al., 2014). This other study (Tucci, Dvorkin-Gheva, Johnson et al., 2014) addressed a different research question but used identical methods as those described here. Therefore, the same NAc lesion and sham lesion animals treated with saline were employed as controls for both studies. Hence, data for these groups are identical between this and the previous study (Tucci, Dvorkin-Gheva, Johnson et al., 2014). NAc lesion and sham lesion animals treated with mCPP employed in the present study comprised completely independent groups as part of the larger experiment.

Subjects were 87 experimentally naïve adult male Long Evans rats weighing 250-300 g at the onset of the experiment. Animals were housed in a rat colony room. They were maintained on a 12 hour light/dark cycle (lights on at 6:00 AM, lights off at 6:00 PM), but testing took place during the light phase. Upon arrival, animals were allowed 7 days to acclimatize to the facility, followed by 5 days of handling before the beginning of the experiment. Each animal was handled for approximately 2-5 minutes each day.

Following handling, animals received stereotaxic surgery, followed by a recovery period of 14 days. During the last 3 days of the recovery period, animals were handled again each day for approximately 2-5 minutes. Food and water were freely available during the experiment. Animals were housed and tested in compliance with the regulations set forth by the guidelines of the Canadian Council on Animal Care and approved by the Animal Research Ethics Board, McMaster University.

Drugs

Drugs were obtained from Sigma-Aldrich, USA. 1-(3-Chlorophenyl)-piperazine hydrochloride (mCPP) was administered to rats at a dose of 0.625 mg/kg. This dose was chosen because a prior study suggested that this dose attenuated measures related to the vigor of checking performance in intact rats (Tucci et al., 2013). mCPP was dissolved in 0.9% physiological saline and administered by a subcutaneous injection under the nape of the neck at a volume of 1 ml/kg. Control animals received an equivalent volume of saline in a similar manner.

Surgery

Animals receiving NAc lesions were administered the excitotoxin, N-methyl-D-aspartate (NMDA; Sigma Aldrich, USA) dissolved in phosphate-buffered saline at a concentration of 12 mg/ml. Animals receiving sham lesions received an equivalent volume of PBS. NMDA and PBS were administered by intracranial injection using a 10 µl non-coring Hamilton syringe (Hamilton Company, U.S.A) mounted to a motorized

Ultra Micro Pump (World Precision Instruments, U.S.A.) that was attached to the arm of a Kopf Stereotaxic Apparatus (David Kopf Instruments, U.S.A.). Animals were anaesthetised using vaporized isofluorane for surgery (Pharmaceutical Partners of Canada, Canada). The local analgesic lidocaine hydrochloride (0.002 mg; Astra Zeneca, Canada) was injected subcutaneously at the surgical site, and the non-steroidal anti-inflammatory analgesic Anafen (0.05 mg/kg; Merial, Canada) was administered 10 minutes prior to the end of surgery by subcutaneous injection. Coordinates for NAc lesion used were: anterior/posterior, +1.2 mm from bregma; medial/lateral, +1.9 mm; dorsal/ventral, -7.0 mm from dura. At the injection site, 0.3 µl of NMDA or PBS was injected bilaterally at a rate of 0.1 µl/min, and the needle was left in place for 5 min to allow fluid to sufficiently diffuse away from the needle tip.

Histology

At the end of behavioral testing, rats were euthanized using carbon dioxide. Brains were extracted and flash frozen in -60° C methylbutane, placed on dry ice for 1 minute, wrapped in aluminum foil, and stored in a -80° C freezer until sectioning. For sectioning, brains were mounted using Tissue-Tek Optimum Cutting Temperature compound and placed in a cryostat for 1 hour to thaw to -18° C. Brains were sectioned in the coronal plane at 12 µm thickness, with approximately every 9th section collected on a gelatine coated slide and stored in a -35° C freezer until immunohistochemistry. Neuronal nuclei (NeuN) protein immunohistochemistry was used to measure the size and location of lesions, and the possibility of any damage in control animals. Coronal

sections were stained using monoclonal mouse anti-neuronal nuclei (1 mg/ml; Chemicon International, U.S.A.) as the primary antibody, followed by a biotinylated monoclonal anti-mouse IgG (0.5 mg/ml; Vector Laboratories, Canada) as the secondary antibody according to a previously described procedure (Jongen-Relo & Feldon, 2002). Sections were analysed using an Axioskope microscope (2.5x objective) and Axiovision 4.3 software system (Carl Zeiss Microimaging Inc., U.S.A). For each section, the region of interest (ROI) was demarcated, and the size of lesion within the ROI was computed. To compute the size of lesion within ROI, brain sections at (or nearest) to pre-determined atlas plates (Paxinos & Watson, 1998) were taken (NAc: plates 12, 14 and 16) and percent of the ROI lesion at these plates were averaged to obtain mean percent of ROI lesion. We set a minimum criterion for lesion size of 55% for inclusion in behavioral analysis, as used in our prior studies (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014).

Apparatus

Animals were tested on a large open field (160 x 160 cm table without walls) that was located in a non-colony experiment room, as described previously (Dvorkin, Perreault, & Szechtman, 2006; Dvorkin et al., 2010). The table was divided virtually into a grid of 25 rectangular places (locales), however there were no actual lines on the table surface. Four small Plexiglas/glass boxes (approximately 8×8×7.5 cm) were located at the same fixed location on the open field throughout the experiment: two were located at corners and two were located at places near the center of the open field. After testing of

each rat, the table and objects were wiped clean with a diluted solution of an antibacterial cleaner (Lysol). A video camera mounted above the open field recorded behavior of the animals (providing a stationary top view of the entire open field and the rat in it). Videotapes were converted to MPEG files (Canopus MPEGPro EMR realtime MPEG-1 MPEG-2 encoder) and from these digitized videos EthoVision 3.1 software (Noldus Information Technology, Netherlands) was used to automatically track the trajectories of locomotion (Noldus, Spink, & Tegelenbosch, 2001; Spink, Tegelenbosch, Buma, & Noldus, 2001).

Data analysis

From the digitized video files, EthoVision 3.1 software was used to extract the time series of *x, y* coordinates of the rat in the open field (Dvorkin et al., 2006). To remove noise, digitized tracking data were pre-processed (by applying appropriate filters to smooth the *x, y* coordinates) (Hen, Sakov, Kafkafi, Golani, & Benjamini, 2004), and the obtained coordinates were divided into episodes of forward locomotion (called progression) and episodes of small movements or immobility (called lingering), as described previously (Drai, Benjamini, & Golani, 2000; Drai & Golani, 2001; Golani, Benjamini, & Eilam, 1993). The coordinate system was mapped onto the 25 open field locales (places) (Szechtman, Sulis, & Eilam, 1998), and the frequency of visits and duration of stops in each locale were computed (the terms ‘visit’ and ‘stop’ are equivalent and are used interchangeably). Checking behavior was defined with reference to the most visited locale (labelled 'key place' or 'key locale'; these terms are equivalent), which in

most instances is also the locale with the longest total duration of stops (Eilam & Golani, 1989; Szechtman et al., 1998). A visit to the key place is also referred to as a 'check' or 'checking', and the following set of four criteria measures of checking behavior were computed. (1) Frequency of checking: total number of visits to the key locale. (2) Length of check: total duration of stay at the key locale divided by the frequency of visits there; this measure is also an indirect index of ritual-like behavior as the appearance of motor rituals in quinpirole-treated rats is associated with a very short duration of stay in the key locale (Ben-Pazi, Szechtman, & Eilam, 2001; Szechtman et al., 1998). (3) Recurrence time of checking: mean duration of return times to the key locale ('return time' is the interval from departure to next arrival at the locale). (4) Stops before returning to check: mean number of places visited between returns to the key locale. Compulsive checking behavior is identified by the presence of a significant difference compared with saline-treated control animals – all four measures need to differ from controls for the claim of compulsive checking (Szechtman et al., 1998), and hence the group of these four measures is termed 'criteria measures' for compulsive checking.

The behavioral profile of compulsive checking behaviour has been empirically dissociated into a set of functional components (Dvorkin et al., 2010). Three functional components have been identified, each of which are greatly exaggerated in compulsive animals: the *vigor* with which checking is performed, the *focus* on the task of checking and the amount of rest or *satiety* following a bout of checking. Lesions to the nucleus accumbens core altered the intensity of checking (as indexed by changes in both the 'frequency of checking' and the 'length of check') and was considered to reflect an

increase in the vigor with which checking is performed. Lesions to the orbitofrontal cortex (OFC) affected the delay between checks (as indexed by change in both the 'time to return to check' and the 'number of stops before returning to check') and was considered to reflect a decrease in attention or focus on the task of checking. Together, the functional roles of the NAc and OFC were considered to control the vigor of checking and the focus on checking, respectively, and deemed relatively independent components of checking behavior.

The third functional component, satiety after a bout of checking, is indexed by the 'time to next checking bout', and has been observed to be greatly reduced in quinpirole sensitized animals (Dvorkin et al., 2010). The authors (Dvorkin et al., 2010) propose that this reduction in satiety reflects the failure to achieve a 'sense of task completion' (Pitman, 1989) or 'feeling of knowing' (Szechtman & Woody, 2004). The method of delineating bouts of checking in the animal model has been described previously (see Dvorkin et al., 2006). Briefly, the computation is based on the logic used to identify the clustering of a bout of eating behavior into a 'meal' and the time between meals into a period of post-ingestion satiety (Tolkamp, Allcroft, Austin, Nielsen, & Kyriazakis, 1998; Tolkamp & Kyriazakis, 1999). Accordingly, a bout of behavior is defined on the basis of the distribution of time intervals between behavioral events (inter-event intervals). This distribution is examined to locate and extract a time-point that will produce a natural split between clusters of inter-event intervals. Specifically, the identified time-point will separate the time intervals into a class of (relatively long) intervals that are between the bouts of behavior (inter-bout intervals) and a class of (relatively shorter) intervals that

belong within a bout of behavior (intra-bout intervals) (Tolkamp et al., 1998; Tolkamp & Kyriazakis, 1999). An algorithm designed with this logic was used to identify bouts of checking behavior (Dvorkin et al., 2006) and extract 'time to next checking bout'. A rat may complete a bout of checking but not start the next bout during the session and hence the number of rats used for 'time to next checking bout' is generally smaller than for the four criteria measures for compulsive checking. Generally, saline-treated rats have 1-2 bouts of checking behavior in a session while quinpirole-treated rats usually perform 2 or more bouts (Dvorkin et al., 2006; Dvorkin et al., 2010). As in Tucci et al. (2013), if more than one bout of checking was performed, only the first 'time to next checking bout' was used for statistical analysis.

Recently, the three separate functional components - vigor, focus and satiety - were simultaneously re-synthesized by non-quinpirole manipulation yielding animals that met full criteria for compulsive checking behavior (Tucci, Dvorkin-Gheva, Johnson et al., 2014), and hence confirming the presence of these constitutive functional components underlying the model OCD behavior (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014).

Design and procedure

The study consisted of a 2 x 2 fully crossed factorial design with one between factor being Type of Lesion (sham vs NAc) and the other being Drug Treatment (saline vs mCPP). Assignment to treatment groups was based on body weight and made 2-3 days before start of behavioral testing. Following recovery from surgery, testing on the

open field began. Rats were weighed in the colony room and transported to the experiment room containing the open field. Rats were administered their assigned treatment, and immediately placed onto the center of the open field. Each trial lasted 55 minutes. Rats received a total of four trials, each separated by 2-3 days.

Statistical analysis

The present study tested whether the exaggerated vigor of checking performance and post-checking satiety induced by NAc lesion is attenuated by mCPP stimulation at the NAc or outside of this structure. A 2-way ANOVA was computed with the between groups factors Type of Lesion (sham or NAc) and Drug Treatment (saline or mCPP) for each of the criteria measures of compulsive checking and for satiety following a bout of checking. To reiterate, the criteria measures of compulsive checking are: 'frequency of checking',' length of check', 'number of stops before returning to check', and 'recurrence time to check'. The former two criteria, together, index the vigor of checking performance, while the latter two, together, index focus on the task of checking. Post-checking satiety is indexed by the 'time to next checking bout'.

The significance level was set at $P < 0.05$. Analysis was computed using SPSS 20 for Windows. Values presented in graphs are the mean and standard error of the mean. To evaluate simple effects, the relevant marginal means and non-overlapping 95% confidence intervals were compared. We analyzed and present data for the fourth trial only. Observations from our lab suggest that mCPP produces a relatively robust effect at this time point, which we also reasoned would be relatively free of any non-specific

effects from the surgery, hence providing a more stable measure of any possible lesion/drug interaction.

Results

Histology

To be included for behavioral analysis, animals with NAc lesion were required to meet a minimum of 55% lesion to the ROI as set in prior studies (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014). The total number of animals included for each treatment condition is shown in Table 1. Overall, an average of approximately 72% cell-body damage to the ROI was observed in NAc lesion animals (Table 1). Figure 1 shows a representative NAc lesion animal across atlas plates 12, 14 and 16 (Paxinos & Watson, 1998) also shown in our previous study (from Tucci, Dvorkin-Gheva, Johnson et al., 2014). The representative animal in Figure 1 had a similar size of cell-body damage in the ROI as the overall mean across NAc lesion groups. Overall, lesions were highly localized to the accumbens core subregion, with only minor damage to the accumbens shell subregion and ventral pallidum at more posterior sections. Animals receiving a sham lesion had no detectable damage to NAc and this is indicated by 0% lesion in Table 1.

Effects of NAc Lesion on Measures of Compulsive Checking

Figure 2 shows criteria measures for compulsive checking and post-checking satiety on the fourth trial. There was a significant main effect of Type of Lesion for 'frequency of checking', $F(1, 83) = 14.781$, $p < .001$, and 'length of check', $F(1, 83) =$

17.516, $p < .001$. That is, NAc lesion increased the 'frequency of checking' and decreased the 'length of check' compared to sham lesion. The 'frequency of checking' and 'length of check' index the vigor of checking performance, and hence, NAc lesion increased vigor. The lesion did not significantly alter the 'recurrence time of checking', $F(1, 83) = 3.854$, $p = .053$, or 'stops before returning to check', $F(1, 83) = 0.068$, $p = .795$. A significant main effect of Type of Lesion was also indicated for satiety, $F(1, 43) = 12.373$, $p < .01$. Figure 2 shows that a lesion to the NAc decreased the amount of satiety compared to sham lesion. Therefore, as expected, in NAc lesion animals there was an increase in the vigor of checking performance and a reduction in post-checking satiety, but no alteration to focus.

Effects of mCPP on Measures of Compulsive Checking

There was a significant main effect of Drug Treatment for 'frequency of checking', $F(1, 83) = 15.225$, $p < .001$, and 'length of check', $F(1, 83) = 22.005$, $p < .001$. mCPP reduced the 'frequency of checking' and increased the 'length of check' compared with saline treatment. However, there was also a significant Type of Lesion X Drug Treatment interaction for 'frequency of checking', $F(1, 83) = 7.999$, $p < .01$. As displayed, mCPP decreased the 'frequency of checking' in NAc lesion animals but not in sham operated animals. There was no significant main effect of Drug Treatment for ‘recurrence time of checking’, $F(1, 83) = 0.134$, $p = .715$. However, there was a significant main effect of Drug Treatment for 'stops before returning to check', $F(1, 83) = 8.438$, $p < .01$. mCPP increased the number of 'stops before returning to check' compared with saline treatment.

This measure is one of the two required indexes for focus on the task of checking. However, because mCPP did not alter the other necessary index of focus, 'recurrence time of checking', the present results do not show an effect of mCPP on focus. Finally, Figure 2 shows a main effect of Drug Treatment on satiety, $F(1, 43) = 14.449$, $p < .001$. mCPP prolonged the duration of post-checking satiety compared to saline treatment. Therefore overall, mCPP treated animals showed a decline in the vigor of checking and a prolongation of post-checking satiety.

Discussion

The present study tested whether the effects of mCPP on vigor and satiety are mediated by an action of mCPP on the NAc or elsewhere in the brain. Similar to our previous findings, bilateral NAc lesion exaggerated the vigor of checking performance and post-checking satiety (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014), and mCPP treatment attenuated these same components of checking (Tucci et al., 2013). Importantly, in the present study, mCPP attenuated the exaggerated vigor and satiety that were produced by a lesion of the NAc. This outcome suggests that stimulation by mCPP of receptors outside of the NAc mediates the mCPP response, given that a large amount of the NAc was destroyed by an excitotoxic lesion, and assuming that the effects of mCPP are not mediated by the small patch of remaining NAc tissue. Below, we consider how these results shed light on a putative neurobiology underlying attenuation of compulsive checking with mCPP.

The present finding is in line with, and contributes to a hypothesis that a location downstream of the NAc mediates the effects of mCPP (Tucci et al., 2013). The putative neural region hypothesized to mediate the mCPP response is the substantia nigra pars reticulata (SNr) (Tucci et al., 2013). The SNr acts as an output mechanism for the basal ganglia, receiving projections from the NAc, and sending projections to the thalamus (Deniau, Menetrey, & Thierry, 1994). Accordingly, it has been suggested that disinhibition of NAc output exaggerates vigor and satiety (Dvorkin et al., 2010). Disinhibition of NAc output can be produced either by a lesion of NAc or by quinpirole inhibiting NAc. Upon reaching the SNr, the NAc output signal may be dampened by mCPP acting on 5-HT receptors located at the SNr. The rationale for suggesting that mCPP acts on 5-HT receptors located at the SNr is as follows: mCPP has activity at several 5-HT receptor subtypes including: 1A, 1B, 1D, 2A, 2C, and 3 (Fiorella et al., 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick et al., 1987). Stimulation of 5-HT receptors located at the SNr has been reported to yield an inhibitory effect on neurons (Collingridge & Davies, 1981; Dray, Gonye, Oakley, & Tanner, 1976; Oberlander, Hunt, Dumont, & Boissier, 1981), which may attenuate nigrothalamic output (Deniau et al., 1994), and therefore, may account for reductions in vigor and satiety. This neurobiological hypothesis may be tested by micro-injecting mCPP into the SNr and observing whether the drug attenuates vigor and satiety exaggerated by NAc lesion or quinpirole treatment as would be predicted according to the hypothesis.

Altogether, results from the present study suggest that NAc is not the critical site of action for mCPP to produce a reduction in compulsive checking. However, using the

same quinpirole animal model as employed in the present study, it has been reported that electrical stimulation of the NAc reduced quinpirole-induced compulsive behavior (Mundt et al., 2009). Therefore, NAc can contribute to the reduction of compulsive behavior in the quinpirole animal model. However, it is not known whether the mechanism by which electrical stimulation of NAc attenuated compulsive checking is functionally equivalent to the effects of mCPP acting outside the NAc, or whether the two experimental procedures reveal that more than one distinct neural structure can mediate attenuation of compulsive checking in the animal model.

In summary, mCPP treatment in NAc lesion animals reduced the vigor of checking and prolonged post-checking satiety. These results suggest that the effect of mCPP on these functional components of checking behavior are not mediated by an action of mCPP on NAc. Rather, the effects on vigor and satiety are likely mediated by a neural structure located downstream of the NAc. Indeed, it is suggested that the attenuation of compulsive checking by mCPP is mediated by 5-HT receptor stimulation at the SNr. Conceivably, the same may apply to attenuation of OCD in human patients.

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Declarations

The authors declare no conflicts of interest.

Abbreviations

OCD, obsessive-compulsive disorder; 5-HT, serotonin; DA, dopamine; mCPP, 1-(3-Chlorophenyl)-piperazine hydrochloride; NA, nucleus accumbens; NAc, nucleus accumbens core; OFC, orbitofrontal cortex; ROI, region of interest; SNr, substantia nigra pars reticulata; CBGTC loops, cortico-striatal-thalamic-cortical loops; DPAT, 8-Hydroxy-2-(di-n-propylamino) tetralin hydrochloride; NMDA, N-methyl-D-aspartate; PBS, phosphate buffered saline

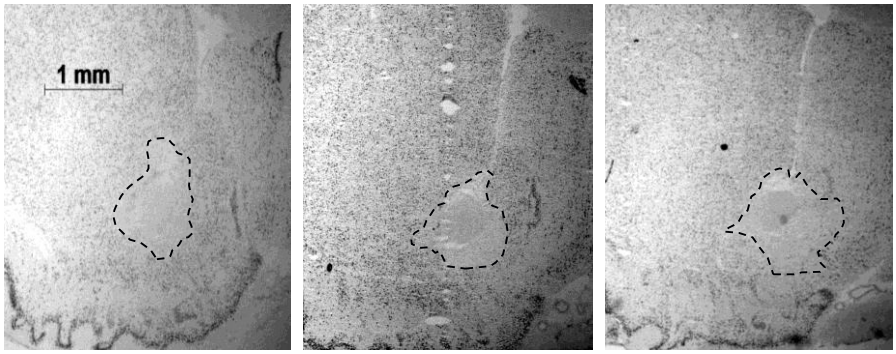
Figure Captions

Figure 1 - A representative neuronal nuclei-stained section for NAc lesion in a rat representative of the average cell-body damage across lesion groups. The left, middle and right panels represent atlas plates 12, 14 and 16 across the NAc, respectively (Paxinos & Watson, 1998). These plates are located 2.76, 2.28, and 2.04 mm from bregma, respectively. The dashed line demarcates the area of cell damage. From Tucci, Dvorkin-Gheva, Johnson et al. (2014).

Figure 2 - Performance on criteria measures of compulsive checking and satiety for NAc and sham lesion animals treated with mCPP or saline. # main effect of Type of Lesion; * main effect of Drug Treatment; ** significantly different than NAc + saline group. All p's < 0.05.

Table 1. Number of rats in each group included for analysis, and the percentage size (mean ± sem and minimum) of lesion to the NAc. A minimum of 55% cell-body damage was required for lesion animals to be included for analysis as in (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014).

	Type of Lesion				
	Sham Lesion		NAc Lesion		
Drug	*N*	Mean (%)	*N*	Mean ± SEM (%)	Minimum (%)
Saline	37	0%	25	73.6 ± 0.0%	55%
mCPP	13	0%	12	71.8 ± 0.9%	57%



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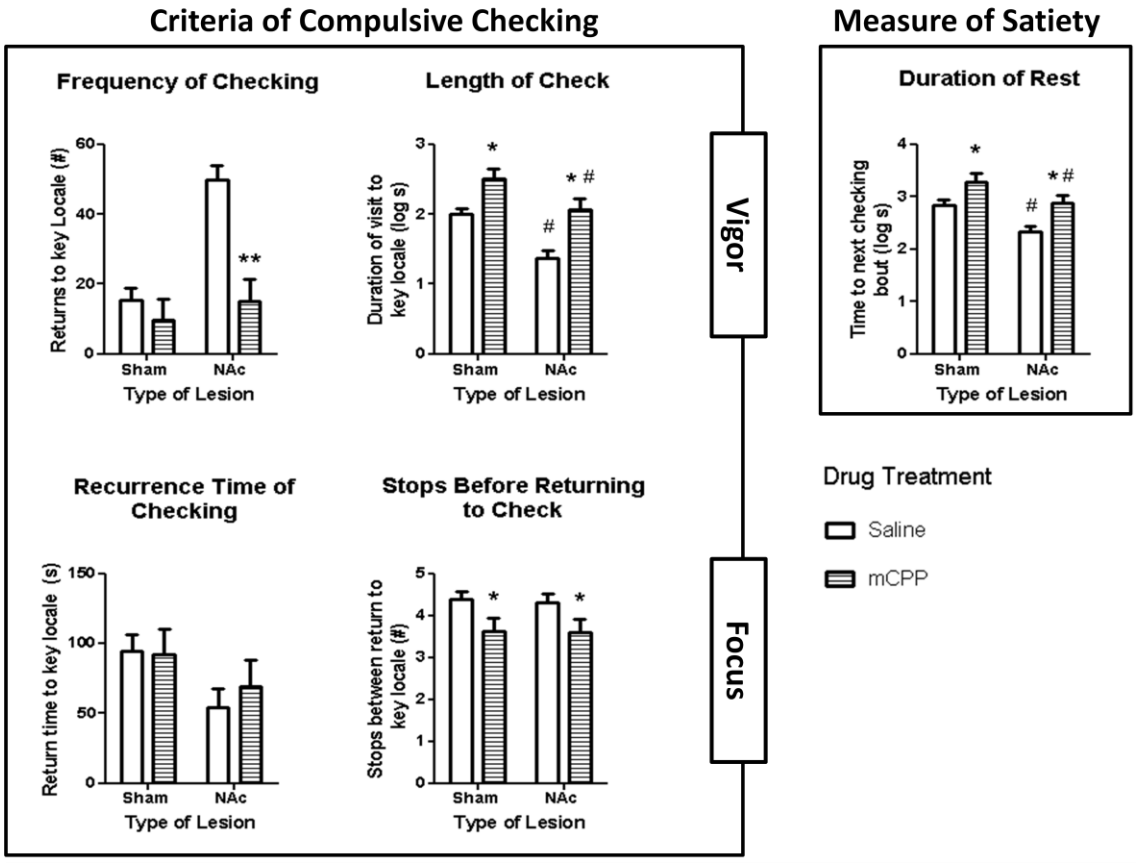

Figure 2 - Performance on criteria measures of compulsive checking and satiety for NAc and sham lesion animals treated with mCPP or saline. # main effect of Type of Lesion; * main effect of Drug Treatment; ** significantly different than NAc + saline group. All *p*'s < 0.05.

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CHAPTER 5: 5-HT2A/C RECEPTORS DO NOT MEDIATE THE ATTENUATION OF COMPULSIVE CHECKING BY MCPP IN THE QUINPIROLE SENSITIZATION RAT MODEL OF OBSESSIVE-COMPULSIVE DISORDER (OCD)

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Mark C. Tucci - PhD candidate. With the assistance and advice of Dr. Henry Szechtman, designed the study, collected data, analyzed results and prepared the manuscript for submission.

Dr. Henry Szechtman - PhD candidate's supervisor. Provided assistance and advice to Mark C. Tucci on all aspects of the study, and contributed in particular to the statistical analysis and manuscript preparation.

Dr. Anna Dvorkin-Gheva - Collaborator. Performed computational analysis on the obtained co-ordinates of the animal on the open field to generate the spatial-temporal form of activity on the open field.

Eric Johnson and Dr. Michael Wong - Laboratory members. Performed a portion of the data collection, assisted with interpretation of the results and edited the manuscript.

Abstract

There is emerging evidence for a dopamine (DA) - serotonin (5-HT) interaction underlying obsessive-compulsive disorder (OCD). In the quinpirole sensitization rat model of OCD, compulsive checking is induced by chronic treatment with the DA agonist quinpirole, and is attenuated by the 5-HT agonist drug mCPP. However, mCPP has affinity for a number of 5-HT receptor subtypes, and it is unknown by which receptors mCPP exerts its effects on quinpirole-treated animals. The present study tested in rats whether mCPP activity at 5-HT2A/C receptors mediates the attenuation of compulsive checking in quinpirole-treated animals. Rats were chronically treated with quinpirole on the open field for the induction of compulsive checking. Following the induction phase, animals were treated with mCPP (1.25 mg/kg) and the selective 5-HT2A/C receptor antagonist ritanserin (1.0 mg/kg or 5.0 mg/kg) to test whether blockade of 5-HT2A/C receptors inhibits attenuation of checking by mCPP. Results showed that as expected, quinpirole induced compulsive checking, and mCPP reduced its performance. However, 5-HT2A/C receptor blockade by ritanserin did not inhibit the attenuation of compulsive checking by mCPP. These results suggest that the reduction in compulsive checking by mCPP is not mediated by activity at 5-HT2A/C receptors, but by another receptor subtype.

Keywords

Obsessive-compulsive disorder (OCD); animal model; compulsive checking behavior; quinpirole; mCPP; ritanserin; 5-HT2A/C

Introduction

While a large body of research implicates both dopamine (DA) and serotonin (5-HT) in obsessive-compulsive disorder (OCD), there is emerging evidence for a possible DA-5-HT interaction underlying the affliction (Goodman et al., 1990; Nikolaus, Antke, Beu, & Muller, 2010; Westenberg, Fineberg, & Denys, 2007; Zohar, Chopra, Sasson, Amiaz, & Amital, 2000). For example, it has been suggested that hyper DA activity in circuits projecting to cortical and subcortical areas may result in obsessional thoughts or compulsive behaviors, and that such hyperactivity could be calmed by blockade of 5-HT2A, or stimulation of 5-HT1A receptors located on pyramidal cells in the prefrontal cortex (Westenberg et al., 2007). According to the authors (Westenberg et al., 2007), such a calming effect may be produced by antipsychotic drugs which in addition to activity at DA D1/D2 receptors, also act as antagonists at 5-HT2A and agonists at 5-HT1A receptors.

In a recent study employing the quinpirole sensitization rat model of OCD, it was reported that acute treatment with the 5-HT agonist drug mCPP reduced the motor vigor of checking and prolonged the post-checking satiety, but did not affect the focus on the task of checking (Tucci et al., 2013). Vigor, focus and satiety had been identified as three separate functional components underlying compulsive checking behavior in the animal model (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014). The effect of mCPP on vigor and satiety rendered the quinpirole-treated animals as no longer meeting criteria for 'compulsive' checking behavior (Tucci et al., 2013). Given the induction of compulsive checking by the DA agonist drug quinpirole and its reduction by the 5-HT

agonist drug mCPP, the authors suggested the presence of a putative DA-5-HT interaction underlying the model compulsive behavior. However, it is unknown which serotonergic receptor subtype(s) stimulated by mCPP mediate the effects of the drug on compulsive checking. Identifying a role for specific 5-HT receptor subtypes would strengthen the argument for a DA-5-HT interaction underlying the performance of compulsive checking behavior in the model.

mCPP acts on several 5-HT receptor subtypes including: 1A, 1B, 1D, 2A, 2C, and 3 (Fiorella, Rabin, & Winter, 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick, Jones, & Tyers, 1987). mCPP has been reported to have high affinity for the 5-HT2A/C receptor subtype (Fiorella et al., 1995; Hamik & Peroutka, 1989; Kilpatrick et al., 1987) and has widely been used as a probe of 5-HT function (Kahn & Wetzler, 1991), despite having also an affinity for a number of non-5-HT receptor subtypes including: adrenergic $\alpha 1/2$ and β receptors, and to a lesser extent, dopamine and cholinergic receptors (Hamik & Peroutka, 1989; Smith & Suckow, 1985). Stimulation of 5-HT2 receptors has been suggested by some to mediate the therapeutic effects produced by selective serotonin reuptake inhibitors (SSRIs) on OCD (Blier & de Montigny, 1998; El Mansari & Blier, 2006). Hence, it is possible that the attenuating effects produced by mCPP on quinpirole-induced compulsive checking may be mediated by stimulation of 5-HT2A/C receptors.

Accordingly, the present study tested whether the selective 5-HT2A/C receptor antagonist drug ritanserin blocks the attenuating effects produced by mCPP on the exaggerated vigor and satiety induced by quinpirole treatment. We hypothesized that

mCPP attenuates these constitutive functional components through stimulation of 5-HT2A/C receptors, and hence predicted that ritanserin co-treatment will block the effects of mCPP. However, if mCPP attenuates these functional components by stimulating other receptor subtypes, then ritanserin co-treatment would not block the attenuating effects of mCPP.

Materials and Methods

Animals

Subjects were 56 experimentally naïve adult male rats of the Long Evans strain that weighed 250-300 g at the onset of the experiment. Animals were housed in a climate controlled colony room with a 12 hour light/dark cycle (6 AM lights on; 6 PM lights off). Testing occurred during the light phase. Food and water were freely available during the experiment. Upon arrival, animals were given a period of 7 days to acclimatize to the facility. This was followed by 5 days of handling for 2-5 minutes each day before the onset of behavioral testing. Animals were housed and tested in compliance with the regulations set forth by the guidelines of the Canadian Council on Animal Care and approved by the Animal Research Ethics Board, McMaster University.

Drugs

All drugs were obtained from Sigma-Aldrich, USA. Quinpirole hydrochloride was administered at a dose of 0.125 mg/kg. This dose was chosen because it was shown in a previous study (Tucci et al., 2013) to induce compulsive checking behavior in rats.

mCPP was administered at a dose of 1.25 mg/kg. This dose was chosen because Tucci et al. (2013) found that it alters the quinpirole response on measures related to the vigor of checking and satiety. Both quinpirole and mCPP were administered in a 0.9% physiological saline vehicle at a volume of 1 ml/kg. For control treatments, animals received 0.9% physiological saline at a similar volume. Ritanserin was administered at doses of either 1 mg/kg or 5 mg/kg in a vehicle containing 67% ethanol and 33% saline at a volume of 0.3 ml/kg. These doses of ritanserin were chosen based on a report in the literature that they significantly reversed hypolocomotion induced by a similar dose of mCPP, and hence, are behaviorally active (Meert, Melis, Aerts, & Clincke, 1997). For control treatments, animals received vehicle at a similar volume. All injections were made sub-cutaneously under the nape of the neck.

Apparatus

Behavioral testing of animals occurred on a large open field (160 x 160 cm table without walls) that was located in a non-colony experiment room, as described previously (Dvorkin, Perreault, & Szechtman, 2006; Dvorkin et al., 2010). The table was divided into a grid of 25 virtual rectangular places (locales); no lines were actually marked on the table surface. Four small Plexiglas/glass boxes (approximately 8×8×7.5 cm) were located at the same fixed location on the open field for the duration of the experiment: two were located at corners and two were located at places near the center of the open field. Following each trial on the open field, the table and objects were wiped clean with a diluted solution of an antibacterial cleaner (Lysol). Behavior of animals on the open field

was videotaped continuously by a camera affixed to the ceiling (providing a stationary top view of the entire open field and the rat in it). Videotapes were converted to MPEG files (Canopus MPEGPro EMR realtime MPEG-1 MPEG-2 encoder) and these digitized videos were used to automatically track the trajectories of locomotion using EthoVision 3.1 (Noldus Information Technology, Netherlands) software (Noldus, Spink, & Tegelenbosch, 2001; Spink, Tegelenbosch, Buma, & Noldus, 2001).

Data analysis

From the digitized video files, EthoVision 3.1 software was used to extract the time series of *x, y* coordinates of the rat in the open field (Dvorkin et al., 2006). To remove noise, digitized tracking data were pre-processed (by applying appropriate filters to smooth the *x, y* coordinates) (Hen, Sakov, Kafkafi, Golani, & Benjamini, 2004), and the obtained coordinates were divided into episodes of forward locomotion (called progression) and episodes of small movements or immobility (called lingering), as described previously (Drai, Benjamini, & Golani, 2000; Drai & Golani, 2001; Golani, Benjamini, & Eilam, 1993). The coordinates were then mapped onto the 25 open field locales (places) (Szechtman, Sulis, & Eilam, 1998), and the frequency of visits and duration of stops in each locale were computed (the terms 'visit' and 'stop' are equivalent and are used interchangeably). Checking behavior is defined with reference to the most visited locale (labelled 'key place' or 'key locale'; these terms are equivalent), which in most instances is also the locale with the longest total duration of stops (Eilam & Golani, 1989; Szechtman et al., 1998). A visit to the key place is also referred to as a 'check' or

'checking'. Four criteria measures of checking behavior are computed. (1) Frequency of checking: total number of visits to the key locale. (2) Length of check: total duration of stay at the key locale divided by the frequency of visits there; this measure is also an indirect index of ritual-like behavior as the appearance of motor rituals in quinpirole-treated rats is associated with a very short duration of stay in the key locale (Ben-Pazi, Szechtman, & Eilam, 2001; Szechtman et al., 1998). (3) Recurrence time of checking: mean duration of return times to the key locale ('return time' is the interval from departure to next arrival at the locale). (4) Stops before returning to check: mean number of places visited between returns to the key locale. Animals are considered to be showing compulsive checking behavior when all four criteria measures differ significantly from saline-treated control animals (Szechtman et al., 1998), and hence the group of these four measures is termed 'criteria measures' for compulsive checking. Because blockade of 5-HT2C receptors has been reported to alter the hypolocomotor effects produced by mCPP (Kennett et al., 1994; Kennett et al., 1997; Lucki, Ward, & Frazer, 1989), we also assessed locomotor activity by measuring 'total distance moved'.

The behavioral profile of compulsive checking behaviour has been empirically dissociated into a set of functional components (Dvorkin et al., 2010). Three functional components have been identified, each of which are greatly exaggerated in compulsive animals: the *vigor* with which checking is performed, the *focus* on the task of checking and the amount of rest or *satiety* following a bout of checking. Lesions to the nucleus accumbens core altered the amount of checking (as indexed by changes in both the 'frequency of checking' and the 'length of check') which reflected an increase in the vigor

with which checking is performed. Lesions to the orbitofrontal cortex (OFC) affected the delay between checks (as indexed by change in both the 'time to return to check' and the 'number of stops before returning to check') which was considered to reflect a decrease in attention or focus on the task of checking. Together, the functional roles of the NAc and OFC are to control the vigor and focus, respectively. Hence, vigor and focus are deemed to be two relatively independent components of checking behavior.

The third functional component, satiety after a bout of checking, is indexed by the 'time to next checking bout', and has been observed to be greatly reduced in quinpirole sensitized animals (Dvorkin et al., 2010). The authors (Dvorkin et al., 2010) propose that this reduction in satiety reflects the failure to achieve a 'sense of task completion' (Pitman, 1989) or 'feeling of knowing' (Szechtman & Woody, 2004). The method of delineating bouts of checking in the animal model has been described previously (see Dvorkin et al., 2006). Briefly, the computation is based off of the logic used to identify the clustering of a bout of eating behavior into a 'meal' and the time between meals into a period of post-ingestion satiety (Tolkamp, Allcroft, Austin, Nielsen, & Kyriazakis, 1998; Tolkamp & Kyriazakis, 1999). Accordingly, a bout of behavior is defined on the basis of the distribution of time intervals between behavioral events (inter-event intervals). This distribution is examined to locate and extract a time-point that will produce a natural split between clusters of inter-event intervals. Specifically, the identified time-point will separate the time intervals into a class of (relatively long) intervals that are between the bouts of behavior (inter-bout intervals) and a class of (relatively shorter) intervals that belong within a bout of behavior (intra-bout intervals) (Tolkamp et al., 1998; Tolkamp &

Kyriazakis, 1999). An algorithm using this logic was used to identify bouts of checking behavior (Dvorkin et al., 2006) and extract 'time to next checking bout'. A rat may complete a bout of checking but not start the next bout during the session and hence the number of rats used for 'time to next checking bout' is generally smaller than for the four criteria measures for compulsive checking. Generally, saline-treated rats have 1-2 bouts of checking behavior in a session while quinpirole-treated rats usually perform 2 or more bouts (Dvorkin et al., 2006; Dvorkin et al., 2010). As in Tucci et al. (2013), if more than one bout of checking was performed, only the first 'time to next checking bout' was used for statistical analysis.

In a recent study, the three separate functional components - vigor, focus and satiety - were simultaneously re-synthesized in rats by NAc lesion and treatment with the selective 5-HT1A agonist drug DPAT, yielding animals to meet full criteria for compulsive checking behavior (Tucci, Dvorkin-Gheva, Johnson et al., 2014). This finding strengthens the hypothesis that these three separate constitutive functional components underlie the model OCD behavior (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014).

Design and procedure

The study consisted of a 2 x 2 x 3 fully crossed factorial design. One between-group factor was Quinpirole Dose (0.0 or 0.125 mg/kg), a second factor was mCPP Dose (0.0 or 1.25 mg/kg), and a third factor was Ritanserin Dose (0.0, 1.0 or 5.0 mg/kg). Animals receiving 0.0 mg/kg of either drug received the respective vehicle treatment.

The study consisted of two phases. The first phase was the induction of compulsive checking by quinpirole. Animals were treated chronically with quinpirole or saline for 9 injections for the development of compulsive checking behavior. Animals were assigned to either chronic quinpirole or saline treatment groups based on body weight 2-3 days before the onset of behavioral testing. The second phase tested whether 5-HT2A/C receptor stimulation by mCPP attenuates the quinpirole-exaggerated vigor and satiety. This phase consisted of 4 tests. For each of these 4 tests, animals continued to receive their typical quinpirole or saline injection. In addition, for the first test, animals were treated with mCPP (0.0 or 1.25 mg/kg) and ritanserin (0.0 or 1.0 mg/kg), yielding 12 independent groups. Animals were assigned to treatment conditions based on body weight so that each group had a relatively similar mean body weight. The second test was a washout test where animals received their respective quinpirole or saline treatment only. For the third test, animals were reassigned based on body weight to receive mCPP (0.0 or 1.25 mg/kg) and the higher dose of ritanserin (0.0 or 5.0 mg/kg). The fourth test was a final quinpirole or saline washout test where rats received their usual quinpirole or saline injection.

Following the 5-day period of handling, testing on the open field began. Rats were weighed in the colony room and transported to the non-colony experiment room containing the open field. Quinpirole and mCPP were administered to rats immediately before being placed gently onto the center of the open field. Ritanserin was administered 1 hour before behavioral testing. Each trial lasted 55 minutes. After the final trial of the last phase, rats were sacrificed by CO2.

Statistical analysis

The present study asked whether 5-HT2A/C receptor stimulation by mCPP attenuates the quinpirole-exaggerated vigor and satiety. Hence, statistical analysis was performed following induction of compulsive checking phase. Because a quinpirole or saline only washout was employed between administrations of mCPP and either dose of ritanserin during the test phase, we collapsed together groups receiving identical drug treatments, and compared across the drug treatment conditions. As a result, graphs show the realigned outcome for the two separate quinpirole + mCPP + ritanserin (1 or 5 mg) test trials. For each of the dependant variables, a 3-way ANOVA was computed. Analysis of the non-overlapping 95% confidence intervals was used to assess simple effects following a significant interaction between drug factors. The significance level was set at $P < 0.05$. Analysis was computed using SPSS 20 for Windows. Values presented in graphs are the mean and standard error of the mean.

Results

Induction of compulsive checking by quinpirole

Figure 1 shows effects for the drug treatments on each of the criteria measures related to compulsive checking. The ANOVA revealed significant main effects for Quinpirole Dose on each of the criteria measures for compulsive checking behavior: 'frequency of checking', $F(1,79) = 123.24$, $p<.001$; 'length of check', $F(1,79) = 101.82$, $p<.001$; 'recurrence time of checking', $F(1,79) = 33.87$, $p<.001$; and 'number of stops

before returning to check', $F(1,79) = 32.24$, $p<.001$. That is, compared to saline treatment, quinpirole (0.125 mg/kg) increased the 'frequency of checking' and decreased the 'length of check', 'recurrence time to check', and 'number of stops before returning to check', rendering animals to meet full criteria for compulsive checking behavior. Figure 2 shows effects for drug treatments on satiety following a bout of checking. A significant main effect of Quinpirole Dose on 'time to next checking bout', $F(1,33) = 20.90$, $p<.001$, was observed. Figure 2 shows that treatment with quinpirole decreased the amount of 'time to next checking bout' compared with saline treatment. Altogether, treatment with quinpirole induced the expected increase in vigor, and decreases in focus and satiety. Figure 3A shows effects for the drug treatments on locomotor activity. A significant main effect of Quinpirole Dose on total distance travelled, $F(1,95) = 207.88$, $p<.001$, was observed. Quinpirole treatment increased the total distance travelled during the test session compared with saline treatment to animals (Figure 3A).

Alterations to quinpirole-induced compulsive checking by mCPP

Significant main effects of mCPP Dose on the criteria measures for compulsive checking were observed for the 'frequency of checking', $F(1,79) = 36.31$, $p<.001$; and 'length of check', $F(1,79) = 41.11$, $p<.001$. Figure 1 shows that mCPP treatment (1.25 mg/kg) reduced the 'frequency of checking' and increased the 'length of check' in animals compared to saline treatment. There was, however, a significant mCPP Dose X Quinpirole Dose interaction, $F(1,79) = 19.07$, $p<.001$, for the 'frequency of checking'. Tests for simple effects revealed that mCPP treatment (1.25 mg/kg) significantly reduced

the 'frequency of checking' in animals treated with quinpirole (quinpirole + mCPP: *M* = 42.70, *SEM* = 4.80; quinpirole + saline: *M* = 98.74, *SEM* = 4.58), but not in animals treated with saline (saline + mCPP: *M* = 6.39, *SEM* = 6.95; saline + saline: *M* = 15.33, *SEM* = 4.90). Overall, mCPP treatment reduced in quinpirole treated rats the two measures related to the vigor of checking performance. There was no main effect of mCPP on 'recurrence time to check', $F(1,79) = 0.50$, $p>.05$, however there was a main effect of the drug on 'number of stops before returning to check', $F(1,79) = 6.48$, $p<.05$. This outcome indicates that mCPP altered one of the two indexes of the focus component. However, the other necessary index of focus, 'recurrence time of checking', was not altered by the drug and hence, the present results do not show an effect of mCPP on focus. There was no significant main effect or interaction effect of mCPP Dose on 'time to next checking bout' (Figure 2), hence, mCPP did not alter satiety. Altogether, mCPP altered the profile of checking behaviour in quinpirole treated animals such that they no longer met full criteria for 'compulsive' checking behavior.

A significant main effect of mCPP Dose on total distance travelled, $F(1,95) = 160.13$, $p<.001$, was observed. As figure 3A shows, compared with saline treatment, mCPP reduced the total distance travelled during the test session. This main effect was explored further in light of a significant mCPP Dose X Quinpirole Dose interaction, $F(1,95) = 97.20$, $p<.001$. Tests for simple effects showed that mCPP treatment (1.25 mg/kg) significantly reduced the total distance travelled in animals treated with quinpirole (quinpirole + mCPP: *M* = 99.91, *SEM* = 17.88 versus quinpirole + saline: *M* = 508.35

SEM = 17.74; p<.05), but not in animals treated with saline (saline + mCPP: *M* = 17.20, *SEM* = 18.33 versus saline + saline: *M* = 67.90, *SEM* = 18.61; p>.05.).

Ability of ritanserin to inhibit the response to mCPP in quinpirole-treated animals

Ritanserin treatment did not alter any of the criteria measures related to compulsive checking (Figure 1) or the amount of satiety (Figure 2) as indicated by the absence of any significant main effects of Ritanserin Dose or interaction effects with the other drug factors. However, a significant mCPP Dose X Ritanserin Dose interaction, $F(1,95) = 97.20$, $p<.001$, was observed for total distance travelled. Figure 3B shows the means of this interaction effect and indicates that the 1.0 mg/kg dose of ritanserin increased the total distance travelled compared with the 0.0 mg/kg dose of ritanserin but only in animals not injected with mCPP.

Discussion

The present study asked whether the attenuation of quinpirole-induced compulsive checking by mCPP, and in particular in vigor and post-checking satiety components, are mediated by the action of mCPP on 5-HT2A/C receptors. It was reasoned that if mCPP effects are mediated by stimulation of these receptors, then co-treatment with the selective 5-HT2A/C antagonist drug ritanserin would block the attenuating effects of mCPP on quinpirole-induced compulsive checking. As would be needed to test this hypothesis, we did observe that mCPP reduced the vigor of checking in quinpirole-treated animals, although surprisingly we did not observe the expected effect of mCPP on post-

checking satiety. Nevertheless, the results were clear as to whether or not ritanserin would reverse the mCPP effect on vigor – it did not. This lack of reversal with ritanserin appears solid because both mCPP and ritanserin were behaviorally active on their own; specifically, acute mCPP attenuated the vigor of quinpirole-induced compulsive checking, as found previously (Tucci et al., 2013) and ritanserin at the 1.0 mg/kg dose significantly increased locomotor activity in quinpirole and saline treated animals (Figure 3B). There are two possible conclusions from the present results: 1) the attenuation of vigor by mCPP is mediated by stimulation of non-5-HT2A/C receptors; 2) the attenuation of vigor by mCPP is mediated by stimulation of 5-HT2A/C receptors but the dose of ritanserin was insufficient to antagonize mCPP stimulation. Below we consider these possibilities in turn.

Does mCPP attenuate vigor through non-5-HT2A/C receptors?

mCPP has affinity for a number of 5-HT receptor subtypes including: 1A, 1B, 1D, 2A, 2C, and 3 (Fiorella et al., 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick et al., 1987). The drug also has affinity for some non-5-HT receptor subtypes including: adrenergic α1/2 and β receptors, and to a lesser extent, dopamine and cholinergic receptors (Hamik & Peroutka, 1989; Smith & Suckow, 1985). Our hypothesis that the attenuation of compulsive checking in quinpirole-treated animals is mediated by mCPP stimulation at 5-HT2A/C receptors was based on reports in the literature that mCPP has high affinity for 5-HT2A/C receptors (Fiorella et al., 1995; Hamik & Peroutka, 1989; Kilpatrick et al., 1987), and that stimulation of these same

receptors may mediate the therapeutic effects produced by SSRI's on OCD (Blier & de Montigny, 1998; El Mansari & Blier, 2006). The present results suggest that alternatively, stimulation by non-5-HT2A/C receptors mediates the behavioral response to mCPP. However, which of the other receptor subtypes, if any, mediate the mCPP response in quinpirole-treated animals remains unknown. It has been reported that mCPP acts as an antagonist at the 5-HT3 receptor subtype (Kahn & Wetzler, 1991), and there is independent evidence in the literature that blockade of the 5-HT3 receptor subtype may produce a therapeutic effect in OCD (Askari et al., 2012; Pallanti, Bernardi, Antonini, Singh, & Hollander, 2014). This may point to the possibility that mCPP mediates the attenuation of vigor in quinpirole-treated animals via blockade of 5-HT3 receptors. However, further testing is needed to address whether this receptor subtype or others mediate the effects of mCPP in the quinpirole model.

Was the dose of ritanserin insufficient to antagonize 5-HT2A/C receptors?

The possibility of insufficient dose to antagonize 5-HT2A/C receptors with ritanserin suggests itself from an inspection of Figure 1 where the higher dose of ritanserin (5 mg/kg) shows a trend for reversal of the mCPP effect on vigor, raising the possibility that doses of ritanserin greater than 5 mg/kg could reverse the mCPP effect in quinpirole-treated animals. Indeed, the literature indicates that higher doses of ritanserin than used here produce more robust effects on reversing hypolocomotion induced by mCPP (Meert et al., 1997). However, in that same study (Meert et al., 1997), doses of ritanserin similar to those used in the present study were sufficient to significantly reverse

the hypolocomotion induced by mCPP. Moreover, in that study (Meert et al., 1997), ritanserin was administered in a similar sub-cutaneous manner and 60 minutes prior to mCPP treatment as in the present study. However, in the Meert et al. (1997) study, mCPP was administered intravenously at 1.0 mg/kg, 15 minutes before testing. Therefore, the effect of mCPP in the Meert et al. (1997) study was likely stronger than in the present study, and hence it is surprising that ritanserin in the present study did not reverse reductions in locomotor activity and vigor by mCPP. Nevertheless, it is possible that a higher dose of ritanserin is necessary to reverse the mCPP effect in the animal model.

Effects of mCPP on post-checking satiety

It remains unknown whether 5-HT2A/C receptor stimulation by mCPP mediates the attenuation of the quinpirole-exaggerated satiety given that in the present study, we did not replicate the attenuation of the quinpirole-exaggerated satiety by mCPP (Tucci et al., 2013). One possible explanation for this unexpected finding may be that the effect of mCPP on satiety is not very robust. There exists in the literature some evidence consistent with this suggestion. For example, the attenuation of satiety by mCPP has been observed to be a transient phenomenon as this effect is present at the beginning of chronic co-treatment with quinpirole, but then tolerates out after repeated injections (Tucci, Dvorkin-Gheva, Sharma et al., 2014). Additionally, at the dose used in the present study (1.25 mg/kg), acute mCPP treatment has been observed to attenuate satiety in quinpirole-treated animals, but does not fully reverse it to the same amount as displayed by saline-controls (Tucci et al., 2013). This suggests that the effect of mCPP

on satiety is not very strong. It is also equally important to consider that in the present study, two groups of mCPP-treated animals did not contribute to the statistical analysis for satiety because too few rats initiated a second bout of checking. This could have contributed to the absence of a main effect for satiety. Therefore, several possibilities may account for the lack of an effect for mCPP on satiety. Nevertheless, it remains unknown whether attenuation of satiety by mCPP is mediated by stimulation at 5-HT2A/C receptors, or elsewhere.

Summary and conclusions

Altogether, the present study revealed that the attenuation of quinpirole-induced compulsive checking by mCPP, and in particular the mCPP-reduction in vigor, was not reversed by ritanserin treatment. This finding suggests that non-5-HT2A/C receptors mediate the effect of mCPP on motor vigor in the animal model. This possibility is strengthened by indications that mCPP has activity at various other 5-HT and non-5-HT receptor subtypes, and that similar doses of ritanserin used in other studies reversed hypolocomotion induced by mCPP, suggesting that they are behaviorally active. Nevertheless, the possibility does exist that a higher dose of ritanserin is necessary to reverse the effect of mCPP on vigor in the animal model given evidence from the present study of a trend towards reversal of the mCPP-mediated attenuation of vigor in quinpirole animals, and indications in the literature that higher doses of ritanserin produce a more robust effect on reversing mCPP-induced hypolocomotion.

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Declaration

The authors declare no conflicts of interest.

Abbreviations

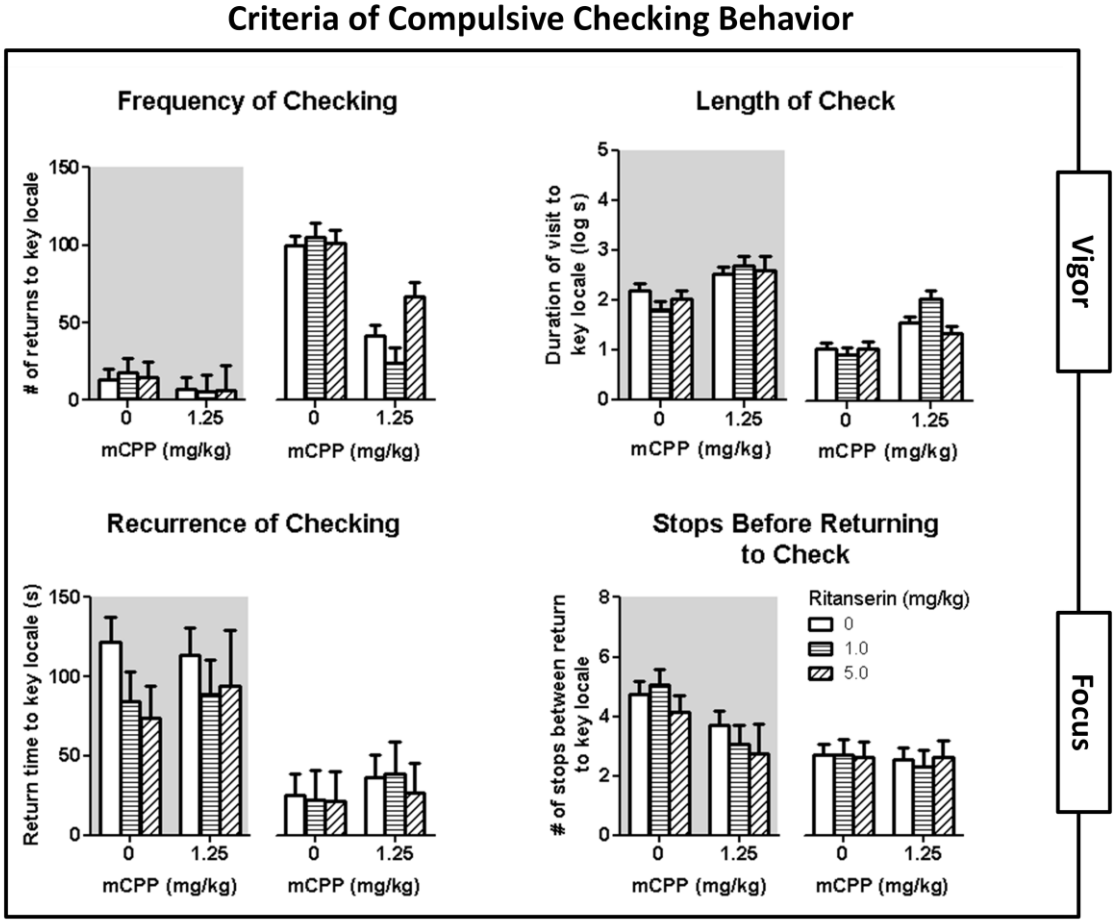
OCD, obsessive-compulsive disorder; 5-HT, serotonin; DA, dopamine; mCPP, 1-(3-Chlorophenyl)-piperazine hydrochloride; NAc, nucleus accumbens core; OFC, orbito frontal cortex; DPAT, 8-Hydroxy-2-(di-n-propylamino) tetralin hydrochloride

Figure Captions

Figure 1 – Effects of quinpirole, mCPP and ritanserin treatment on criteria measures of compulsive checking behaviour. *Vigor* of checking performance is indexed by the 'frequency of checking' and 'length of check', and *focus* is indexed by 'recurrence time of checking' and 'stops before returning to check'. Saline treatment (left, grey panels); quinpirole treatment (right, white panels).

Figure 2 – Effects of quinpirole, mCPP and ritanserin on satiety following a bout of checking. Saline treatment (left, grey panel); quinpirole treatment (right, white panel).

Figure 3A – Effects of quinpirole, mCPP and ritanserin on locomotor activity. Saline treatment (left, grey panel); quinpirole treatment (right, white panel). **3B -** Simple effects collapsing across saline and quinpirole-treated animals following a significant 2-way interaction between mCPP Dose and Ritanserin Dose on locomotor activity. * significantly different than mCPP (0.0 mg/kg) + ritanserin (0.0 mg/kg) group.



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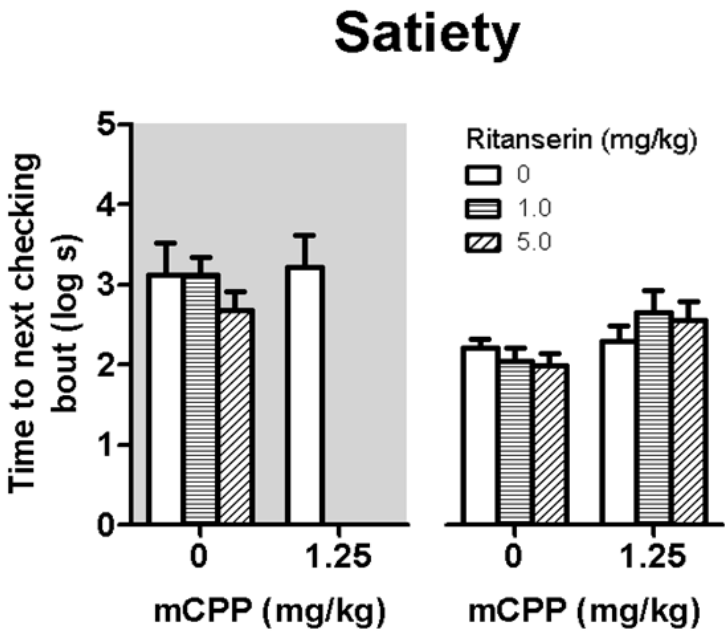

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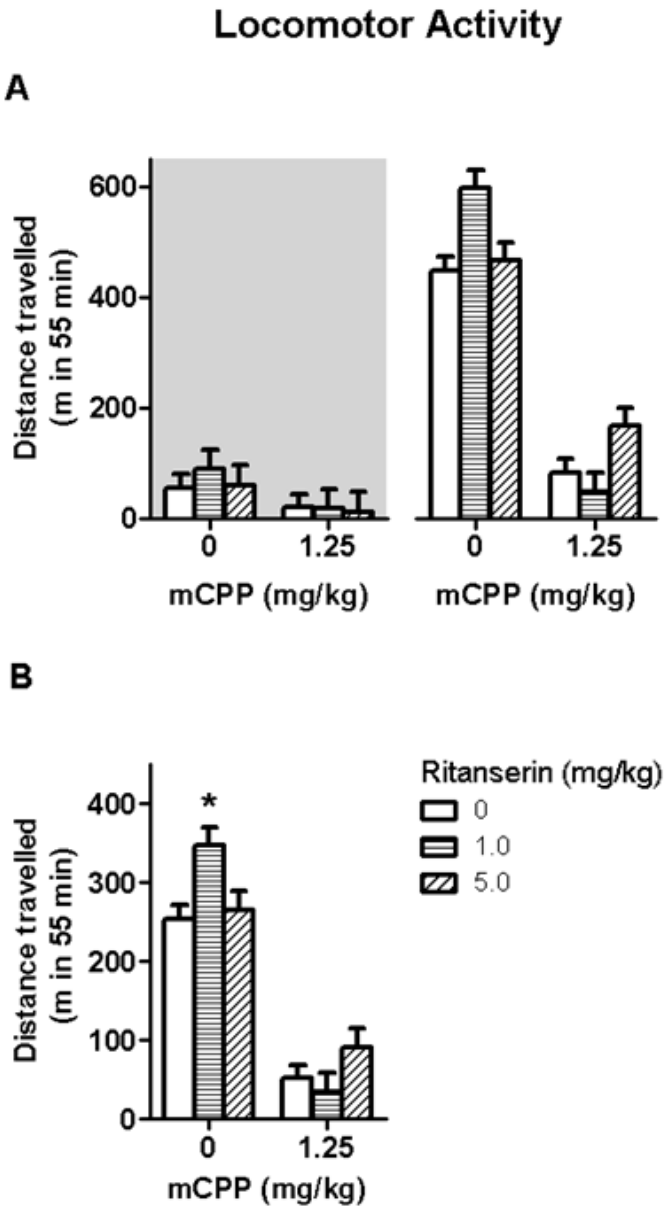

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CHAPTER 6: DISCUSSION

The non-selective 5-HT agonist drug mCPP contributed to the 5-HT hypothesis of OCD (Murphy et al., 1989) based on studies that the drug exacerbated OCD symptoms in patients (Broocks et al., 1998; Gross-Isseroff et al., 2004; Hollander et al., 1991; Pigott et al., 1993; Zohar et al., 1987) . However, an almost equal number of studies reported that mCPP does not exacerbate symptoms (Charney et al., 1988; de Leeuw & Westenberg, 2008; Goodman et al., 1995; Ho Pian et al., 1998; Khanna et al., 2001; Pigott et al., 1993), yielding conflicting results in the literature. Studies using animal models of OCD also report somewhat discrepant results, with the drug decreasing model compulsive behaviour in some studies (Korff et al., 2008), and increasing it in others (Kreiss et al., 2013; Kontis et al., 2008; Tsaltas et al., 2005). It remains unknown why treatment with mCPP has yielded such inconsistent effects on OCD.

The objective of this thesis was to shed light on the behavioural and neurobiological effects of mCPP using the experimental process of analysis to reduce and isolate behavioural phenomena to their constitutive components (Teitelbaum, 2012; Teitelbaum & Pellis, 1992). This method provides the opportunity to observe functional neurobiological and behavioural components that may otherwise remain unidentified when a phenomenon is studied as being a unitary entity. The quinpirole rat model of OCD provides the ability to utilize this approach by observing the effects of the drug on three constitutive functional components identified to underlie the model OCD behaviour (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014). To reiterate, these functional components are: vigor, focus and satiety. Accordingly, it was reasoned that by

employing this approach, we may better identify behavioural and neurobiological effects of the drug on OCD behaviour that may in part shed light on the inconsistent effects produced by mCPP in the human and animal literature.

Figure 1 restates the plan of study for the thesis and provides a brief overview of the results and conclusion.

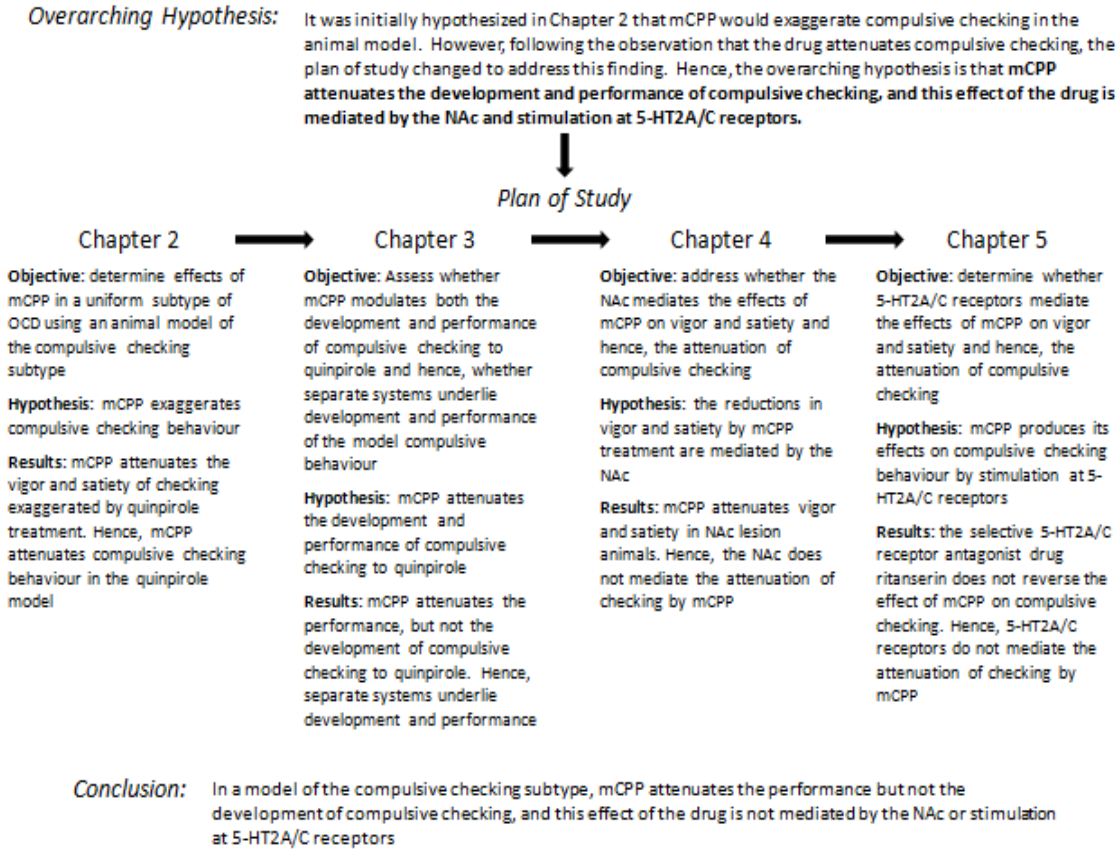

Figure 1. Plan of study with results and conclusion

Below, results from each of the studies are discussed in detail in terms of their specific objectives, hypotheses and potential implications. This is followed by a

hypothesis for a putative DA-5-HT interaction underlying compulsive checking in the animal model, and implications for future clinical work. The thesis is ended with a conclusion discussing the outcome of the overarching hypothesis, and a final comment reiterating the overarching objective and summarizing the novel contributions to the literature.

Chapter 2: Effects of the serotonergic agonist mCPP on male rats in the quinpirole sensitization model of obsessive-compulsive disorder (OCD); (Tucci et al., 2013)

To address whether the discrepant findings reported in the literature that mCPP either exacerbates or has no effect on OCD symptoms is at least partially attributable to heterogeneity of OCD symptoms, the objective of Chapter 2 was to test in rats the effects of mCPP treatment on compulsive checking using the quinpirole sensitization model. We tested the hypothesis that **mCPP exaggerates compulsive checking behaviour.** In doing so, we analysed whether the drug differently affects the separate constitutive components of compulsive checking—vigor, focus and satiety (Dvorkin et al., 2010; Tucci, Dvorkin-Gheva, Johnson et al., 2014)—to assess whether the presence of such a phenomenon may contribute to an explanation for the inconsistent findings from the human clinical studies.

Results showed that acute mCPP (1.25 mg/kg) treatment altered the profile of compulsive behaviour in quinpirole-treated animals such that there was a decrease in the vigor of checking performance, normalization in satiety following a bout of checking, and no alteration to focus on the task of checking compared to quinpirole controls. The lower

dose of mCPP (0.625 mg/kg) produced similar effects as the higher dose (1.25 mg/kg) except that it did not normalize satiety. Hence, at both doses, animals no longer met criteria for full-blown compulsive checking behaviour. When mCPP was administered to naïve animals, results indicated that compared to saline control animals, neither dose of mCPP potentiated any of the measures of checking behaviour. Overall, mCPP treatment dose-dependently decreased activity on the open-field, suggestive of the absence of compulsive behaviour. Taken together, the findings suggest that mCPP has ameliorative effects on compulsive checking behaviour.

It was hypothesized that mCPP would exaggerate compulsive checking behaviour. However, results demonstrate that mCPP ameliorated the model OCD. When we reduced compulsive checking to its three constitutive functional components, it was observed that the amelioration produced by mCPP was by the selective attenuation of some, but not all, functional components. This outcome was not consistent with our hypothesis. While we report an ameliorative effect for mCPP on compulsive checking in the animal model, findings from human clinical studies do not report attenuation of OCD symptoms produced by the drug.

In accounting for this discrepancy, we consider three possible explanations. First, it is important to consider that in the human studies, the subtype of OCD was not uniform; hence, such an ameliorative effect may be reserved to the compulsive checking subtype only and may not have been captured when measuring the effect of the drug across the heterogeneous patient group. The possibility that heterogeneity of the patient

population may contribute to inconsistent findings has been suggested by others (Goodman et al., 1995; Khanna et al., 2001).

Second, patients were always tested while in a quiet room without, to the extent possible, any OCD provoking stimuli. That is, patients with the OCD trait were not necessarily provoked into the OCD state during testing. However in the present study, mCPP treatment was administered to rats in the OCD state induced by quinpirole treatment. Accordingly, it is unknown whether treating animals with the quinpirole-induced OCD trait but during a period of rest would exacerbate compulsive behaviour. Addressing this possibility using the quinpirole model would be difficult as exposure to the open field produces a potential threat that activates the security motivation system, and accordingly, the performance of checking behaviour (Szechtman and Woody, 2004). Hence exposure to potentially threatening stimuli is a necessary component of the model in addition to sensitization by quinpirole. Nevertheless, the possibility exists that whether OCD is active or at rest may mediate the response to mCPP.

The third explanation stems from the fact that in the present study, the effect of mCPP on a rich set of constitutive functional components underlying compulsive behaviour were analysed, while in the human studies OCD behaviour was measured as a unitary phenomenon. The advantage of our approach is that it allows us to reduce OCD behaviour to the separate behavioural entities that may underlie the disorder, and assess whether the drug differentially modulates them. The existence of all three behavioural components has not been identified in the human disorder, hence it is at present not possible to address whether mCPP would similarly modulate them. However the

literature reports that in some human clinical studies mCPP decreased 'energy' and increased 'drowsiness' (Charney et al., 1988), symptoms which may be akin to a reduction in the vigor of checking performance. This observation, then, is not necessarily discrepant with past findings, but rather consistent with them, and suggests that more subtle effects of the drug may be captured by reducing OCD to its underlying behavioural processes, highlighting the utility of this approach.

While the present findings bear some consistency with the human literature, they are more in line with reports from the animal literature where mCPP attenuated the model compulsive behaviour (Korff et al., 2008). It is difficult, though, to suggest what similarities exist between models to account for this outcome. The model compulsive behaviour in the Korff et al. (2008) study is not of a specific subtype of OCD behaviour, and it is not known whether the same functional components identified in the quinpirole model are present in the other model. Therefore, it remains unknown by what processes mCPP produces an amelioration of the compulsive behaviour in this other model. Hence, where possible, animal models with strong face validity for OCD subtypes, and the identification of behavioural processes underlying model OCD behaviour, is needed to further address how mCPP reduces compulsive behaviour. This would also address how mCPP alters other subtypes of OCD.

Altogether, the results of Chapter 2 indicate that in a model of the compulsive checking subtype, mCPP ameliorates OCD behaviour. This effect of mCPP is not consistent with any of the previous human studies, but none of these human studies assessed the effects of the drug on the compulsive checking subtype only. This highlights

the importance of testing the drug on a uniform subtype of OCD behaviour. Furthermore, the rich set of measures employed to assess the drug's effects on compulsive checking reveal more subtle effects of the drug on behavioural processes underlying OCD behaviour not captured when OCD is measured as a unitary phenomenon. Both of these observations may shed some light on the discrepant findings for the effect of mCPP on OCD behaviour reported in the literature.

Chapter 3: Separate mechanisms for development and performance of compulsive checking in the quinpirole sensitization rat model of obsessive-compulsive disorder (OCD); (Tucci, Dvorkin-Gheva, Sharma et al., 2014)

The objective for Chapter 3 was to utilize mCPP to identify whether separate mechanisms exist for the development and for the performance of compulsive checking. As shown in Chapter 2, acute mCPP treatment to quinpirole-sensitized animals attenuates compulsive checking. However, it is unknown whether a similar effect would be observed on the development of compulsive checking if mCPP was co-treated during the induction of compulsive checking by quinpirole, and whether it would carry over to a reduction of performance on a quinpirole-only challenge following the induction phase. Given evidence from Chapter 2 that mCPP reduces checking, and that sensitization of checking by quinpirole during the induction phase is necessary for its full-blown development and performance, we hypothesized that **mCPP attenuates the development and performance of compulsive checking to quinpirole.** It was predicted that mCPP would attenuate the development of checking during the induction phase to quinpirole,

and as a function of the reduced development of checking, we would observe a decrease in performance of compulsive checking on the quinpirole-only challenge. This outcome was suggested to reflect the presence of a unitary mechanism mediating development and performance of the model OCD behaviour.

Results during the induction phase indicated that chronic mCPP co-treatment inhibited compulsive checking behaviour; animals co-treated with quinpirole (0.25 mg/kg) and mCPP (0.625 or 1.25 mg/kg) showed an attenuation of vigor related to checking as compared to animals treated with quinpirole-only. However when the same animals were challenged with quinpirole-only following the induction phase, they displayed full-blown compulsive checking behaviour. mCPP also affected satiety following a bout of checking, but in a somewhat different manner. Upon acute administration during the induction phase, satiety appeared to normalize; however towards the end of this phase and during the quinpirole-only challenge, satiety was greatly reduced to similar levels as quinpirole-only controls. This observation suggests that the mCPP effect on satiety tolerates after multiple treatments and hence, may not be as robust on this functional component as on vigor.

At first glance, findings from the induction phase would suggest that mCPP inhibits development of compulsive checking behaviour to quinpirole. However, this appearance is deceptive because in the absence of mCPP during the quinpirole-only challenge, animals showed full-blown compulsive checking behaviour. Consequently, the combined results suggest the presence of partially non-overlapping mechanisms mediating the development and performance of the model OCD behaviour, with mCPP

modulating performance but not development of compulsive checking. The finding that mCPP does not inhibit the response to quinpirole suggests that mCPP does not necessarily ameliorate compulsive behaviour, but rather, attenuates its performance.

These results further suggest that the development of compulsive checking in the animal model is not just a function of motor practice, but rather, reflects neuroplastic changes mediated by chronic DA D2/3 receptor stimulation by quinpirole, without much involvement of 5-HT receptor stimulation. Consistent with this view, a number of brain changes have been reported in quinpirole-sensitized rats. For example, using the 2-deoxyglucose technique which is used to measure local cerebral glucose utilization, decreases in glucose utilization were reported in cortical (the cingulate cortex-area 1, frontal cortex-area 3, lateral orbital cortex, medial/ventral orbital cortex, and parietal cortex) and subcortical areas (ventral pallidum and nucleus accumbens) of quinpirole-sensitized rats (Carpenter, Pazdernik, & Levant, 2003; Richards, Pazdernik, & Levant, 2005). Others report increased high-affinity states of dopamine D2 receptors ($D2^{High}$) in quinpirole-sensitized animals (Culver, Szechtman, & Levant, 2008; Perreault et al., 2007; Seeman et al., 2006) and decreased dopamine levels in the left pre-frontal cortex (Sullivan, Talangbayan, Einat, & Szechtman, 1998). However, it is unclear which of these alterations, if any, mediate the development of compulsive checking in the animal model.

On the other hand, there appears to be oppositional roles for DA and 5-HT on the performance of compulsive checking. That is, DA hyperactivity produced by quinpirole sensitization facilitates the performance of compulsive checking, while 5-HT

hyperactivity produced by mCPP reduces it. This effect is consistent with the hypothesized neurochemical contributions of DA and 5-HT by the security motivation theory of OCD to modulate OCD behaviour (Szechtman & Woody, 2004). Accordingly, the theory suggests that the motivational drive to perform compulsive behaviour is mediated by DA activity that, unabated, gives rise to 'compulsive' checking. The attenuation of compulsive behaviour was suggested to be produced by a 5-HT-mediated satiety signal, and hence together, reflects oppositional roles for DA and 5-HT on compulsive behaviour.

In summary, we used mCPP to identify the presence of partially non-overlapping mechanisms underlying the development and the performance of OCD behaviour in the quinpirole sensitization model. In doing so, we observed oppositional roles for DA and 5-HT on compulsive checking, with the former inducing neuroplastic changes mediating the development of compulsive behaviour, and the latter reducing its performance but not necessarily ameliorating it.

Chapter 4: The nucleus accumbens core does not mediate the attenuation of compulsive checking produced by mCPP treatment in a rat model of obsessive-compulsive disorder (OCD); Tucci, Dvorkin-Gheva, Johnson, Cheon et al. (submitted to *The European Journal of Neuroscience* on 2014-07-18)

Given findings from Chapters 2 and 3 that mCPP reduces in rats compulsive checking exaggerated by quinpirole treatment, the objective for Chapter 4 was to attempt to localise where in the brain mCPP produces its effects on the model compulsive

behavior. Previous work from our lab showing that NAc lesion exaggerates vigor and satiety contributed to the hypothesis that **reductions in vigor and satiety by mCPP are mediated by the NAc.** Accordingly, it was predicted that if the NAc mediates reductions in vigor and satiety by mCPP stimulation, animals with NAc lesion would not show reductions in these functional components in response to mCPP treatment. However, if another neural region mediates reductions in vigor and satiety, NAc lesion animals would continue to display reductions in these functional components in response to mCPP treatment.

Results showed that as expected, NAc lesion produced a robust change only in measures indexing motor vigor and post-checking satiety as compared with sham-operated controls. Treatment with mCPP (0.625 mg/kg) significantly altered measures such that vigor was decreased and satiety was normalized in lesion animals compared with saline-treated controls. Together, these results suggest that NAc lesion exaggerates vigor and satiety in rats, and mCPP treatment attenuates the exaggeration of these components.

These results suggest that the reduction in checking by mCPP is not mediated by the NAc. This suggestion is solid because the NAc was destroyed by excitotoxic cell-body lesion. If the NAc was the site of mCPP activity reducing checking, then it follows that the drug should not produce its effects because of destruction of this neural region. However, this was not observed. Hence, mCPP activity at receptors outside of the NAc must mediate reductions in checking.

Chapter 4 does not isolate and identify the neural region mediating the effects of mCPP on compulsive checking. However, the findings contribute to a hypothesis for the underlying neurobiological mechanism mediating the effects of mCPP on compulsive checking. To build the neurobiological hypothesis, we start at the neural site which was just suggested not to mediate the response to mCPP - that is, the NAc.

The NAc is located in the basal ganglia and as such, is a component of CBGTC loops which have been suggested to be hyperactive, contributing to OCD (Aouizerate et al., 2004; Baxter, 1992; Graybiel & Rauch, 2000; Huey et al., 2008; Insel & Winslow, 1992; Modell et al., 1989; Saxena et al., 2001; Stein, 2002; Szechtman & Woody, 2004; Vermeire et al., 2012; Wise & Rapoport, 1989). In the quinpirole model, it has been suggested that the NAc exerts inhibitory control on vigor and satiety, and that quinpirole or NAc lesion disinhibits this control resulting in exaggerations of these functional components and hence, compulsive behaviour (Dvorkin et al., 2010). This suggestion is in line with hyperactivity in CBGTC circuitry yielding OCD. As a result, in building the neurobiological hypothesis, the NAc is an important starting point because it may be involved in the 'release' of two components related to compulsive checking: vigor and satiety. Therefore, the attenuation of these functional components by mCPP would appear to be located downstream of the release point - that is, downstream of the NAc.

The NAc projects to several structures including: caudate putamen, dorsolateral and dorsomedial ventral palidum, globus pallidus, lateral hypothalamus, substantia nigra pars compacta and the substantia nigra pars reticulata (Usuda, Tanaka, & Chiba, 1998). Of these targets, the substantia nigra pars reticulata (SNr) is hypothesized to be the site of

mCPP activity producing a reduction in compulsive checking. The rationale for this suggestion follows.

The SNr has been considered to be an output site for the basal ganglia (Alexander et al., 1986). It projects to areas of the thalamus that mediate motor activity (Zahm & Brog, 1992), and to the prefrontal cortex (Deniau, Menetrey, & Thierry, 1994) which is involved in emotion and motivational processes (Kolb, 1984). The NAc normally exerts inhibitory influence over nigrothalamic circuitry (Deniau et al., 1994). NAc lesion or quinpirole treatment has been suggested to release inhibitory control over vigor and satiety produced by the NAc, yielding compulsive checking (Dvorkin et al., 2010). The SNr is a reasonable location for mCPP activity reducing compulsive behaviour resulting from NAc disinhibition because 5-HT stimulation here has been reported to inhibit neuronal activity (Collingridge & Davies, 1981; Dray, Gonye, Oakley, & Tanner, 1976; Invernizzi et al., 2007), and that mCPP stimulation of 5-HT2C receptors here may stimulate GABA neurons (Di Giovanni, Di Matteo, La Grutta, & Esposito, 2001). Together, the inhibition of SNr firing and/or increasing GABA activity may inhibit nigrothalamic output by the SNr, resulting in a reduction of compulsive checking. This hypothesis may be tested by micro-infusing mCPP into the SNr and assessing whether targeted infusion of the drug produces a similar attenuating effect on these functional components as when it is systemically administered.

Altogether, this neurobiological hypothesis may also shed light on why mCPP inhibits performance but does not completely ameliorate compulsive checking. The neuroplastic changes hypothesized to underlie development of compulsive checking to

quinpirole in Chapter 3 may occur upstream of the SNr. It is not presently known where upstream this may occur, but the NAc may be a possible site given the suggestion that disinhibition of this structure releases negative control on checking (Dvorkin et al., 2010). Disinhibition of the NAc may be suppressed downstream by mCPP activity at the SNr. mCPP activity at the SNr may reduce performance of checking, but, if the drug does not act on the upstream neuroplastic changes driving checking, it follows that checking would not be reversed or ameliorated. This may account for why in Chapter 3 chronic quinpirole + mCPP co-treatment reduced the development of compulsive checking, but upon the quinpirole-only challenge where mCPP treatment was removed, animals showed full-blown compulsive checking.

In summary, Chapter 4 revealed that mCPP activity at receptors outside of the NAc mediates the attenuation in vigor and satiety by mCPP. A neurobiological hypothesis was put forward suggesting that the effects of mCPP may be mediated by the SNr which may reduce nigrothalamic output yielding a reduction in compulsive checking. Downstream blockade of hyperactive signalling from the NAc may partially account for why mCPP reduces performance of compulsive checking, but does not completely ameliorate it.

Chapter 5: 5-HT2A/C receptors do not mediate the attenuation of compulsive checking by mCPP in the quinpirole sensitization rat model of obsessive-compulsive disorder (OCD); Tucci, Dvorkin-Gheva, Johnson, Wong et al. (accepted in *Behavioural Brain Research*)

As shown in Chapters 2, 3 and 4, mCPP selectively reduces in rats the vigor and satiety related to compulsive checking exaggerated by quinpirole treatment. Neurochemically, mCPP has affinity for a number of 5-HT and non-5-HT receptor subtypes, and it remains unknown which receptor(s) stimulated by mCPP mediate the effects of the drug on vigor and satiety. Hence, the objective for Chapter 5 was to use the process of analysis to identify which 5-HT receptor subtypes mediate the effects of mCPP. Given reports that mCPP has high affinity for the 5-HT2A/C receptor subtype, and suggestions from the literature that stimulation of these same receptors may underlie the therapeutic effect produced by SSRIs on OCD symptoms (Blier & de Montigny, 1998; El Mansari & Blier, 2006), we hypothesized that **mCPP produces its attenuating effects on compulsive checking behaviour in the rat model by stimulation at 5-HT2A/C receptor subtypes.** Accordingly, we predicted that blockade of 5-HT2A/C receptors by the selective 5-HT2A/C receptor antagonist drug ritanserin would reverse the attenuating effects of mCPP in quinpirole treated rats.

Results showed that rats treated with quinpirole (0.125 mg/kg) displayed the expected exaggerations in vigor, focus and satiety, and hence full-blown compulsive checking behavior when compared with saline controls. mCPP (1.25 mg/kg) treatment reduced in quinpirole-sensitized rats motor vigor, but neither doses of ritanserin (1.0 and

5.0 mg/kg) reversed this effect of mCPP. Ritanserin at the 1.0 mg/kg dose did however increase locomotor activity in quinpirole and saline treated rats. This last observation suggests that ritanserin was behaviourally active, but did not produce the predicted effect on vigor. There are two possible explanations for this outcome: 1) motor vigor is mediated by non-5-HT2A/C receptors, or; 2) the dose of ritanserin was not sufficient to reverse the effect of mCPP on vigor. These possibilities are considered below.

Our hypothesis that mCPP produces its effects on compulsive checking behaviour in the rat model by stimulation at 5-HT2A/C receptor subtypes was based on reports in the literature that the drug has high affinity for these receptors (Fiorella et al., 1995; Hamik & Peroutka, 1989; Kilpatrick et al., 1987), and that stimulation of these receptors has been suggested to underlie the therapeutic effects of SSRIs on OCD symptoms (Blier & de Montigny, 1998; El Mansari & Blier, 2006). If, according to our results, mCPP does not reduce vigor and hence compulsive checking by stimulation at 5-HT2A/C receptors, then what receptors mediate this mCPP response?

mCPP has affinity for several 5-HT receptor subtypes: 1A, 1B, 1D, 2A, 2C, and 3 (Fiorella et al., 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick et al., 1987). Although mCPP is classically used as a probe of the serotonergic system (Kahn & Wetzler, 1991), the drug does have some activity at non-5-HT receptors including: adrenergic alpha-1/2 and beta receptors, and dopamine and cholinergic receptors (Hamik & Peroutka, 1989; Smith & Suckow, 1985). Which of these receptors mediate the mCPP response, if not by 5-HT2A/C receptors, is not known from the present study. However,

the present thesis as well as another study may rule out the possibility for activity at certain receptors in mediating the effect of mCPP on motor vigor.

The possibility that attenuation of vigor is mediated by mCPP activity at DA D2/3 receptors seems unlikely. The rationale behind this suggestion is as follows. In Chapter 2, it was shown that mCPP alone treated to naive animals does not induce compulsive checking. On the other hand, the DA D2/3 receptor agonist drug quinpirole was shown to induce compulsive checking. Furthermore, none of the studies in this thesis reveal that mCPP produces a synergistic effect on vigor when administered along with quinpirole as would be the predicted observation if the mCPP effect on vigor were mediated by stimulation at DA D2/3 receptors.

In another study, it was reported that in NAc lesion animals showing exaggerations in both vigor and satiety, the addition of systemic treatment with the selective 5-HT1A agonist drug DPAT exaggerated the focus component (Tucci, Dvorkin-Gheva, Johnson et al., 2014). This simultaneous re-synthesis of each of the separate functional components rendered animals to display full-blown compulsive checking by non-quinpirole means, and revealed a role for 5-HT1A stimulation in mediating the focus component. Importantly, 5-HT1A receptor stimulation did not reduce vigor in NAc lesion animals. Together, these results suggest that mCPP activity at 5-HT1A receptors is probably weak and does not mediate the reduction in vigor. Overall, it appears unlikely that the mCPP reduction in vigor is mediated by activity at DA D2/3 or 5-HT1A receptors, in addition to the finding of the present study of a non-effect for 5-HT2A/C receptors.

In considering other possible receptor subtypes that may mediate the reduction in vigor by mCPP, it has been reported that blockade of the 5-HT3 receptor by granisetron or ondansetron enhances the therapeutic effects by the SSRI fluvoxamine (Askari et al., 2012; Pallanti et al., 2014). As discussed above, mCPP has antagonist activity at the 5-HT3 receptor subtype, and it is possible activity here may mediate the attenuation of vigor. Another report suggests that stimulation of the 5-HT1D receptor may decrease OCD symptoms (Zohar et al., 2004), and mCPP has stimulatory properties at this receptor subtype as well. Therefore, it is possible that activity at 5-HT1D receptors may mediate the decrease in vigor by mCPP. Additional studies using a similar design as the present one, but of course using different pharmacological agents, may be used to further address which receptor subtype(s) mediates the mCPP reduction in vigor.

A final consideration is whether the dose of ritanserin was not sufficient to reverse the effects of mCPP on motor vigor. This suggestion arises from the observation of a trend towards reversal by ritanserin of the attenuating effect produced by mCPP on vigor in quinpirole-treated animals. Hence, would a higher dose of ritanserin be necessary to produce a significant reversal on the attenuating effects of mCPP on motor vigor? The literature does indicate that doses of ritanserin that are higher than the ones used in the present study (1.0 & 5.0 mg/kg) produce a greater amount of reversal of the hypolocomotion induced by mCPP (Meert, Melis, Aerts, & Clincke, 1997). Conceivably, higher doses of ritanserin could produce a more robust behavioural effect. However, in the Meert et al. (1997) study, doses of ritanserin similar to those used in the present study still produced significant reversals on the hypolocomotion induced by mCPP. Together,

this finding along with evidence from the present study that at least the 1.0 mg/kg dose of ritanserin was observed to be behaviourally active, suggests that it is unlikely that the dose of ritanserin was insufficient to reverse the reduction in vigor by mCPP.

One limitation of the present study was that it remains unknown whether mCPP activity at 5-HT2A/C receptors mediates the normalization in satiety produced by the drug. Surprisingly, mCPP did not reverse the decrease in satiety produced by quinpirole as was observed in Chapters 2 and 3. This outcome may reflect a somewhat weaker effect for mCPP on satiety given evidence from Chapter 2 that the same dose of mCPP (1.25 mg/kg) normalizes satiety in quinpirole-treated animals but does not fully reverse it to the same amount as displayed by saline-controls. Additionally, evidence from Chapter 3 suggests that the normalizing of satiety by mCPP is a transient phenomenon as this effect is present at the beginning of chronic co-treatment with quinpirole, but then tolerates out after repeated injections. Together, this may reflect a less robust effect for mCPP on satiety compared with vigor. However, it is also important to consider that in the present study, not enough mCPP-treated animals initiated a second bout of checking, and as a result, two groups of mCPP-treated animals did not contribute to the statistical analysis for satiety. This may have also contributed to the absence of an effect for mCPP on normalizing satiety. Altogether, because mCPP did not reverse the quinpirole-exaggerated satiety, it remains unknown whether ritanserin would reverse the effect of mCPP, and hence whether 5-HT2A/C receptors mediate this functional component.

In summary, Chapter 5 revealed that ritanserin blockade of 5-HT2A/C receptors does not appear to reverse the reduction in vigor by mCPP. It seems unlikely that the

dose of ritanserin was insufficient to reverse the effect of mCPP on vigor. This suggests that mCPP activity at non 5-HT2A/C receptors mediates the attenuation of motor vigor related to compulsive checking.

Possibility of a DA-5HT interaction underlying the model OCD behaviour

Neuroanatomical and neurochemical interconnections between DA and 5-HT systems give rise to the occurrence of interactions between the two systems (Albert & Francois, 2010; Barnes & Sharp, 1999; Di Matteo et al., 2008; Dray, Davies, Oakley, Tongroach, & Vellucci, 1978; Filip & Bader, 2009; Fink & Gothert, 2007; Hayes & Greenshaw, 2011; Herve, Pickel, Joh, & Beaudet, 1987; Leger, Charnay, Hof, Bouras, & Cespuglio, 2001; Navailles & De Deurwaerdere, 2011). For example, early neuroanatomical studies demonstrated that 5-HT projections innervate dopamine producing cells in the substantia nigra and VTA (Dray et al., 1978; Herve et al., 1987). Functionally, it has been reported that mCPP and the similar non-selective 5-HT agonist drug MK 212 inhibits mesolimbic DA function by 5-HT2C receptor stimulation at the VTA and SNr (Di Giovanni, Di Matteo, Di Mascio, & Esposito, 2000). Hence, the two systems may work in concert to yield a given effect.

The presence of a DA-5-HT interaction underlying OCD has been suggested by others (Goodman et al., 1990; Westenberg et al., 2007; Zohar et al., 2000). For example, Westenberg and colleagues (2007) suggest that antipsychotics may produce therapeutic effects through a DA-5-HT interaction. More specifically, the authors indicate from reports in the literature that in addition to inhibitory effects on DA receptors,

antipsychotics yield excitatory effects at 5-HT1A receptors and inhibitory effects at 5-HT2A receptors (Bortolozzi et al., 2005). Accordingly, they review findings that DA release in the prefrontal cortex and nucleus accumbens in response to antipsychotic treatment may be mediated in part by stimulation at 5-HT1A receptors that project to the DA-producing cells in the VTA (Denys, Klompmakers, & Westenberg, 2004; Hallbus, Magnusson, & Magnusson, 1997). Hence, interactions between the two neurochemical systems appear to underlie at least in part the neurobiology of OCD.

The results of the present study are also suggestive of a DA-5-HT interaction underlying the model compulsive behaviour. More specifically, results from Chapters 2, 3 and 5 indicate that compulsive checking induced by the DA agonist drug quinpirole can be reduced by treatment with the 5-HT agonist drug mCPP. This suggestion seems to be solid because quinpirole is a selective DA D2/3 receptor agonist (Levant, Grigoriadis, & DeSouza, 1993), and mCPP has affinity for several 5-HT receptors (Fiorella et al., 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick et al., 1987) and is used as a probe of 5-HT function (Kahn & Wetzler, 1991). However, the suggestion of a DA-5-HT interaction would be strengthened if we could identify which 5-HT receptor subtype(s) mediate the mCPP response in quinpirole-sensitized animals.

It also remains unknown whether the nature of the putative interaction between the two systems is direct or whether a third intermediary component is involved. An example of a more direct interaction would be the ability of 5-HT to modulate DA activity by stimulating receptors located on the DA producing neurons as described above. An example of a role for an intermediary component is demonstrated by one

study which showed that increases in DA at the nucleus accumbens shell produced by MDMA treatment are reduced by 5-HT2B/C-mediated activation of the VTA, but that the 5-HT2B/C stimulation first increases VTA GABA which in turn reduces DA at the nucleus accumbens shell (Bankson & Yamamoto, 2004). Hence, GABA yields an intermediary role modulating the interaction between the DA and 5-HT systems. According to the neurobiological hypothesis we proposed for the reduction of compulsive checking by mCPP, we suggested that the SNr is the neuroanatomic site that mediates the response to mCPP. The literature indicates that a large proportion of SNr cells are GABA-containing neurons (Herrero, Barcia, & Navarro, 2002; Nair-Roberts et al., 2008; Richards, Shiroyama, & Kitai, 1997). Hence, it is possible that mCPP activity at 5-HT receptors on GABA neurons of the SNr mediates the attenuation of compulsive checking exaggerated by quinpirole-induced DA hyperactivity. Therefore, such a DA-5-HT interaction may be more complex and involve an intermediary component.

Nevertheless, the present finding of an oppositional effect for DA and 5-HT hyperactivation on compulsive behaviour, that is, that DA drives while 5-HT reduces it, is consistent with a neurobiological hypothesis put forth by the security motivation theory of OCD. According to the authors (Szechtman & Woody, 2004), the security motivation system is activated by potential threat resulting in a motivational drive mediated by midbrain dopaminergic neurons projecting to cortical and subcortical regions. In the animal model, exposure to the open field presents a potential threat, and treatment with the DA agonist quinpirole sensitizes the motivational drive. This motivational drive results in the performance of species typical behaviour in an attempt to generate a satiety

signal resulting in the shut-down of the behaviour. In the animal model, the behaviour is checking. The satiety response, according to the hypothesis, was predicted to be mediated by brainstem 5-HT producing cells to cortical and subcortical structures. Although it is unknown where precisely in the brain mCPP produces its effects, it is probably by activity at 5-HT receptors given that the drug has affinity for several 5-HT receptors (Fiorella et al., 1995; Hamik & Peroutka, 1989; Hoyer & Neijt, 1988; Kilpatrick et al., 1987) and is used as a probe of 5-HT function (Kahn & Wetzler, 1991). Taken together, we show that both DA and 5-HT activity may interact to modulate a security motivation system underlying compulsive behaviour. This finding contributes neurobiologically to a growing literature on the motivational basis of OCD (for example, see Boyer & Lienard, 2006; Szechtman & Woody, 2004), and provides a solid rationale to further elucidate the underlying neurobiological circuitry to develop targeted therapeutics and behavioural interventions that reflect the affliction's motivational underpinnings.

Implications for future clinical studies and novel therapeutics

The results of the present thesis have implications for future studies using mCPP and other potential pharmacological treatments for OCD in the human. The results from Chapter 2 provide a solid rationale to address what the effect of mCPP is in human OCD. This is because we identified two methodological factors that may better shed light on the effect of the drug in humans: 1) mCPP should be tested on a homogeneous symptom

subtype of OCD, and; 2) OCD should not be measured as a uniform behavioural phenomenon. The importance of both of these factors is discussed below.

The results from Chapter 2 suggest that any further studies testing the effect of mCPP in the human should control for symptom subtype. That is, effort should be made to include only a homogeneous type of OCD symptom in future studies. Accordingly, because the effect of the drug was well characterized in an animal model of the compulsive checking subtype, it is suggested that future human studies employ patients diagnosed with this subtype of OCD behaviour.

However, if OCD is measured as a uniform phenomenon in humans, controlling for symptom subtype might not be sufficient to identify effects of mCPP given that in the animal model, the drug has selective effects on functional components. However, the presence of the same or similar functional components present in the animal model have not been fully identified in the human. A reasonable next step in human OCD research may be to address whether such functional components exist. This may be accomplished by applying the same criteria measures used to index behaviour of rats in the quinpirole model to the human form of the disorder. The rationale for this approach stems from the fact that the criteria measures for the rat model were based on the spatial temporal performance of OCD behaviour of the human (Szechtman et al., 1998). Indeed, there are even some studies that report the form of compulsive checking performed by the human (Eilam, Zor, Szechtman, & Hermesh, 2006). If, as in the animal model functional components of OCD behaviour are observed, assessing the effects of mCPP by reducing OCD behaviour to its functional underlying components may provide the ability to

capture selective effects of the drug on human OCD behaviour. Indeed, this approach to further elucidate the effects of mCPP on OCD would be useful to better understand the effect of any pharmacological agent on OCD.

The observation from Chapter 3 that separate neurobiological mechanisms mediate the development versus performance of OCD, and that mCPP reduces the performance of compulsive checking but does not actually ameliorate the developed behaviour has significant implications for the treatment of OCD. These results suggest that it is possible that current and future pharmacological treatments may merely block or 'mask' OCD symptoms but not actually reverse the neurobiological mechanisms underlying them. Hence, while the OCD state may be attenuated, the OCD trait remains intact. Somewhat consistent with this, the literature indicates that relapse of OCD symptoms may occur following the discontinuation of medication (Koran, Hackett, Rubin, Wolkow, & Robinson, 2002; Leonard & Rapoport, 1987; Maina, Albert, & Bogetto, 2001; Ravizza, Barzega, Bellino, Bogetto, & Maina, 1996; Thoren, Asberg, Cronholm, Jornestedt, & Traskman, 1980). It is not known whether in these studies pharmacological treatment merely blocked OCD, with discontinuation of treatment effectively 'releasing' OCD symptoms. Nevertheless, our data suggests that such a possibility could exist. Hence, the quinpirole model, by the identification of separate systems mediating development and performance of compulsive behaviour, may be a useful tool to test current and novel therapeutics to address whether they merely block performance of OCD, or whether they produce a more desirable effect and reverse the neuroplastic changes underlying the developed behaviour.

Conclusion

Following the observation from Chapter 2 that mCPP attenuates compulsive checking and does not exaggerate it as was initially hypothesized, the plan of study for the thesis changed to address this finding and hence, the overarching hypothesis tested was that **mCPP attenuates the development and performance of compulsive checking, and this effect of the drug is mediated by the NAc and stimulation at 5-HT2A/C receptors.** Overall, the results lead us to fully reject the initial hypothesis. Below, the overarching hypothesis is broken-down to indicate how this conclusion was arrived at.

- **mCPP exaggerates compulsive checking** - from initial plan of study

  Rejected - Chapter 2 revealed that in a model of the compulsive checking subtype, mCPP attenuates compulsive behaviour induced by quinpirole treatment

- **mCPP attenuates the development and performance of compulsive checking...**

  Rejected - Chapter 3 showed that mCPP attenuates the performance, but not the development of compulsive checking to quinpirole

- **...this effect of the drug is mediated by the NAc...**

  Rejected - Chapter 4 demonstrated that mCPP attenuates vigor and satiety in NAc lesion animals, hence, the NAc does not mediate the effects of mCPP

- **...and by stimulation of 5-HT2A/C receptors.**

Rejected - Chapter 5 showed that the selective 5-HT2A/C receptor antagonist drug ritanserin does not reverse the effect of mCPP on compulsive checking, hence, 5-HT2A/C receptors do not mediate the effect of mCPP

In conclusion, the present thesis reveals that in a model of the compulsive checking subtype, mCPP attenuates the performance but not the development of compulsive checking, and this effect of the drug is not mediated by the NAc or stimulation at 5-HT2A/C receptors.

Final comment

OCD is a complex psychiatric affliction and efforts to design more effective treatment strategies will require progress in basic research. The process of analysis by experimentation allows us to break down a complex phenomenon such as OCD to its underlying constitutive components (Teitelbaum, 2012; Teitelbaum & Pellis, 1992). This approach reveals neurobiological and behavioural features of experimental manipulations that may otherwise not be captured when OCD is measured as a unitary phenomenon. Using the process of analysis by experimentation, the present thesis reduced and identified behavioural and neurobiological correlates of mCPP in a model of compulsive checking behaviour. In doing so, the behavioural and neurobiological correlates of the model compulsive behaviour were also further elucidated.

The utility of the approach of analysis by experimentation is underscored by the implications for our original findings. To reiterate, we suggest that mCPP has attenuating

effects on the compulsive checking subtype of OCD, but that this effect of the drug has not been captured in previous clinical studies because OCD was measured as a unitary phenomenon across different subtypes of OCD symptoms in patients. This finding is important because it contributes to a plausible explanation for the discrepant effects mCPP has been reported to produce in the human literature. We also used mCPP to identify separate mechanisms for the development and the performance of compulsive checking in the quinpirole model, and suggest that mCPP reduces the performance of compulsive behaviour but not its development. This finding is important for the possible use of mCPP or other pharmacological agents in the treatment of OCD because it suggests the possibility that some drugs may merely block OCD symptoms, but not actually ameliorate the underlying neurobiology driving them. In observing that mCPP probably attenuates compulsive checking behaviour downstream of the NAc, we put forth a hypothesis for the neurobiological mechanisms underlying the behavioural response to mCPP and the attenuation of compulsive checking. Finally, although the attenuating effects of mCPP do not appear to be mediated by 5-HT2A/C receptors, the putative opposing roles for DA and 5-HT identified in the present thesis are consistent with the neurobiology hypothesized for a security motivation theory of OCD, and hence are consistent with a motivational basis for the affliction.

There still remains more work to be done to further reduce and identify the behavioural and neurobiological effects of mCPP on OCD. The most important next experiments need to address which 5-HT receptor subtypes mediate the effects of mCPP

on compulsive checking, and localize where in the brain mCPP acts to modulate compulsive checking.

The purpose of this thesis was to shed light on the behavioural and neurobiological effects of mCPP using the quinpirole sensitization rat model of OCD, and in a reciprocal manner, to use the drug to dissect and further understand behavioural and neurobiological components of compulsive checking in the quinpirole sensitization rat model of OCD. It is respectfully suggested that to this end, the present thesis has achieved this goal.

CHAPTER 7: REFERENCES FOR CHAPTERS 1 AND 6

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