Univerzita Karlova v Praze
Přírodovědecká fakulta

Studijní program: Biologie
Studijní obor: Fyziologie živočichů- Neurobiologie

Bc. Dominika Radostová

Effects of clomipramine and risperidone on learning and flexibility in an animal model of obsessive-compulsive disorder

Vliv klomipraminu a risperidonu na učení a flexibilitu u animálního modelu obsedantně-kompulzivní poruchy

Diplomová práce

Vedoucí diplomové práce: doc. RNDr. Aleš Stuchlík, Ph.D.

Praha, 2015

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovávala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 14. 8. 2015

Podpis

Acknowledgments:

I would like to thank to my supervisor doc. Aleš Stuchlík, Ph.D. for immense patience and time spent preparing this thesis. Great thanks also belongs to Mgr. Hana Hatalová for assistance with data processing and for valuable advice and comments on writing this thesis. Also I wish to thank my family and friends for their patience and support throughout my studies.

ABSTRAKT

Chronická sensitizace dopaminových D2/D3 receptorů jejich agonistou quinpirolem (QNP) indukuje u potkanů kompulzivní kontrolování, které je považováno za model obsedantně-kompulsivní poruchy (OCD). Předchozí studie odhalila deficit v kognitivní flexibilitě u QNP sensitizovaných potkanů. Tato práce se zaměřila na určení, zda je tento deficit kognitivní flexibility zmírněn společným podáváním klomipraminu (CMI), risperidonu (RIS) nebo kombinací obou (CMI+RIS) a QNP léčby.

Byla použita averzivně motivovaná úloha aktivního vyhýbání se místu na kolotočovém bludišti s tzv. přeučením. K měření výkonu byl hodnocen počet vstupů do šokového sektoru, kterému je zapotřebí se vyhýbat. Bylo využito šesti skupin léčených různými látkami: kontrolní skupina, QNP skupina, CMI skupina, skupina s kombinací QNP/CMI, skupiny s kombinacemi QNP/RIS a QNP/CMI/RIS. Překvapivě byla u QNP skupiny ve srovnání s kontrolní pozorováno horší akviziční učení pokud byly tyto dvě skupiny porovnávány samostatně. Nicméně, i tak předvedla podobně jako v předchozí studii QNP skupina ve srovnání s kontrolní horší výkon v první den přeučení. Při porovnání všech skupin bylo zhoršené počáteční učení pouze u skupiny dostávající kombinaci QNP/CMI ve srovnání s kontrolní skupinou. První den přeučení měla pouze QNP skupina výrazně vyšší počet vstupů než skupina kontrolní. Výsledky naznačují, že léčba CMI celkově snižuje učení, zatímco léčba kombinací RIS a CMI zlepšuje přeučování.

KLÍČOVÁ SLOVA: quinpirol, klomipramin, risperidon, OCD, potkan, chování, rotující aréna

ABSTRACT

Chronic sensitization of dopamine D2/D3 receptors by agonist quinpirole (QNP) induces compulsive checking behaviour in rats, which is considered an animal model of obsessive-compulsive disorder (OCD). Previous study revealed deficit in cognitive flexibility in QNP sensitized rats. This thesis focused on determining if this cognitive flexibility deficit is ameliorated by co-administration of clomipramine (CMI), risperidone (RIS) or combination of both (CMI+RIS) to QNP treatment.

Aversively motivated active place avoidance task on a Carousel maze with reversal was used. The number of entrances into a to-be-avoided shock sector was evaluated as measure of performance. Six treatment groups were used: control group, QNP group, CMI group, QNP/CMI combination, QNP/RIS combination and QNP/CMI/RIS combination. Surprisingly, when compared alone, significantly worse acquisition was observed for QNP group compared to control group. However, similarly to previous study, QNP group had a worse performance in a first reversal session compared to control group. When all groups were compared, only QNP/CMI group had worse initial learning compared to control group. In reversal learning, only QNP treated group had a significantly more entrances than control group in first reversal session. Results suggest that co-treatment with CIM reduces overall learning, while co-treatment with RIS or CMI combined with RIS improves reversal learning.

KEY WORDS: quinpirole, clomipramine, risperidone, OCD, rat, behaviour, rotating arena

LIST OF ABREVIATIONS

5HT	5-hydroxy-tryptamine, Serotonin
AAPA	Active allothetic place avoidance task
ACC	Anterior cingulate cortex
ACQ	Acquisition learning phase
ALDH	Aldehyde dehydrogenase
AMPA receptor	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CANS	The Childhood Acute Neuropsychiatric Symptoms
CANTAB	The Cambridge Neuropsychological Test Automated Battery
CBT	Cognitive behavioural therapy
CMI	Clomipramine hydrochloride?
COMT	Catechol-O-methyltransferase
CSF	Cerebrospinal fluid
DAT	Dopamine transporter
DBS	Deep brain stimulation
DLPC	Dorsolateral prefrontal cortex
DSM-5	The fifth edition of The Diagnostic and Statistical Manual of Mental Disorders
ED	Extradimensional set shifting
EPS	Extrapyramidal side-effects
EX/RP	Expose and response prevention in CBT
GABA	γ-aminobutyric acid
GABHS	Group-A β-haemolytic streptococcal infections
GM	Gray matter
GPe	Globus pallidus, external segment
GPi	Globus pallidus, internal segments
HAB	Habituation phase
HSF	High frequency stimulation
ICD	The International Statistical Classification of Diseases and Related Health Problems
ID	Intradimensional set shifting
MAO	Monoamine oxidase
mCPP	m-chlorophenylpiperazine

MDmc	Medial dorsal part of the magnocellular nucleus of thalamus
MRI	Magnetic resonance imaging
NAC	Nucleus accumbens
NMDA receptor	N-methyl-D-aspartate receptor
OCD	Obsessive-compulsive disorder
OFC	Orbitofrontal cortex
PANDAS	Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections
PET	Positron emission tomography
PFC	Prefrontal cortex
QNP	Quinpirole hydrochloride
REV	Reversal learning phase
RIS	Risperidone
rTMS	Repetitive transcranial magnetic stimulation
SAL	Saline solution
SEM	Standard error of the mean
SERT	Serotonin transporter
SNc	Substantia nigra, pars compacta
SNr	Substantia nigra, pars reticulata
SNRI	Serotonin and noradrenaline reuptake inhibitors
SPECT	Single positron emission computed tomography
SRI	Serotonin reuptake inhibitors
SSRI	Selective serotonin reuptake inhibitors
STN	Subthalamic nucleus
TCA	Tricyclic antidepressants
TNFα	Tumor necrosis factor α
VAmc	Ventral anterior part of the magnocellular nucleus of thalamus
VTA	Ventral tegmental area
WCST	Wisconsin Card Sorting Test
WM	White matter
Y-BOCS	Yale-Brown Obsessive Compulsive Scale

LIST OF CONTENTS

1 INTRODUCTION

When several treatment options are available prediction of treatment outcome prior to start of a treatment is very advantageous. Effectiveness of any single treatment can be affected by genetic factors, symptom dimensions, neurophysiological parameters and possibly neurocognitive intermediate phenotype. Such a priori prediction of likely treatment effectiveness or ineffectiveness can significantly reduce time of suffering of patients especially in diseases where effect of treatment is evident only after a prolonged time. One of these diseases is obsessive-compulsive disorder. There are several treatment options available but they have to be each tested for a long time, lengthening the patients suffering, and psychological load.

Obsessive compulsive disorder is a psychiatric disorder with a lifetime prevalence of 2-3% of the population. Disorder is characterized by intrusive thoughts (obsessions) which are often accompanied by rigid, repetitive and time consuming behaviours (compulsions). At the present, first line of treatment is administration of high doses of SRIs which helps about 60% of patients. Some of the patients who are unresponsive to the SRI treatment respond to complementation of treatment with neuroleptics. Of these, most often are used low doses of haloperidol and risperidone, but the nature of this interaction is yet unclear. Due to the long time for SRI to become effective, attempts had been made to predict which treatment will be efficient beforehand based on varied parameters including symptom dimensions, MRI indicators and cognitive intermediate phenotypes.

Animal models are very useful in testing these types of hypotheses. One of the animal models of OCD displays compulsive checking when placed into open-field after sensitization with D2/D3 agonist quinpirole. These animals were also shown to display cognitive flexibility deficit in reversal learning. Indeed, deficits in cognitive flexibility were often reported in OCD patients, especially for patients with checking symptoms. The cognitive flexibility is an ability to react appropriately to changing environment and is tested by many experimental tasks including reversal learning in both human subjects and animals.

The aim of this study was to determinate an effect of clomipramine and risperidone and their combination on impaired cognitive flexibility in quinpirole induced compulsive checking model of OCD. Clomipramine is a tricyclic antidepressant which was selected because it is effective in OCD treatment, and also reduces checking behaviour in quinpirole sensitized animals. Risperidone is an antipsychotic drug often used as an augmentation therapy to SRI treatment in OCD. Experimental apparatus Carousel maze is a good tool for assessment of cognitive flexibility performance, thanks to a demanding reversal set up possibility. We hypothesize, that if there is an effective treatment for treating cognitive flexibility deficit in these animals, it could be likely effective treatment for a subgroup of patients that display reduced cognitive flexibility.

2 REVIEW OF LITERATURE

2.1 Obsessive-compulsive disorder (OCD)

Obsessive compulsive disorder is a psychiatric disorder, which can disrupt life of patients and their relatives. The prevalence of obsessive-compulsive disorder is about 2 – 3 % (Ruscio *et al.*, 2008). OCD is the fourth most frequent psychiatric disorder. These numbers are high even without taking into the account the claims that there are not many cases identified in psychiatrist's office (Wahl *et al.*, 2010). Prevalence of OCD is even higher than prevalence of much better known schizophrenia (American Psychiatric Association, 2013). Obsessive-compulsive disorder is encompasses two groups of symptoms, obsessions and compulsions, as the name of disorder can suggest. Obsessions are characterized by uncontrollable intrusive thoughts, which are recurrent and debilitating for patients. Patients often suffer obsessive thoughts for many years, which can lead to anxiety or depression. Patients try to suppress obsessive states by forming compulsions (but see Robbins *et al.*, 2012). Compulsions are ritualistic motor acts or, uncommonly, repetitive mental acts. Compulsions represent way to relieve obsessions and are the visible manifestation of symptoms in patients. In some cases, obsessions occur alone, but the most often patients suffer from obsession acompanied by compulsions.

OCD is heterogeneous disorder in many ways. Firstly, there are many ways how the OCD can manifest in patients. Also, there is not a specific age of onset or single aetiology. Moreover, patient's treatment response and comorbidities vary. The manifestation of OCD is divided in several symptom dimensions, which were designed for better understanding of symptom structure. The first symmetry dimension includes symmetry obsessions followed by compulsions such as ordering, counting or repeating. Dimension of forbidden thoughts describes fear of death or harm happening to a patients or to loved one or, sexual and/or religious obsessions. These fears are usually accompanied by checking compulsions. Lastly, cleaning dimension is based on obsessional fear of contamination or infection and is followed by cleaning and washing rituals (Bloch *et al.*, 2008). The last mentioned form of OCD is also the most frequent (Prabhu *et al.*, 2013). Though symptoms stay constant throughout life in most cases, the change of symptoms within a dimension may occur (Mataix-Cols *et al.*, 2002). Patients are aware of the absurdity of their acts but do not have a power to control and stop. With time, symptoms become increasingly time consuming and debilitating.

Age of onset of the disorder divides patients into two groups. In main group of patients symptoms take shape at about the age of twenty years but second group consists of children patients (Heyman *et al.*, 2001). It was found children patients suffer from the same types of symptoms as adult patients with a same intensity/range (Delorme *et al.*, 2006). In adult onset

patients males and females are equally affected. However in paediatric patients boys are affected more often (Geller, 2006). In adult onset patients there was no gender preference was found.

OCD patients commonly exhibit range of comorbid diseases. These can include depression, anxiety, social phobia, ticks, eating disorders or others (Torresan *et al.*, 2013). And conversely, obsessive and compulsive symptoms can be comorbidity of other psychiatric disorders, for example Tourette syndrome, trichotillomania (Aouizerate *et al.*, 2004), schizophrenia (de Haan *et al.*, 2013) or Sydenham's chorea (Hounie *et al.*, 2004).

The clinical psychiatrics can gain insight for identification and diagnosis of this disorder from diagnosis manuals. Very recent fifth edition of The Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013) is the most used diagnostic tool in the United States. Other diagnostic manual is The International Statistical Classification of Diseases and Related Health Problems (World Health Organization, 1992) which is commonly used in the Czech Republic. Additionally, Yale-Brown Obsessive Compulsive Scale (Goodman *et al.*, 1989) helps psychiatrists with severity rating. These documents contain description of symptoms, epidemiology and sets of diagnostic codes.

2.1.1 Pathophysiology of obsessive-compulsive disorder

The heterogeneity of the OCD is reflected not only by symptoms, comorbidities and age of onset but also thought its causes. Several causes of disorder were suggested and they can be divided into genetic and environmental. Specifically, it was demonstrated that approximately 47 % of variance in OCD is explained by genetic factors and the remaining part of variance is dependent on environment (van Grootheest *et al.*, 2005). Amply discussed cause is pathophysiological effect of the infection and traumas in childhood and subsequent outbreaks of the disorder in later life. All these influences have an effect on central nervous system, on specific anatomical circuits and on neurotransmitter systems.

2.1.1.1 Genetic background of OCD

Years of research found that at least some of OCD forms may be genetically determined or partly dependent. But there is an indication that OCD is probably dependent on a number of genes with small effect. A lot of genetic studies investigated obsessive-compulsive disorder also in families of diagnosed patients. Higher prevalence of OCD patients was detected among already diagnosed patient's relatives compared to relatives of healthy controls (Goodman *et al.*, 1995; Grabe *et al.*, 2006). Genetic causes of OCD were indicated also thanks to twin studies. Although twin studies have a long history only small number of these studies estimated a heritability of OCD. For now, heritability of children obsessive compulsive symptoms seems to

be about 45-65 % and the level of heritability in adult patients seems to be about 27-47 % (van Grootheest *et al.*, 2005).

Up to today, there are numerous studies which try to specify exact genes as a basis of OCD. The problem is that many results and many are mutually exclusive. Studies interested in findings specific genes and loci crucial for understanding genetic background of the OCD, are targeted to definite genes or to specific neurotransmitter system abnormality, but there is not any special identified yet. Many studies suggest large amount of candidate genes of small effect within the serotonergic, glutamatergic and dopaminergic systems. Especially suspicious are genes for the receptors, transporters or enzymes or even whole loci, which encompass a large number of genes (Willour *et al.*, 2004; Shugart *et al.*, 2006; Pauls, 2010). For example, association between OCD and genes for monoamine oxidase A (MAOA) and catechol-O-methyltransferase (COMT), which are important in inhibition of biogenic amine signalization, is widely studied but with ambiguous findings (Schindler *et al.*, 2000; Sampaio *et al.*, 2015). There are a lot of other genes, which are studied for their putative role in OCD development such as genes for transporters (variations in polymorphisms in dopaminergic DAT1, glutamatergic SLC1A1, or serotonergic SLC6A4) or receptors (variations in frequencies of alleles in dopaminergic DRD3 and DRD4, or polymorphism of serotonergic HTR2A) (Pauls, 2010; Sampaio *et al.*, 2013). Their association with OCD is not clearly confirmed yet. Except these there are studies which are interested in genes involved in immune system or in hormones such as transcription factor tumor necrosis factor α (TNFα), brain-derived neurotrophic factor (BDNF) or estrogen receptor α (Sampaio *et al.*, 2013; Zai *et al.*, 2015). For now the association of these genes with OCD is not convincing.

Unfortunately, so far the association studies do not reveal consistent results to confirm specific genes involved in OCD pathophysiology. Inconsistent results suggest that there is not a unique gene which may cause OCD, but many genes of small effect have a role in outbreak of the disorder. At the same time importance of environmental effects must not be forgotten, because as twin studies had shown, understanding of OCD aetiology is dependent on combination polygenic factors and the environment.

2.1.1.2 Environmental factor- traumas and infections

The second component of OCD aetiology, an environment, also has an impact on outbreak of the disorder. There are two common non-genetic etiological factors, traumas and infections, which can be cause of OCD in patients without family history of the illness. The possible influence of trauma and stressful life events are considered for both OCD childhood and adulthood onset OCD (Pollitt, 1957; Cath *et al.*, 2008; Fontenelle *et al.*, 2012). It was suggested that increased frequency of stressful life events like serious illness, traumatic injury, car

accident, victimization or death are associated with increased rate and/or severity OCD (McKeon *et al.*, 1984; Storch *et al.*, 2006)**.** There is a group of adult patients, in which the OCD occurred after traumatic events in their childhood, such as physical and emotional abuse or neglect (Lochner *et al.*, 2002).

The second environmental factor, affecting primarily paediatric patients, is related to various infectious pathogens. Form of abrupt onset of obsessive compulsive symptoms commonly with tics is often associated with group-A beta-haemolytic streptococcal infections (GABHS). This form of OCD is now classified as belonging to Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS) (Swedo *et al.*, 1998). GABHS is primarily discussed as related to Sydenham's chorea, the neurologic manifestation of rheumatic fever. Indeed, symptoms of Sydenham's chorea also include obsessive compulsive behaviours (Hounie *et al.*, 2004). Moreover, pathogens like *Mycoplasma pneumonia* (Müller *et al.*, 2004) or *Borrelia burgdorfei* (Riedel *et al.*, 1998) are also studied for modulating effect on development of tics and obsessive-compulsive behaviour.

Motor and behaviour symptoms of the PANDAS and Sydenham's chorea are both caused by basal ganglia damage through autoimmune action of antibodies or cross-reacting immune cells (Bronze and Dale, 1993). It is not yet certain what immunological marker is specific for PANDAS, but there are two putative candidates. The first, D8/17, is monoclonal antibody, which reacts with epitopes expressed on B lymphocytes and with striatal neurons (Swedo *et al.*, 1997). The second proposed marker is antibody to dopamine D1 and D2 receptors. Its binding to striatal neurons may change dopaminergic transmission and affect behaviour (Brimberg *et al.*, 2012). The most affected regions are caudate nucleus, putamen or globus pallidus (Giedd *et al.*, 2000) and damage of these areas is often manifested as tics, motor hyperactivity and obsessive compulsive symptoms (Swedo *et al.*, 1998; Snider and Swedo, 2004).

Although authors of PANDAS phenomena defined evaluation criteria, such as early rapid onset of the disease and previous streptococcal infection, some studies described diagnostic system of PANDAS as insufficient and ambiguous (Singer *et al.*, 2012). More recently, the new and broader view was introduced. The Childhood Acute Neuropsychiatric Symptoms (CANS) imply whole spectrum of acute neuropsychiatric states, which are characterized by young onset. CANS include diseases caused not only by GABHS infection but also by other pathogens and vascular, hypoxic or drugs induced mechanisms (Singer *et al.*, 2012). For better understanding and diagnosis of CANS and especially PANDAS it will be necessary to study microbiology and immune system in detail during GABHS infection (Kurlan and Kaplan, 2004).

We still do not know exact cause of the obsessive compulsive disorder, but we have a lot of evidences for both genetic and environmental causes. Both genetics and environment act on structure and function of central nervous system and manifest as different symptoms of the obsessive compulsive disorder.

2.1.2 Neuroanatomy of OCD

Even if exact cause of obsessive-compulsive disorder is still not known, it was demonstrated that symptoms observed in patients are associated with certain abnormalities in central nervous system. Structures that were found to be associated with OCD include orbitofrontal cortex, anterior cingulate cortex, prefrontal and parietal cortices, and caudate nucleus (Menzies *et al.*, 2008). Neuronal abnormalities are related to structural changes in volume, white matter density and also to activation during both during rest and during symptom provocation. Unfortunately, imaging methods don't provide simple results and clear identification neuronal substrate of the disorder. From the varied results it appears, that heterogeneity of this disorder is reflected in neuronal substrates, too.

Current findings agree with theoretical view of pathophysiology in cortico-striatal loops in the OCD (Milad and Rauch, 2012). According to imaging studies it was indeed confirmed that OCD is associated with abnormality in orbitofronto-striatal circuits (Saxena *et al.*, 1998; Menzies *et al.*, 2008). This loop is one of the five parallel circuits which connect cortical areas with basal ganglia (Alexander, 1986). All of these loops have basic common scheme of projection (*Figure 1*) and comprise of two differently directed paths each of which has a divergent effect on target cortical area. When the first, direct pathway, is activated its disinhibiting effect on thalamus causes activation of the cortex. The second, indirect pathway, increases inhibition of the thalamic nuclei and thereby causes an inhibition of the cortex (Alexander and Crutcher, 1990).

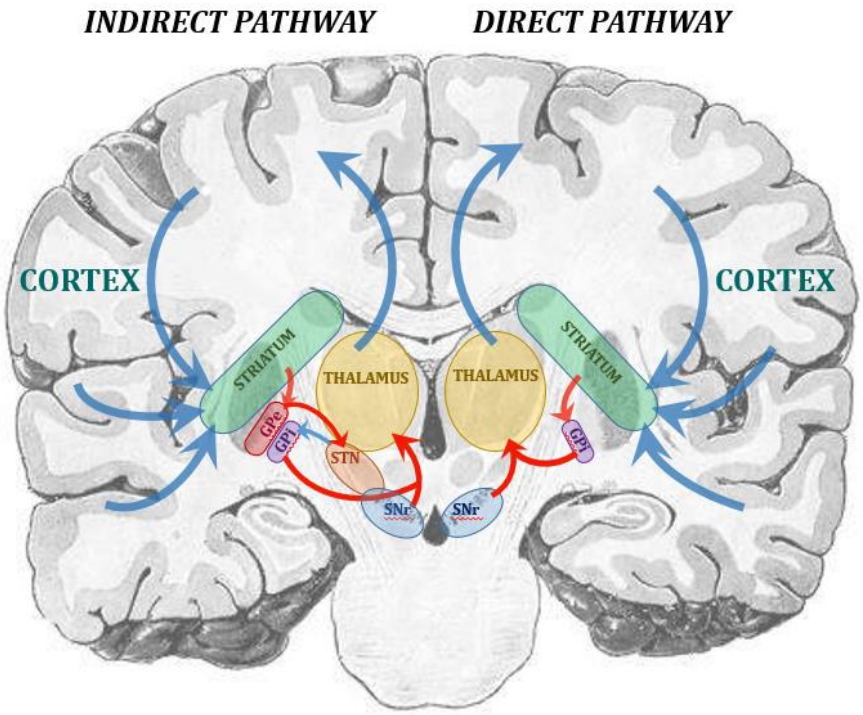


Figure 1: Basic scheme of the cortico-striatal loops. (Blue arrow means excitatory glutamatergic projection, red arrow means inhibitory GABAergic projection.)
The start of each of circuits between cortical areas and basal ganglia is specific cortex area and using excitatory glutamatergic signalization continues to the striatum. Striatal GABAergic inhibitory neurons project to two separate circuits, direct and indirect. Direct outputs from striatum head to internal segment of globus pallidus (GPi) and substantia nigra pars reticulata (SNr). This complex sends second GABAergic neurons to thalamic nuclei and from here the last connections end back in cerebral cortex. In indirect pathway striatal neurons project to external segment of globus pallidus (GPe) and then to subthalamic nucleus (STN), both connections are GABA inhibitory. The next projection head to GPi/SNr complex, joins and in parallel continues with direct pathway. Direct pathway causes activation of cortex, because inhibitory projection from GPi/SNr is blocked by striatal neurons. Indirect pathway works just the opposite, active projection from STN causes support of GABAergic signalization and attenuation of the thalamus and subsequently cortex. Moreover, dopamine and other neurotransmitters may modulate these pathways (Adjusted according to Alexander and Crutcher, 1990)

The orbitofronto-striatal circuit is composed of activating glutamatergic projection from the lateral orbitofrontal cortex (*Brodmann's area 10, Walker's area 12*) to ventromedial part of the caudate nucleus. The pathway continues with inhibitory connections to the internal segment of the globus pallidus (GPi) and rostromedial section of the substantia nigra pars reticulata (SNr). Next inhibitory outputs project from caudate nucleus and SNr to magnocellularis part of the thalamus, especially to the ventral anterior (VAmc) and medial dorsal (MDmc) nuclei. The last component of this circuit is activation projection from thalamus back to lateral orbitofrontal cortex. Previously described direct pathway of orbitofronto-striatal circuit has complementary

indirect pathway projecting from caudate nucleus through external segment of globus pallidus and subthalamic nucleus to GPi, where both pathways merge (Alexander, 1986).

When we compare mentioned brain regions involved in OCD pathophysiology and course of described pathway, there is not a complete overlap. Though most of these structures are parts of the orbitofronto-striatal circuit, some structures are not part of this circuit such as anterior cingulate cortex or prefrontal and parietal cortices. These brain regions are part of parallel cortico-striatal circuits and therefore it was suggested anterior cingulate (Rotge *et al.*, 2008) and dorsolateral prefrontal circuits (Menzies *et al.*, 2008) are involved in OCD pathophysiology too. All these circuits connect to different cortical targets but pass through the same striatal regions (*Figure 2*). Therefore aforementioned basic circuit organization showed in Figure 1 is the same in each of this circuit.

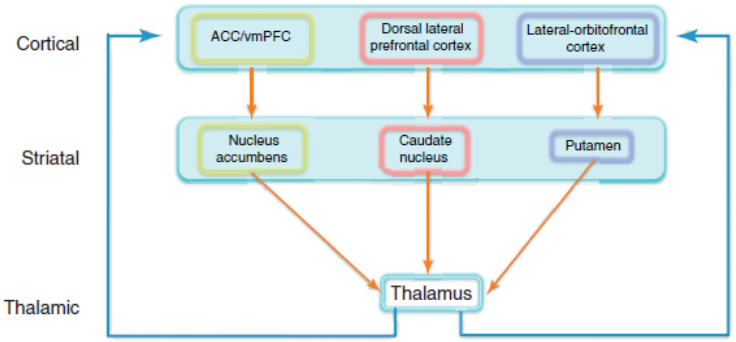


Figure 2: Illustration of three cortico-striatal circuits involved in OCD pathophysiology (Milad and Rauch, 2012)

In more detail, three models have been proposed for dysfunction of these circuits in OCD. First, Modell's model is based on abnormalities and dysregulation of the basal ganglia, which are not able to modulate connections between cortical area and medial dorsal (MDmc) nucleus of thalamus. Modell suggested that if GABAergic inhibitory effect of striatal projection is decreased, then positive-feedback loop between OFC and MDmc develops (Modell *et al.*, 1989). Disadvantage of this model is that it does not include specific role and function of ACC in OCD symptoms. A later, Baxter's model deals with aforementioned imbalance between direct and indirect pathway. Orbitofrontal and anterior cingulate cortices project to caudate nucleus and activate direct pathway and DLPC preferentially projects through caudate nucleus to indirect pathway (Baxter, 1995; Saxena *et al.*, 2001). The important part of the Schwartz's, the third, model is based on a disturbance in cholinergic interneuronal projections. Normally, these cells select significant information and generate appropriate response as a pattern of activity. This activity information reaches the OFC or ACC, the regions which have role in error detection. Hyperfunction in cholinergic interneurons can cause hyperactivity of OFC and ACC and thus may cause OCD symptoms (Schwartz, 1999).

These theories about cortico-striatal circuit involvement in pathophysiology of OCD are supported by known roles of these regions in human behaviour. Figee and colleagues (2013) summarized previous studies which describe cases of patients, who developed OCD after lesions

in different areas of brain. These structures included caudate nucleus, putamen, globus pallidus, nucleus accumbens, frontal cortex, temporal or parietal cortices but also cerebellum and amygdala. Some of these structures are part of cortico-striatal loops. But also some findings showed that lesions of structures such as putamen, internal capsule and fronto-parietal lobe resulted in improvement or disappearance of obsessive-compulsive symptoms and they are proposed to possible targets of treatment techniques such as deep brain stimulation (Figee *et al.*, 2013).

It would seem that imaging studies will agree among themselves about what brain regions are involved in pathophysiology of the OCD. However, although studies agree on involvement of structures within cortico-striatal structures in OCD, they do not observe anomalies in exactly the same structures within these circuits. Commonly though hyperactivity and/or increase of volume in caudate nucleus was observed, higher activity and decrease of grey matter in OFC and ACC and increase of activity in GPe (Menzies *et al.*, 2008). The anomaly in cortico-striatal circuits may indeed be only common nominator that connects patients with OCD. Additionally, differences in methods and oodles of variability in patient's medication and comorbidities may add to discrepancies between studies.

One of the imaging methods commonly used is magnetic resonance imaging (MRI). MRI allows imaging of grey (GM) and white matter (WM). Several MRI studies found abnormalities in gray matter volume caudate nuclei (increased volume), orbitofrontal cortex (decreased volume, increased WM) and anterior cingulate gyrus (decreased GM), amygdala (decreased volume, decreased WM) and thalamus (increased volume) (Szeszko *et al.*, 1999; Atmaca *et al.*, 2007). But even these findings are not conclusive, for example the studied volume of the caudate nucleus was also found to be unchanged (Aylward *et al.*, 1996) as well as reduced (Robinson *et al.*, 1995). This example of the caudate nuclei provides insight into the complex and difficult situation of identifying substantial structures in OCD. The meta-analysis of the several studies has showed a summary of volumetric changes in patients with OCD and it has found only anterior cingulate and orbitofrontal cortices consistently showing decreased volume and increase in GM volume conversely size of GM in thalamus (Rotge *et al.*, 2009).

Activation of neural structures may be imaged and measured by positron emission tomography (PET) or single positron emission computed tomography (SPECT). These techniques use radioactive labelled ligands and measure metabolism activity or blood flow. In OCD patients there was found a consistent increased in regional cerebral blood flow in frontal regions. Increased metabolic rate in OCD patient was found specifically in orbitofrontal gyri and caudate nuclei and decreased metabolism was found in parietal regions (Saxena *et al.*, 1998). But there were a lot of different studies which presented multiple outcomes for a wide range of the brain regions and several meta-analyzes summarized their results. Meta-analysis by Whiteside

and colleagues (Whiteside *et al.*, 2004) found abnormal activity only in a head of the caudate nuclei and left orbitofrontal gyrus. Later exploration of literature was focused on abnormality nervous tissue during provocation OCD symptoms. Ultimately authors have found many significant differences between patients and healthy controls. Affected regions include orbitofrontal gyrus, ACC, prefrontal cortex, temporal gyrus, premotor cortex, globus pallidus or hippocampus and parietal lobe (Rotge *et al.*, 2008). These two mentioned comprehensive studies point to differences in results which may be caused by varying conditions during measurement (relaxed state vs. symptom provocation).

2.1.3 Treatment of OCD

The treatment of the obsessive compulsive disorder is not simple. Today medicine has a choice of range of treatments with varied effectiveness and associated risks. One of the most often chosen ways of treatment is pharmacological intervention. Prescribed drugs belong to antidepressants – serotonin reuptake inhibitors (SRI). Increasingly common non-invasive part of treatment of newly diagnosed patients is cognitive behavioural therapy (CBT). Studies invent constantly new approaches for the cases when improvement of symptoms is not observed. These include augmentation with antipsychotics, use of novel drugs acting on glutamate or acetylcholine systems and in very serious treatment resistant cases even deep brain stimulation or lesions of brain structures within cortico-striatal loop.

The oldest and also the most widely used today way of treatment is administration of the serotonin reuptake inhibitors (SRI). This effect of SRI in OCD was trigger to study an involvement of the serotonergic system in OCD pathophysiology. The SRI include three subgroups of the substances which inhibit serotonin transporter (SERT) but can interact with most of neurotransmitter systems. In OCD most important are two classes of SRI: tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitors (SSRI). One of the tricyclic antidepressants, which are effective in treatment of the OCD, is clomipramine (Cartwright and Hollander, 1998). Clomipramine is serotonin reuptake inhibitor and antagonist/inverse agonist of histamine H1 receptors, muscarinic acetylcholine receptors and α1 adrenergic receptors and also dopamine D2 receptors (*see 2.2.1.3 Antidepressive drugs*). However, in treatment of the OCD SSRI were a big progress, since they produce less side effects than clomipramine. The most commonly used are fluoxetine, fluvoxamine or paroxetine.

Unfortunately, there are limits to efficiency of therapeutic influence of the serotonin reuptake inhibitors. It was found that approximately 40 % of the OCD patients do not show symptoms improvement following SRI monotherapy (Skoog and Skoog, 1999; Pallanti *et al.*, 2002). If this first attempt fails the different pharmacological procedures can be tried. For

example, there is possibility of change of dosing (Ninan *et al.*, 2006), change of route/method of drug administration (Fallon *et al.*, 1998) or switching to another serotonin reuptake inhibitor (Fineberg *et al.*, 2015). In some patients, co-administration of the neuroleptic drugs, classic or also atypical improves efficiency of the treatment. Haloperidol and risperidone are the most often used antipsychotics to augment SSRI treatment in OCD (Mcdougle *et al.*, 1994; Hollander and Rossi, 2003).

Pharmacological treatment achieves the highest efficacy when it is combined with cognitive behavioural therapy (CBT) (O'Connor *et al.*, 2006). During this psychotherapy procedure patient is exposed to feared situation in a company of a therapist. At present a goal of CBT is exposure and response prevention (EX/RP). In other words, it is a procedure involving withdrawal of performing compulsions, repetitive motor or mental acts, and teaching patients how appropriately respond to unpleasant thoughts and urges. This therapy is long-term but, when successful, each session helps to decrease compulsive symptoms (Franklin *et al.*, 2000; Chamberlain *et al.*, 2005) and reduces hyperactivity in OFC observed in OCD patients (Baxter *et al.*, 1988; Rauch *et al.*, 1994).

Very serious cases of OCD are often resistant to aforementioned ways of treatment. In these cases repetitive transcranial magnetic stimulation (rTMS) or deep brain stimulation (DBS) can be helpful. Rarely, lesions are performed.

Repetitive transcranial magnetic stimulation is relatively novel non-invasive procedure in treating of the OCD. It is based on inhibiting neuronal activity by pulse of a magnetic field. The method of rTMS is dependent on the stimulation frequency and target region, but because of the limited reach of the stimulation, only cortical areas can be accessed (Berlim *et al.*, 2013). Cortical targets that are currently being inhibited with successful treatment outcomes in OCD are motor cortical regions (Mantovani *et al.*, 2006) and OFC (Nauczyciel *et al.*, 2014). Meta-analysis also confirmed OFC and motor areas as promising targets of rTMS (Jaafari *et al.*, 2012; Berlim *et al.*, 2013).

Compared to rTMS DBS can additionally reach subcortical structures. This method is based on high-frequency stimulation of the brain region, which have inhibitory effect on nerve tissue and mimics lesion effect (Bourne *et al.*, 2012). Mian and colleagues observed improvement of obsessions and overall mood after DBS in ventral striatum and improvement of compulsions after stimulation of subthalamic nucleus (Mian *et al.*, 2010).

The most invasive and irreversible way to treat highly refractory/resistant OCD patients is neurosurgical treatment. The significant improving patient's condition was described for anterior cingulotomy, anterior capsulotomy and others (Mundus and Jenike, 1992). The procedure mechanism lies in disruption of the reciprocal connections between cortical areas and subcortical structures. Surprisingly, a reduction of the symptoms severity is observed with a

delay of 3-6 months (Doshi, 2009). Both, DBS and lesions, are very accurate techniques thanks to stereotactic apparatus. Assessment of effectivity all of these three methods (rTMS, DBS, neurosurgical) is affected by fact that only nonresponding patients undergo this treatment and ethics does not allow for randomized controls in studies.

Despite clear heterogeneity of obsessive-compulsive disorder in form of symptoms, age of onset and treatment response there is a consensus in involvement of cortico-striatal circuit in OCD pathophysiology - although there is not a clear answer as to which structures are impaired in these circuits. Even though symptoms between patients vary, all OCD patients share a presence of intruding unbiddable thoughts and motoric acts that at least partially relieve the patient. Obsessions and compulsions are not randomly paired but can be divided into clusters where certain obsessions are accompanied by a specific set of compulsions. It was proposed that this variation in symptom presence is related to which brain structures are affected in cortico-striatal loops (Saka *et al.*, 2004). It was also suggested that OCD is associated not only with anatomy abnormalities but also with abnormalities in neurotransmitter systems, upon which the function of brain structures is dependent.

2.2 Brain neurochemistry

Brain neurochemistry is the basis of proper functioning of the brain and adequate responsiveness. As it is known from other examples (Parkinson‘s or Alzheimer‘s disease) abnormalities in neurochemistry can have devastating effects. It seems that obsessive-compulsive disorder is no exception because, according to available findings, abnormalities of cortico-striatal circuits may be due to altered functioning of neurotransmitter systems. The first indication of neurotransmitter system abnormality in OCD was suggested by a positive effect after administration of tricyclic antidepressant, clomipramine (Renynghe de Voxrie, 1968). Because main target of clomipramine is serotoninergic system this finding lead to extensive interest in study of serotoninergic involvement in OCD. However, it was soon clear that OCD pathology is more complex and studies started to focus also on other neurotransmitter system involved in cortico-striatal circuits such as dopaminergic, glutamatergic and on modulating effects of oxytocin and parvalbumine.

2.2.1 Serotonin

5-hydroxy-tryptamine (5HT), the serotonin, is biogenic amine, belonging to indolamines. In the body of living organism serotonin fulfils many roles. In the vessels serotonin functions as a vasoconstriction substance and has a role in immune reactions and in muscle system. Serotonin in the brain can have a role either of classical neurotransmitter via actions on synaptic membrane or have a role of neuromodulator (Murphy *et al.*, 1998). Serotonergic transmission may affects many behaviours such as sexual, stereotypic or aggressive (Murphy *et al.*, 1998). Serotonin also regulates stress, anxiety, mood and a body temperature (Stahl, 1998b).

5HT is synthetized by hydroxylation of tryptophan by enzyme tryptophan hydroxylase to 5-hydroxy-tryptophan followed by decarboxylation by L-amino acid decarboxylase to 5-hydroxy-tryptamine. Conversely after reuptake into presynaptic neurons through transporters serotonin is degraded by monoamine oxidase type A and B (Aouizerate *et al.*, 2005). Serotonin is synthesized in the brainstem raphe nuclei wherefrom 5HT innervation directs to limbic regions such as prefrontal and cingulate cortices, the amygdala, hippocampus, ventral striatum, thalamus and hypothalamus (Azmitia and Whitaker-Azmitia, 1995; Murphy *et al.*, 1998; Aouizerate *et al.*, 2005). Many of these structures are also part of cortico-striatal loops, which are considered to be a basis of OCD pathophysiology (see *Figure 3*).

The target cells are affected by serotonin through 5HT receptors, which are divided in 7 classes. Of these, first three classes – 5HT1, 5HT2 and 5HT3- are the most studied ones. Members of 5HT1 receptor family are autoreceptors on serotoninergic neurons in raphe nuclei. There are two members within this receptor class, 5HT1A and 5HT1D receptors. They are activated by extracellular serotonin and are responsible for feedback inhibition of 5HT release (Pineyro and Blier, 1999). 5HT2 receptors are distributed throughout brain and mechanism of action of antidepressants and neuroleptics is mediated through these receptors. 5HT3 receptors play role in facilitation of serotonin release on nerve terminals (Meneses, 1999) . Other groups of serotoninergic receptors (5HT4, 5HT5, 5HT6 and 5HT7) are much less researched, but it is known that all of serotonin receptors, except 5HT3, are coupled with different G proteins (Meneses, 1999; Aouizerate *et al.*, 2005).

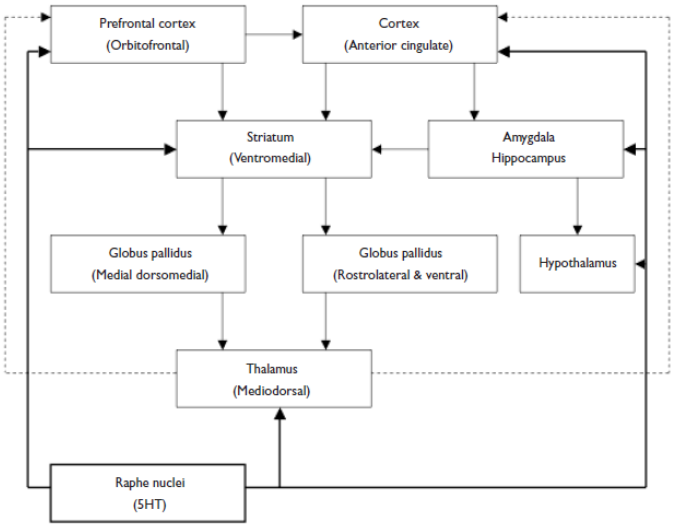


Figure 3: Association of serotonergic projections with cortico-striatal loops (Aouizerate *et al.*, 2005)

2.2.1.1 Serotonin in cortico-striatal circuits and OCD

The serotonin was the first neurotransmitter associated with pathophysiology of OCD. After discovery that serotonin reuptake inhibitors reduce OCD manifestation abnormalities in brain serotonin system became the most studied possible cause of obsessive-compulsive disorder. Nevertheless effective treatment target does not have to mean evidence of serotonergic dysfunction (Murphy *et al.*, 1998). It is possible that serotoninergic system can serve simply as an instrument for pharmacotherapeutic intervention (Baumgarten and Grozdanovic, 1998).

However, as was already mentioned, role of serotonin in OCD was also indicated by genetic studies. The most frequent targets of these studies are gene variations of receptors, serotonin transporters and enzyme MAO (Pauls, 2010; Sampaio *et al.*, 2013). Apart from consensus about the variations in genes related to serotoninergic function in OCD other research directions which studied role of serotonin in OCD patients, do not yet yield conclusive results. For example analysis of cerebrospinal fluid has shown increased brain 5HT levels in OCD patients (Insel *et al.*, 1985), but Leckman (1994) did not find any differences between patients and control healthy people.

Studies using pharmacological approach to assess role of serotonin in OCD pathophysiology also report mixed results. Ritanserin used only in scientific research is an antagonist of serotonergic receptors and its application causes exacerbation of OCD or OC symptoms. Authors suggested a possible positive effect of serotoninergic agonists in treatment of OCD (Delgado and Moreno, 1997). However, administration of m-chlorophenylpiperazine (mCPP), a 5HT agonist, also showed an exacerbation of OC symptoms (Hollander *et al.*, 1992). The explanation may lie in specificity of drugs to subtypes of serotonergic receptors. For example mCPP is a nonselective agonist of 5HT1A, 1D, 2A and 2C receptors, but is an antagonist of 5HT3 and α2 receptors, while ritanserin has pure 5HT antagonist effect (Delgado and Moreno, 1997; Baumgarten and Grozdanovic, 1998).

2.2.1.2 Interactions of serotonin with other neurotransmitters

Brain tissue is a very complex structure. No neurotransmitter system is independent of other neurotransmitter systems- they influence each other. Synthesis and secretion of serotonin in raphe nuclei is influenced and regulated by a range of neuroactive substances. Raphe nuclei received different afferent inputs such as GABAergic, glutamatergic, dopaminergic substance P, acetylcholine or noradrenalin.

Similarly to many structures in the brain raphe nuclei also receive GABAergic signals through habenulo-raphe pathway causing the decrease of 5HT release (Becquet *et al.*, 1993). However, high frequency stimulation of this pathway surprisingly increased 5HT release in projection areas. These contradictory result can be explained by direct excitatory effect of glutamate, the main neurotransmitter in habenulo-raphe pathway, on serotonergic cells during stimulation and indirect effect of glutamate on GABAergic interneurons in raphe nuclei in case of low frequency stimulation (Kalén *et al.*, 1989). GABAergic action in raphe nuclei is predominantly mediated through $GABA_A$ and to lesser extent through $GABA_B$ receptors, while glutamate stimulates almost exclusively NMDA receptors (Becquet *et al.*, 1993). Other important input to raphe nuclei comes from dopaminergic innervation. Through the use of dopamine receptor blockade and activation by selective antagonists and agonists it was shown that D2 receptors exert a main effect on raphe serotoninergic neurons. Stimulation of D2 receptors causes two distinct actions, first 5HT release in raphe nuclei is increased locally but second striatal release of 5HT from raphe innervation is decreased (Ferré *et al.*, 1994). Noradrenergic and histaminergic innervation suppress 5HT release from raphe nuclei (Fink *et al.*, 1990) while substance P (Reisine *et al.*, 1982) and acetylcholine enhance 5HT release (Ribeiro *et al.*, 1993).

2.2.1.3 Antidepressive drugs

Antidepressants are drugs which are familiar even to people without medical education. This group of drugs is used for treatment of depressive disorder (Masand and Gupta, 1999), panic disorder (Barlow *et al.*, 2000), obsessive-compulsive disorder (Fineberg *et al.*, 2012) and even for treatment of bulimia (Walsh *et al.*, 1997). The main superfamily of antidepressants is serotonin reuptake inhibitors (SRI). SRI therapeutic effect is mediated by blocking serotonin reuptake on neuron terminals and thereby increases the level of serotonin on synapses. According to their other targets (apart from serotonergic function) SRI are divided into several subgroups. The best-known group are selective serotonin reuptake inhibitors (SSRI) which selectively inhibits reuptake of serotonin by serotonin transporter (SERT). Older, less selective, group are tricyclic antidepressants (TCA). These, apart from inhibiting SERT also inhibits reuptake of noradrenaline. Other, similar class, of SRI are serotonin and noradrenaline reuptake inhibitors (SNRI). In all above mentioned classes of SRIs there are many minority affected systems such as acetylcholinergic, histaminergic or adrenergic systems. Indeed, types of antidepressants differ mainly in affinities to these other neurotransmitter systems.

Mechanism of SSRI action has acute and chronic effect. Acute binding of molecule of SSRI decreases transporter affinity for serotonin because of negative allosteric modulation of SSRI binding site and therefore increases extracellular concentration of serotonin (Stahl, 1998b). This increased level of extracellular serotonin stimulates 5HT1A and 5HT2A autoreceptors and negative feedback causes slowing firing rate of 5HT projections.

If antidepressants are applied chronically, autoreceptors become desensitized and therefore both 5HT release and level of 5HT in target structures is increased (Stahl, 1998b). One of these structures is orbitofrontal cortex (Mansari *et al.*, 1995). Desensitization of autoreceptors is a proposed mechanism of action of SSRIs and also can account for delay in effect of treatment. Onset of action of SSRI is delayed in terms of several weeks after first administration of SSRIs (Stahl, 1998a).

2.2.1.3.1 Antidepressive clomipramine in treatment of OCD

Even if we are not sure about cause of pathophysiology of OCD and neither about precise role of serotonin, it is clear that antidepressive drugs have positive effect on OCD symptoms at least for 60 % of patients (Skoog and Skoog, 1999; Pallanti *et al.*, 2002). Pharmacological treatment is primarily based on antidepressants administration. Using of SSRIs and tricyclic antidepressants is common because their efficacy has been established in placebo-controlled studies (Fineberg and Gale, 2005; Fineberg *et al.*, 2012). Clomipramine has a special place in treatment of OCD since it was a first drug discovered to treat it. Clomipramine is 3-chlorinated

derivative of other known tricyclic antidepressant imipramine (*Figure 4*). After taking clomipramine orally it is well absorbed from gastrointestinal tract converts to pharmacologically active dimethyl-clomipramine (Stern *et al.*, 1980). Apart from treating of OCD, clomipramine is effective in treating major depressive disorder, panic disorder, catalepsy, chronic pain and others. The selective target of clomipramine action is serotonin reuptake but it also acts as antagonist of histamine H1 receptor, muscarinic acetylcholine or α1 adrenergic receptors (Raisman *et al.*, 1979). These antagonistic effects are blamed for some unwanted side effects such as sedation and nausea. Compared to clomipramine, SSRIs exhibit lower rate of side effects and therefore the SSRIs are currently a first line treatment of OCD. Clomipramine is only elected in patients who failed to respond SSRIs (Mundo *et al.*, 2000; Fineberg *et al.*, 2012).

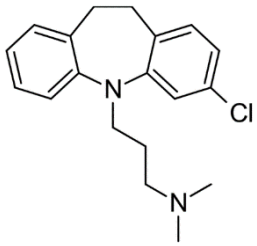


Figure 4: Schematic structure of clomipramine or in systematic name 3-(3-chloro-10,11-dihydro-5H-dibenzoazepin-5-yl)-N,N-dimethylpropan-1-amine

Precisely because of differences in specificity to more than one neurotransmitter systems it was not known what neurotransmitter system is responsible for therapeutic effect of SSRI. Ineffectiveness of desipramine, which acts only as an inhibitor presynaptic noradrenergic reuptake indicates that anti-obsessional effect, is dependent on increasing 5HT levels (Hoehn-Saric *et al.*, 2000). Different serotonin selective reuptake inhibitors were examined with regard to efficiency and tolerability in OCD patients, but no differences were detected (Mundo *et al.*, 2000; Bergeron *et al.*, 2002).

2.2.2 Dopamine

Neurotransmitter dopamine, 4-(2-aminoethyl) benzene-1,2-diol, is a catecholamine which plays an important role in the body and brain circuits. In the body dopamine modulates vasodilatation, secretion of ions and hormones but it also plays role in immune system (Iversen and Iversen, 2007). Best known function of dopamine as a neurotransmitter in the brain is in reward and motivation system. During reward dopamine levels are increased in reward circuit, similar to addictive drugs which activate the same reward system in brain (Di Chiara and Bassareo, 2007). Additionally, dopamine is an important modulator of motor control and inhibitor of prolactin release (Iversen and Iversen, 2007).

The synthesis of dopamine starts by hydroxylation of tyrosine by tyrosine hydroxylase to L-DOPA. Last step of synthesis creates dopamine using amino acid decarboxylase. After dopamine action at synapses it is transferred back into neuron terminals through the dopamine transporters and degraded. Degradation efficiency is dependent on activity of MAO, aldehyde dehydrogenase (ALDH) and COMT (Feldman *et al.*, 1997).

There are four dopamine pathway in mammalian brain. One pathway starts from substantia nigra, two from ventral tegmental are (VTA) and last starts in hypothalamus. Nigro-striatal pathway starts in substantia nigra pars compacta in midbrain and projects to dorsal striatum, to caudate nucleus and to putamen. Nigrostriatal pathway plays a role in motor control in basal ganglia. Disruption of dopaminergic signalization in this pathway causes Parkinsonism (Girault and Greengard, 2004). The second dopaminergic mesocortical pathway projects from VTA in midbrain to frontal cortex. It appears that this pathway plays a role in learning and memory (Feldman *et al.*, 1997). Mesolimbic pathway also starts in ventral tegmental area but its projections head to limbic system: mainly to bulbus olfactorius, ventral striatum and nucleus accumbens. This is the circuit of reward and motivation (Di Chiara and Bassareo, 2007). The last short tuberoinfundibular projection supplies dopamine from infundibular nucleus in hypothalamus to pituitary gland and modulates prolactin secretion (Iversen and Iversen, 2007).

The cells respond to dopamine signalization only if they carry dopamine membrane receptors. The five dopaminergic receptors were characterized and, based on similarities, divided into two subgroups - D1 and D5 belong to D1-like receptors and D2, D3 and D4 belong to D2-like receptors. All of these receptors are G protein–coupled receptors with a different effect on adenylyl cyclase activity during dopaminergic signalization. Activation of D1- like receptors stimulates cAMP production. D1-like receptors are preferentially localized postsynaptically in striatum. D2-like receptors inhibit adenylate cyclase activity and decrease level of cAMP in target cells. These receptors are expressed both postsynaptically as well as presynaptically on dopaminergic neurons (Sokoloff *et al.*, 2006; Rankin *et al.*, 2010; Rondou *et al.*, 2010).

2.2.2.1 Dopamine in cortico-striatal circuits and OCD

The role of dopaminergic system in pathophysiology obsessive-compulsive disorder is still unclear as is the precise role of serotonin. Dopamine theory of OCD was first postulated by Goodman (Goodman *et al.*, 1990) who proposed that serotonergic deficiency may result in increased dopaminergic tone which in turn could lead to downregulation of dopamine receptors. Eventually, dopamine involvement in OCD was supported by findings on human subjects. Yet, exact mechanism of dopamine dysregulation, and its interaction with serotonin system in OCD is not elucidated to this date.

The role of dopamine in OCD pathophysiology is supported by evidence from different studies. It was observed that administration of dopamine agonists may cause exacerbation of stereotypes and OC symptoms and conversely dopamine antagonists may reduce these symptoms in OCD patients (Goodman *et al.*, 1990). These findings are also supported by facts that antidepressive drugs augmented with dopamine blockers are more effective in OCD

treatment than antidepressants alone (Mcdougle *et al.*, 1990). Experimental findings support dopamine involvement in OCD. Increased binding of radioligand to DAT was shown (Kim *et al.*, 2003; Wee, 2005 but see Hesse *et al.*, 2005), possibly indicating increase in dopamine tone (Jaber *et al.*, 1997). Denys shown decreased D2/D3 receptors availability in OCD patients which can be result of hyperdopaminergic state and competition of dopamine and radiotracer in studies (Denys *et al.*, 2004, 2013). Genetic studies showed that OCD was associated with low activity of catechol-O-methyl transferase allele and A2 allele of D2 receptor Taq1 A gene (Denys *et al.*, 2006). Despite these studies indicating hyperdopaminergic state, biochemical studies suggest normal dopamine tone in OCD patients (Hollander *et al.*, 1992)

Dopaminergic system is closely linked to cortico-striatal circuits discussed in the first chapter. Localization of dopaminergic receptors on striatal structures is not homogeneous, D1 receptors are preferentially located on striatal neurons belonging to direct pathway and D2 receptors are located on neurons belonging to indirect pathway. When dopamine is released, D1 receptors activate direct pathway, and target cortical structures are activated. Activating D2 receptors inhibits inhibiting indirect pathway, resulting in same overall activation of target cortical structure (Gerfen and Surmeier, 2012). To summarise, the overall effect of dopamine in cortico-striatal circuits is facilitating.

2.2.2.2 Antipsychotic drugs

Antipsychotic drugs are most used to attenuate psychotic states of patients suffering from schizophrenia or bipolar disorder. It is common that antipsychotics in combination with antidepressants are used to treat also other psychiatric disorders including depression. Antipsychotics are dopamine-serotonin antagonists. Moreover, antipsychotics bind histamine, muscarinic acetylcholinergic or adrenergic receptors (Miyamoto *et al.*, 2012). Antipsychotic effect is apparently mediated by combination of serotonin and dopamine receptor blockade, because serotonin nor dopamine receptor antagonists alone do not have beneficial treatment effect (Kapur and Seeman, 2001).

Antipsychotics can be divided into typical and atypical antipsychotics or, in other words, into first- and second-generation antipsychotics. These two types differ in several aspects including chemical structure and pharmacological effects. The most expressive difference between them is a higher efficiency and lower level of extrapyramidal side-effect symptoms (EPS) followed by atypical compared to typical antipsychotic administration (Hippius, 1999). The term ‘extrapyramidal’ pertains to the motor pathway which includes basal ganglia and nigrostriatal dopamine system, too. EPS are composed of acute and tardive part. Acute

syndromes are dystonia, akathisia and Parkinsonism and those that occur after prolonged treatment are tardive, and include dyskinesia a tardive dystonia (Pierre, 2005).

We do not know the exact cause of the difference in efficiencies between these two groups of antipsychotics. Some researchers suggested dissimilarities in potency of binding or in location of the action as a cause. Potency of typical antipsychotics at 5-HT2 and D2 receptors antagonism is equal whereas atypical antipsychotics block 5-HT2 receptors with much higher potency than D2 receptors. Additionally, atypical antipsychotics bind D2 receptors more specifically in mesolimbic than nigrostriatal system (Meltzner *et al.*, 1989). This difference in D2 binding can be at the root of higher level extrapyramidal side-effects first-generation antipsychotics administration, which bind D2 receptors equally in striatum and in mesolimbic system (Stockmeier *et al.*, 1993).

Moreover, both, the efficiency and level of extrapyramidal symptoms, are dose-dependent. According to application dose of drug there is different rate of serotonin and dopamine receptors occupancy. PET (positron emission tomography) clinical studies of schizophrenic patients shown that clinical response to classic neuroleptics, in common clinical doses, is associated with roughly 70% occupancy of the D2 dopamine receptors an level where EPS emerged, in contrast with atypical neuroleptic administration, in which roughly 50% occupancy of D2 receptors together with 40% occupancy of D1 receptors were observed with a much lower incidence of EPS (Farde *et al.*, 1992). Similar results were demonstrated in animal model (Wadenberg, 1993).

Risperidone (64 766) is one of the second-generation antipsychotic drugs (*Figure 5)* (Leysen, 1988) which has relatively low EPS potencial (Gerlach and Peacock, 1995). Similarly to other atypical antipsychotics, risperidone mainly acts as antagonist of 5-HT2 and D2 receptors while showing the higher binding affinity for serotonin receptors. None the less, its D2 affinity is relatively high (in comparison with other second-generation drugs) but still is 20-times lower than 5-HT2 affinity (Leysen, 1988). Except these two binding targets it was observed that risperidone may also block histaminergic H1 and adrenergic α2 receptors (Janssen *et al.*, 1988).

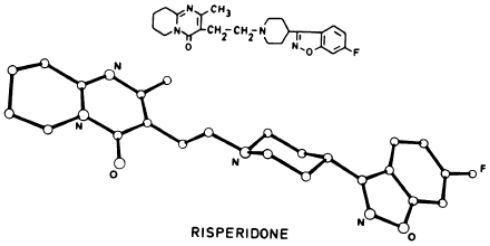


Figure 5. Chemical structure and perspective drawing of the X-ray structure of risperidone (R 64 766), or 3-{2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl}-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one (Janssen *et al.*, 1988)

2.2.2.2.1 Antipsychotic risperidone in treatment of OCD

There are numerous clinical studies which compare efficiency of classic SSRI treatment supplemented by individual antipsychotic drugs in OCD. Although not univocal, positive effect of this treatment method is observed in OCD patients (reviewed in Bloch *et al.*, 2006). Discrepancies arose probably due to the fact that not every antipsychotic drug is effective in OCD treatment. The most significant improvement was showed in studies using risperidone and haloperidol as augmentation therapy to SSRI treatment (Kawahara *et al.*, 2000; Skapinakis *et al.*, 2007). Results of studies testing this new treatment are complicated by the fact that these studies work with patients, who did not respond to classical SSRIs or clomipramine administration, and therefore are not representative of all OCD patients (Ravizza *et al.*, 1996; Hollander and Rossi, 2003).

Interestingly, there is another effect of risperidone application. Administration of risperidone to patients, with different diagnosis than OCD, may cause appearance of previously absent OCD symptoms (Alevizos *et al.*, 2002; Lykouras *et al.*, 2003). It was suggested that this contradictory effect of administration of risperidone can be dependent on high inhibition of dopamine transport causing serotonin-dopamine imbalance (Duggal, 2003).

2.2.3 Glutamate

The glutamic acid belongs to non-essential amino acids. Glutamate, a salt of glutamic acid, is the main excitatory neurotransmitter in a brain acting through both ionotropic and metabotropic receptors. Thanks to activation of ionotropic AMPA and NMDA receptors glutamate is important for synaptic plasticity mechanism during memory formation and many other functions (Erecinska and Silver, 1990).

2.2.3.1 Glutamate in OCD

There is mounting evidence that hyperactivity in glutamatergic system is involved in pathophysiology of OCD. The first suggestive findings were elevated glutamate levels in cerebrospinal fluid (CSF) and overall hyperactivity in system (Chakrabarty *et al.*, 2005). Further studies were focused on abnormalities in glutamate related genes in OCD. There are a lot of genes that can affect function of glutamatergic system, whose deletion or expression inhibition causes compulsive behaviour in animals. For example SLC1A1, mentioned in first chapter, is often studied gene for glutamate transporter called excitatory amino acid carrier 1 (EAAC1) (Dickel *et al.*, 2006). Other research focused on gene variations in GRIN2B, which encodes a

subunit 2B of glutamate N-methyl-D-aspartate receptor (NMDA). A significant association between OCD and polymorphisms was found in the 3′ untranslated region of GRIN2B. This pioneering result needs to be confirmed in a larger sample (Arnold *et al.*, 2004). In animal studies the other glutamate related gene considered to be involved in OCD codes for a very familiar scaffolding protein SAPAP3. Deletion of this gene causes silencing of glutamate transmission via AMPA receptors (Wan *et al.*, 2013) and increases anxiety and compulsive self-grooming in rats (Welch *et al.*, 2008).

Important parts of research of glutamate role in OCD are clinical studies using riluzole, which reduces glutamate transmission (Wang *et al.*, 2004). This drug is often used in treatment of amyotrophic lateral sclerosis, but its positive effect was observed also in OCD patients (Coric *et al.*, 2005; Pittenger *et al.*, 2008). Although riluzole causes improvements in only about half of patients, these findings are very helpful, because patients in these studies were mostly resistant to classic methods of treatment.

2.2.4 Other less studied modulators

Thanks to complexity of brain circuits and interactions between specific neurotransmitter and neuromodulator systems it is not surprising that OCD research also focuses on less extensive neurotransmitter systems. For example it was found that levels of arginine vasopressin (Altemus *et al.*, 1992) and oxytocin (Leckman *et al.*, 1994) are elevated in CSF of OCD patients. Moreover, there are studies which are focused on involvement of parvalbumin, prolactin, opioids or steroids in OCD pathophysiology (Burguiere *et al.*, 2014). Unfortunately, scarcity of studies focused on these substances makes these results far from conclusive.

Today, research of neurotransmitter system is essential to our understanding of OCD pathophysiology. These is a strong evidence of abnormal functioning of dopaminergic, serotonergic and glutamatergic systems in OCD. Understanding these abnormalities may help to understand changes accompanying abnormalities in brain structures, brain connectivity and clarify an exact role of cortical-striatal circuits in OCD. It was proposed that symptom manifestation vary depending on which brain structures within cortico-striatal circuit are altered. Many of these brain structures are also important to overall cognitive functioning. Therefore it is possible that, apart from OCD symptom, alteration within certain region can also manifest as damage to cognitive function. Indeed, cognitive impairment in OCD is a subject of numerous studies for many years, but so far these studies presents only mixed results.

2.3 Cognitive function

Research of OCD symptoms is focused on pathology of the disorder as well as on detailed cognitive state of patients. There are many cognitive functions studied in patients with OCD in comparison with healthy controls. Rigidity and inflexibility describes pathology of obsessive and compulsive behaviour which prompted researchers to question if these descriptions can also be extended into cognitive domain. Many cognitive functions were tested in OCD patients with focus on decreased cognitive flexibility. Nowadays there is tendency to develop tests usable for animal as well as for human subjects, to ensure that the results are interspecies comparable.

The neuroanatomy section described anatomical knowledge of OCD which supports the inclusion of the frontal-striatal circuits in OCD pathology (see 2.1.2 Neuroanatomy of OCD). Many of these structures are involved in behavioural flexibility – changing of behaviour based on changing environment. Today, studies focus mainly on testing cognitive flexibility by reversal learning and two types of set shifting. Other, less often used, cognitive flexibility tests include conflict monitoring and error detection, motor response inhibition, decision making, reinforcement learning by reward and avoidance of risky choices. Cognitive flexibility in many different forms was associated with OCD symptoms.

2.3.1 Cognitive flexibility

Cognitive flexibility is ability of all successfully living organisms including humans. It represents the ability to inhibit behavioural or mental response, which is no longer optimal in the current situation, and to use other alternative behaviour. Decision to change a strategy is dependent on dopaminergic system, which controls the behaviour through reward or punishment. It allows responding to new situations and even entirely new environments. Thanks to cognitive flexibility, we can adapt our behaviour according to changes in environment, imminent danger or rewarding outcomes.

2.3.1.1 Testing of cognitive flexibility

Cognitive flexibility is tested in three different variations of discrimination tasks (*Figure 6*), in which the selection one option from several others is controlled by motivation by reward or punishment. These tasks include reversal learning, intra-dimensional set shifting (ID) and extra-dimensional set shifting (ED). The correct solving of these discriminative tasks is dependent on more capabilities than just flexibility and each of task tests different aspect of cognitive flexibility: reversal learning task is focused on ability to alter specific stimulus-reward

association, ID tests the ability to maintain a set of rules and thus successfully shift performance to novel set of rules of the same dimension and ED is based on ability to alter set of rules and decide based on completely new rules in a different dimension (Lawrence *et al.*, 1998).

In each of these three tasks, there are two stimuli displayed and a patient's task is to choose one of them. The initial rue, after certain sequence of correct answers, is changed without notice of the patient. The patient has to detect following change and the new relevant rule using only "right" or "wrong" feedback. Difference between these tasks is in ambitiousness of degree to which the switch of the rule occurs. In reversal learning, patient needs to switch response to the second option, which was already presented. In ID shift patient has to select between two new stimuli but preserve one dimension (one specific parameter of the stimulus: shape, colour, number), or during ED shift patient needs to change the rule across the dimensions (Bissonette *et al.*, 2014) *(Figure 6).*

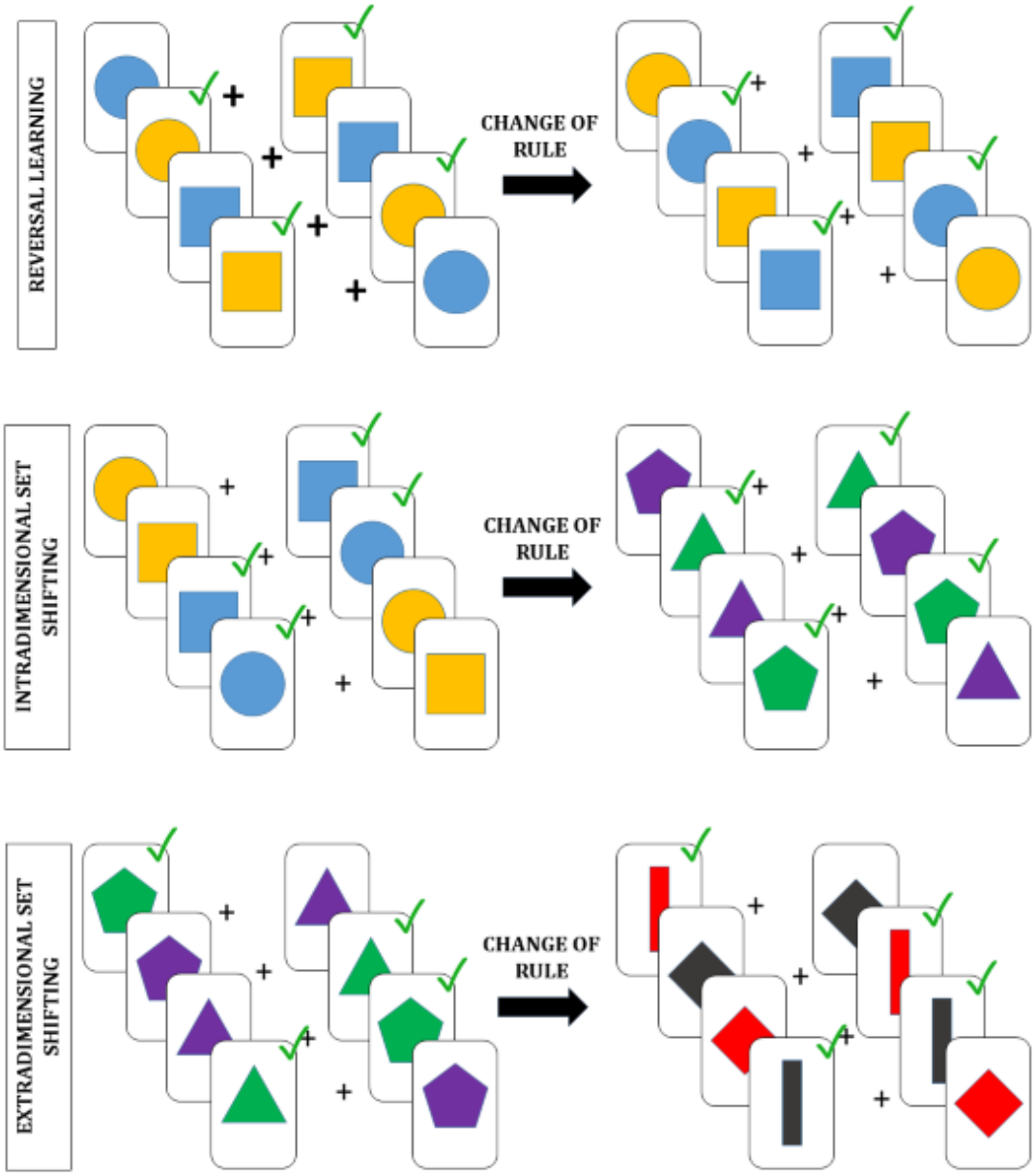


Figure 6: Schematic representation of different types of set shifting task between colour and shape.
✓ represents correct response. One pair of cards represents two options of choices in one trial.
In initial acquisition of discrimination is correct dimension colour (yellow), not shape of objects. The reversal task reverses previously correct stimuli to second stimulus included in the same stimulus dimension of colour, the correct answer is newly blue colour independently on shape of stimuli. Intradimensional set shifting task submit completely new exemplars, but the rule of colour is preserved and green colour is newly correct answer. Extradimensional set shifting task also presents completely new exemplars but at the same time rule changes from previously correct colour to newly correct shape dimension and „stick" is the right choice regardless of its colour.

Reversal learning tasks test a basic form of cognitive flexibility. During acquisition stage the subject has a task of selecting one of the two presented stimuli to acquire reward. For example blue colour is the right choice and therefore the subject should choose blue circle when the second option is yellow circle (or square). The second presented dimension - shape - is irrelevant. Reversal stage itself starts by switching of the rewarded stimulus to the second, previously unrewarded, stimulus - yellow colour. Previously rewarded blue colour becomes irrelevant similarly to the always irrelevant shape dimension (Rolls, 2004; Bissonette *et al.*, 2014).

The second form of discrimination tasks is the intradimensional set shifting (ID) or in other words also affective set shifting. ID shift is similar to reversal learning in some aspects. In this task subject learns that one of the two options is the correct response (for example, one of two colours) and second dimension (shape) is irrelevant just as in reversal task. But the second phase of test is indicated by a changing to different options in both dimensions, although still one of the colours remains a relevant factor. This means that blue and yellow stimuli are replaced by red and green different shapes, shapes are still irrelevant and subject would only have to identify which colour is rewarded. ID is concerned with changing rewarded stimulus within one stimulus dimension (Rolls, 2004; Bissonette *et al.*, 2014).

Much more different is extradimensional set shifting (ED) or attentional set shifting task. Similarly to ID shift following a successful acquisition stage completely new stimuli are presented to testing subject. But the difference is that subject attention has to focus on the second stimulus dimension (for example shape) and be diverted from the previously relevant dimension (colour). Therefore, new stimuli are presented but also it is necessary to change discrimination rule and to learn and choose different stimulus dimension. So in our example, colour is irrelevant from now on and shape of stimulus object is important. The attention to formerly relevant dimension has to be inhibited and again focused on a new previously irrelevant stimulus dimension, which is from now important to correct solution (Rolls, 2004; Bissonette *et al.*, 2014).

ID and ED set shifting are often tested together as IDED set shifting task and in human psychiatric diagnostics IDED set shifting task is part of the Cambridge Neuropsychological Test Automated Battery (CANTAB)(Sahakian and Owen, 1992; Robbins *et al.*, 1994). These two different forms of set shifting were developed from well-known Wisconsin Card Sorting Test (WCST) (Robinson *et al.*, 1980), which was inherently test of extradimensional set shifting. WCST is still widely used test today. In WCST there are 60 cards in pack, each card depicting specific number (one – four) of one of the four symbols (triangle, circle, star and cross) in one of the four colour (yellow, green, blue and red). None of cards are identical. Four stimulus cards are placed before patient on the table. The patients have to place cards from pack one by one under

specific stimulus cards and according to examiner responses („right“ or „wrong“) the patient has to deduce the correct rule – sorting by either colour, count or shape. When the patient places ten cards correctly, the examiner changes the sorting rule. The patient recognizes this shift only based on changed examiner´s responses (Lezak, 2004).

2.3.1.1.1 Analogical testing of cognitive flexibility in animals

Previously mentioned reversal learning and two forms of the set shifting tasks were modified also for monkeys (Dias *et al.*, 1996), rats (Birrell and Brown, 2000) and mice (Garner *et al.*, 2006). Similarly to human tests, animal is required to shift an initially learned rule. Challenging is that these tasks must be adapted to testing animal cognitive flexibility without a verbal guidance. Animals are motivated during testing by a of food reward or, in some cases, by avoidance of punishment. Testing designs are adapted based on animal species tested, in rodents it is studied the ability of association of olfactory and texture stimuli (McAlonan and Brown, 2003) or easily dissociable visible cues (Chudasama and Robbins, 2003) but in primates tests can be adapted to their advanced visual and spatial systems (Clarke *et al.*, 2005).

In the common reversal task for rodents, animal is trained to dig for a food reward in specific material according to previously learned association with odour or texture of bowl-covering (McAlonan and Brown, 2003). Boulougouris *and colleagues* (2009) introduced a reversal learning task for rodents as an operant conditioning in a chamber with two levers, in which animal gained the sucrose pellets as a reward if animal pressed one lever three times. More difficult rat reversal training tasks were also developed. For example, visual discrimination in chambers with touch screen was successfully used in rats (Chudasama and Robbins, 2003). More cognitively demanding for rats is reversal learning phase of active place avoidance task. In this task animal must learn to avoid certain sector in rotating arena to avoid a mild electric shock and during reversal learning, this location is rotated 180 degrees (Lobellova *et al.*, 2013; Hatalova *et al.*, 2014).

Animal testing of ID and ED are very similar to previously mentioned testing of reversal learning. In rodents, many tasks are based on association of odour with specific material on the mat that indicates a location of reward (Birrell and Brown, 2000; Garner *et al.*, 2006). Primates are tested using touch screen and they must learn to discriminate only between visual stimuli (Walton *et al.*, 2010). ID and ED tasks often follows reversal phase and all of these three stages are tested together in IDED testing battery manner.

2.3.1.2 Neuroanatomy of cognitive flexibility

Studies focused on basis of cognitive flexibility found several structures allegedly involved in different types of this cognitive ability. Number of these structures is discussed in connection with brain circuits, which are considered to be an anatomical background of OCD. These include OFC, basolateral amygdala and striatum.

Orbitofrontal cortex is most often associated with cognitive flexibility. Involvement of OFC was studied in humans (Hornak *et al.*, 2004), monkeys (Clarke *et al.*, 2008) as well as rodents (McAlonan and Brown, 2003) and results show direct connection between functional OFC and successful reversal learning. Except OFC also involvement of striatum is also discussed as a possible important element in reversal learning. Lesion of medial striatum as well as OFC in marmosets caused perseverative behaviour in primates and in rats (Clarke *et al.*, 2008; Ghahremani *et al.*, 2010). Lesion of cholinergic interneurons in striatum affects ED set shifting, while does not affect reversal learning (Aoki *et al.*, 2015) Also, there is some evidence that lesion of basolateral amygdala disrupts reversal learning (Schoenbaum *et al.*, 2003).

2.3.1.3 Cognitive flexibility in OCD patients

There are many overlaps between brain structures involved in mediation of cognitive flexibility and those in cortico-striatal circuits involved in pathophysiology of OCD. Indeed, repetitive and perseverative acts and invariable strict rules suggest involvement of dysfunction of cognitive flexibility in OCD pathophysiology. Even if the results are not convincing for all of tests, results suggest abnormalities in cognitive flexibility in OCD patients. It appears that during reversal testing, patients with OCD show longer delay to response, and abnormal activation of OFC. Deficits in OCD patients were observed also during ED and ID set shifting tasks.

OCD patients did not show any performance deficit compared with healthy controls when subjects were alerted to changing rules (Fenger *et al.*, 2005) as well as in tests without notice to changing rules (Remijnse *et al.*, 2006). However, both of these studies found out a longer response delays in patients compared to controls. Patients probably need more processing time to orient themselves to new situation after rule change. Although the results did not show the behavioural difference in solving tasks, observed changed recruitment of orbitofronto-striatal circuit points to change in cognitive processing of these tests (Remijnse *et al.*, 2006).

Chamberlain and col. (Chamberlain *et al.*, 2006) found out impaired cognitive flexibility at a time when was necessary to shift attentional focus on newly relevant stimulus dimension in extradimensional shift task. The same stage of test was critical for OCD patient also in other studies (Okasha *et al.*, 2000; Watkins *et al.*, 2005; Tükel *et al.*, 2012). Another study found

impairment of cognitive flexibility only in ID stage (Veale *et al.*, 1996). Nevertheless although set shifting deficits were found in many cases, other studies were unable to find differences between OCD patients and heathy controls in cognitive flexibility requiring tests (Abbruzzese *et al.*, 1995; Purcell *et al.*, 1998; Henry, 2006).

Cognitive flexibility complex and its research is not simple, but there are many options to test it, which, on the other hand, does not allow for comparability of the findings. Also, reversal tasks may be too simple in comparison with animal test analogues. Simplicity of reversal learning is sometimes criticized for their low ability to detect reversal deficits in patients (Hatalova *et al.*, 2014).

2.3.2 Response inhibition

Another form of flexible behaviour is an ability to stop previously automated action. This ability is also a part of reversal and other learning tasks in which it is important to withhold previously learned response to correctly solve the task.

Logan and col. introduced the model of the reaction time task, where the stop signal presented. In this task, subjects respond to visually presented letters by pressing one of two buttons. When the infrequent stop signal is presented, subjects should stop pressing the button (Logan *et al.*, 1984). This stop-signal task explores the development of inhibitory response. Time required to stop an action and structures involved in this cognitive mechanism are most common output variables. Based on similarities with inhibition during discriminative tasks, there were studies looking for brain activity in the same structures as during reversal and set shifting tasks. However, Ghrahremani and colleagues (2010) suggested that inhibitory reaction is a distinct brain processes. The main role was ascribed to right inferior frontal cortex (Aron *et al.*, 2003; Buchsbaum *et al.*, 2005). Involvement of other structures including temporal and parietal cortices, anterior cingulate cortex, basal ganglia and cerebellum was also detected (Godefroy *et al.*, 1996; Rubia *et al.*, 2001; Horn *et al.*, 2003; Aron *et al.*, 2004).

Disruption in response inhibition can manifest as susceptibility to motor or mental repetition or perseveration, which are words also describing symptoms of obsessive-compulsive disorder (Chamberlain *et al.*, 2005). Testing of OCD patients again demonstrated slower response time, but the total number of errors did not differ from healthy controls (Watkins *et al.*, 2005; Chamberlain *et al.*, 2006).

2.3.3 Conflict monitoring and error detection

Error detection and conflict monitoring play important role in fear of ours own mistakes and awareness of ours errors, which may affect our lives. Ability to realize one's own mistake make these two processes closely related to response inhibition. It was proposed, that in OCD this fear of error is a driving force of obsessive thoughts (Pitman, 1987).

Testing error detection is based on conflict between automatic reaction and reaction required to successfully solve a task. The Stroop task is the best known example for assessing this capability. In this test there are words for colour names written in different colour fonts (for example the word "red" written in green font). The name of word is dominant response, but the task requires saying aloud a colour of the word font (Deckersbach *et al.*, 2000). This ability is dependent on dorsal medial frontal cortex including anterior cingulate and associated motor cortices (Tükel *et al.*, 2012).

Overactive error detection and conflict monitoring, were associated with OCD pathology because of often reported feeling ¨something is wrong¨ by patients (Abbruzzese *et al.*, 1997). Compulsions are than visible consequences of such concerns, patient's attempts to reduce conflict signals or to correct presumed errors. Indeed, the OCD patients have increased response times when solving Stroop test than healthy controls (Tükel *et al.*, 2012; Stern and Taylor, 2014).

Previously mentioned cognitive functions are not the only ones which are studied in OCD patients. OCD patients were also tested on cognitive capabilities such as attention, planning and decision making and working and long term memory. Specific tasks are used alone or within testing batteries. Results of these studies are not often conclusive. For example, some studies observed deficit in form of slower responses in attention in OCD patients (Jurado *et al.*, 2001; Tükel *et al.*, 2012) but other did not (Moritz *et al.*, 2002). However, it is possible that slower performance during attention tasks dependent on current treatment with SRIs (Moritz *et al.*, 2002). Another common part of cognitive testing of patients is testing of planning using Tower of London and Tower of Hanoi tasks. Most of studies did not showed any significant differences in planning between OCD patients and controls in classic parameters except longer latencies to make a choice (Veale *et al.*, 1996; Rowe *et al.*, 2001; Bohne *et al.*, 2005; Cavedini, 2009). Memory, both long term and short term, is a very important cognitive function. The ability to store new memory seems to be unaffected but several studies found impairment in recall (Deckersbach *et al.*, 2000; Kim *et al.*, 2002). Observed memory deficit could have been caused by difficulties in the use of organizational strategies when patients are focused on irrelevant details during testing (Deckersbach *et al.*, 2000). Although OCD patients appear to be impaired in many cognitive domains, results remain at this time very inconclusive, sometimes even mutually exclusive.

In conclusion, recurrent result from many different cognitive tests suggests slower response times of OCD patients. This may be caused by anxiety of patients not to make a mistake (Stern and Taylor, 2014), or because task is in fact more demanding for patients than for control subjects. It is possible, that to observe actual mistakes in OCD patients in these tasks, they have to be more cognitively demanding, or time constrained. Testing of cognitive may be affected by many factors. Contradictions and conflicting results between studies can be influenced for example by different manipulation of neurotransmitter system by having patients treated with different antidepressants or antipsychotics. It was found that intervention in serotonergic system may change cognitive functions (Meneses, 1999). Moreover, findings can be affected by different comorbidities. For example it was found that comorbid depression in OCD patients contributes to cognitive deficit (Moritz *et al.*, 2001). Some cognitive and other symptoms or research of neuronal background of disorders are not possible to study in patients, due to aforementioned variability in patients or, importantly, ethical viewpoint. This research area reaps benefits from animal studies. In such cases valid animal model is very advantageous for scientific research.

2.4 Animal model of obsessive-compulsive disorder

Animal models of psychiatric disorders are a very useful tool to study many facets of neuropsychiatric diseases. Novel treatments, hypotheses about causes and neurological substrates all can be tested more readily in an animal model. Apart from the impossibility of sacrificing human subjects during the research if needed, the main advantage of animal models lies in sheer numbers of animals with no comorbidities - or at least with homologous ones. Disadvantage lies in a fact that many aspects of mental disease occur only in the mind - which becomes tricky when it comes to looking into thinking of a rat. What is left is an inference of a mental state of an animal from its behavior.

Animal model usually does not mimic real disease in all of its aspects. Aspects of a disease which animal model mimics were characterized by Willner in 1986. He called them validities - set of criteria in which animal model is similar or same to a real disease. These can be divided into three categories: face, construct and predictive (Willner, 1986). Face validity corresponds to observable behavioral homologies; construct validity corresponds to involvement of the same brain structures and neurotransmitter systems and includes similarity in cognitive deficits (Albelda and Joel, 2012); lastly, predictive validity corresponds to predictability of the treatment outcomes. To this date this division is useful in describing an animal model although some refinements of these criteria were presented as well (Belzung and Lemoine, 2011).

2.4.1 Quinpirole induced compulsive checking

Animal models developed for examination of obsessive-compulsive symptoms are induced by genetic changes, behavioral manipulation, intervention in neonatal development or pharmacological administration of drugs. All animal models of OCD have in common manifestation of wide range of stereotypical behaviors by animals. These behaviors are regarded as analogues of human compulsions. Unfortunately, second equally important aspect of disease, obsessive thoughts, cannot be observed in any animal model. One of pharmacological animal models of obsessive-compulsive disorder - quinpirole induced animal model of compulsive checking - induces behavior that is most similar to human compulsive checking rituals.

Administration of quinpirole, the agonist of dopaminergic D2 and D3 receptors was observed to model of OC symptoms in 1998 by research group of Prof. Szechtman (Szechtman *et al.*, 1998). Uniqueness of this animal model lies in modeling one concrete form of OCD symptom - obsessive checking. Since it is challenging to directly compare human and animal behavior, set of ethological criteria of compulsive behavior was devised by Prof. Szechtman. These criteria can describe both animal and human behaviour in ethological terms: First, there is one or two

particular (key) places/objects to which the subject returns excessively more often than to other places/objects in the subject's living place. Second, these particular places/objects are visited more often than to others. Third, limited count of places is visited in between returns to the key places/objects. Fourth, a characteristic set of acts is performed at the particular place/object. Fifthly, set of acts is dependent on particular places/objects and it is changed when the environmental properties of the places/objects are altered (Szechtman *et al.*, 1998). All these five criteria were shown to be true for both OCD patients and animals sensitized with QNP.

When quinpirole sensitized animal is repeatedly placed in an open field with several (usually four) objects placed at fixed locations, it quickly pays attention to only selected few (usually two) objects and a "home base", a corner where animal returns most often (Szechtman *et al.*, 1998)(See *figure 7* acquired from personal communication). Animal visits comparatively smaller number of available objects more often, analogous to a patient who during checking focuses on checking mostly objects of his obsession. Similarly, patient can withhold checking behavior, but eventually will resume checking when his/her willpower wanes. If home cage is placed in the open field arena where animal conducts its checking behavior, it remains in the home cage for a while before coming out to resume checking activities again (Zor *et al.*, 2011). Environmental dependence can be readily observed in patients where they can withhold compulsive behavior in environment outside their usual checking environment (e.g. home).

Figure 7: Open-field table used in original study of animal model quinpirole-induced compulsive checking. Movement of rats between four different objects on the table is recorded by camera (required from personal communication with Prof. Henry Szechtmann)

2.4.1.1 Validity of quinpirole sensitization as an animal model of OCD

The quinpirole model of OCD has high face validity, predictive validity and some constructive validity. High face validity is due to the above mentioned striking resemblance between behaviour of animal sensitized by quinpirole and of patients suffering from. Additionally, OCD behaviour is often described as perseverative (Yadin *et al.*, 1991). This type of behaviour was observed in spontaneous alteration in T maze (a natural tendency of animal to enter a different arm of a maze on a next trial) where quinpirole sensitized animals alternated much less than control animals (Einat and Szechtman, 1995).

Animals sensitized by quinpirole are also similar in their treatment response to OCD patients: hence have a good predictive validity. First of all, quinpirole checking is attenuated when clomipramine is coadministered with quinpirole (Szechtman *et al.*, 1998). Clomipramine treatment is effective in only about 50 % patients (Leonard *et al.*, 1989), which may mean that quinpirole compulsive checking models only specific form of disorder sensitive to clomipramine.

Additionally, clomipramine is also effective in preventing another type of OCD related behaviour in quinpirole sensitized rats – contrafreeloading (De Carolis *et al.*, 2011). This term describes a natural phenomenon where animal chooses to work for a reward instead of choosing a freely available source (Jensen, 1963; Koffer *et al.*, 1971). In an excess, contra-freeloading is considered as a manifestation of compulsive behaviour (De Carolis *et al.*, 2011). In this paradigm water deprived animals are at first trained to press the lever to receive a small amount of water. This part is called operant conditioning phase. This phase is followed by a choice phase where animals are offered water by means of lever pressing and from freely available source. Quinpirole sensitized animals chose to obtain water significantly more by means of lever pressing compared to saline treated animals. Also, quinpirole sensitized animals did not drink all water they obtained but actually drank less water than saline treated animals. This suggested that increased lever pressing was not driven by increased thirst. Contrafreeloading and hypodipsia was not attenuated by coadministration of D2/D3 inverse agonist haloperidol or dopamine stabilizer aripiprazole. On the other hand clomipramine in dose of 10 mg/kg was effective in reducing both contrafreeloading and hypodipsia to the level of control treated animals (De Carolis *et al.*, 2011).

Secondly, nicotine was shown to reduce compulsions in OCD patients (Salín-Pascual and Basañez-villa, 2003; Lundberg *et al.*, 2004) as well as in quinpirole checking animals (Tizabi *et al.*, 2002).

High frequency stimulation (HFS) is another method of OCD treatment, especially in treatment resistant patients (Nuttin *et al.*, 1999; Sturm *et al.*, 2003; Greenberg *et al.*, 2009). Main target structures in OCD patients are anterior limb of the internal capsule, nucleus accumbens, ventral capsule/ventral striatum, subthalamic nucleus (Kohl *et al.*, 2014). Of these, HFS of NAC and subthalamic nucleus (STN) was tested in quinpirole sensitization model of OCD. HFS of nucleus accumbens (NAC) shell and core decreased checking behaviour in quinpirole sensitized rats (Mundo *et al.*, 2000). High frequency stimulation subthatamic nucleus also decreased checking behaviour in quinpirole sensitized rats (Winter *et al.*, 2008). Lesion studies revealed that lesion of NAC increases checking behaviour in saline treated rats to a level of quinpirole treated rats, while NAC lesion has no effect on quinpirole sensitized rats (Dvorkin *et al.*, 2010). This suggests that quinpirole exerts it effect on checking behaviour via inhibiting NAC. However, recent study showed that lesion of NAC does not prevent development of compulsive checking in

quinpirole treated rats, it just reduced the speed by which checking develops (Ballester González *et al.*, 2015). HFS of NAC and subthalamic nucleus, similarly to positive effect of clomipramine and nicotine on compulsive checking in quinpirole treated animals support predictive validity of this animal model.

Constructive validity of quinpirole induced animal model of OCD is supported by involvement of D2 dopamine receptors, involvement of striatum but is hindered by lack of evidence of OFC involvement. Additionally, reduced cognitive flexibility helps to strengthen constructive validity of the model.

Current animal model is based on sensitization of D2 receptors with D2/D3 agonist quinpirole. Following sensitization, changes in striatal structures were observed in QNP treated animals. Increased of D2 receptor binding (Culver *et al.*, 2008) and decrease of glucose utilization was observed in NAC after sensitization with quinpirole, but, importantly, not after acute treatment with QNP (Carpenter *et al.*, 2003). Another brain region of interest in OCD is orbitofrontal cortex. Interestingly, lesion of OFC has no effect on checking behaviour in quinpirole sensitized animals (Dvorkin *et al.*, 2010). This is very interesting in a light that HFS of NAC has an inactivating effect on OFC (McCracken and Grace, 2007) and quinpirole is thought to have also an inhibitory effect on NAC (Dvorkin *et al.*, 2010). Lack of effect of OFC lesion detracts from the constructive validity of this animal model since OFC hyperactivity is considered as one of the most prominent intermediate phenotypes of OCD (Ursu and Carter, 2010).

Cognitive impairments, if associated with the psychiatric disease, are also considered intermediate phenotypes and therefore, if present in animal model, can support model's construct validity. Although sparse, there were several studies observing similar cognitive impairment in quinpirole treated animals as are observed in OCD patients. Spontaneous alternation was decreased in quinpirole treated rats (Einat and Szechtman, 1995) as well as defect in reversal learning (Boulougouris *et al.*, 2009; Hatalova *et al.*, 2014). These findings also suggest involvement of D2/D3 receptors in flexible behaviour.

3 AIMS OF THE THESIS AND FORMULATION OF EXPERIMENTAL QUESTIONS

- Is it possible to reproduce deficit in cognitive flexibility in reversal task in Carousel maze as it was observed in previously study?

- Is deficit in cognitive flexibility observed in Carousel maze sensitive to antidepressant (clomipramine) treatment as it was observed in compulsive checking symptoms in open field in original study of Szechtman (Szechtman *et al.*, 1998)?

- Is co-application of clomipramine and antipsychotic drug risperidone helpful in alleviation of detected cognitive deficit in quinpirole animal model of obsessive-compulsive disorder?

4 EXPERIMENTAL METHODS

4.1 Animals

Adult male Long-Evans rats aged 11-15 weeks from breeding colony of the Institute of Physiology AS CR were used with a start body weight of 300-400 g. Animals were housed in transparent plastic cages, four animals per cage. Animals were housed on a 12-h light cycle (lights on at 6 a.m.) at stabile temperature of 22 °C and humidity of 50 %. Animals had a free access to water and to standard laboratory chow pellets. Experimental procedures were conducted during the light phase and animals were assigned into groups in pseudorandom manner, so each group had a roughly same average body weight at beginning of experiments. Upon arrival, animals were handled for 2 minutes daily for a week. All animal care was in accordance with the Animal protection Code of the Czech Republic and a corresponding directive of the European Community Council on the use of laboratory animals (2010/63/EC).

4.2 Chemical substances and regime of their application

Quinpirole hydrochloride (QNP, Sigma-Aldrich, Czech Republic, Cat. No. Q102), D2 and D3 agonist, was diluted with saline (0.9% NaCl, Sal) to the concentration 0.5 mg/ml. Each animal received QNP (0.5 mg/kg) by subcutaneous injection 30 minutes prior to the behavioural testing.

Clomipramine (CMI, Sigma-Aldrich, Czech Republic, Cat. No. C7291), a tricyclic antidepressive drug, was diluted with saline to the concentration 10 mg/ml. Administered dose of clomipramine was 10 ml/kg intraperitonealy 1.5 h prior to the behavioural testing.

Risperidone (RIS, kindly supplied by IOCB), atypical antipsychotic drug, was dissolved in a drop of acetic acid and diluted with saline to 0.25 mg/ml concentration. Administered dose was 0.25 ml/kg 1.5 h prior to the behavioural testing.

All animals (total n = 68) were divided into one of six groups based on future treatment regime: saline treated control group (0.9% NaCl, Sal, n =10), quinpirole treated group (Qnp, n=16), clomipramine treated group (Cmi, n= 9), group treated by combination of quinpirole and clomipramine (Qnp/Cmi, n=11), group treated by combination quinpirole and risperidone (Qnp/Ris, n=11) and the last one group treated by combination of quinpirole, clomipramine and risperidone as well (Qnp/Cmi/Ris, n=11). Drugs were administered according to schedule, 30 minutes (QNP and SAL) or 1.5 h (CMI and RIS), before actual behavioural testing in Carousel maze.

4.3 Experimental apparatus- Carousel maze

Carousel maze is an apparatus for testing active allothetic place avoidance (AAPA), it is a tool to study working memory, long term episodic memory, cognitive coordination and cognitive flexibility. Successful solving of this task is hippocampally dependent. Apparatus is an elevated circular metal arena (diameter 80 cm) surrounded by 60 cm Perspex wall (*Figure 8*). The wall has conical shape and is adapted to prevent the escape of rats. Transparency of the material allows orientation of animal on arena based on external cues in the experimental room. The movement of animal is recorded by a camera, which is suspended from the ceiling above the arena. For better visibility of animal movement in dimly lit room, camera records infrared light from led diode, which is placed on an animal's back.

During arena rotation (about 1rpm), tracking system evaluates location of animal relative to the room coordinates and relative to the arena itself based on a position of led diode placed on the edge of the arena and another on an animal (*Figure 9*). AAPA task is based on presence of the invisible shock sector 60 ° (in this task stable in room coordinates, therefore does not rotate with the arena), which is defined in the computer software iTrack (Bio-Signal Group, DE, USA). If the camera captures an animal movements in this putative sector, animal receives a mild electrical shock (0.2 mA – 0.6 mA) through subcutaneous needle attached on the nape of its neck (*Figure 10*). A mild shock was titrated to motivate an animal to escape, but not to elicit freezing or other inappropriate escape reaction (like jumping). This form of punishment is administered automatically every 3 seconds up to the time an animal leaves the shock sector.

Figure 8: Carousel maze

Figure 9: Laboratory rat carrying mounted diode

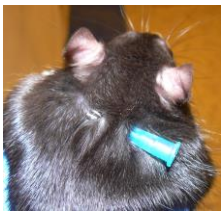


Figure 10: Subcutaneous needle chip attached on the nape of rat

Coordinates of animal movement are processed by complementary software TrackAnalysis (Bio-Signal Group, DE, USA) into many different parameters of its movement. These parameters include number of entrances to forbidden sector, total number of shocks, maximum time of avoidance or time to first entrance, total walked distance and speed.

4.4 Experimental procedure

Experimental design has three principal phases: habituation with sensitization, acquisition and reversal phase. The first phase - habituation and sensitization - took place in 10 sessions (HAB1-HAB10) and the procedure was performed every other day. Every session in rotating Carousel maze was 30 minutes long (same as subsequent acquisition and reversal testing). During habituation the shock sector was not active. Animals got used to the new environment and because of administration of drugs prior to every session in maze according to group assignment (see regime of drug application). Brain dopamine receptors were sensitized in animals where QNP was a part of a treatment regime. The duration of habituation length is given by minimal number of quinpirole application before animals develop compulsive checking symptoms in open field maze (Szechtman *et al.*, 1998). In carousel maze, sensitization process is recognizable by increased locomotion.

Habituation was followed by five acquisition sessions (ACQ1-ACQ5). 24 h prior the first acquisition session subcutaneous needle was attached on the rats' nape, to carry electrical current. This time, in the Carousel maze, there was a defined the shock sector on the north side of arena. Every session took again 30 minutes, every other day. When a rat entered the shock sector it was administered mild electric shock, which motivated animals to learn the position of shock sector and subsequently avoid it. After the fifth acquisition session only animals which entered to sector ten times and less during the last session, were selected to advance to the next phase of experiment, the reversal. Threshold of ten entries was empirically established to signify that animal successfully avoided the shock sector. It was essential that only animals that learned the acquisition entered a reversal phase. The reason is that animal cannot reverse learns learned rule if it was unable to learn it in the first place.

In following reversal phase of experiment, the shock sector was rotated by 180 degrees - to the south side of arena. This phase included 3 sessions every other day (REV1-REV3), each 30 minutes long same as previous sessions.

4.5 Parameters used and statistical analysis

Although in Carousel maze there are many parameters which can be analysed, for example number of entrances into shock sector, total number of shocks, time to first entrance, time spend in specific sectors, thigmotaxis or maximum avoidance time, our statistical analysis is focused only on entrances into shock sector. This parameter most straightforwardly reflects the ability of learning during acquisition and reversal learning after shock sector relocation. Time to the first entrance is another important parameter which reflects between the session memory. None the less, number of entrances will be used as a parameter for the main analysis, because analysis of more parameters could result in increase of chance findings.

Statistical analysis of all the collected data was performed using SPSS program (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 23.0. Chicago: SPSS Inc.). Acquisition phase and reversal learning were analysed and interpreted separately.

Normality was tested by Shapiro-Wilk test and homogeneity of variances by Levene's test. If data did not have normal distribution or variances were not homogenous, appropriate transformation was used. When data distribution met parametric assumptions they were analysed using two way repeated measures ANOVA, which investigated effect of session, (testing whether individual session differs from each other), effect of treatment (testing whether any group of rats receiving one type of drug differ from another group), and interactions (which tested it groups displayed different response trend). When appropriate, planned comparisons, simple effect analysis and Hochberg's GT2 and REGWQ post hoc tests were used.

5 RESULTS

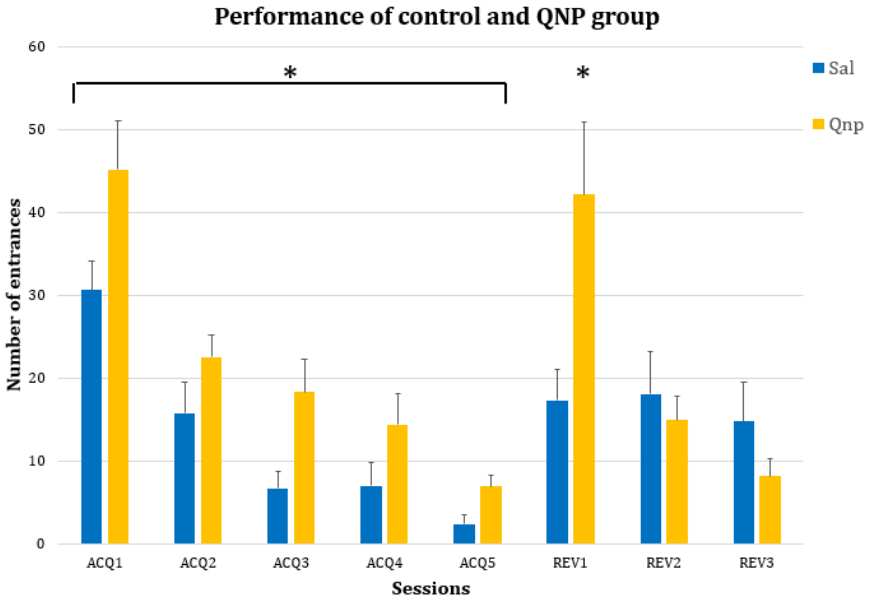
5.1 Comparison of learning abilities of QNP and control rats with previous results

First of all, we decided to check if these findings reproduce our previous study and compared number of entrances only between control and quinpirole group in both acquisition and reversal stages of experiment. In the previous study difference between these two groups was not found in acquisition. In reversal phase quinpirole sensitized rats showed a significant but transient impairment of learning (Hatalova *et al.*, 2014).

5.1.1 Acquisition analysis of number of entrances into the shock sector

Values of entrances from acquisition were not normally distributed and variances were homogeneous. Exploration of box plots revealed a positive skew and therefore the logarithmic transformation was selected to adjust normality of data. Unfortunately, data from ACQ5 session of control animals did not reached normal distribution ($df = 10$, $p = 0.27$), which was caused by high kurtosis due to highly uniform values in control group with a total of single entrance during last acquisition day. Moreover, one outlier was found in fifth day of acquisition in control animals, but no problem in session performance or irregularities in data were not found and therefore there is no reason for deletion of this outlier observation. Cautiously, we proceeded with parametric testing, risking type 2 error.

Two way repeated ANOVA revealed, that overall, control group had significantly less entrances than quinpirole group $F(1,22) = 10.845$, $p = 0.003$. Also, an effect of session was significant $F(4,88) = 65.012$, $p < 0.001$. Planned contrasts revealed significant decrease of number of entrances between each consecutive two sessions except between sessions ACQ3 and ACQ4 (ACQ1/ACQ2 $F(1,22) = 41.441$, $p < 0.001$, ACQ2/ACQ3 $F(1,22) = 24.707$, $p < 0.001$, ACQ3/ACQ4 $F(1,22) = 3.344$, $p = 0.081$, ACQ4/ACQ5 $F(1,22) = 13.347$, $p = 0.001$). Effect of interaction was not significant $F(4.88) = 1.836$, $p = 0.129$. Results are shown in *Graph 1*. Current experiment therefore did not confirm lack of difference between quinpirole and control group in acquisition learning (Hatalova *et al.*, 2014).



Graph 1: Comparison of number of entrances into the shock sector between control group of animals (Sal) and quinpirole group of animals (Qnp) during both acquisition and reversal learning. Animals receiving QNP had a greater number of entrances during all acquisition sessions together compared to control group. Also in the first reversal session Qnp group showed significant worse performance than control group. The average number of entrances is completed showing a standard error of the mean (SEM). * denotes a significant difference from a control group at $p < 0.05$

5.1.2 Reversal analysis of number of entrances into the shock sector

Data from reversal learning stage of experiment were also not normally distributed and in addition variances were not equal. Logarithmic transformation corrected both of these issues. Two way repeated ANOVA showed significant effect of session $F(2,34) = 16.270$, $p < 0.001$ which was followed by testing of planned contrasts which shown a significant decrease in number of entrances between each consecutive pair of sessions (REV1/REV2 $F(1.17) = 11.276$, $p = 0.004$, REV2/REV3 $F(1,17) = 6.913$, $p = 0.006$). Effect of treatment did not demonstrate a significant difference $F(1,17) = 0.445$, $p = 0.514$. However, there was a significant interaction between session and treatment $F(2,34) = 5.164$, $p = 0.011$ and therefore simple effect analysis was used and found significant higher number of entrances of QNP group $F(1,17) = 8.34$, $p = 0.010$ in first reversal session but lack of difference into subsequent reversal sessions. Results are shown in *Graph 1*.

5.2 Statistical analysis of all treatment groups

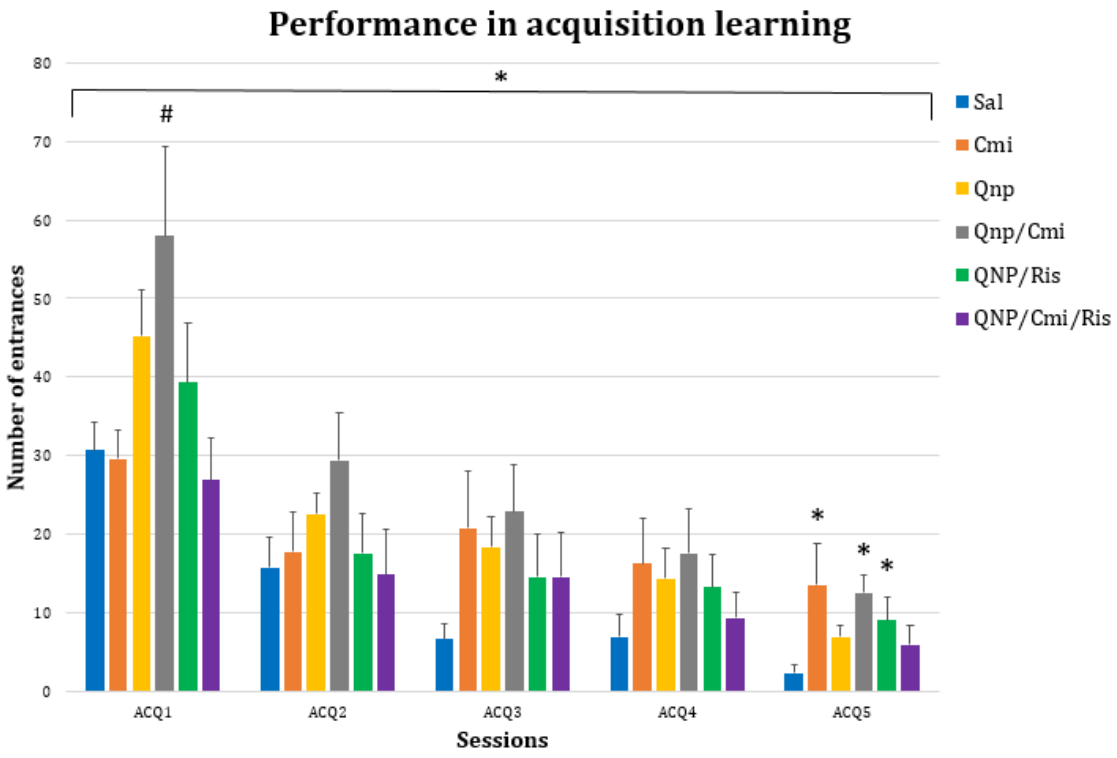
5.2.1 Analysis of entrances during acquisition

Again, two way repeated measures ANOVA was used to compare all treatment groups. Data from entrances during acquisition of experiment were not normally distributed and were not homogeneously distributed. Therefore logarithmic transformation was used to correct normality, but data of control group and group Qnp/Cmi/Ris in session ACQ5 remained non-normal distributed. We did not find a transformation which was able to correct normality in these groups. Since, from the observation of boxplot graphs, the deviation from normality was not extreme, two way repeated measure ANOVA was used. ANOVA showed a significant effect of treatment $F(1,59) = 941.880$, $p = 0.019$. Hochberg's post hoc test revealed significant difference only between control group and group of rats which received combination QNP/CMI ($p = 0.038$). Also, an effect of session was revealed $F(4,236) = 101.599$, $p < 0.001$. Following repeated planned contrasts were significant in each set of comparisons (ACQ1/ACQ2 $F(1,59) = 92.862$, $p < 0.001$, ACQ2/ACQ3 $F(1,59) = 14.513$, $p < 0.001$, ACQ3/ACQ4 $F(1,59) = 5.899$, $p = 0.018$, ACQ4/ACQ5 $F(1,59) = 20.530$, $p < 0.001$). The effect of interaction was significant $F(20.236) = 1.741$, $p = 0.028$ but following planned contrasts did not reveal any significant differences there. Detailed simple effect analysis explained this interaction by differences between groups in first session $F(5,59) = 2.25$, $p = 0.061$ of acquisition and in the fifth session $F(5,59) = 4.81$, $p = 0.001$. Hochberg's post hoc test of the first acquisition session revealed worse performance of QC group compared with QCR group ($p = 0.039$). And in data from the fifth session Hochberg's post hoc test revealed worse performance of Qnp/Ris group ($p = 0.038$), Cmi group ($p = 0.015$) and Qnp/Cmi group ($p = 0.001$) each compared to control group of animals.

Some animals, apart from those that were excluded because acquisition criterion ($n = 16$), were excluded due to jumping on the walls or even out of arena desperately trying to escape ($n = 7$). These individuals were taken out of experiment, as not to cause them more distress, and their records were not statistically analysed because their movements were dependent on rotating of arena and they did not get shocks correctly. Their counts and group memberships were recorded. Heightening of the plexiglas wall surrounding the arena in subsequent animal runs corrected the problem.

All data from acquisition were included in statistical analysis but results from reversal learning were processed without two entire group of rats, one which received only clomipramine and the second which received a combination of QNP/CMI. Clomipramine group of animals was included in the experiment due the need to exclude possible effect of clomipramine alone on memory and learning itself. Statistical analysis of acquisition learning did not demonstrate such outcome. For that reason clomipramine group was no longer to necessary to have this group in experiment in reversal learning phase. The second, group

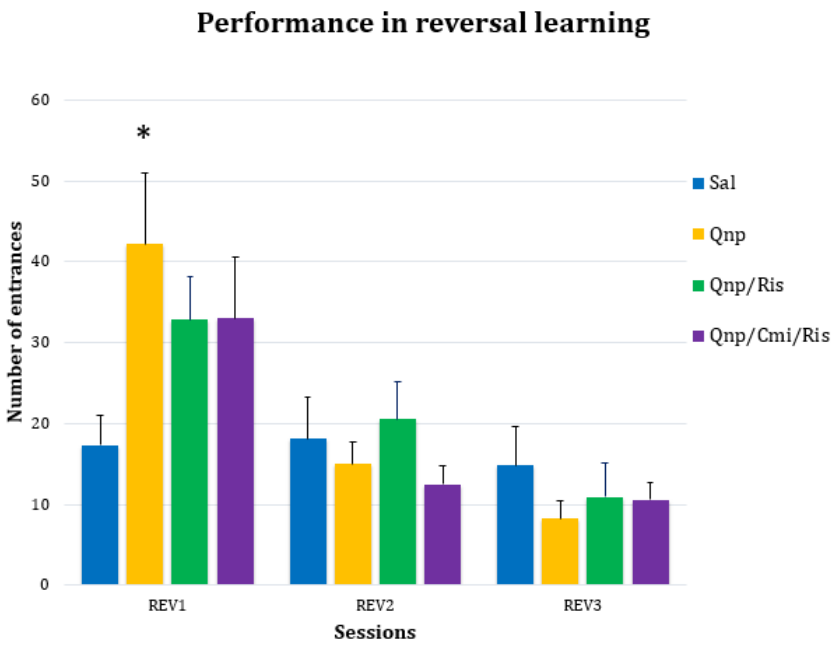
receiving a combination of QNP/CMI had worse performance with higher number of entrances and therefore, too few animals met the criterion of ten entrances (n = 4) to correct statistical analysis of their reversal learning.



Graph 2: Comparison of number of entrances into the shock sector between all treated groups of animals during acquisition only. Throughout all sessions together group of animal receiving combination Qnp/Cmi was significantly worse in comparison with control group. Moreover in the first session Qnp/Cmi group had worse performance than group receiving full combination of Qnp/Cmi/Ris. Also there were other significant differences between Cmi group, Qnp/Ris group and Qnp/cmi group always in comparison with control group. The average number of entrances is completed showing a standard error of the mean (SEM). * denotes a significant difference from a control group at $p < 0.05$. # denotes a significant difference from a Qnp/Cmi/Ris group at $p < 0.05$.

5.2.2 Analysis of entrances during reversal phase

Data from reversal learning were not normally distributed, but after logarithmic transformation normality was achieved. Two way repeated measure ANOVA was used for on all remaining groups. Effect of session was significant $F(2,66) = 46.081$, $p < 0.001$, with planned comparisons showing a significant difference between REV1 and REV2 session $F(1,33) = 38.297$, $p < 0.001$ as well as between REV2 and REV3 session $F(1,33) = 19.774$, $p < 0{,}001$. Surprisingly, effect of treatment was not significant $F(3,33) = 0.296$, $p = 0.828$, indicating that there was no difference in reversal performance between the groups. However, effect of interaction was significant $F(6,66) = 2.817$, $p = 0.017$. Simple effect analysis explained this interaction by the difference between tested animal groups during first reversal session $F(3,33) = 3.89$, $p = 0.017$. Detailed dissection of results using Hochberg's post hoc test revealed that significant difference in first reversal session is caused by higher number of entrances of QNP group compared to control group ($p = 0.017$).



Graph 3: Comparison of number of entrances into the shock sector between all treated groups during reversal only. In the reversal phase of experiment there was only one significant impairment of group of animals receiving quinpirole alone compared with control group. The average number of entrances is completed showing a standard error of the mean (SEM). * denotes a significant difference from control group at $p < 0.05$.

6 DISCUSSION

The cognitive flexibility is an important ability of all animals including humans. Findings, which showed deficits in cognitive flexibility in patients suffering obsessive-compulsive disorder, sparked a wave of new studies focused on these predispositions in OCD patients and also in animal models. Currently, cognitive flexibility is considered to be an inseparable from OCD and, if present in an animal model, it contributes positively to its validity. Quinpirole sensitization rat model of compulsive checking was also verified this way. Our previous results showed a significant deficit in cognitive flexibility in reversal task in Carousel maze (Hatalova *et al.*, 2014). Current study can be considered its extension.

The first objective of this study was to replicate previous results. For this purpose the ability of acquisition and reversal learning of quinpirole sensitized rats was compared with control rats alone. The number of entrances by rats into the shock sector was analysed as a measure of their ability to learn. Current results show a difference between control group of animals and quinpirole group in number of entrances during acquisition phase of experiment. This result is inconsistent with previous finding which did not find a difference in acquisition learning between groups (Hatalova *et al.*, 2014).

Surprisingly main analysis of reversal learning of control animals and QNP animals did not reveal any difference between control and quinpirole sensitized animals during all reversal sessions together. However, from the graph it is apparent that control group had a better initial performance but did not improve in following sessions, whereas quinpirole sensitized animals had worse initial performance but improved throughout two following reversal sessions. This accounted for lack of apparent difference between groups. This assumption was confirmed by a significant interaction and following simple effect analysis. This analysis showed a significantly better performance of control group in first reversal session and trend towards superior performance of quinpirole group in third reversal session. Similar result of stable performance of control animals with not much improvement during reversal sessions compared with steep improvement of performance of quinpirole group was already observed in the previous study (Hatalova *et al.*, 2014). To conclude, even though QNP animals demonstrated worse performance than control group in reversal learning, cognitive flexibility deficit cannot be confirmed because of impaired learning observed in acquisition.

The main part of analysis included all acquired data from all treated groups of animals. The analysis of acquisition learning sessions found impaired performance only in animals receiving QNP/CMI compared with a control group. Additionally, detailed analysis of individual sessions revealed differences between groups in the first acquisition session and the fifth (last) acquisition session. In a first day of acquisition animals receiving QNP/CMI made more

entrances compared to group treated by combination QNP/CMI/RIS. More importantly, other three groups – QNP/RIS, CMI, and QNP/CMI - were impaired compared to control group in the last reversal session. To note, when all groups were included in an analysis, there was no difference between quinpirole sensitized animals and a control group anymore. From these results it appears that not only animals receiving the combination of quinpirole and clomipramine have damaged learning. None the less, QNP/CMI group was the only one that had more entrances than control group in overall performance which suggests it is the worst performing group. Moreover, this group could not be analysed in reversal learning because only four animals met the criterion of less than 10 entrances into the shock sector in the last acquisition day. According to visual inspection, administration of combination of clomipramine and risperidone administration to animals treated with quinpirole tended to produce a better performance of acquisition learning than QNP administration alone. This would agree with clinical studies revealing better treatment outcomes of using risperidone co-application with antidepressant treatment (Kawahara *et al.*, 2000; Skapinakis *et al.*, 2007). However, our study did not find this trend significant.

Significantly worse performance of animals receiving clomipramine in addition to quinpirole was surprising because former reports described that the clomipramine can reduce compulsive behaviour both in clinical studies in OCD patients (Cartwright and Hollander, 1998; Fineberg *et al.*, 2012) and in quinpirole animal model studies (Szechtman *et al.*, 1998). For this reason it is surprising that our finding did show impaired acquisition learning in QNP/CMI group of animals. It appears that in this animal model of OCD cognition and compulsive behaviour is mediated by different, may be even antagonising, systems.

Although main analysis did not reveal any differences in reversal learning between groups, follow up analysis of each session separately found significantly more errors in QNP group compared to the control group in the first reversal session. Although it may appear that both treatment with risperidone and risperidone and clomipramine improved reversal learning, the visual inspection of the graph reveals it is not so. Trend line of these groups is more alike to quinpirole alone treated animals than to control group. Moreover, not all animals from RIS-QNP group were included, since acquisition learning was mildly impaired in acquisition. In summary, best co-treatment option to improve cognitive flexibility is co-administration of CMI and RIS, since this is the only group that was neither impaired in acquisition nor reversal compared to control group.

In general, negative effect of clomipramine in current study was a most interesting finding. Results of acquisition learning of the group receiving clomipramine for the purpose of suppression of quinpirole induced behaviour lead to suspicion that clomipramine in combination with quinpirole affects memory or cognitive coordination. Thus it is possible that

clomipramine reduces compulsive and stereotypic behaviour in animal tests in open field (Szechtman *et al.*, 1998) by means of impairing cognition. Simply put, the reason for reduction of stereotypes in open field may be due fact that animal forgot stereotypes performed in previous session.

Several earlier studies found some indications of adverse tricyclic antidepressant drugs, including clomipramine, on memory. For example, memory functions dependent on hippocampal processing, but not frontal lobe dependent functions, were impaired in three weeks long therapy of clomipramine (Bartfai *et al.*, 1991). Another study involving long term (6 month and more) administration of clomipramine found impaired memory functions compared with healthy controls (Gorenstein *et al.*, 2006). Finally, also in other animal model of obsessive-compulsive disorder (Andersen *et al.*, 2010) and depression (Bhagya *et al.*, 2008), based on neonatal administration of clomipramine, deficit of memory was revealed. For these reasons we added a group receiving only clomipramine without quinpirole administration in our experiment. We detected that performance of this group was impaired only during the fifth acquisition session, which could indicate that clomipramine alone impairs the memory or learning. None the less, from results it appears that much more detrimental to memory is a combination of clomipramine and quinpirole. Further study is mandatory to answer this issue.

7 CONCLUSIONS

This study only partly reproduced the cognitive deficit in quinpirole-induced model of compulsive checking in rats. Moreover, even though clomipramine alone may not impair acquisition learning, clomipramine administration alongside quinpirole administration caused acquisition learning deficits. Reversal learning task did not reveal any convincing evidences about effectiveness of any of used drug combination. However, reversal deficit was most sensitive to combination of risperidone and clomipramine treatment. Quinpirole animal model presents specific checking form of obsessive-compulsive disorder which means that results may not be generally applicable. Possibly, this model could represent a specific group of patients with impaired cognitive flexibility. It may be that exactly these patients would benefit from direct augmentation of SRI treatment with neuroleptics.

8 REFERENCES

Abbruzzese, M., Ferri, S., and Scarone, S. (1995). Wisconsin Card Sorting Test performance in obsessive-compulsive disorder: no evidence for involvement of dorsolateral prefrontal cortex. Psychiatry Research *58*, 37–43.

Abbruzzese, M., Ferri, S., and Scarone, S. (1997). The selective breakdown of frontal functions in patients with obsessive-compulsive disorder and in patients with schizophrenia: A double dissociation experimental finding. Neuropsychologia *35*, 907–912.

Albelda, N., and Joel, D. (2012). Current animal models of obsessive compulsive disorder: an update. Neuroscience *211*, 83–106.

Alevizos, B., Lykouras, L., Zervas, I., and Christodoulou, G. (2002). Risperidone-induced Obsessive-Compulsive symptoms : A series of six cases. Journal of Clinical Psychopharmacology *22*, 461–467.

Alexander, G., and Crutcher, M. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends in Neurosciences *13*, 266–271.

Alexander, G. E. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annual Review of Neuroscience *9*, 357–381.

Altemus, M., Pigott, T., Kalogeras, K., Demitrack, M., Dubbert, B., Murphy, D., and Gold, P. (1992). Abnormalities in the regulation of vasopressin and corticotropin releasing factor secretion in obsessive-compulsive disorder. Archives of General Psychiatry *49*, 9–20.

American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Pub.

Andersen, S. L., Greene-Colozzi, E. a, and Sonntag, K. C. (2010). A novel, multiple symptom model of obsessive-compulsive-like behaviors in animals. Biological Psychiatry *68*, 741–747.

Aoki, S., Liu, A., Zucca, S., and Wickens, J. (2015). Role of striatal cholinergic interneurons in set-shifting in the rat. The Journal of Neuroscienceournal of Neuroscience *35*, 9424–9431.

Aouizerate, B., Guehl, D., Cuny, E., Rougier, A., Bioulac, B., Tignol, J., and Burbaud, P. (2004). Pathophysiology of obsessive-compulsive disorder: a necessary link between phenomenology, neuropsychology, imagery and physiology. Progress in Neurobiology *72*, 195–221.

Aouizerate, B., Guehl, D., Cuny, E., Rougier, A., Burbaud, P., Tignol, J., and Bioulac, B. (2005). Updated overview of the putative role of the serotoninergic system in obsessive-compulsive disorder. Neuropsychiatric Disease and Treatment *1*, 231–243.

Arnold, P., Rosenberg, D., Mundo, E., Tharmalingam, S., Kennedy, J., and Richter, M. (2004). Association of a glutamate (NMDA) subunit receptor gene (GRIN2B) with obsessive-compulsive disorder: a preliminary study. Psychopharmacology *174*, 778–785.

Aron, A. R., Fletcher, P. C., Bullmore, E. T., Sahakian, B. J., and Robbins, T. W. (2003). Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. Nature Neuroscience *6*, 115–116.

Aron, A. R., Robbins, T. W., and Poldrack, R. a (2004). Inhibition and the right inferior frontal cortex. Trends in Cognitive Sciences *8*, 170–177.

Atmaca, M., Yildirim, H., Ozdemir, H., Tezcan, E., and Poyraz, a K. (2007). Volumetric MRI study of key brain regions implicated in obsessive-compulsive disorder. Progress in Neuro-Psychopharmacology & Biological Psychiatry *31*, 46–52.

Aylward, E., Harris, G. J., Hoehn-Saric, R., Barta, P., Machlin, S. R., and Pearlson, G. D. (1996). Normal caudate nucleus in obsessive-compulsive disorder assessed by quantitative neuroimaging. Archives of General Psychiatry *53*, 577–584.

Azmitia, E., and Whitaker-Azmitia, P. (1995). Anatomy, cell biology, and plasticity of serotonergic system. Neuropsychopharmacological omplications for the actions of psychotropic drugs. Psychopharmacology: The Fourth Generation of Progress, 443–449.

Ballester González, J., Dvorkin-Gheva, A., Silva, C., Foster, J. a., and Szechtman, H. (2015). Nucleus accumbens core and pathogenesis of compulsive checking. Behavioural Pharmacology *26*, 200–216.

Barlow, D. H., Gorman, J. M., Shear, M. K., and Woods, S. W. (2000). Cognitive-Behavioral Therapy, Imipramine, or Their Combination for Panic Disorder. Jama *283*, 2529–2536.

Bartfai, A., Åsberg, M., Mårtensson, B., and Gustavsson, P. (1991). Memory effects of clomipramine treatment: relationship to CSF monoamine metabolites and drug concentrations in plasma. Biological Psychiatry *30*, 1075–1092.

Baumgarten, H., and Grozdanovic, Z. (1998). Role serotonin in obsessive-compulsive disorder. The British Journal of Psychiatry. Supplement *35*, 13–20.

Baxter, L. R. (1995). Neuroimaging studies of human anxiety disorders: cuttin paths of knowledge through the field of neurotic phenomena. In: Psychopharmacology: The Foourth Generation of Progress, New York: Raven Press.

Baxter, L. R., Schwartz, J. M., Mazziotta, J. C., Phelps, M. E., Pahl, J. J., Guze, B. H., and Fairbanks, L. (1988). Cerebral glucose metabolic rates in nondepressed patients with obsessive-compulsive disorder. The American Journal of Psychiatry *145*, 1560–1563.

Becquet, D., Hery, M., Francois-Bellan, A., Giraud, P., Deprez, P., and Faudon, M. (1993). Glutamate, GABA, glycine and taurine modulate serotonin synthesis and release in rostral and caudal rhombencephalic raphe cells in primary cultures. Neurochemistry International *23*, 269–283.

Belzung, C., and Lemoine, M. (2011). Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. Biology of Mood & Anxiety Disorders *1*, 9–23.

Bergeron, R., Ravindran, A., Chaput, Y., Goldner, E., Swinson, R., and van Amerigen, M. (2002). Sertraline and fluoxetine treatment of obsessive-compulsive disorder: results of a double-blind, 6-month treatment study. Journal of Clinical Psychopharmacology *22*, 148–154.

Berlim, M. T., Neufeld, N. H., and Van den Eynde, F. (2013). Repetitive transcranial magnetic stimulation (rTMS) for obsessive-compulsive disorder (OCD): an exploratory meta-analysis of randomized and sham-controlled trials. Journal of Psychiatric Research *47*, 999–1006.

Bhagya, V., Srikumar, B. N., Raju, T. R., and Shankaranarayana Rao, B. S. (2008). Neonatal clomipramine induced endogenous depression in rats is associated with learning impairment in adulthood. Behavioural Brain Research *187*, 190–194.

Birrell, J. M., and Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. The Journal of Neuroscience *20*, 4320–4324.

Bissonette, G., Powel, E., and Roesch, M. (2014). Neural structures underlying set-shifting: roles of medial prefrontal cortex and anterior cingulate cortex. Behavioural Brain Research *1*, 91–101.

Bloch, M. H., Kelmendi, B., Coric, V., Bracken, M. B., and Leckman, J. F. (2006). A systematic review : antipsychotic augmentation with treatment refractory obsessive-compulsive disorder. Molecular Psychiatry *11*, 622–632.

Bloch, M. H., Landeros-Weisenberger, A., Rosario, M. C., Pittenger, C., and Leckman, J. F. (2008). Meta-analysis of the symptom structure of obsessive-compulsive disorder. The American Journal of Psychiatry *165*, 1532–1542.

Bohne, A., Savage, C., Deckersbach, T., Keuthen, N., Jenike, M., Tuschen-Caffier, B., and Wilhelm, S. (2005). Visuospatial abilities, memory, and executive functioning in trichotillomania and obsessive-compulsive disorder. Journal of Clinical and Experimental Neuropsychology *27*, 385–399.

Boulougouris, V., Chamberlain, S. R., and Robbins, T. W. (2009). Cross-species models of OCD spectrum disorders. Psychiatry Research *170*, 15–21.

Bourne, S. K., Eckhardt, C. A., Sheth, S. A., and Eskandar, E. N. (2012). Mechanisms of deep brain stimulation for obsessive compulsive disorder : effects upon cells and circuits. Frontiers in Integrative Neuroscience *6*, 1–14.

Brimberg, L. *et al.* (2012). Behavioral, pharmacological, and immunological abnormalities after streptococcal exposure: a novel rat model of Sydenham chorea and related neuropsychiatric disorders. Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology *37*, 2076–2087.

Bronze, M. S., and Dale, J. B. (1993). Epitopes of streptococcal M proteins tahta evoke antibodies that cross-react with human brain. The Journal of Immunology *151*, 2820–2828.

Buchsbaum, B. R., Greer, S., Chang, W.-L., and Berman, K. F. (2005). Meta-analysis of neuroimaging studies of the Wisconsin card-sorting task and component processes. Human Brain Mapping *25*, 35–45.

Burguiere, E., Monteiro, P., Mallet, L., Feng, G., and Graybiel, A. M. (2014). Striatal circuits, habits, and implications for obsessive-compulsive disorder. Current Opinion in Neurobiology *30*, 59–65.

De Carolis, L., Schepisi, C., Milella, M. S., and Nencini, P. (2011). Clomipramine, but not haloperidol or aripiprazole, inhibits quinpirole-induced water contrafreeloading, a putative animal model of compulsive behavior. Psychopharmacology *218*, 749–759.

Carpenter, T. L., Pazdernik, T. L., and Levant, B. (2003). Differences in quinpirole-induced local cerebral glucose utilization between naive and sensitized rats. Brain Research *964*, 295–301.

Cartwright, C., and Hollander, E. (1998). SSRIs in the treatment of obsessive-compulsive disorder. Depression and Anxiety *8*, 105–113.

Cath, D. C., van Grootheest, D. S., Willemsen, G., van Oppen, P., and Boomsma, D. I. (2008). Environmental factors in obsessive-compulsive behavior: evidence from discordant and concordant monozygotic twins. Behavior Genetics *38*, 108–120.

Cavedini, P. (2009). Decisional processes in obsessive-compulsive spectrum disorders: From neuropsychology to clinical implications.

Clarke, H. F., Robbins, T. W., and Roberts, A. C. (2008). Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience *28*, 10972–10982.

Clarke, H. F., Walker, S. C., Crofts, H. S., Dalley, J. W., Robbins, T. W., and Roberts, a C. (2005). Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience *25*, 532–538.

Coric, V. *et al.* (2005). Riluzole augmentation in treatment-resistant obsessive-compulsive disorder: An open-label trial. Biological Psychiatry *58*, 424–428.

Culver, K. E., Szechtman, H., and Levant, B. (2008). Altered dopamine D2-like receptor binding in rats with behavioral sensitization to quinpirole: effects of pre-treatment with Ro 41-1049. European Journal of Pharmacology *592*, 67–72.

Deckersbach, T., Savage, C., Baer, L., and Jenike, M. (2000). The relationship between semantic organization and memory in obsessive-compulsive disorder. Psychotherapy and Psychosomatics *69*, 101–107.

Delgado, P., and Moreno, F. (1997). Different roles for serotonin in anti-obsessional drug action and the pathophysiology of obsessive-compulsive disorder. The British Journal of Psychiatry. Supplement *35*, 21–25.

Delorme, R., Bille, A., Betancur, C., Mathieu, F., Chabane, N., Mouren-Simeoni, M. C., and Leboyer, M. (2006). Exploratory analysis of obsessive compulsive symptom dimensions in children and adolescents: a prospective follow-up study. BMC Psychiatry *6*, 1–10.

Denys, D. *et al.* (2013). Dopaminergic activity in Tourette syndrome and obsessive-compulsive disorder. European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology *23*, 1423–1431.

Denys, D., Van Nieuwerburgh, F., Deforce, D., and Westenberg, H. G. M. (2006). Association between serotonergic candidate genes and specific phenotypes of obsessive compulsive disorder. Journal of Affective Disorders *91*, 39–44.

Denys, D., Wee, N. Van Der, Janssen, J., Geus, F. De, and Westenberg, H. G. M. (2004). Low Level of Dopaminergic D 2 Receptor Binding in Obsessive-Compulsive Disorder. Biological Psychiatry *55*, 1041–1045.

Dias, R., Robbins, T., and Roberts, A. (1996). Dissociation in prefrontal cortex of affective and attentional shifts. Nature *380*, 69–72.

Dickel, D., Veenstra-VanderWeele, J., Cox, N., Wu, X., Fischer, D. J., and Van Etten-Lee, M. (2006). Association testing of the positional and functional candidate gene SLC1A1/EAAC1 in early-onset obsessive-compulsive disorder. Archives of General Psychiatry *63*, 778–785.

Doshi, P. K. (2009). Surgical treatment of obsessive-compulsive disorders: Current status. Indian Journal of Psychiatry *51*, 216–221.

Duggal, H. (2003). Risperidone-Induced Obsessive-Compulsive Symptoms : Serotonin- Dopamine Imbalance ? Journal of Clinical Psychopharmacology *23*, 681–682.

Dvorkin, A., Silva, C., McMurran, T., Bisnaire, L., Foster, J., and Szechtman, H. (2010). Features of compulsive checking behavior mediated by nucleus accumbens and orbital frontal cortex. The European Journal of Neuroscience *32*, 1552–1563.

Einat, H., and Szechtman, H. (1995). Perseveration without hyperlocomotion in a spontaneous alternation task in rats sensitized to the dopamine agonist quinpirole. Physiology & Behavior *57*, 55–59.

Erecinska, M., and Silver, I. (1990). Metabolism and role of glutamate in mammalian brain. Progress in Neurobiology *35*, 245–296.

Fallon, B., Liebowitz, M., Campeas, R., Schneier, F., Marshall, R., Davies, S., Goetz, D., and Klein, D. (1998). Intravenous clomipramine for obsessive-compulsive disorder refractory to oral clomipramine. Archives of General Psychiatry *55*, 918–924.

Farde, L., Nordstöm, A., Wiesel, F., Pauli, S., Halldin, C., and Sedvall, G. (1992). Positron Emission Tomographic Analysis of Central D1 and D2 Dopamine Receptor Occupancy in Patients Treated With Classical Neuroleptics and Clozapine Relation to. Archives of General Psychiatry *49*, 538–544.

Feldman, R., Meyer, J., and Quenzer, L. (1997). Principles of neuropsychopharmacology, Sunderland, MA: Sinauer Associates, Inc.

Fenger, M. M., Gade, A., Adams, K. H., Hansen, E. S., Bolwig, T. G., and Knudsen, G. M. (2005). Cognitive deficits in obsessive-compulsive disorder on tests of frontal lobe functions. Nordic Journal of Psychiatry *59*, 39–44.

Ferré, S., Cortés, R., and Artigas, F. (1994). Dopaminergic Regulation of the Serotonergic Pathway : Microdialysis Studies in Freely Moving Rats. The Journal of Neuroscience *14*, 4839–4846.

Figee, M., Wielaard, I., Mazaheri, A., and Denys, D. (2013). Neurosurgical targets for compulsivity: what can we learn from acquired brain lesions? Neuroscience and Biobehavioral Reviews *37*, 328–339.

Fineberg, N. a, Brown, A., Reghunandanan, S., and Pampaloni, I. (2012). Evidence-based pharmacotherapy of obsessive-compulsive disorder. The International Journal of Neuropsychopharmacology *15*, 1173–1191.

Fineberg, N. a, and Gale, T. M. (2005). Evidence-based pharmacotherapy of obsessive-compulsive disorder. The International Journal of Neuropsychopharmacology *8*, 107–129.

Fineberg, N. a, Reghunandanan, S., Simpson, H. B., Phillips, K. a, Richter, M. a, Matthews, K., Stein, D. J., Sareen, J., Brown, A., and Sookman, D. (2015). Obsessive-compulsive disorder (OCD): Practical

strategies for pharmacological and somatic treatment in adults. Psychiatry Research *227*, 114–125.

Fink, K., Schlicker, E., Neise, A., and Göthert, M. (1990). Involvement of presynaptic H3 receptors in the inhibitory effect of histamine on serotonin release in the rat brain cortex. Naunyn-Schmiedeberg's Archives of Pharmacology *342*, 513–519.

Fontenelle, L. F. *et al.* (2012). Towards a post-traumatic subtype of obsessive-compulsive disorder. Journal of Anxiety Disorders *26*, 377–383.

Franklin, M. E., Abramowitz, J. S., Kozak, M. J., Levitt, J. T., and Foa, E. B. (2000). Effectiveness of exposure and ritual prevention for obsessive-compulsive disorder: randomized compared with nonrandomized samples. Journal of Consulting and Clinical Psychology *68*, 594–602.

Garner, J. P., Thogerson, C. M., Würbel, H., Murray, J. D., and Mench, J. a (2006). Animal neuropsychology: validation of the Intra-Dimensional Extra-Dimensional set shifting task for mice. Behavioural Brain Research *173*, 53–61.

Geller, D. A. (2006). Obsessive-compulsive and spectrum disorders in children and adolescents. Psychiatric Clinics of North America *29*, 353–370.

Gerfen, C. R., and Surmeier, D. J. (2012). Modulation of striatal projection systemy by dopamine. Annual Review of Neuroscience *34*, 441–466.

Gerlach, J., and Peacock, L. (1995). New antipsychotics: the present status. International Clinical Psychopharmacology *10*, 39–48.

Ghahremani, D. G., Monterosso, J., Jentsch, J. D., Bilder, R. M., and Poldrack, R. A. (2010). Neural components underlying behavioral flexibility in human reversal learning. Cerebral Cortex *20*, 1843–1852.

Giedd, J. N., Rapoport, judith L., Garvey, M. A., Perlmutter, S., and Swedo, S. E. (2000). MRI Assessment of Children With Obsessive-Compulsive Disorder or Tics Associated With Streptococcal Infection. The American Journal of Psychiatry *157*, 281–283.

Girault, J., and Greengard, P. (2004). The Neurobiology of Dopamine Signaling. Archives of Neurology *61*, 641–644.

Godefroy, O., Lhullier, C., and Rousseaux, M. (1996). Non-spatial attention disorders in patients with frontal or posterior brain damage. Brain *119*, 191–202.

Goodman, W. K., Mcdougle, C. J., Price, L., Riddle, M. A., Pauls, D. L., and Leckman, J. (1990). Beyond the serotonin hypothesis: a role for dopamine in some forms of obsessive compulsive disorder? Journal of Clinical Psychiatry *51*, 36–43.

Goodman, W. K., Price, L., Rasmussen, S. a, Mazure, C., Fleischmann, R. L., Hill, C. L., Heninger, G., and Charney, D. S. (1989). The yale-Brown Obsessive Compulsive Scale. Archives of General Psychiatry *46*, 1006–1011.

Goodman, W. K., Rasmussen, S. a, and Leckman, J. F. (1995). A family study of obsessive-compulsive disorder. American Journal of Psychiatry *152*, 76–84.

Gorenstein, C., de Carvalho, S. C., Artes, R., Moreno, R. A., and Marcourakis, T. (2006). Cognitive performance in depressed patients after chronic use of antidepressants. Psychopharmacology *185*, 84–92.

Grabe, H. J. *et al.* (2006). Familiality of obsessive-compulsive disorder in nonclinical and clinical subjects. The American Journal of Psychiatry *163*, 1986–1992.

Greenberg, B. D., Rauch, S. L., and Haber, S. N. (2009). Invasive circuitry-based neurotherapeutics : Stereotactic ablation and deep brain stimulation for OCD. Neuropsychopharmacology *35*, 317–336.

Van Grootheest, D. S., Cath, D. C., Beekman, A. T., and Boomsma, D. I. (2005). Twin Studies on Obsessive–Compulsive Disorder: A Review. Twin Research and Human Genetics *8*, 450–458.

De Haan, L., Sterk, B., Wouters, L., and Linszen, D. H. (2013). The 5-year course of obsessive-compulsive symptoms and obsessive-compulsive disorder in first-episode schizophrenia and related disorders. Schizophrenia Bulletin *39*, 151–160.

Hatalova, H., Radostova, D., Pistikova, A., Vales, K., and Stuchlik, A. (2014). Spatial reversal learning in chronically sensitized rats and in undrugged sensitized rats with dopamine D2-like receptor agonist quinpirole. Frontiers in Behavioral Neuroscience *8*, 122.

Henry, J. (2006). A meta-analytic review of Wisconsin Card Sorting Test and verbal fluency performance in obsessive-compulsive disorder. Cognitive Neuropsychiatry *11*, 156–176.

Hesse, S., Müller, U., Lincke, T., Barthel, H., Villmann, T., Angermeyer, M. C., Sabri, O., and Stengler-Wenzke, K. (2005). Serotonin and dopamine transporter imaging in patients with obsessive-compulsive disorder. Psychiatry Research *140*, 63–72.

Heyman, I., Ombonne, E. F., Simmons, H., Ord, T. F., Meltzer, H., and Goodman, R. (2001). Prevalence of obsessive-compulsive disorder in the British nationwide survey of child mental health. British Journal of Psychiatry *179*, 4–10.

Hippius, H. (1999). A historical perspective of clozapine. Journal of Clinical Psychiatry *60*, 22–23.

Hoehn-Saric, R., Ninan, P., and Black, D. (2000). Multicenter double-blind comparison of sertraline and desipramine for concurrent obsessive-compulsive and major depressive disorders. Archives of General Psychiatry *57*, 76–82.

Hollander, E., DeCaria, C., Nitescu, A., Gully, R., Suckow, R., and Cooper, T. (1992). Serotonergic function in obsessive-compulsive disorder: behavioral and neuroendocrine responses to oral m-chlorophenylpiperazine and fenfluramine in patients and healthy volunteers. Archives of General Psychiatry *49*, 21–28.

Hollander, E., and Rossi, N. (2003). Risperidone augmentation in treatment-resistant obsessive-compulsive disorder: a double-blind, placebo-controlled study. International Journal of Neuropsychopharmacology *6*, 397–401.

Horn, N. R., Dolan, M., Elliott, R., Deakin, J. F. W., and Woodruff, P. W. R. (2003). Response inhibition and impulsivity: an fMRI study. Neuropsychologia *41*, 1959–1966.

Hornak, J., O'Doherty, J., Bramham, J., Rolls, E. T., Morris, R. G., Bullock, P. R., and Polkey, C. E. (2004). Reward-related reversal learning after surgical excisions in orbito-frontal or dorsolateral prefrontal cortex in humans. Journal of Cognitive Neuroscience *16*, 463–478.

Hounie, A. G., Pauls, D. L., Mercadante, M., Rosário-Campos, M., Shavitt, R., de Mathis, M., and de Alvarenga, P. (2004). Obsessive-compulsive spectrum disorders in rheumatic fever with and without Sydenham's chorea. The Journal of Clinical Psychiatry *65*, 994–999.

Chakrabarty, K., Bhattacharyya, S., Christopher, R., and Khanna, S. (2005). Glutamatergic dysfunction in OCD. Neuropsychopharmacology *30*, 1735–1740.

Chamberlain, S. R., Blackwell, a D., Fineberg, N. a, Robbins, T. W., and Sahakian, B. J. (2005). The neuropsychology of obsessive compulsive disorder: the importance of failures in cognitive and behavioural inhibition as candidate endophenotypic markers. Neuroscience and Biobehavioral Reviews *29*, 399–419.

Chamberlain, S. R., Fineberg, N. A., Blackwell, A. D., Ph, D., Robbins, T. W., and Sahakian, B. J. (2006). Motor inhibition and cognitive flexibility in obsessive- compulsive disorder and trichotillomania. American Journal of Psychiatry *163*, 1282–1284.

Di Chiara, G., and Bassareo, V. (2007). Reward system and addiction: what dopamine does and doesn't do. Current Opinion in Pharmacology *7*, 69–76.

Chudasama, Y., and Robbins, T. W. (2003). Dissociable contributions of the orbitofrontal and infralimbic cortex to Pavlovian autoshaping and discrimination reversal learning : Further evidence for the functional heterogeneity of the rodent frontal cortex. The Journal of Neuroscience *23*, 8771–8780.

Insel, T. R., Muller, E., Alterman, I., Linnoila, M., and Murphy, D. (1985). Obsessive-compulsive disorder and serotonin: is there a connection? Biological Psychiatry *20*, 1174–1188.

Iversen, S. D., and Iversen, L. L. (2007). Dopamine: 50 years in perspective. Trends in Neurosciences *30*, 188–193.

Jaafari, N., Rachid, F., Rotge, J.-Y., Polosan, M., El-Hage, W., Belin, D., Vibert, N., and Pelissolo, A. (2012). Safety and efficacy of repetitive transcranial magnetic stimulation in the treatment of obsessive-compulsive disorder: a review. The World Journal of Biological Psychiatry *13*, 164–177.

Jaber, M., Jones, S., Giros, B., and Caron, M. (1997). The dopamine transporter: a crucial component regulating dopamine transmission. Movement Disorders *12*, 629–633.

Janssen, P., Nemegeers, J., Awouters, F., Schellekens, K., Megens, A., and Meert, T. (1988). Pharmacology of risperidone (R64 766), a new antipsychotic with serotonin-D2 and dopamine-D2 antagonistic properties. The Journal of Pharmacology and Experimental Therapeutics *244*, 685–693.

Jensen, G. (1963). Preference for bar pressing over" freeloading" as a function of number of rewarded presses. Journal of Experimental Psychology *65*, 451–454.

Jurado, M., Junque, C., Vallejo, J., and Salgado, P. (2001). Impairment of incidental memory for frequency in patients with obsessive-compulsive disorder. Psychiatry *104*, 213–220.

Kalén, P., Strecker, R., Rosengren, E., and Bjo, A. (1989). Regulation of striatal serotonin release by the lateral habenula-dorsal raphe pathway in the rat as demonstrated by in vivo microdialysis: role of excitatory amino acids and GABA. Brain Research *492*, 187–202.

Kapur, and Seeman (2001). Does fast dissociation from the dopamine D (2) receptor explain the action of atypical antipsychotics?: A new hypothesis. The American Journal of Psychiatry *158*, 360.

Kawahara, T., Ueda, Y., and Mitsuyama, Y. (2000). A case report of refractory obsessive – compulsive disorder improved by risperidone augmentation of clomipramine treatment. Psychiatry and Clinical Neurosciences *54*, 599–601.

Kim, C.-H., Koo, M.-S., Cheon, K.-A., Ryu, Y.-H., Lee, J.-D., and Lee, H.-S. (2003). Dopamine transporter density of basal ganglia assessed with [123I]IPT SPET in obsessive-compulsive disorder. European Journal of Nuclear Medicine and Molecular Imaging *30*, 1637–1643.

Kim, M.-S., Park, S.-J., Shin, M. S., and Kwon, J. S. (2002). Neuropsychological profile in patients with obsessive-compulsive disorder over a period of 4-month treatment. Journal of Psychiatric Research *36*, 257–265.

Koffer, K., Coulson, G., and Hospital, W. P. (1971). Feline indolence : Cats prefer free to response-produced food *. Psychonomic Science *24*, 41–42.

Kohl, S., Schönherr, D. M., Luigjes, J., Denys, D., Mueller, U. J., Lenartz, D., Visser-Vandewalle, V., and Kuhn, J. (2014). Deep brain stimulation for treatment-refractory obsessive compulsive disorder: a systematic review. BMC Psychiatry *14*, 214.

Kurlan, R., and Kaplan, E. L. (2004). The pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS) etiology for tics and Obsessive-compulsive symptoms: hypothesis or entity? Practical considerations for the clinician. Pediatrics *113*, 883–886.

Lawrence, A. D., Sahakian, B. J., and Robbins, T. W. (1998). Cognitive functions and corticostriatal circuits: insights from Huntington's disease. Trends in Cognitive Sciences *2*, 379–388.

Leckman, J. F. *et al.* (1994). Elevated cerebrospinal fluid levels of oxytocin in obsessive-compulsive disorder: Comparison with Tourette's syndrome and healthy controls. Archives of General Psychiatry *51*, 782–792.

Leonard, H., Swedo, S., Rapoport, J., Koby, E., Lenane, M., Cheslow, D., and Hamburger, S. (1989). Treatment of obsessive-compulsive disorder with clomipramine and desipramine in children and adolescents: a double-blind crossover comparison. Archives of General Psychiatry *46*, 1088–1092.

Leysen, J. E. (1988). Profile of risperidon, a new antipsychotic. The Journal of Pharmacology and Experimental Therapeutics *247*, 661–670.

Lezak, M. D. (2004). Neuropsychological assessment, Oxford University Press.

Lobellova, V., Entlerova, M., Svojanovska, B., Hatalova, H., Prokopova, I., Petrasek, T., Vales, K., Kubik, S., Fajnerova, I., and Stuchlik, A. (2013). Two learning tasks provide evidence for disrupted behavioural flexibility in an animal model of schizophrenia-like behaviour induced by acute MK-801: a dose-response study. Behavioural Brain Research *246*, 55–62.

Logan, G. D., Cowan, W. B., and Davis, K. A. (1984). On the Ability to Inhibit Simple and Choice Reaction Time Responses : A Model and a Method. Journal of Experimental Psychology *10*, 276–291.

Lochner, C., du Toit, P. L., Zungu-Dirwayi, N., Marais, A., van Kradenburg, J., Seedat, S., Niehaus, D. J. H., and Stein, D. J. (2002). Childhood trauma in obsessive-compulsive disorder, trichotillomania, and controls. Depression and Anxiety *15*, 66–68.

Lundberg, S., Carlsson, A., Norfeldt, P., and Carlsson, M. L. (2004). Nicotine treatment of obsessive-compulsive disorder. Progress in Neuro-Psychopharmacology & Biological Psychiatry *28*, 1195–1199.

Lykouras, L., Alevizos, B., Michalopoulou, P., and Rabavilas, A. (2003). Obsessive – compulsive symptoms induced by atypical antipsychotics . A review of the reported cases. Progress in Neuro-Psychopharmacology & Biological Psychiatry *27*, 333–346.

Mansari, M. El, Bouchard, C., and Blier, P. (1995). Alteration of serotonin release in the guinea pig orbito-frontal cortex by selective serotonin reuptake inhibitors. Neuropsychopharmacology *13*, 117–127.

Mantovani, A., Lisanby, S. H., Pieraccini, F., Ulivelli, M., Castrogiovanni, P., and Rossi, S. (2006). Repetitive transcranial magnetic stimulation (rTMS) in the treatment of obsessive-compulsive disorder (OCD) and Tourette's syndrome (TS). The International Journal of Neuropsychopharmacology *9*, 95–100.

Masand, P., and Gupta, S. (1999). Selective serotonin-reuptake inhibitors: an update. Harvard Review of Psychiatry 7.2, 69–84.

Mataix-Cols, D., Rauch, S. L., Baer, L., Eisen, J. L., Shera, D. M., Goodman, W. K., Rasmussen, S. a, and Jenike, M. a (2002). Symptom stability in adult obsessive-compulsive disorder: data from a naturalistic two-year follow-up study. The American Journal of Psychiatry *159*, 263–268.

McAlonan, K., and Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. Behavioural Brain Research *146*, 97–103.

McCracken, C. B., and Grace, A. a (2007). High-frequency deep brain stimulation of the nucleus accumbens region suppresses neuronal activity and selectively modulates afferent drive in rat orbitofrontal cortex in vivo. The Journal of Neuroscience *27*, 12601–12610.

Mcdougle, C. J., Goodman, W. K., Leckman, J. F., Lee, N., Heninger, G., and Price, L. (1994). Haloperidole addition in fluvoxamine-refractory obsessive-compulsive disorder: A double-blind, placebo-controlled study in patients with and without tics. Archives of General Psychiatry *51*, 302–308.

Mcdougle, C. J., Goodman, W. K., Price, L., Delgado, P., and Krystal, J. (1990). Neuroleptic addition in fluvoxamine-refractory obsessive-compulsive disorder. The American Journal of Psychiatry *147*, 652–654.

McKeon, J., Roa, B., and Mann, A. (1984). Life events and personality traits in obsessive-compulsive neurosis. The British Journal of Psychiatry *144*, 185–189.

Meltzner, H., Matsubara, S., and Lee, J. (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. Journal of Pharmacology and Experimental Therapeutics *251*, 238–246.

Meneses, A. (1999). 5-HT system and cognition. Neuroscience & Biobehavioral Reviews *23*, 1111–1125.

Menzies, L., Chamberlain, S. R., Laird, A. R., Thelen, S. M., Sahakian, B. J., and Bullmore, E. T. (2008). Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited. Neuroscience and Biobehavioral Reviews *32*, 525–549.

Mian, M. K., Campos, M., Sheth, S. a, and Eskandar, E. N. (2010). Deep brain stimulation for obsessive-compulsive disorder: past, present, and future. Neurosurgical Focus *29*, E10.

Milad, M. R., and Rauch, S. L. (2012). Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways. Trends in Cognitive Sciences *16*, 43–51.

Miyamoto, S., Miyake, N., Jarskog, L. F., Fleischhacker, W. W., and Lieberman, J. a (2012). Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. Molecular Psychiatry *17*, 1206–1227.

Modell, J., Mountz, J., Curtis, G., and Greden, J. (1989). Neurophysiologic dysfunction in basal ganglia/limbic striatal and thalamocortical circuits as a pathogenetic mechanism of obsessive-compulsive disorder. The Journal of Neuropsychiatry and Clinical Neurosciences *1*, 27–36.

Moritz, S., Birkner, C., Kloss, M., Jahn, H., Hand, I., Haasen, C., and Krausz, M. (2002). Executive functioning in obsessive-compulsive disorder, unipolar depression, and schizophrenia. Archives of Clinical Neuropsychology *17*, 477–483.

Moritz, S., Fricke, S., Wagner, M., and Hand, I. (2001). Further evidence for delayed alternation deficits in obsessive-compulsie disorder. The Journal of Nervous and Mental Disease *189*, 557–570.

Müller, N., Riedel, M., Blendinger, C., Oberle, K., Jacobs, E., and Abele-Horn, M. (2004). Mycoplasma pneumoniae infection and Tourette's syndrome. Psychiatry Research *129*, 119–125.

Mundo, E., Maina, G., and Uslenghi, C. (2000). Multicentre, double-blind, comparison of fluvoxamine and clomipramine in the treatment of obsessive-compulsive disorder. International Clinical Psychopharmacology *15*, 69–76.

Mundus, S., and Jenike, M. a (1992). Neurosurgical treatment of malignant obsessive-compulsive disorder. Psychiatric Clinics of North America *15*, 921–938.

Murphy, D. L., Andrews, A. M., Wichems, C. H., Li, Q., Tohda, M., and Greenberg, B. D. (1998). Brain serotonin neurotransmission: An overview and update with an emphasis in serotonin subsystem heterogeneity, multiple receptors, interactions with other neurotransmitter systems, and consequent implications for understanding the actions of serotonergi. Journal of Clinical Psychiatry *59*, 4–12.

Nauczyciel, C., Le Jeune, F., Naudet, F., Douabin, S., Esquevin, a, Vérin, M., Dondaine, T., Robert, G., Drapier, D., and Millet, B. (2014). Repetitive transcranial magnetic stimulation over the

orbitofrontal cortex for obsessive-compulsive disorder: a double-blind, crossover study. Translational Psychiatry *4*, e436.

Ninan, P., Koran, L., Kiev, A., Davidson, J., Rasmussen, S., Zajecka, J., Robinson, D., Crits-Christoph, P., Mandel, F., and Austin, C. (2006). High-dose sertraline strategy for nonresponders to acute treatent for obsessive-compulsive disorder: a multicenter double-blind trial. The Journal of Clinical Psychiatry *67*, 15–22.

Nuttin, B., Cosyns, P., Demeulemeester, H., and Gybels, J. (1999). Electrical stimulation in anterior limbs of internal capsules in patients with obsessive-compulsive disorder Separating in-utero and postnatal influences on later disease. The Lancet *354*, 13353.

O'Connor, K. P., Aardema, F., Robillard, S., Guay, S., Pélissier, M.-C., Todorov, C., Borgeat, F., Leblanc, V., Grenier, S., and Doucet, P. (2006). Cognitive behaviour therapy and medication in the treatment of obsessive-compulsive disorder. Acta Psychiatrica Scandinavica *113*, 408–419.

Okasha, a., Rafaat, M., Mahallawy, N., Nahas, J., Seif El Dawla, A., Sayed, M., and El Kholi, S. (2000). Cognitive dysfunction in obsessive-compulsive disorder. Acta Psychiatrica Scandinavica *101*, 281–285.

Pallanti, S., Hollander, E., Bienstock, C., Koran, L., Leckman, J., Marazziti, D., Pato, M., Stein, D., and Zohar, J. (2002). Treatment non-response in OCD: methodological issues and operational definitions. The International Journal of Neuropsychopharmacology *5*, 181–191.

Pauls, D. L. (2010). The genetics of obsessive-compulsive disorder: a new review. American Journal of Medical Genetics. Part C: Seminars in Medical Genetics *148C*, 149–163.

Pierre, J. M. (2005). Extrapyramidal Symptoms with Atypical Antipsychotics Incidence , Prevention and Management. Drug Safety *28*, 191–208.

Pineyro, G., and Blier, P. (1999). Autoregulation of Serotonin Neurons : Role in antidepressant drug action. Pharmacological Reviews *51*, 533–591.

Pitman, R. (1987). A cybernetic model of obsessive-compulsive psychopathology. Comprehensive Psychiatry *28*, 334–343.

Pittenger, C., Kelmendi, B., Wasylink, S., Bloch, M. H., and Coric, V. (2008). Riluzole augmentation in treatment-refractory obsessive-compulsive disorder: a series of 13 cases, with long-term follow-up. Journal of Clinical Psychopharmacology *28*, 363–367.

Pollitt, J. (1957). Natural history of obsessional states. British Medical Journal *26*, 194–198.

Prabhu, L., Cherian, A. V., Viswanath, B., Kandavel, T., Bada Math, S., and Janardhan Reddy, Y. C. (2013). Symptom dimensions in OCD and their association with clinical characteristics and comorbid disorders. Journal of Obsessive-Compulsive and Related Disorders *2*, 14–21.

Purcell, R., Maruff, P., Kyrios, M., and Pantelis, C. (1998). Cognitive deficits in obsessive-compulsive disorder on tests of frontal-striatal function. Biological Psychiatry *43*, 348–357.

Raisman, R., Briley, M., and Langer, S. (1979). Specific tricyclic antidepressant binding sites in rat brain. Nature *281*, 148–150.

Rankin, M., Hazelwood, L., Fre, R., Namkung, Y., Rex, E., Roof, R., and Sibley, D. (2010). Molecular Pharmacology of the Dopamine Receptors. In: Dopamine Handbook, Oxford University Press.

Rauch, S., Jenike, M., Alpert, N., Baer, L., Breiter, H., Savage, C., and Fischman, A. (1994). Regional cerebral blood flow measured during symptom provocation in obsessive-compulsive disorder using oxygen 15-labeled carbon dioxide and positron emission tomography. Archives of General Psychiatry *51*, 62–70.

Ravizza, L., Barzega, G., Bellino, S., Bogetto, F., and Maina, G. (1996). Therapeutic effect and safety of adjunctive risperidone in refractory obsessive-compulsive disorder (OCD). Psychopharmacology Bulletin *32*, 677–682.

Reisine, T., Soubrié, P., Artaud, F., and Glowinski, J. (1982). Involvement of lateral habenula-dorsal raphe neurons in the differential regulation of striatal and nigral serotonergic transmission in cats. The Journal of Neuropsychiatry and Clinical Neurosciences *2*, 1062–1071.

Remijnse, P., Nielen, M. M. A., van Balkom, A. J. L. M., Cath, D. C., van Oppen, P., and Uylings, H. B. M. (2006). Reduced orbitofrontal-striatal activity on a reversal learning task in obsessive-compulsive disorder. Archives of Clinical Neuropsychology *63*, 1225–1236.

Renynghe de Voxrie, G. (1968). Anafranil (G34586) in obsessive neurosis. Archives of Neurology *68*, 167–173 In Cartwright, C., and Hollander, E. (1998). SSRIs in the treatment of obsessive-compulsive disorder. Depression and Anxiety *8*, 105–113.

Ribeiro, E. B., Bettiker, R. L., Bogdanov, M., and Wurtman, R. J. (1993). Effects of systemic nicotine on serotonin release in rat brain. Brain Research *621*, 311–318.

Riedel, M., Straube, A., Schwarz, M. J., Wilske, B., and Müller, N. (1998). Lyme disease presenting as Tourette's syndrome. The Lancet *351*, 1997–1998.

Robbins, T., James, M., Owen, A., Sahakian, B., McInnes, L., and Rabbitt, P. (1994). Cambridge Neuropsychological Test Automated Battery (CANTAB): A Factor Analysis Study of a Large Sample of Normal Elderly Volunteers. Dementia, 266–281.

Robbins, T. W., Gillan, C. M., Smith, D. G., Wit, S. De, and Ersche, K. D. (2012). Neurocognitive endophenotypes of impulsivity and compulsivity : towards dimensional psychiatry. Trends in Cognitive Sciences *16*, 81–91.

Robinson, A., Heaton, R., Lehman, R., and Stilson, D. (1980). The utility of the Wisconsin Card Sorting Test in detecting and localizing frontal lobe lesions. Journal of Consulting and Clinical Psychology *48*, 605–614.

Robinson, D. G., Wu, H., Munne, R., Ashtari, M., Alvir, J. M. J., Lerner, G., Koreen, A., Cole, K., and Bogerts, B. (1995). Reduced caudate nucleus volume in obsessive-compulsive disorder. Archives of General Psychiatry *52*, 393–398.

Rolls, E. T. (2004). The functions of the orbitofrontal cortex. Brain and Cognition *55*, 11–29.

Rondou, P., Haegeman, G., and Van Craenenbroeck, K. (2010). The dopamine D4 receptor: biochemical and signalling properties. Cellular and Molecular Life Sciences : CMLS *67*, 1971–1986.

Rotge, J., Guehl, D., Dilharreguy, B., Cuny, E., Tignol, J., Bioulac, B., Allard, M., Burbaud, P., and Aouizerate, B. (2008). Examen critique Provocation of obsessive – compulsive symptoms : a quantitative voxel-based meta-analysis of functional neuroimaging studies. Journal of Psychiatry & Neuroscience *33*, 405–412.

Rotge, J.-Y., Guehl, D., Dilharreguy, B., Tignol, J., Bioulac, B., Allard, M., Burbaud, P., and Aouizerate, B. (2009). Meta-analysis of brain volume changes in obsessive-compulsive disorder. Biological Psychiatry *65*, 75–83.

Rowe, J. B., Owen, a. M., Johnsrude, I. S., and Passingham, R. E. (2001). Imaging the mental components of a planning task. Neuropsychologia *39*, 315–327.

Rubia, K., Russell, T., Bullmore, E. T., Soni, W., Brammer, M. J., Simmons, A., Taylor, E., Andrew, C., Giampietro, V., and Sharma, T. (2001). An fMRI study of reduced left prefrontal activation in schizophrenia during normal inhibitory function. Schizophrenia Research *52*, 47–55.

Ruscio, A., Stein, D., Chiu, W., and Kessler, R. (2008). The epidemiology of obsessive-compulsive disorder in the National Comorbidity Survey Replication. Molecular Psychiatry *15*, 53–63.

Sahakian, B., and Owen, A. (1992). Computerized assessment in neuropsychiatry using CANTAB : discussion paper. Journal of Royal Society of Medicine *85*, 399–402.

Saka, E., Goodrich, C., Harlan, P., Madras, B. K., and Graybiel, A. M. (2004). Repetitive behaviors in monkeys are linked to specific striatal activation patterns. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience *24*, 7557–7565.

Salín-Pascual, R. J., and Basañez-villa, E. (2003). Changes in compulsion and anxiety symptoms with nicotine transdermal patches in non-smoking obsessive-compulsive disorder patients. Revista de Investigación Clinica *55*, 650–654.

Sampaio, A., Lins, R., Daltro-Oliveira, R., Quarantini, L., do Rosário, M. C., Miguel, E. C., and Hounie, A. G. (2013). Genetic association studies in obsessive-compulsive disorder. Revista de Psiquiatria Clinica *40*, 177–190.

Sampaio, A. S. *et al.* (2015). COMT and MAO-A polymorphisms and obsessive-compulsive disorder: a family-based association study. PloS One *10*, e0119592.

Saxena, S., Brody, a L., Ho, M. L., Alborzian, S., Ho, M. K., Maidment, K. M., Huang, S. C., Wu, H. M., Au, S. C., and Baxter, L. R. (2001). Cerebral metabolism in major depression and obsessive-compulsive disorder occurring separately and concurrently. Biological Psychiatry *50*, 159–170.

Saxena, S., Brody, A., Schwarz, M. J., Baxter, L. R., and Hohagen, F. (1998). Neuroimaging and frontal-subcortical circuiry in obsessive-compulsive disorder. British Journal of Psychiatry. Supplement *173*, 26–37.

Shugart, Y. Y. *et al.* (2006). Genomewide linkage scan for obsessive-compulsive disorder: evidence for susceptibility loci on chromosomes 3q, 7p, 1q, 15q, and 6q. Molecular Psychiatry *11*, 763–770.

Schindler, K. M., Richter, M. A., Kennedy, J. L., Pato, M. T., and Pato, C. N. (2000). Association between homozygosity at the COMT gene locus and obsessive compulsive disorder. American Journal of Medical Genetics *724*, 721–724.

Schoenbaum, G., Setlow, B., Nugent, S. L., and Saddoris, M. P. (2003). Lesions of Orbitofrontal Cortex and Basolateral Amygdala Complex Disrupt Acquisition of Odor-Guided Discriminations and Reversals. Learning and Memory *10*, 129–140.

Schwartz, J. M. (1999). A Role for Volition and Attention in the Generation of New Brain Circuitry. Journal of Consciousness Studies *6*, 115–142.

Singer, H. S., Gilbert, D. L., Wolf, D. S., Mink, J. W., and Kurlan, R. (2012). Moving from PANDAS to CANS. The Journal of Pediatrics *160*, 725–731.

Skapinakis, P., Papatheodorou, T., and Mavreas, V. (2007). Antipsychotic augmentation of serotonergic antidepressants in treatment-resistant obsessive-compulsive disorder: a meta-analysis of the randomized controlled trials. European Neuropsychopharmacology *17*, 79–93.

Skoog, G., and Skoog, I. (1999). A 40-year follow-up of patients with obsessive-compulsive disorder. Archives of General Psychiatry *56*, 121–127.

Snider, L., and Swedo, S. E. (2004). PANDAS: current status and directions for research. Molecular Psychiatry *9*, 900–907.

Sokoloff, P., Diaz, J., Foll, B. Le, Guillin, O., Leriche, L., Bezard, E., and Gross, C. (2006). The dopamine D3 receptor : A therapeutic target for the treatment of Neuropsychiatric disorders. CNS and Neurological Disorders-Drug Targets *5*, 25–43.

Stahl, S. (1998a). Basic psychopharmacology of antidepressants , Part 1 : Antidepressants have seven distinct mechanisms of action. Journal of Clinical Psychiatry *59*, 5–14.

Stahl, S. M. (1998b). Mechanism of action of serotonin selective reuptake inhibitors Serotonin receptors and pathways mediate therapeutic effects and side effects. Journal of Affective Disorders *51*, 215–235.

Stern, E. R., and Taylor, S. F. (2014). Cognitive neuroscience of obsessive-compulsive disorder. The Psychiatric Clinics of North America *37*, 337–352.

Stern, R., Marks, I., Mawson, D., and Luscombe, D. (1980). Clomipramine and exposure for compulsive rituals: II. Plasma levels, side effects and outcome. The British Journal of Psychiatry *136*, 161–166.

Stockmeier, C., DiCarlo, J., Zhang, Y., Thompson, P., and Y, M. (1993). Characterization of Typical and Atypical Antipsychotic Drugs Based on in Vivo Occupancy of Serotonin2 and Dopamine2 schizophrenia. The Journal of Pharmacology and Experimental Therapeutics *266*, 1374–1384.

Storch, E. A., Ledley, D. R., Lewin, A. B., Murphy, T. K., Goodman, W. K., Johns, N. B., and Geffken, G. R. (2006). Peer victimization in children with obsessive- compulsive disorder : relations with symptoms of psychopathology . Peer Victimization in Children With Obsessive – Compulsive Disorder : Relations With Symptoms of Psychopathology. Journal of Clinical Child and Adolescent Psychiatry *35*, 446–455.

Sturm, V., Lenartz, D., Koulousakis, A., Treuer, H., Herholz, K., Klein, J. C., and Klosterkötter, J. (2003). The nucleus accumbens: a target for deep brain stimulation in obsessive–compulsive- and anxiety-disorders. Journal of Chemical Neuroanatomy *26*, 293–299.

Swedo, S. E., Leonard, H. L., Garvey, M., Mittleman, B., Allen, A. J., Perlmutter, S., Dow, S., Zamkoff, J., Dubbert, B. K., and Lougee, L. (1998). Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. American Journal of Psychiatry *155*, 264–271.

Swedo, S. E., Leonard, H. L., Mittleman, B., Allen, A. J., Rapoport, J. L., Dow, S., Kanter, M. E., Chapman, F., and Zabriskie, J. (1997). Identification of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. The A Journal of Psychiatry Journal of Psychiatry *154*, 110–112.

Szechtman, H., Sulis, W., and Eilam, D. (1998). Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD). Behavioral Neuroscience *112*, 1475–1485.

Szeszko, P. R., Robinson, D., Alvir, J. M. J., Bilder, R. M., Lencz, T., Ashtari, M., Wu, H., and Bogerts, B. (1999). Orbital Frontal and Amygdala Volume Reductions in Obsessive-compulsive Disorder. Archives of General Psychiatry *56*, 913.

Tizabi, Y., Louis, V. a, Taylor, C. T., Waxman, D., Culver, K. E., and Szechtman, H. (2002). Effect of nicotine on quinpirole-induced checking behavior in rats: implications for obsessive-compulsive disorder. Biological Psychiatry *51*, 164–171.

Torresan, R. C., Ramos-Cerqueira, A. T. a, Shavitt, R. G., do Rosário, M. C., de Mathis, M. A., Miguel, E. C., and Torres, A. R. (2013). Symptom dimensions, clinical course and comorbidity in men and women with obsessive-compulsive disorder. Psychiatry Research *209*, 186–195.

Tükel, R., Gürvit, H., Ertekin, B. A., Oflaz, S., Ertekin, E., Baran, B., Kalem, S. A., Kandemir, P. E., Ozdemiroğlu, F. A., and Atalay, F. (2012). Neuropsychological function in obsessive-compulsive disorder. Comprehensive Psychiatry *53*, 167–175.

Ursu, S., and Carter, C. S. (2010). An initial investigation of the orbitofrontal cortex hyperactivity in obsessive-compulsive disorder: exaggerated representations of anticipated aversive events? Neuropsychologia *47*, 2145–2148.

Veale, D., Sahakian, B., and Marks, I. (1996). Specific cognitive deficits in tests sensitive to frontal lobe dysfunction in obsessive-compulsive disorder. Psychological Medicine *26*, 1261–1269.

Wadenberg (1993). Dopamine D 2 Receptor Occupancy Is a Common Mechanism Underlying Animal Models of Antipsychotics and Their Clinical Effects. Neuropsychopharmacology *25*, 633–641.

Wahl, K., Kordon, A., Kuelz, K. a, Voderholzer, U., Hohagen, F., and Zurowski, B. (2010). Obsessive-Compulsive Disorder (OCD) is still an unrecognised disorder: a study on the recognition of OCD in psychiatric outpatients. European Psychiatry *25*, 374–377.

Walsh, B., Wilson, G., Loeb, K., Devlin, M., Pike, K., Roose, S., Fleiss, J., and Waternaux, C. (1997). Medication and psychotherapy in the treatment of bulimia nervosa. American Journal of Psychiatry *154*, 523–531.

Walton, M. E., Behrens, T. E. J., Buckley, M. J., Rudebeck, P. H., and Rushworth, M. F. S. (2010). Separable learning systems in the macaque brain and the role of orbitofrontal cortex in contingent learning. Neuron *65*, 927–939.

Wan, Y., Ade, K. K., Caffall, Z., Ilcim Ozlu, M., Eroglu, C., Feng, G., and Calakos, N. (2013). Circuit-Selective Striatal Synaptic Dysfunction in the Sapap3 Knockout Mouse Model of Obsessive-Compulsive Disorder. Biological Psychiatry, 1–8.

Wang, S., Wang, K., Wang, W., and Jen, F. (2004). Mechanisms underlying the riluzole inhibition of glutamate release from rat cerebral cortex nerve terminals (synaptosomes). Neuroscience *125*, 191–201.

Watkins, L., Sahakian, B. J., Robertson, M., Veale, D., Rogers, R., Pickard, K., Aitken, M., and Robbins, T. (2005). Executive function in Tourette 's syndrome and obsessive-compulsive disorder. Psychological Medicine *35*, 571–582.

Wee, N. J. A. Van Der (2005). Windows on the brain Functional neuroimaging studies in obsessive-compulsive disorder, UFB Grafische Media.

Welch, J. M. *et al.* (2008). Cortico-striatal synaptic defects and OCD-like behaviors in SAPAPs mutant mice. Nature *448*, 894–900.

Whiteside, S., Port, J., and Abramowitz, J. (2004). A meta–analysis of functional neuroimaging in obsessive–compulsive disorder. Psychiatry Research: Neuroimaging *132*, 69–79.

Willner, P. (1986). Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. Progress in Neuro-Psychopharmacology & Biological Psychiatry *10*, 677–690.

Willour, V. L. *et al.* (2004). Replication study supports evidence for linkage to 9p24 in obsessive-compulsive disorder. American Journal of Human Genetics *75*, 508–513.

Winter, C., Mundt, A., Jalali, R., Joel, D., Harnack, D., Morgenstern, R., Juckel, G., and Kupsch, a (2008). High frequency stimulation and temporary inactivation of the subthalamic nucleus reduce quinpirole-induced compulsive checking behavior in rats. Experimental Neurology *210*, 217–228.

World Health Organization (1992). International Statistical Classification of Diseases and Related Problems, Geneva: World Health Organization.

Yadin, E., Friedman, E., and Bridger, W. (1991). Spontaneous alternation behavior: an animal model for obsessive-compulsive disorder? Pharmacology Biochemistry and Behavior *40*, 311–315.

Zai, G., Zai, C., Arnold, P., Freeman, N., Burroughs, E., Kennedy, J., and Richter, M. (2015). Meta-analysis and association of brain-derived neurotrophic factor (BDNF) gene with obsessive-compulsive disorder. Psychiatric Genetics *25*, 95–96.

Zor, R., Szechtman, H., Hermesh, H., Fineberg, N. a, and Eilam, D. (2011). Manifestation of incompleteness in obsessive-compulsive disorder (OCD) as reduced functionality and extended activity beyond task completion. PloS One *6*, e25217.