Potentiel thérapeutique de JJ-3-42, un nouvel agoniste sélectif des récepteurs 5-HT2C, dans le traitement des maladies psychiatriques

Thèse

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Résumé

Introduction: Dernièrement, différents éléments de preuve soutiennent les avantages potentiels de l'activation des récepteurs 5-HT2C dans divers troubles psychiatriques. À cet égard, JJ-3-42, un agoniste puissant et sélectif des récepteurs 5-HT2C, a démontré un profil prometteur dans des études préliminaires chez l'animal. Cependant, l'étendue des réponses centrales de ce nouveau composé dans diverses dimensions du comportement des animaux n'est pas encore claire. Dans cette étude, nous avons étudié les implications thérapeutiques possibles de l'activation des récepteurs 5-HT2C après l'administration de JJ-3-42 à l'aide d'un large éventail de modèles animaux de troubles mentaux.

Méthodes: Dans un premier temps, nous avons examiné les résultats principaux de JJ-3-42 dans une batterie de tests comportementaux chez le modèle de souris TPH2-KI avec une déficience importante de sérotonine dans le cerveau. Plusieurs tests standards liés à la cognition, à l'anxiété, aux interactions sociales et au comportement répétitif/compulsif des animaux ont été utilisés. Dans cette étude et pour la première fois, nous avons comparé JJ-3-42 à un ligand de sérotonine endogène non sélectif, le 5-HTP, et à un agoniste puissant et sélectif des récepteurs 5-HT2C, CP809.101, dans une cohorte de souris de type sauvage et homozygote (HO) TPH2-KI R439H. Deuxièmement, nous avons examiné les propriétés modulatrices de JJ-3-42 sur le système de la dopamine en utilisant des indices d'activité locomotrice d'animaux de type sauvage dans différents paradigmes. À cet égard, l'efficacité de JJ-3-42 à résister à l'activité hyperlocomotrice des animaux suite à l'administration de différents psychostimulants a été évaluée. Par ailleurs, la réponse d'une injection aiguë de JJ-3-42 sur l'activité locomotrice innée de DAT-KO HO ainsi que la locomotion de souris DAT-KO HO épuisées en dopamine ont été testées. Enfin, l'effet du prétraitement quotidien de JJ-3-42 sur la réponse locomotrice d'une dose ‘challenge‘ de cocaïne chez des animaux sensibilisés a été étudié.

Résultats: Nous avons observé une réduction significative des tendances comportementales répétitives et agressives des souris TPH2-KI HO après l'administration de divers agents sérotoninergiques. En utilisant deux agonistes sélectifs du récepteur 5-HT2C, CP809.101 et JJ-3-42, nous avons démontré que ces réponses bénéfiques étaient véhiculées par l'activation des récepteurs 5-HT2C dans le cerveau. De plus, nous avons enregistré un fort caractère pro-cognitif et anti-compulsif pour le nouvel agoniste de 5-HT2C, JJ-3-42, dans plusieurs paradigmes comportementaux du modèle murin TPH2-KI. Nos résultats indiquent le profil favorable de JJ-3-42 par rapport à l'autre agoniste sélectif de la 5-HT2C, CP809.10, dans les tests liés à l'anxiété. En fait, nos résultats indiquent qu'à une dose puissante et thérapeutique de 10 mg/kg, JJ-3-42 est dépourvu de toute réponse anxiogène significative chez l'animal. Dans la deuxième partie de nos expériences, JJ-3-42 a montré un profil antipsychotique robuste et dose-dépendant en résistant à l'hyperactivité normalement induite par l'administration d'apomorphine et d'amphétamine. Nos résultats démontrent qu'à une dose de 10 mg/kg, ce composé n'a aucun effet sur l'état hyperdopaminergique inné ou induit des animaux, mais qu'à 20 mg/kg, il diminue considérablement l'activité locomotrice des souris DAT-KO HO et des animaux traités par psychostimulants. Contrairement, JJ-3-42 à 10 mg/kg a potentialisé les réponses locomotrices de la cocaïne et du MK-801 chez des souris, soulignant la complexité de l'interaction des récepteurs de la sérotonine 5-HT2C et du système dopaminergique dans le cerveau. Enfin, le prétraitement quotidien des animaux avec JJ-3-42 à 10 mg/kg n'a pas modifié la réponse d'une dose de 'challenge' de cocaïne chez les souris sensibilisées par rapport à une solution saline dans un protocole de sensibilisation de sept jours.

Conclusion: Ces résultats confirment les effets positifs de l'agoniste du récepteur 5-HT2C JJ-3-42 dans la régularisation de différentes dimensions comportementales chez les animaux. Nos résultats démontrent des propriétés antipsychotiques et procognitives convaincantes pour JJ-3-42 dans divers modèles animaux de troubles mentaux. Ce composé a permis de réduire l'impulsivité et d'améliorer la sociabilité des animaux, sans signe de réponse anxiogène. Ensemble, nos résultats suggèrent que JJ-3-42 pourrait posséder des indications thérapeutiques dans plusieurs dimensions de troubles mentaux telles que la schizophrénie, la toxicomanie ou les troubles obsessionnels compulsifs. Notre étude

implique que ce médicament pourrait éventuellement entraîner moins d'effets secondaires et un meilleur contrôle des symptômes négatifs et cognitifs chez les patients psychiatriques. Enfin, nos résultats indiquent que JJ-3-42 pourrait réduire les indices d'agression et d'impulsivité sans provoquer d'anxiété dans la population cible. Considérant un besoin crucial de développer de nouveaux médicaments ayant une meilleure efficacité thérapeutique et des effets secondaires moindres, les résultats de cette thèse pourraient éventuellement conduire à une amélioration substantielle du traitement des maladies psychiatriques.

Abstract

Introduction: Recently, various lines of evidence support the potential benefits of 5-HT2C receptor activation in different psychiatric disorders. In this regard, JJ-3-42 a potent and selective 5-HT2C serotonin receptor agonist has demonstrated a promising profile in pre-clinical animal studies. However, the extent of central responses of this new compound in various dimensions of animal behaviour is not clear yet. In this study, we investigated the possible therapeutic implications of 5-HT2C receptor activation following the administration of JJ-3-42, using a wide range of animal models of mental disorders.

Methods: Firstly, we examined the central outcomes of JJ-3-42 in a battery of behavioural tests in the brain-serotonin deficient TPH2-KI mouse model. Several standard tests related to cognition, anxiety, social interaction and repetitive/compulsive behaviour were used. In this study and for the very first time, we tested the JJ-3-42 molecule, side-by-side with a non-selective endogenous serotonin ligand, 5-HTP, and a potent and selective 5-HT2C receptor agonist, CP809.101, in a cohort of wild type and homozygote (HO) TPH2-KI R439H mice. Secondly, we examined the potential of JJ-3-42 to modulate the dopamine system using indexes of locomotor activity in wild type animals using different paradigms. In this regard, the efficiency of JJ-3-42 to resist the induced hyperlocomotor activity following the administration of different psychostimulants was evaluated. Moreover, the response of acute injection of JJ-3-42 on the innate locomotor activity of DAT-KO HO as well as the locomotion of dopamine depleted DAT-KO HO mice was tested. Finally, the effect of daily pre-treatment of JJ-3-42 on the locomotor response following cocaine administration in sensitized animals was studied.

Results: We observed a significant reduction of repetitive and aggressive behavioural tendencies of TPH2-KI HO mice following the administration of various serotonergic agents. Using two selective 5-HT2C agonists CP809,101 and JJ-3-42, we showed that these beneficial responses were most likely carried via activation of 5-HT2C receptors in the brain. Moreover, we recorded a strong pro-cognitive and anti-compulsive character for the

new 5-HT2C agonist JJ-3-42 across related behavioural paradigms in the TPH2-KI mouse model. Our results, also, indicate the favourable profile of JJ-3-42 compared to the other selective 5-HT2C agonist CP809.10 in anxiety-related tests. In fact, our findings show that, in contrast to the compounds, at a potent and therapeutic dose of 10 mg/kg, JJ-3-42 is devoid of any significant anxiogenic response in animals. In the second part of our experiments, JJ-3-42 shows a robust and dose-dependent antipsychotic profile by preventing hyperactivity of animals induced by the administration of apomorphine and amphetamine. Our results demonstrates that, at a lower dose of 10 mg/kg, this compound has no effect on the innate or induced hyperdopaminergic state of the animals while at 20 mg/kg, it reduces the locomotor activity in DAT-KO HO mice and psychostimulant-treated WT animals. On the other hand, JJ-3-42, at 10 mg/kg, potentiated the locomotor responses of cocaine and MK-801, pointing out a complex interaction of the serotonin 5-HT2C receptors and the dopamine system. Finally, our results indicate that the daily pre-treatment of animals with JJ-3-42 at 10 mg/kg, does not change the locomotor response of a challenge dose of cocaine in sensitized mice compared to saline in a seven-days sensitization protocol.

Conclusion: These results confirm the positive behavioural outcomes of 5-HT2C receptor agonist JJ-3-42 administration in the regulation of different mouse behaviours. Our results indicate compelling antipsychotic and pro-cognitive properties of JJ-3-42 in various mouse models of mental disorders. This compound successfully reduced the impulsivity and improved the sociability of the animals with no evidence of anxiogenic response. Taken together, our findings suggest that JJ-3-42 might possess therapeutic indications in several dimensions of mental disorders such as schizophrenia, drug addiction or obsessive-compulsive disorders. Our study implies that this drug could conceivably lead to fewer side effects and better control of negative and cognitive symptoms in psychiatric patients. Finally, our results indicate that JJ-3-42 might reduce the aggression and impulsivity indexes in animals, which might point out to the clinical implication of this drug in the future. Considering the crucial need for the development of new drugs possessing better therapeutic efficiency and less side effects, the results presented in this thesis should be

considered a substantial progress towards the advancement of the use of 5-HT2C drugs in the treatment of psychiatric disorders.

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5-HIAA, 5-hydroxyindoleacetic acid
5-HT, 5-hydroxytryptamine, serotonin
5-HT1C, serotonin receptor type 1C
5-HT1Rs, serotonin receptor type 1
5-HT2, serotonin receptor type 2
5-HT2A, serotonin receptor type 2A
5-HT2B, serotonin receptor type 2B
5-HT2C, serotonin receptor type 2C
5-HT3Rs, serotonin receptor type 3
5-HT4Rs, serotonin receptor type 4
5-HT6Rs, serotonin receptor type 6
5-HT7Rs, serotonin receptor type 7
5-HTP, 5-Hydroxytryptophane
5,7-DHT, 5,7-Dihydroxytryptamine
8-OH-DPAT, (±)-8-hydroxy-2-(di-n-propylamino) tetralin
ADAR, adenosine deaminase acting on RNA
BDNF, brain derived neurotropic factor
cAMP, Cyclic Adenosine Monophosphate
CHO Chinese hamster ovary
CNS, central nervous system
COMT, catechol-o-methyltransferase
CSF, cerebrospinal fluid
D1, Dopamine receptor type 1
DA, Dopamine
DAG, diacylglycerol
DOI, 2,5-dimethoxy-4-iodoamphetamine
DSM, Diagnostic and Statistical Manual
ERK, extracellular signal-regulated kinase
FC, frontal cortex
FDA, Food and Drugs Administration
FRL, Flinders Resistant Line
FSL, Flinder sensitive line
GABA, gamma-aminobutyric acid
GPCR, G protein-coupled receptor
GRK, G protein receptor kinase
GSK, glycogen synthase kinase
GWAS, genome-wide association study
Gαs, the Gs protein alpha subunit
Gβ-γ, the G protein beta-gamma complex
HEK293, human embryonic kidney 293 cells
HO, homozygote
ICD, International Classification of Disorders
INI/VSV, INI/VGV and VSV/VGV
IP3, inositol 1,4,5-triphosphate

LSD, lysergic acid diethylamide
MAO, monoamine oxidase
MDMA, 3,4methylenedioxymethamphetamine
mPFC, medial prefrontal cortex
mRNA, messenger ribonucleic acid
NA, nucleus accumbens
OCD, obsessive-compulsive disorder
PCP, Phencyclidine
pCPA, 4-Chloro-DL-Phenylalanine, tryptophan hydroxylase inhibitor
PET, positron emission tomography
PFC, prefrontal cortex
PI3K, phosphoinositide 3 kinase
PKC, protein kinase C
PLA2, phospholipase A2
PLC, phospholipase C
PLD, phospholipase D
PPI, Pre-pulse inhibition test
RNA, ribonucleic acid
SNc, substantia nigra pars compacta
SNP, single-nucleotide polymorphisms
SSRIs, serotonin-specific reuptake inhibitors
TCA, tricyclic antidepressant
TFMPP, 3-Trifluoromethylphenylpiperazine
US, United States
VTA, ventral tegmental area
WT, wild type
Δ9–THC, tetrahydrocannabinol

Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a multipurpose neurotransmitter in the brain that plays an essential role in the regulation of many basic physiological states like sleep, appetite, body temperature as well as more complex functions including mood, emotion, cognition and memory in animals [1-8]. Based on such vast and complex involvement in central responses, the implication of serotonin in the presentation of various mental diseases is not surprising. The fundamental role of serotonin in the pathophysiology of many psychiatric illnesses like anxiety, mood, and psychotic disorders has been demonstrated in the scientific literature [9-12]. Serotonin is synthesized in the body from essential amino acid L-tryptophan by tryptophan hydroxylase (TPH) enzyme [13, 14]. Two isoforms of this enzyme in humans have been identified, TPH1 and TPH2 [13, 14]. TPH1 is the predominant form in the peripheral tissues and the pineal gland. TPH2 is mainly responsible for making serotonin in the brain [13, 14]. The synthesis of serotonin in the brain mostly occurs at dorsal and medial raphe nuclei in the brainstem [15]. The serotonergic neurons originating from these structures reach all major parts of the brain through a vast branching and complex network of axons [16, 17]. Serotonin produced in these neurons is transported and stored in synaptic vesicles at pre-synaptic endings. Neuronal electrical activity leads to release of the content of these vesicles in the synaptic cleft and subsequent binding of serotonin to pre- and post-synaptic receptors (Figure 1.2.) [18]. These receptors carry out multiple central and peripheral functions of serotonin [18].

The vast effects of serotonin in the brain are mediated through seven distinct families of receptors, 5-HT1 to 5-HT7 with at least 14 receptor subtypes (5-HT1A, 1B, 1D, 1E, 1F; 2A, 2B, 2C, 3, 4, 5A, 5B, 6 and 7) [18, 19]. These receptors are all G-protein-coupled receptors (GPCRs), except for 5-HT3, which is a ligand-gated cation channel. The signalling of serotonin receptors is extremely diverse and serotonin receptor subtypes recruit all three canonical pathways through Gαi, Gαq/11, and Gαs proteins [18, 19]. In addition to multiple classes of receptors, serotonin transporter (SERT) plays an essential role in serotonergic neurotransmission [20]. After release in synaptic cleft, SERT reuptakes serotonin back into pre-synaptic neurons where it can either be

restored in synaptic vesicles or metabolised to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase A (MAO-A) enzyme. SERT is located both at the soma and the terminal axons of serotonin-containing neurons [21, 22]. By determining the synaptic serotonin concentration in the synaptic cleft, SERT activity moderates the response of serotonin receptors (Figure 1.2.).

Recently, the role of distinct serotonin receptors in the pathophysiology of different mental disorders has been studied extensively [18]. Among various serotonin receptors, 5-HT2C receptors (5-HT2CRs) have been at the center of the latest research in the field of neuroscience [9]. There are compelling scientific arguments indicating the central involvement of these receptors in mental disorders. Various psychopathologies like mood and anxiety disorders have been linked to the occurrence of different mutations in the gene encoding 5-HT2CRs in the population [17, 23-25]. Furthermore, it has been shown that such genetic variations can modulate the therapeutic as well as adverse responses of various medications in different groups of patients [26-28]. Besides genetic evidence, the involvement of 5-HT2C receptors in mediating the beneficial effects of neuroleptics and antidepressants has been repeatedly documented in pharmacological studies [9, 29]. In recent years, compounds with activity at the level of 5-HT2C receptors have been examined in several clinical trials for the management of addiction, mood and psychotic disorders [9, 30]. Related to this, successful marketing of Agomelatine for the treatment of obsessive-compulsive and mood disorders marks the trend of the current pharmaceutical industry to harvest the therapeutic potential of these receptors. Finally, 5-HT2CRs are the only known GPCRs that go through pre-mRNA editing process, which allows the presence of a distinguished set of 5-HT2CRs isomers at the cell surface of various regions of the brain [31]. This unique phenomenon is believed to have clinical importance in the neurotransmission of serotonin neurons in the brain as well as the response of medication in patients [32]. However, to elucidate the exact implications of this process, further investigations are needed in the upcoming years. Taken together, these results point out the distinctive and significant involvement of 5-HT2CRs in the pathophysiology of mental disorders and endorse the future research to probe in their therapeutic potentials.

The aim of the present thesis is to examine the implications of a new 5-HT2CRs agonist JJ-3-42 as a potential treatment of various dimensions of mental disorders in pertinent animal models. The following chapters will be discussing the relevant genetic, molecular and pharmacological aspects of 5-HT2C receptors in connection with some of the most common psychiatric disorders. Further, available results of different 5-HT2CRs active drugs in related dimensions of human and non-human animal behaviour will be reviewed. In this regard, we will particularly examine the recently published results of new and selective 5-HT2CRs agonist JJ-3-42. We will debate the pertinence of our animal model, TPH2-KI brain serotonin-deficient mice, compared to other models available to study the central responses of serotonin. Then, we will present the outcomes of our new 5-HT2CRs agonist in a battery of behavioural tests conducted in our transgenetic animal model. Finally, we will discuss our findings in the light of current knowledge of central responses of 5-HT2C receptors in the field of psychopharmacology.

1. 5-HT2C receptors

1.1. Genetics of 5-HT2C receptors

The gene encoding 5-HT2C receptors, HTR2C, is located on chromosome X in both human (position q24) and mice (position d-F4) [33, 34]. This means that, since the male has just one copy of the gene, even a recessive allele would be expressed in phenotype while in female different variants are expressed in cells, hence the mosaic polymorphism [33, 34]. The entire HTR2C gene in humans consists of six exons and five introns [35]. This genetic structure possesses three cutting sites that are recognized in the process of alternative splicing resulting in three splice variants of the 5-HT2CRs in the cell [35]. The full length functional receptor is the product of cutting the amino acid chain on the first site while the other two create truncated non-functional 5-HT2C receptors [36]. Though the significance of such splicing in case of 5-HT2CRs is not clear yet, some proposed that it operates as a mechanism of quality control in the cellular transcription machinery [35, 37]. In fact, there is some new evidence showing that the two severely truncated forms may also serve as chaperon molecules and seem to be affected in certain disease [36, 38, 39].

Besides alternative splicing, which is a common feature in various proteins, 5-HT2CRs undergo a unique molecular process called mRNA editing. RNA editing is the substitution or modification of the sequence of pre-mRNA due to the activity of cytidine deamination (which converts cytidine to uracil) or adenosine deamination (which converts adenosine to inosine) in the cell [40]. Among various classes of GPCRs, 5-HT2C receptors are the only known receptors that go through pre-mRNA editing. This is important since this process results in the generation of multiple functional variants of the receptor in post-transcriptional modifications [41]. In the case of 5-HT2CRs, adenosine deaminase acting on RNA 1 and 2 (ADAR1 and 2) replaces adenosine with inosine in pre-mRNA in various points of the sequence [41]. The translation of this new RNA leads to the synthesis of a protein with a new amino acid sequence. In the HTR2C gene in humans, RNA editing occurs at five positions (A, B, C, D and E) that eventually could change the amino acid sequence [42]. In this case, alteration of the sequence due to RNA editing

can produce 32 different transcript combinations, which can generate 24 different proteins from the non-edited isoform INI, to fully edited isoform VGV [43, 44].

The significance of these sequence modifications lies in the fact that this happens in the critical second intracellular loop region of the receptor (Exon 5). This could eventually modify receptor activity by shifting the interaction of the receptor and corresponding G-protein [45]. The example of such variation in the cytosolic signalling cascade has been shown in the decreased constitutive activity of edited RNA isoforms toward the phospholipase C (PLC) pathway in comparison to the non-edited RNA isoforms [46]. Although, fully edited VGV isoform has a very high cell surface expression, it has lost almost all of its constitutive activity while non-edited INI is the most efficient isomer in recruiting the G-protein [47]. Moreover, this reduction has been observed to be in correlation with the degree of the edition in RNA isoform [48]. This is noteworthy since it has been demonstrated that the abundance of serotonin results in the higher expression of the isoforms with lower activity upon stimulation as a negative feedback loop [31]. Such direct regulation of the expression of the receptor by serotonin concentration leads to the presentation of various profiles of 5-HT2C isoforms in a regional and temporal manner in the brain. As a result, a varied concentration of 5-HT2C receptor isomers in different parts of the animal's brain has been documented [41]. For example, in the hippocampus of the rat, 36 percent of isoforms consisted of VNV type, however, this was just 6 percent in choroid plexus [49]. This observation was also carried across species as VSV variant was the most frequent isoform of 5-HT2CRs in the human brain, in contrast to VNV in rats [49]. So far, 5-HT2CRs are the only known GPCRs that possess such particular characteristics [31].

Since the discovery of these modifications, researchers discussed whether pre-RNA editing of the 5-HT2CRs had any physiological and pathological significance in the brain [44]. Therefore, the importance of such post-transcriptional editing and the consequent altered innate activity of these receptors has been the focus of different studies [31, 36]. Some of these relevant animal studies observed the alteration of RNA editing profile following the drastic changes in serotonin neurotransmission [50]. For example, depletion of the brain serotonin by pCPA, an inhibitor of tryptophan hydroxylase, was associated with alteration of RNA editing and increase of the VNV isoform of 5-HT2CRs in animals [31]. On the other hand, in this study serotonin 5-HT2A/2C

receptor agonist DOI induced an opposite RNA editing profile [31]. In parallel, some behavioural models of acute and chronic anxiety and depression led to alteration of RNA profile in animals [51]. For instance, changes in RNA profiles have been observed as soon as 1 hour after water-maze challenge test [52]. More importantly, the beneficial effects of some psychoactive drugs like Fluoxetine, Imipramine and Risperidone in some of the behavioural tests were connected to the alteration of corresponding RNA editing profile in animals [52, 53]. For instance, chronic administration of Fluoxetine has been reported to change the mRNA expression pattern of 5-HT2CRs in the cortex and hippocampus of rats, reflecting its positive response [54].

It is postulated that the constitutive outcome of the 5-HT2CRs might alter the activity of various brain networks related to psychiatric disorders. It has been reported that varied degrees of RNA editing in different sub-groups of 5-HT2C positive cells led to different behavioural outcomes in animals [55]. For example, in animals expressing only the fully edited VGV isoform, higher aggression and anxiety level, compared to the controls, were recorded [38]. Further, this has been associated with reduced serotonin neurotransmission as the result of altered constitutive activity of 5-HT2CRs in these mice [38]. Similarly, some evidence indicates that 5-HT2CRs RNA profile determines behavioural responses of animals in related animal settings. For instance, comparison of three different strains of mice showed that the constitutive higher anxiety level in two strains of mice (BALB/CJ and DBA/2J) to C57BL/6J was related to a higher functional RNA editing profile in these mice [56, 57]. Similarly, the degree of the edition of 5-HT2CRs mRNA correlated with the behavioural outcome of animals in the high-impulsive and low-impulsive strain of rats [58]. The same group also demonstrated that certain 5-HT2CRs profiles reduced the vulnerability to cocaine-associated cues, probably due to alteration of serotonin signalling capacity in the brain cells of the animals [58, 59]. Considering these new findings, the contribution of RNA editing to psychiatric disorders in humans has been the subject of recent studies. As a result, the RNA editing profile in various groups of mental disorder patients had been compared to the normal population [51, 60-62]. In this respect, a significant difference in RNA editing profile in the prefrontal cortex (PFC) of patients suffering from the mood disorder and their counterpart controls has been demonstrated [61]. Other studies confirmed that the presence of major depression is related to an increased level of RNA editing particularly at D site of PFC in patients [32, 60]. This regional variation of 5-HT2C mRNA editing has been also

shown in the brain of suicide victims [31, 32]. However, due to relatively small sample sizes and variations in genetic background, disagreements in these studies are not unusual. For instance, while altered RNA editing status, like significant decrease of VGV isoform, has been detected in bipolar disorder patients, other studies did not report any difference to the control group [39, 60].

Similarly, several studies have tried unsuccessfully to establish an association between the presence of schizophrenia and the degree of 5-HT2CRs editing in the PFC of the patients [36, 45]. In one study, however, the global decrease of RNA editing and the increase of non-edited INI isoform at the B site in the PFC of schizophrenic patients were reported [62]. While the contradictory results make it difficult to come to a definite conclusion about the significance of the RNA editing in the pathology of bipolar disorders and schizophrenia, there is reliable data linking such variations to the occurrence of suicide. In most of the studies, there is an increased rate of RNA editing in the PFC of the suicide victims independent of underlying mental pathology [32, 61]. Lastly, other CNS pathologies including Prader–Willi syndrome, anxiety disorders and obesity have also been connected to RNA editing variations in human populations showing the extensive nature of the implications of this mechanism [38, 63, 64]. To better understand the significance of RNA profile editing of 5-HT2C receptors in the brain and its contribution to the pathophysiology of mental disorders more studies in the future are needed.

Besides the role of RNA editing, the importance of variations of HTR2C gene in the population affected by psychiatric disorders has been studied recently. As in the case of any other gene, HTR2C also is the subject of multiple mutations during the process of evolution. Recent advancement of the genetic science has helped us to better comprehend the contribution of these receptors to the pathophysiology of various dimensions of mental diseases in humans. Historically, most of variations of HTR2C gene have been identified in the context of feeding disorders and obesity in the normal population [65]. Some of these alleles have been related to the likelihood of metabolic syndrome and obesity as the outcome of treatment with antipsychotics [26, 27, 66]. This is important since obesity and other metabolic side effects like dyslipidemia and resistance to insulin are major sources of fatal comorbidities and causes of noncompliance in psychiatric patients [67, 68]. Related to this, the role of certain variants of HTR2C in metabolic responses of medication, especially the weight gain due to the treatment

with the new generation of the antipsychotics, has been studied [26, 69]. For example, the amount of weight gain observed after the treatment of antipsychotics was associated with the presence of certain alleles of -759C/T, -997G/A, and -1165A/G polymorphisms in the population [70, 71]. These mutations have been particularly associated with Clozapine-induced weight gain in multiple studies, although some reports have observed no connection [72-74]. Interestingly, T allele of -759C/T and C allele of -697G/C have rendered the subjects resistant to antipsychotic-induced obesity compared to the control group [26, 27, 66]. General accounts of review studies, however, suggest a definitive role for the HT2CR gene polymorphisms in weight gain and metabolic syndrome experienced by the various subgroups of people in response to antipsychotics [67, 68].

Besides metabolic response, there has been a long debate in the scientific community whether the presence of HTR2C mutations contributes directly to the occurrence of mental disorders in the population or not. Interestingly, though most of the HTR2C mutations have been studied in the perspective of their implication in obesity, some polymorphisms of HTR2C gene have been correlated to a variety of psychopathologies in recent years [9, 25, 75]. Among the single-nucleotide polymorphisms (SNPs) of HTR2C, Cys23Ser rs6318 (C/G) in human has probably been studied more than any others [9]. This single nucleotide polymorphism (SNP) leads to the substitution of a serine for cysteine at 23rd amino acid of the receptor [25, 75]. Though initial investigation of HTR2C gene polymorphisms could not identify any association of Cys23Ser allele with the presence of mental disorders, the involvement of this mutation in the incidence and the course of various diseases has been revealed in recent years [25, 75]. For example, Cys23Ser polymorphism was a consistent risk factor for multiple hospitalizations and lengthier admissions in schizophrenic patients [24]. Moreover, increased frequency of bipolar and major depressive disorder (MDD) in the population has been in a positive correlation with the presence of Cys23Ser polymorphism [23-25]. Also, Cys23Ser and other HTR2C gene mutations, have been related to a higher probability of impulsivity, as well as anxiety and obsessive-compulsive disorder (OCD) in different subgroups of patients [26, 65, 69, 76]. Further, the presence of the psychotic feature in Alzheimer's disease patients has been significantly associated with the occurrence of this allele [24, 77]. This mutation was related to the development of orofacial

tardive dyskinesia in female schizophrenic patients after long-term treatment with neuroleptics [24, 75].

Interestingly, Cys23Ser polymorphism does not appear to be associated with the classical weight gain observed in patients after the treatment with antipsychotics [72]. Whether the therapeutic response of antipsychotics and this specific polymorphism in the HTR2C gene were related has been debated for many years. In fact, up until recently, there were a series of conflicting reports regarding implications of Cys23Ser allele in positive therapeutic responses in patients [25]. In a recent meta-analysis compiling different populations in various centers, a weak association of occurrence of the Cys23Ser polymorphism and positive Clozapine response in patients has been shown [78]. However, other studies could not find any relationship between antipsychotic efficacy and the profile of this HTR2C polymorphism [79, 80]. It is notable that genetic studies of various mutations involved in the pathophysiology of mental disorder are a relatively new and expanding field of research. The considerable amount of replication failure and contradictory data, especially in mental disorders, are not infrequent [81, 82]. Variance in the size of the studied population, genetic background, and different treatment or diagnostic criteria across these investigations have been suggested as explanations of such disparity among studies [81, 82].

While genetic research in humans focuses on natural variations of the gene in the population, the study of the consequences of a more drastic genetic manipulation has been the basis of genetically modified animal models. In fact, the behavioural studies of mice lacking 5-HT2CRs shaped our understanding of biological function of these receptors in various psychiatric dimensions. In this regard, the study of 5-HT2CRs knock-out (5-HT2C-KO) mice has confirmed the metabolic profile of these receptors. These mice showed a plethora of metabolic symptoms from insulin resistance and type 2 diabetes to various degrees of late-onset obesity due to hyperphagia [83-87]. Similarly, these mutants were resistant to the weight-losing effect of Fenfluramine, a serotonin-releasing agent, and other comparable appetite suppressant drugs in contrast to the control animals [87, 88]. On top of the metabolic profile, abnormal behaviours of these mice in various related paradigms imply the involvement of these receptors in the pathophysiology of mental disorders. For instance, 5-HT2C-KO adult mice showed repetitive

and compulsive behaviours including a distinct and “neat” pattern of chewing non-nutritive clay or plastic materials [84, 89]. Also, these mice were hypersensitive to repeated stress and displayed altered responses in body weight and level of stress hormones following exposure to stressful conditions [84, 89]. Remarkably, an anxiolytic phenotype in elevated zero-maze, open-field and novel object recognition test, despite normal locomotor capacity, have been documented in these animals [57]. Spontaneous convulsive attacks and lower threshold for audio-induced seizures are, among others, pathological features in these mice [90, 91]. Furthermore, abnormal responses of 5-HT2C-KO mice to psychostimulants have been observed across various studies. For instance, exaggerated cocaine-induced hyperactivity and motor impulsivity have been reported in this model [92-94]. Finally, an imbalanced neuroendocrine regulatory mechanism along the malfunctions of various neural networks as the consequent of the absence of 5-HT2CRs has been proposed in these mice [84, 87, 89, 94]. The ensemble of these results points to the important implications of 5-HT2CRs in regulation of a wide range of behaviours in animals.

The results of genetic studies in humans and animals offered a valuable understanding of the role of these receptors in pathophysiology of mental disorders in recent years. Advancing the comprehension of the central functions of 5-HT2C receptors, these studies have recently guided several clinical drug studies. For example, trials of drugs acting on these receptors led to the introduction of Lorcaresin as a new treatment of obesity to the market. Currently, there are new clinical trials trying to examine the benefits of 5-HT2CRs ligands in the treatment of addiction to various drugs of abuse in humans [34, 65]. Lastly, new lines of investigations have shown the clinical implications of these receptors in controlling the resistant cases of epilepsy based on the findings of animal models [34, 65]. Taken together, the genetic studies pointed out to the involvement of the 5-HT2CRs in the pathophysiology, treatment response and the course of various mental disorders in the afflicted population in recent years. Furthermore, these findings have outlined the potential therapeutic benefits of this serotonin receptors in the treatment of various psychiatric and non-psychiatric disorders.

1.2. Pharmacology of 5-HT2C receptors

5-HT2CRs were initially termed 5-HT1C receptors after their discovery in 1984 [95, 96]. In 1993, a new nomenclature was adopted, organising serotonin receptors into 7 subfamilies. Subsequently, 5-HT1C receptors were renamed 5-HT2C receptors [97]. With the exception of 5-HT3Rs, serotonin receptors are all GPCRs [18]. These receptors are all composed of an extracellular N-terminal domain, a cytosolic C-terminal domain, and seven-trans-membrane alfa-helices in the middle. The extracellular loops and trans-membrane core are involved in ligand binding at the surface of the cell [98]. On the other hand, the three intracellular loops and C terminus are critical for binding of the receptor to G protein. This interaction is essential for the subsequent signal transmission, trafficking and desensitization/resensitization processes in the cytosol [99]. The activation of 5-HT2CRs at the surface of the cell is the first step of mediating the various responses of the ligand by activation of a network of intracellular signalling proteins. Presently, 14 subgroups of serotonin receptors have been identified and cloned [100]. The serotonin subtypes 5-HT1Rs (5-HT1ARs, 5-HT1BRs, 5-HT1DRs, 5-HT1ERs, and 5-HT1FRs) and 5-HT5Rs usually couple to Gαi protein, which ultimately leads to decrease of intracellular cAMP through inhibition of adenylyl cyclase [18]. On the contrary, 5-HT4Rs, 5-HT6Rs, and 5-HT7Rs are Gαs-coupled, so they positively modulate adenylyl cyclase and increase the levels of cAMP [18, 101]. However, 5-HT2ARs, 5-HT2BRs, and 5-HT2CRs, are Gαq/11-coupled receptors, so following their activation, recruitment of phospholipase C leads to augmentation of the calcium level and production of inositol triphosphate (IP3) and diacylglycerol (DAG) in the cytoplasm (Figure 1.2.) [19, 102]. Subsequently, the activation of several proteins in the endoplasmic reticulum, particularly calmodulin, is responsible for several cellular responses following the production of IP3 (Figure 1.2.). On the other hand, DAG can activate protein kinase C (PKC) leading to a distinct cascade of intracellular responses [19]. In both of these pathways, activation of a chain of reactions eventually leads to the transcription of target genes and production of corresponding proteins in the cell [101].

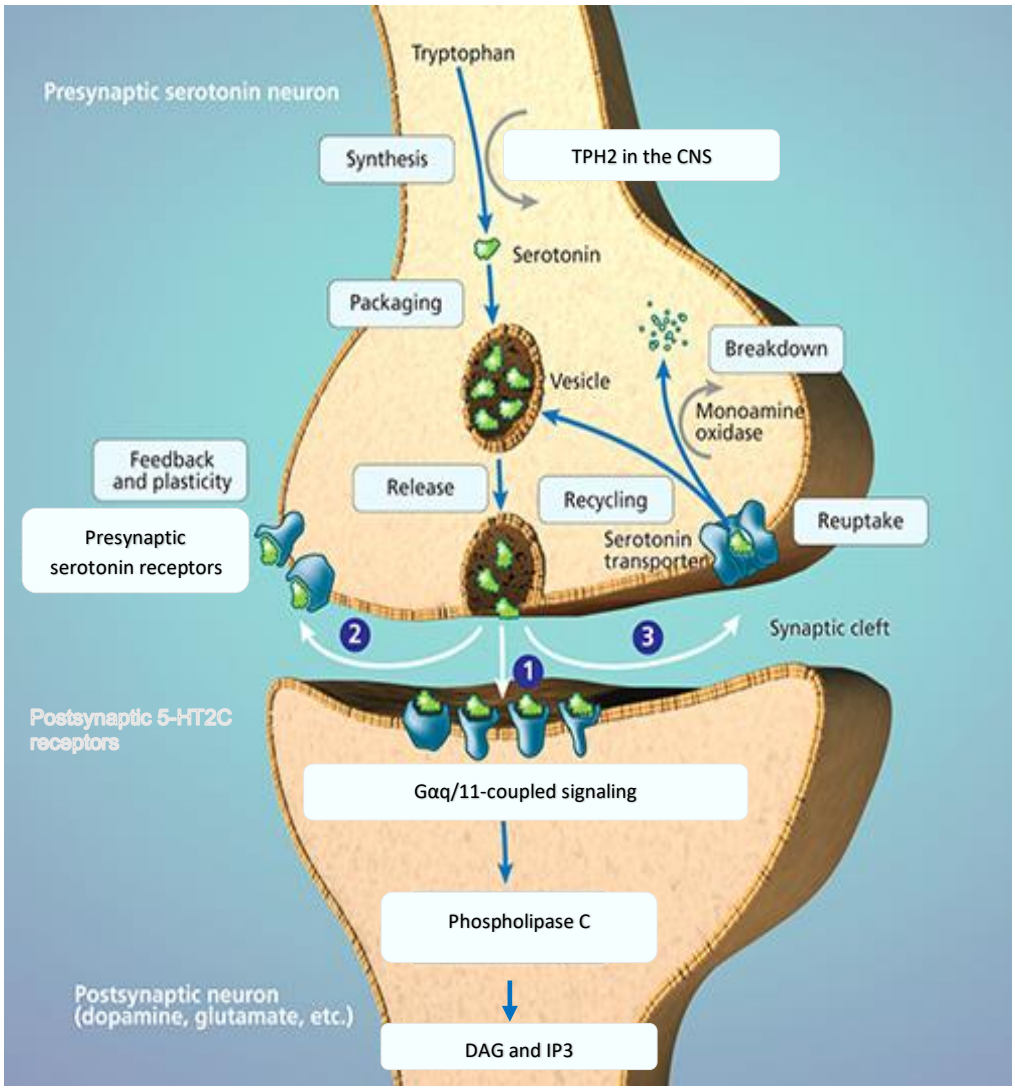

Figure 1.2. Schema of serotonin cycle in the synaptic cleft, Marije aan het Rot et al. CMAJ 2009 (Modified)

Besides the well-known canonical pathway that represents the basal activity of the 5-HT2C receptors in the majority of cells, there are reports of engagement of other signalling pathways in different settings downstream of these receptors. For example, 5-HT2CRs activation can also recruit phospholipase A2 (PLA2), thus releasing arachidonic acid through a pertussis toxin-sensitive G protein as a signal mediator [103, 104]. In addition, through Gα13 protein coupling, 5-HT2CRs binding can activate phospholipase D (PLD), which releases Gβ-γ complex and recruits extracellular signal-regulated kinase (ERK) 1 and 2 [104, 105]. In Chinese hamster

ovary (CHO) cells, it has been observed that 5-HT2CRs even coupled to Gαi protein leading to the reduction of cAMP concentration [104, 106]. Finally, a calmodulin-β Arrestin pathway, a G-protein independent interaction, has been described conveying various cellular responses after the activation of 5-HT2C receptors [107, 108]. The importance of these multiple signal transduction pathways and their significance in regards to the different cell types expressing 5-HT2C receptors in different physiological or pathological conditions remains unclear. However, various factors have been shown to be involved in determining the dynamics of intracellular signalling pathways downstream of 5-HT2CRs.

It has been shown that 5-HT2CRs RNA editing and isomer arrangement at the surface of the cell could impact signal transduction pathways downstream of these receptors [61]. In fact, the employment of different editing variants results in, not only the modulation of the degree of affinity for corresponding G-protein, but also in the determination of the type of G protein employed [55]. For example, the unedited receptor isomer couples to both Gαq/11 and Gα13 proteins, whereas it has been observed that increased editing reduces or eliminates coupling to Gα13 pathway [47]. Related to this, the impact of RNA editing on altering the signalling capacity of 5-HT2CRs by forming homodimers and heterodimers has been discussed [63, 111, 112]. Homodimers are made of two identical receptors while heterodimers are the complex of two different proteins [113]. Endogenously, on the plasma membrane of epithelial cells of choroid plexus, 5-HT2CRs exist in a dimer form [113]. These homodimers are found in the endoplasmic reticulum of the cells and, thereafter, are transported to the plasma membrane through Golgi apparatus [114, 115]. Formation of these dimers is essential for 5-HT2CRs function as two molecules of ligand bind to one dimer complex that interacts only with one G-protein [116]. It is worth mentioning that higher order complexes of 5-HT2C receptors have not yet been reported in the natural state, but they have been documented following their artificial overexpression in Human embryonic kidney (HEK) 293 cells [117, 118]. Interestingly, dimers of 5-HT2CRs can occur with both non-edited and fully edited isoforms [115, 119]. For example, INI/VSV, INI/VGV and VSV/VGV isoform pairs have been reported in HEK293 cells [113, 119]. The prevalence of such dimers and their physiological significance in the innate activity of 5-HT2C receptors in the brain is not fully understood. Moreover, while the formation of

heterodimers of 5-HT2CRs and different classes of receptors has been reported in many in various in vitro studies, these formations have not been observed in vivo yet [113, 119].

Nonetheless, there are some speculations about the possible function of such heterodimers in various contexts. For instance, the functional imbalance of 5-HT2C and 5-HT2A receptors in the medial prefrontal cortex (mPFC) has been suggested to be involved in impulsive and aggressive behaviour of animals [92]. Similar functional interaction through heterodimers of 5-HT2C and 5-HT2A receptors regarding the addictive and food intake behaviour of animals has been suspected as well [120, 121]. In line with this, the heterodimers of 5-HT2CRs and other groups of receptors, like ghrelin binding receptors, have been demonstrated in vitro [63]. Diminished ghrelin-mediated calcium mobilization in interaction with the fully active unedited form of 5-HT2C receptors, but not partially edited isoform, has been observed [63]. In addition, Lorcaserin, 5-HT2CRs agonist prescribed in the treatment of obesity, prevented ghrelin-induced food intake in mice [111]. Following this, working heterodimer between ghrelin receptors and 5-HT2CRs was proposed to mediate some of the observed effects of Lorcaserin in rat neurons [111]. Such complexes are also believed to play a role in appetite signalling and in stress-induced feeding behaviour in animals [111]. Related to this, the presence of functional heterodimer complexes formed by 5-HT2C and melatonin receptors has been proposed to be involved in mood disorders [122]. In fact, it is hypothesized that Agomelatine, as an antidepressant with an affinity for both melatonin and 5-HT2C receptors, interacts with such heterodimers in the brain [85]. To reach a concrete conclusion regarding the final role of these heterodimers in the pathophysiology and treatment of mental disorders, more research in future is required. Nevertheless, the ensemble of these studies indicates that RNA editing, through determination of innate activity of the 5-HT2C receptors, might be responsible in the pathogenesis as well as the treatment response of some mental disorders.

In addition to the inherent properties of the receptor, the particularities of a certain ligand can also modulate the intracellular signalling pathway downstream of the receptor. As such the “agonist-directed trafficking” concept is used to explain the signalling variation induced by different ligands downstream a distinctive receptor [123, 124]. Theoretically, following the ligand-receptor binding, the distinct spatial conformation of the receptor intracellular domain

plays a determining role in activation of specific G protein [125, 126]. This leads to recruitment of various scaffolding molecules and controls the ultimate intracellular response of the ligand [125, 126]. It is noteworthy that this notion opposes the classical interpretation of ligand-receptor interaction, as an "on or off" switch, in place for so many years [123, 127]. With that in mind, various agonists or antagonists specific for a given receptor are potentially capable of recruiting or blocking different signalling pathways downstream of that receptor distinctively. The importance of this process, which has also been called "biased agonism" or "functional selectivity" in the framework of central functions of 5-HT2C receptors has been investigated recently. It has been observed that PLC and PLA2 signalling pathways are preferentially activated in the constitutive state of 5-HT2CRs positive cells [128, 129]. However, this might change with the presence of different ligands [128, 129].

A good example of such complex interaction has been shown in cultured cells by using various 5-HT2C antagonists. For example, SB 206553 displayed strong inverse agonist properties on PLC, PLA2 and Gαi responses [130]. While SB 243213 (an antagonist at PLC) demonstrated partial inverse agonist response at PLA2 and a full inverse agonist property against Gαi activation [130]. Finally, SB 242084, another 5-HT2CRs antagonist, was equally effective as an inverse agonist toward PLA2 and Gαi activation but showed low efficacy agonism for PLC pathway [131]. These examples illustrate the intricacy of signalling mechanism downstream of 5-HT2CRs in the presence of distinct ligands. In a similar way, it has been shown that certain ligands could shift the response of GPCRs toward the Arrestin-dependant or G-protein-independent signalling pathways in cells. The importance of such biased responses in mediation of the side effects of some medications and development of tolerance in patients has been debated [124]. Interestingly, for the 5-HT2C receptors, G-protein-independent pathway has been linked to the innate activity of receptors in the brain [109, 132]. G-protein-independent pathway depends on the recruitment of different proteins, especially Arrestins, as stabilizing protein scaffold [133]. Arrestins are a small family of proteins (4 members) implicated in regulating signal transduction of GPCRs. Arrestin-1, Arrestin-2 (also called β-Arrestin-1), Arrestin-3 (also called β-Arrestin-2) and Arrestin-4 are the only known members of this family [123, 126]. Briefly, following the binding of a ligand to the receptor, phosphorylation of the C terminal of the receptor at specific locations starts a chain of intracellular reactions [124]. This leads to

recruitment of Arrestin molecules which usually function as scaffolding and chaperon proteins for the ligand-receptor complex. The Arrestin molecule employment stops sterically further G-protein coupling to the receptor and usually marks the end of ligand-receptor interaction [127]. Arrestin addition stabilizes the ligand-receptor complex in endoplasm. This formation may or may not lead to either desensitization, resensitization or termination of the original response depending on the presence of different factors [124]. Moreover, such complex might even activate a totally different signalling pathway by recruiting alternative proteins in the cells [123].

The modulatory role of scaffolding proteins, especially the Arrestin family, in the regulation of central responses of different receptors has been shown lately [123, 127]. It is now known that 5-HT2C receptors activate a variety of G-protein independent intracellular pathways, at least in experimental settings [107]. Interestingly, the degree of constitutive activity of various 5-HT2CRs isomers has been associated with their predisposition of G-protein-independent signalling [109]. This means that RNA editing affects the addition of Arrestin molecules to the intracellular protein complex and determines the outcome of biological response through desensitization and trafficking processes [109]. For example, the fully edited VGV isoform does not recruit Arrestin molecule and solely goes through agonist-dependent endocytosis [109]. However, the non-edited form binds to Arrestin in an agonist-independent manner. This conveys the constitutive activity of these receptors in intracellular compartment [109]. Besides the involvement in the innate activity of the receptors, G-protein independent pathway has been implied in the final responses of activation of 5-HT2CRs . For example, in HEK 293 cells, the proximal region of 5-HT2CRs mediated a calmodulin-β-Arrestin dependant signal [132]. It has been shown that such Arrestin dependant signalling is responsible for the induced neurogenesis and antidepressant response of 5-HT2CRs receptors [132]. Despite such results, the understanding of full implications of such G-protein independent pathway in various dimensions of mental disorder demands additional investigations. The ensemble of these reports briefly depicts the intricacy of regulating factors involved in 5-HT2CRs intracellular signalling. These results point out also to the pertinence of studying 5-HT2C receptors signalling as a possible treatment modality in some of the mental disorders. Further studies, in order to clarify the details of these signalling regulatory mechanisms and their therapeutic potentials in psychiatric disease are required.

1.3. Interaction of serotonin and dopamine systems

The central interaction of serotonin and dopamine systems is an important component of the pharmacology of 5-HT2C receptors in the brain. The essential implications of both dopaminergic and serotonergic system in the pathophysiology of various mental disorders have been known for many years [66]. As the result, the aberrant communication of serotonergic and dopaminergic systems is a shared characteristic of psychiatric diseases like psychotic, mood and eating disorders [66, 134, 135]. In this regard, a strong regulatory function for serotonin system over dopaminergic tone in major compartments of the brain and in various physiological settings has been suggested [66, 134, 135].

Several lines of evidence, including the anatomical distribution, support the involvement of 5-HT2CRs in the mediation of modulatory responses of serotonin system. The expression of 5-HT2CRs in almost all of brain cell populations except astrocytes has been detected [136, 137]. 5-HT2CRs are mainly post-synaptic receptors, although there are some reports addressing their pre-synaptic distribution in the anterior olfactory nucleus and the choroid plexus [138]. The highest density of these receptors has been reported in the epithelial cells of choroid plexus, however, cingular cortex, basal ganglia, amygdala, and hippocampus also show a decent amount of 5-HT2CRs [139]. Further, the presence of 5-HT2CRs among other serotonin receptors in major dopaminergic elements of the animal brain like ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) has been shown by various methods [15, 140, 141]. For example, using the radio-ligand binding and in situ hybridization technic, 5-HT2CRs mRNA, along both mesolimbic and nigrostriatal pathways, has been detected [142]. In human as well, in situ hybridization studies have shown the varying level of 5-HT2CRs mRNA in the choroid plexus, cerebral cortex, hippocampus, amygdala and substantia nigra, [144]. However, in contrast to rodents, in which both dopaminergic and gamma-aminobutyric acid (GABA)ergic neurons of VTA and SNc contained 5-HT2CRs mRNA and protein, in human brain counterparts, 5-HT2CRs mRNA did not appear in dopaminergic cells [145, 146]. This leaves the possibility of GABAergic cells to account for 5-HT2C positive cells in these regions [144]. In most of these regions, a somatodendritic pattern of distribution for 5-HT2CRs has been shown [143]. In fact, the presence of 5-HT2CRs on GABAergic that make direct contact with dopaminergic cell

bodies in SNc and VTA has been a consistent finding in various studies [145, 146]. In line with this, the co-expression of 5-HT2CRs with glutamic acid decarboxylase (GAD), the enzyme responsible for the synthesis of GABA in GABAergic interneurons of the cortex, striatum, and amygdala has been shown [146-148]. The presence of 5-HT2CRs on these interneurons positions them ideally to influence the dopamine output of both mesocorticolimbic and nigrostriatal pathways of the brain [148]. This evidence supports the modulatory role of 5-HT2CRs on dopaminergic system through GABAergic projections in major dopamine centers of the brain [16, 17, 34, 134]. Altogether, these findings are consistent with the reported inhibitory role of 5-HT2CRs in controlling the dopamine concentration in different brain compartments [34, 134].

Apart from the neuroanatomical evidence, genetic and pharmacological studies also support the interaction of 5-HT2C receptors and the dopaminergic system of the brain. For instance, 5-HT2C-KO mice demonstrated a higher dopaminergic activity in the nucleus accumbens (NA) and dorsal striatum, which was related to hyperactivity of SNc neurons [85]. Also, it has been shown that the absence of the 5-HT2C receptors in the brain made these animals more sensitive to the response of dopaminergic drugs. For example, exaggerated grooming and stereotypical behaviour following administration of psychostimulants including amphetamine and GBR 12909 were detected in these mice [85]. Similar to this a rare missense mutation in HTR2C was associated with alteration of positron emission tomography (PET) readings indicating a higher release of dopamine in the NA, caudate nucleus, and putamen during a standardized stress challenge in carriers [154]. This points out to the close interaction of 5-HT2C receptors activity and the central responses of dopamine in the brain.

In pharmacological studies, it is well known that dopamine release in the basal ganglia is regulated by serotonin receptors especially 5-HT2CRs in SNc and VTA [153, 155]. It has been shown that the activation of 5-HT2CRs by agonists leads to inhibition of dopamine release in these regions while the antagonists have the opposite response [156, 157]. Though the amplitude of these changes varies depending on the agonist activity and its selectivity, several similar results have been replicated across various studies. For example, Ro-60-0175 a 5-HT2CRs agonist inhibited dopamine neuronal activity in different experimental settings to the same degree while pre-treatment with 5-HT2CRs antagonists blocked this effect [131, 157]. In line

with this, inverse agonists of 5-HT2CRs increased dopamine release by VTA and SNc neurons in most studies [157-159]. Moreover, the effect of various inverse agonists was blocked by selective antagonists of 5-HT2C receptors showing the specificity of such response in these studies [157, 160]. The induced release of dopamine in the brain structures following 5-HT2CRs inverse agonists reveals the prominence of constitutive activity of 5-HT2CRs in modulating the tonic discharge of dopaminergic neurons [157, 160]. In fact, it has been shown that while antagonists of 5-HT2CRs increased the dopamine concentration up to 30 percent, inverse agonists boosted such dopamine release by up to 75 percent more than the baseline [130, 131]. This means that the innate state of 5-HT2CRs applies a constant impediment to lower the dopamine release in the brain. These results are in line with the long-standing theory of the regulatory role of serotonin in the activity of dopaminergic systems of the brain [17].

Concerning this, suboptimal dopamine neurotransmission in various brain components of depressed patients as the result of a possible excessive activity of 5-HT2CRs has been discussed before [29, 161]. In fact, the altered innate activity of 5-HT2CRs due to abnormal patterns of RNA editing profile in depressed patients has been a constant finding [51, 61]. Interestingly, the therapeutic response of chronic treatment with selective serotonin reuptake inhibitors (SSRIs) has been associated with correcting some of these abnormalities in animal models [51, 53]. In accordance, other central dopamine pathologies have been linked to the altered levels of RNA editing and basal activity of 5-HT2C receptors in the NA and the VTA [162]. For example, in an animal model of movement disorder, the intrinsic activity of 5-HT2C receptors in dopaminergic regions has been proposed as a determining mechanism of purposeless oral movements in rats [163]. This is significant since the alteration of the release of dopamine in the NA by VTA neurons is a critical part of the pathophysiology of various mental diseases like schizophrenia and bipolar disorders [162, 163]. Similarly, it has been demonstrated that the response of patients to various psychoactive drugs, including antipsychotics and antidepressants, at least partly depended on the level of basal activity of 5-HT2CRs and their degree of editing [164-166]. This is remarkable since many of current efficacious neuroleptics act on both dopamine and 5-HT2C receptors [164]. Altogether, these results demonstrate the clinical significance of the control of central 5-HT2C receptors over major dopaminergic structures of the brain in the context of mental disorders.

5-HT2CRs, by increasing the GABAergic inhibitory tone, reduce the dopamine release in the central dopaminergic pathways of the brain [167, 168]. Though this seems to be the case in most studies, recent investigations show major differences between the final outcome of 5-HT2CRs on nigrostriatal and mesolimbic systems [131, 140, 157, 160]. In other words, there is convincing evidence of asymmetry of the inhibitory responses of 5-HT2CRs on the neuronal activity of SNc and the one of VTA. For example, although in most studies, acute administration of SSRIs decreased the dopamine neurotransmission of the VTA, this was not the case for the SNc [169, 170]. Similarly, mCPP, a mixed 5-HT2 receptors agonist, reduced the activity of dopamine-containing neurons of the VTA but not the SNc [171]. Further, selective 5-HT2CRs antagonist, SB 242084 was able to block this effect showing the exclusiveness of 5-HT2CRs in the manifestation of these outcomes [171]. Consequently, a preferential inhibitory control on the mesolimbic dopamine neurotransmission by 5-HT2CRs has been suggested [152, 172]. Noteworthy is the fact that the inhibition of nigrostriatal pathway is recognized as the underlying mechanism of the detrimental motor side effects of various neuroleptics [152]. In the light of recent research proposing 5-HT2CRs agonists as emerging new antipsychotic drugs, this is really promising for the field of psychopharmacology.

Despite these positive results, contradictory findings point to a crucial lack of understanding regarding the detailed mechanisms by which dopamine release is controled by serotonin receptors in the brain. For example, decreased VTA dopaminergic neuronal activity following SSRIs administration has not been a consistent observation [173, 174]. Also, in some studies, acute injection of Citalopram was devoid of any effect on dopamine neurons of the VTA [173, 175]. Similarly, there are indications that some serotonergic drugs do not attenuate or potentiate dopamine activity in some regions of the brain after the treatment with stimulants [176, 177]. In addition, acute Fluoxetine, among other SSRIs, was not able to change the concentration of dopamine in the striatum of animals in different studies [178, 179]. Interestingly, Fluoxetine increased dopamine neurotransmission in the PFC and decreased dopamine release in the striatum and NA in other studies [180, 181]. This profile has been proposed as a unique therapeutic feature of Fluoxetine since no other SSRIs had a similar influence on the content of dopamine in the PFC and the basal ganglia [180]. Apart from the responses of serotonergic drugs, the reports of dopamine concentration changes following the elevation or depletion of

brain serotonin level has been an important source of discrepancy in the literature. In this respect, depending on the site and method of measurement, dopamine content of the brain reported increased or decreased after the total brain serotonin reduction [182, 183]. This is especially true for the older studies that used non-selective enzyme inhibitors or mechanical damage to raphe nuclei as a method of brain serotonin depletion and the replenishment with the substrate to increase the serotonergic tone in animals [182, 183]. Similarly, these contradictions extend to the measurements of dopamine release after increasing the serotonergic tone of the brain using electrical stimulation of raphe nuclei or various non-selective serotonergic agents [131, 179, 184, 185].

Today, thanks to recent advancements in the understanding of the biological interaction of dopamine and serotonin systems, some of these observed contradictions have been explained. For instance, we know that serotonin can modulate dopamine neurotransmission via a variety of mechanisms including reducing the electrical activity of dopaminergic neurons, inhibiting the actual release of dopamine in the tissue or modulating the postsynaptic dopamine receptor positive cells [186]. It has been documented that recorded effects of serotonin on dopaminergic neurons in electrophysiological studies might not agree with the measurements of the concentration of dopamine in the tissue by voltammetry or microdialysis [186]. Following this, some of the reported conflicting results are believed to be due to the non-linear relationship of the electrical firing of dopaminergic neurons and the actual release of dopamine in the tissue [9, 34]. Further, it has been suspected that the activation of 5-HT2BRs and 5-HT2ARs, especially in the case of administration of older non-selective 5-HT2 agonists, might explain some of these inconsistencies [9, 34]. In fact, 5-HT2CRs share a high degree of homology in their amino acid sequence with the other two members of the 5-HT2 family: 5-HT2A and 5-HT2B receptors [187]. This emphasizes the challenge of selectivity in many of designed compounds since most of the older drugs bind with high affinity to all of these three receptors. This lack of discrimination against other subtypes especially 5-HT2ARs (with some opposite central responses) may be the source of some of the existing uncertainty about the final impact of 5-HT2CRs in dopaminergic pathways [28, 188].

Lastly, it is important to notice the distinction of modulatory action of the serotonergic system in fine-tuning of both “tonic” and “phasic” dopamine neurotransmission. In general, in tonic neurotransmission, increase of endogenous serotonergic input moderates the innate activity of dopaminergic neurons [46]. The output of such regulation is normally revealed after the treatment with antagonists or inverse agonists of serotonin receptors [46]. As previously stated, it is observed that 5-HT2CRs inverse agonists induced an increase of the accumbal and striatal dopamine release, even after the depletion of serotonin [131]. So, it has been suggested that basal and constitutive release of dopamine at least in mesocorticolimbic pathway involves the constant contribution of 5-HT2C receptors [172]. On the other hand, the state dependent or phasic control of serotonin over dopamine release varies with the degree of activity of dopamine neurons [186, 189]. This means in certain excitatory conditions, the action of serotonin neurons might intervene with the outcome of dopaminergic system [186, 189]. In this perspective, assumptions about the role of 5-HT2CRs in the modulation of the tonic activity of the dopaminergic system might not apply to the phasic release of dopamine [190]. This has been recognized as a possible cause of the confusion concerning the interaction of serotonin and dopamine system in the literature [9].

Altogether, the available anatomical, genetic, and pharmacological evidence supports the contribution of 5-HT2C receptors in regulation of physiological and pathological dopamine function in the brain. Moreover, since abnormal dopamine neurotransmission is a shared pathophysiology of many mental disorders, 5-HT2C drugs might hold scientific implications in the future to regulate the dopamine activity in the various brain structures like the cerebral cortex and the striatum. In the following section, some evidence of the close interaction between 5-HT2CRs and the dopamine system in the context of several CNS disorders will be briefly reviewed.

1.3.1. Schizophrenia

Excessive dopaminergic activity in the mesolimbic pathway and its projections to ventral striatum is the hallmark of the pathophysiology of schizophrenia [191]. Naturally, it is known that the blockade of dopamine D2 receptors in these brain compartments is the shared

pharmacodynamics feature of most of antipsychotics [192]. Recently, regulatory properties of 5-HT2C receptors over the activity of dopaminergic cells, in various contexts of mental disorders, have been avidly studied [191]. Namely, various researchers investigated the antipsychotic properties of 5-HT2C receptor agonists in related experimental paradigms [191]. Considering the limitations of research in humans, psychostimulant-induced models of schizophrenia are one of the most common tools for the screening of new antipsychotics [193]. Higher levels of dopamine in the brain following the administration of these drugs can induce a psychosis-like state in healthy subjects and exacerbate positive symptoms in schizophrenic patients [193]. In fact, the exaggerated dopamine neurotransmission in patients is echoed in animal models by the administration of these psychostimulant drugs [193]. Based on such observations, investigations of sensory-motor disturbances like increased psychomotor activity, abnormal pre-pulse inhibition and cognitive disruptions following the injection of these drugs, are the ideas behind many of the current animal models in this field [193]. Briefly, the ability to resist the central responses of these dopaminergic drugs in animals has been viewed as a valuable screening tool to predict the antipsychotic efficacy of freshly developed therapeutic compounds [34].

Since the discovery of antipsychotics, the implication of the various serotonin receptors in their efficacy and mechanism of action has been the topic of numerous studies [17]. Remarkably, high affinity for 5-H2CRs has been observed in different antipsychotic drugs, ranging from Chlorpromazine to Clozapine, as a potential site of action as well as an explanation of distinct metabolic profiles of these drugs [66]. Moreover, different authors reported the antipsychotic responses of activation of 5-HT2CRs in various animal models [194]. For instance, both short-term responses, like stimulant-induced hyperlocomotion, or long-term changes, due to neuronal adaptations after repeated and intermittent administration of dopaminergic drugs, recorded beneficial outcomes for 5-HT2CRs agonists [194]. Selective 5-HT2C agonists like RO 60-0175 and WAY 161503 resisted the DOI-induced head twitch in animals [195, 196]. This model is believed to represent the central response of hallucinogenic drugs in humans. The agonists of 5-HT2C receptors like WAY-163909 hindered behaviours related to psychotic features, including stereotypy and climbing induced by NMDA antagonists or amphetamine, in various models [197, 198]. Moreover, activation of 5-HT2C receptors reduced cocaine-induced locomotor activity and inhibited the development of sensitization to this drug in animals [135, 199].

Similarly, various 5-HT2C receptors agonists like CP809,101, RO 60-0175, WAY 161503 and mCPP, counteracted the induced horizontal locomotor activity by MK-801 and amphetamine in animals exhibiting the antipsychotic outline of these receptors [195, 196, 200]. Resisting the increased phasic dopaminergic activity in the mesolimbic pathway has been proposed as the mechanism of action of 5-HT2CRs agonists in afore mentioned studies [155, 201].

Besides investigation of therapeutic properties, screening designed compounds for the possible threatening adverse effects is an essential part of development of novel medications. Motor side effects are one of the major adverse effects of current antipsychotics due to the excessive blockage of dopamine receptors in nigrostriatal pathway [66]. That is why the probable detrimental response of 5-HT2CRs agonists in various dimensions of motor functions has been studied. Though in the literature, reduction in locomotor indexes has been witnessed after administration of some 5-HT2C agonists, recent reports show that this happens in much higher than therapeutic doses [196]. For example, in rotarod, a classic test to evaluate motor coordination in animals, motor impairment has been described only after administration of higher doses of non-selective mCPP and Ro-60-01755 [202, 203]. On the other hand, 5-HT2CRs partial agonist MBP failed to affect the locomotion of the animals at doses that counteracted the increased locomotor activity induced by MK-801 or amphetamine [196]. Moreover, 5-HT2C ligands do not induce catalepsy, a measure of motor dysfunction in the preclinical settings, even at very high doses [197, 204]. Further, JJ-3-42, a new 5-HT2C agonist, produced very little catalepsy in comparison to Haloperidol, at a dose that resisted amphetamine-induced hyperactivity [205]. Moreover, some selective 5-HT2C agonists like WAY 163909 augmented the positive responses of Haloperidol and Clozapine but failed to increase the chances of catalepsy in combination with these drugs in animals [197, 204]. On the other hand, the antagonists of 5-HT2CRs like SB 228357 inhibited Haloperidol-induced catalepsy in the animal models [198, 206]. As the result of these studies, it has been proposed that newer generations of 5-HT2CRs ligands possess potential antipsychotic properties without disturbing the locomotor activity of animals [196].

Information processing deficit is a typical phenomenal occurring in schizophrenia patients. This pathological feature is due to disruption of pontine sensory reflex. In the normal population, the

startle response is inhibited when the startling stimulus (pulse) is preceded by a weaker, non-startling one (pre-pulse). However, this inhibition, signifying the capacity to filter out the unnecessary information, is absent in the schizophrenic patients [193]. This is the core of the pre-pulse inhibition (PPI) test, which is believed to have a great predictive validity in screening new antipsychotics [193]. Most of the stimulants like amphetamine and apomorphine or hallucinogenic drugs including NMDA antagonists like MK-801 and phencyclidine, induce a deficit in the PPI test [193]. On the other hand, neuroleptics, especially Haloperidol, demonstrate efficacy in this model and rescue this reflex [193]. Of note, the agonists of 5-HT2CRs showed beneficial response against the abnormal MK-801 or apomorphine-induced pre-pulse inhibition in animals [197, 204]. Likewise, JJ-3-42, a new 5-HT2CRs agonist, restored amphetamine-disrupted pre-pulse inhibition in mice [205]. Moreover, administration of 5-HT2C receptors agonists potentiated the antipsychotic activity of neuroleptics in this paradigm [197]. For example, the 5-HT2CRs agonist WAY 163909 increased the efficacy of various antipsychotics against stimulants in PPI [197].

Despite the relative efficiency of antipsychotics in controlling positive symptoms like hallucinations, delusions and thought disorders in the clinic, their efficacy against negative symptoms, especially the detrimental cognitive decline in psychotic patients, is very limited [191]. Therefore, future antipsychotics are expected to enhance the cognitive function and reduce the negative symptoms in animal models [191]. The impact of 5-HT2CRs ligands in various dimensions of cognitive capacity has been tested but the results of such studies show a great amount of contradiction. For instance, older and non-selective agonists like mCPP reduced consolidation of memory, while non-selective 5-HT2 antagonists like Ritanserin and Ketanserin enhanced the memory function in mice [207-209]. However, a selective 5-HT2A ligand, M100907, was able to block the pro-cognitive effect of Ketanserin in some studies, suggesting the involvement of 5-HT2ARs in this response [207-209]. In contrast to this, positive and pro-cognitive properties have been observed in newer and more selective 5-HT2CRs agonists. For instance, CP809,101 has proven a significant cognitive enhancement impact in the H-Maze test in brain serotonin-deficient mice [210]. Moreover, JJ-3-42 enhanced the cognitive capacity of the animals in the novel object recognition test in NMDA receptor hypo-functioning NR1-knockdown mice [200, 205]. However, this compound failed to enhance reversal learning in

MK-801-treated mice [205, 211]. Taken together, despite some positive reports, the exact cognitive profile of 5-HT2CRs and their capacity to improve the negative symptoms of schizophrenia remains ambiguous. Further studies in the various dimensions of cognitive function are needed to address some of these contradictions.

In favour of promoting 5-HT2CRs agonists as potential new antipsychotics, various authors have put forward several scientific arguments. It has been mentioned that the dominant central expression of these receptors would limit the chances of peripheral side effects of drugs acting on these receptors [144, 211]. Also, it has been argued that the preferential selectively in inhibition of mesolimbic dopamine release over the nigrostriatal pathway by 5-HT2CRs agonists predicts fewer extrapyramidal side effects for such antipsychotics [211]. Finally, the proven appetite-suppressing property of these agonists makes them a great choice, alone or in combination with other drugs, to address the metabolic abnormalities seen in schizophrenic patients [212, 213]. These arguments along with the promising preclinical results of these compounds led to a very optimistic view regarding the antipsychotic property of 5-HT2CRs drugs in the field of schizophrenia. However, the clinical trials of such drugs proved not very successful. Vabicaserin by Pfizer, an agonist of 5-HT2CRs and potential antipsychotic and anorectic compound, improved positive symptoms in the subjects. However, it did not meet the primary efficacy endpoints in the clinical trial [214, 215]. On the other hand, a potent 5-HT2CRs inverse agonist, and dopamine D2/α1-adrenergic/5-HT2ARs antagonist Sertindole was withdrawn from the market in Europe, due to cardiovascular side effects [50, 216, 217]. Nonetheless, additional studies showed that this drug was effective in reducing anxiety, improving cognition and brain plasticity, most likely through 5-HT2C receptors [216]. Altogether, while the recent results show a favourable outline for the clinical implications of 5-HT2C drugs in the field of schizophrenia, further investigations are needed to clarify the final outcomes of newer 5-HT2C drugs in cognitive and social paradigms of the animals.

1.3.2. Drugs of abuse

Similar to schizophrenia, the potential therapeutic responses of 5-HT2CRs, in the context of addiction and dependency to drugs of abuse, have been studied. Drugs of abuse are a diverse

group of natural or synthetic molecules that can evoke different psychological or behavioural responses by stimulating the CNS [218]. This could include euphoria, alertness and enhanced psychomotor activity mostly due to the temporary and transient increased action of various dopaminergic brain centers [219]. On the other hand, chronic drug abuse and dependency can result in significant morbidity, potential mortality, and decline of the quality of life in humans. United Nations Office of Drugs and Crime estimated the abuse of stimulants like cocaine and amphetamine affects more than 50 million people worldwide [220, 221].

In the search for an effective treatment, a wide range of pharmacological animal models have been used to test the beneficial reaction of 5-HT2C receptors in addiction to various drugs [222-225]. The protecting responses of 5-HT2CRs agonists against the addictive properties of different groups of drugs of abuse have been confirmed [216]. For example, selective 5-HT2CRs agonists, RO 60-0175 and WAY 163909, suppressed voluntary intake of cocaine in self-administration tests in animals [222-225]. Moreover, 5-HT2CRs activation reduced the consumption of ethanol and inhibited reinstatement of ethanol addiction in rodents [226-228]. Such protective effect of 5-HT2CRs agonists has been extended to resisting the central responses of morphine and Δ9–THC in various models [177, 229-235]. In addition, 5-HT2CRs agonists reduced intravenous self-administration of nicotine and weakened the reaction to nicotine cues in rats trained to discriminate nicotine from saline [236]. Following these positive results, clinical responses of various 5-HT2CRs agonists against addiction to stimulants and other groups of drugs of abuse are under investigation [237]. Recently, the clinical efficacy of Lorcaserin in the treatment of nicotine addiction in the population has been described [238, 239]. As earlier stated, increased concentration of dopamine in the brain, especially in the mesolimbic pathway, is the chief mechanism of action of many substances with the potential of abuse [240, 241]. Therefore, the reduction of dopamine concentration in the mesolimbic pathway and resisting the central impact of dopamine releasing drugs in the VTA, NA, and cingulate has been connected to the beneficial responses of 5-HT2CRs agonist [134, 145].

On the contrary, selective 5-HT2CRs antagonists produced opposite effects and enhanced the reinstatement of drug-seeking behaviour in animal models of addiction [223, 242]. This is in agreement with the recorded outcome of central responses of antagonism of 5-HT2CRs in the

brain. In this respect, administration of antagonists of 5-HT2CRs resulted in an increase of the dopamine neuronal activity and dopamine release in the striatum and NA in a receptor-specific manner [134, 140]. Also, the antagonists of 5-HT2CRs boosted the increase of central dopamine concentration following the administration of an extended list of drugs including morphine, phencyclidine, MK-801 and cocaine [243-245]. Similarly, 5-HT2C-KO mice showed an increased amount of self-administered cocaine compared to wild types [94]. Also, enhanced cocaine-induced dopamine release in the NA has been recorded in these mice in contrast to control animals [94].

In these studies, the central response of 5-HT2C receptors in the brain seems straightforward. However, some ambiguity regarding the changes in level of dopamine following activation of these receptors exists. For example, bimodal and dose-dependent control of 5-HT2C receptors antagonist SB 206553 over cocaine-induced dopamine release in the mesolimbic pathway has been reported [246]. While injections of 5-HT2C agonists into the VTA or the PFC resulted in a reduction of cocaine-induced locomotion, intra-NA microinjections of the same agonist led to the enhancement of such responses [241, 247, 248]. Interestingly, intra-NA and intra-PFC injections of 5-HT2C antagonists abolished and increased the cocaine-induced locomotor activity in the rodents respectively, pointing out to the regional differences of dopamine regulation by 5-HT2C receptors [241, 251]. Similarly, the injection of SB 242084 ,an antagonist of 5-HT2CRs, in the NA attenuated the response of systemically administered cocaine on the dopamine level of NA [252]. Remarkably, most of these conflicting results were reported after the injection of cocaine or drugs with similar mechanisms of action. To explain these results, some authors have proposed that 5-HT2CRs modulate only the exocytotic release of dopamine following the dopamine neuron electrical activity [179, 190, 244, 253]. Such control manifests in the case of drugs like Haloperidol, morphine, nicotine, phencyclidine and MK-801 and is dependent on the degree of activity of dopaminergic neurons [162, 179, 253]. In contrast to this, in the case of non-exocytotic impulse-independent dopamine release, the modulatory response of 5-H2CRs is not prominent [162, 179, 253]. It is noteworthy that such non-exocytotic dopamine release mostly occurs after the administration of cocaine and amphetamine in the brain [162, 179, 253]. It is worth mentioning that in contrast to the neuronal impulse dependant release of the

neurotransmitter in the synaptic clef, non-exocytotic dopamine release happens generally after the administration of the “transporter blockers” or “releaser” in the neurons [162, 179, 253].

Moreover, the aforementioned results point out to a significant difference between the outcome of the activation of 5-HT2CRs in the NA compared to other dopaminergic receiving regions of the brain like PFC [241, 247, 248]. Conversely, these results demonstrate the difficulty of interpretation of the behavioural outcome of systemic administration of 5-HT2CRs ligands as it may involve activation of different neuronal populations involved in various neuronal pathways. Variances in the central responses of different subpopulations of 5-HT2CRs positive cells should be accounted for in analysis of the final outcome of drugs of abuse in the brain [254].

Despite such discrepancies, the ensemble of these results is in favour of the modulatory profile of 5-HT2C receptors of the abnormal neurotransmission of dopamine in the CNS following the administration of drugs of abuse. Further, the influence of 5-HT2CRs on the release of dopamine in brain structures might indicate their therapeutic value in the treatment of addiction and drug dependence in the clinic [141, 158]. In this regard, Lorcaserin has demonstrated protective responses against consumption of various drugs of abuse like alcohol and nicotine in self-administered test in laboratory animals [227, 236]. Furthermore, Lorcaserin has shown promising results in treatment of food addiction in various groups of patients in the clinical setting [212, 238]. Correspondingly, in combination with Varenicline, Lorcaserin displayed additive effects against nicotine self-administration, and nicotine-seeking behaviour in rodents [237, 255]. Consequently, successful clinical trials of Lorcaserin in combination with Varenicline showed the practical weight of 5-HT2CRs modulation in the treatment of nicotine addiction in patients [141, 237]. Presently, the therapeutic consequences of various agonists of 5-HT2CRs to treat addiction disorders in the vulnerable population are under investigation [34, 140, 237].

1.3.3. Parkinson’s disease

Parkinson’s disease (PD) is one of the areas of ongoing research into interaction of dopamine and serotonin systems. PD is a neurodegenerative disease that manifests with bradykinesia, rigidity and tremor at rest due to a considerable loss of nigrostriatal dopaminergic neurons [256].

Administration of the metabolic precursor of dopamine, L-DOPA, accompanied by peripheral inhibitors of the aromatic amino acid decarboxylase inhibitors or dopamine agonists is the foundation of PD treatment [256]. Despite many clinical attempts to find a better treatment, L-DOPA is still the most effective medication to treat Parkinson's disease. However, this drug normally loses its clinical efficiency quickly due to a variety of side effects, including motor fluctuations and L-DOPA-induced dyskinesia (LID) [257, 258]. Animal models have been used for many years to better understand the pathological mechanism involved in these side effects and for testing the new treatments against them. The injection of 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl 1, 2,3,6-tetrahydropyridine (MPTP) leads to the destruction of nigrostriatal dopaminergic neurons and are traditionally used to model Parkinson’s disease in rodents [259, 260]. These models are believed to replicate the state of LID following long-term dopamine replacement by L-DOPA in PD patients [257, 258].

Following the intimate interaction of dopamine and serotonin systems, various serotonergic drugs have been tested to ameliorate the symptoms of PD in patients. A collection of pharmacological studies supports the significant contribution of the serotonin tone in the expression of LID both in animal models and in PD patients [261, 262]. Respecting this, the positive response of modulation of serotonergic tone in controlling the symptoms of LID is a common finding in various studies [261, 263-266]. In these studies, targeting the 5-HT1 family of receptors rescued the exaggerated L-DOPA response in animal models [261]. For example, 5-HT1A and 5-HT1B receptors agonists reduced the serotonergic neurotransmission and improved L-DOPA-induced dyskinesia in animals [267]. Various 5-HT1A agonists like 8-OH-DPAT, Buspirone or Sarizotan, 5-HT1B agonists like CP-94253 or 5-HT1A/1B agonists like Eltoprazine resisted the development of LID in hemiparkinsonian rats and Parkinsonian macaques [261, 263-266]. As a result of these studies, modulation of 5-HT1 serotonin receptors has emerged as a potential therapeutic target for movement disorders and the control of treatment side effects in PD patients [261, 263-266]. This has led to some successful clinical trials in PD patients. For example, chronic administration of Buspirone in advanced PD patients with neural transplants has been proven to significantly diminish graft-induced dyskinesia [268]. However, such positive responses were of limited practical use in the clinic due to their undesired side effects. Among others, it has been shown that stimulation of 5-HT1A receptors can affect the efficacy of L-

DOPA, despite the initial favourable outcomes [269]. Furthermore, some proposed that serotonin neurotransmission facilitates the conversion of L-DOPA into dopamine, and augments the neurotransmission of dopamine in sensitive sections of the brain leading to the pathological symptoms [270]. The excessive stimulation of dopamine receptors type 1 (D1) in these structures is the principal biological mechanism of a variety of movement disorders observed in PD patients [270]. Subsequently, it is postulated that the reduction of serotonin neurotransmission inhibits such excess of L-DOPA-derived dopamine, and leads to the improvement of the symptoms in patients [270]. Despite these limitations, the modulation of serotonin neurotransmission is one of the current strategies for the management of side effects of long-term L-DOPA administration [269]. This is inevitable since interaction with serotonin system is a shared factor of various psychoactive drugs that are used to treat the psychiatric comorbidities or control the side effects of PD treatment in the clinic [270].

Respecting this, the outcome of SSRIs in relationship to the symptoms of Parkinson's disease has been studied. Some SSRIs including Citalopram and Paroxetine alleviated partially LID symptoms in animal models of dyskinesia and maintained L-DOPA efficacy for a longer time [271, 272]. In addition, some SSRIs provided a dose-dependent protection against the development of motor side effects of the PD treatment in rodents [273]. More importantly, these effects were achieved at doses that produced the antidepressant-like outcomes in rats [273]. Likewise, other studies confirmed that SSRIs could lessen motor symptoms and reduce side effects of the treatment even at the lower therapeutic doses in various PD animal models [273, 274]. It is argued that indirect activation of the 5-HT1A receptors is responsible for the mentioned positive responses of SSRIs in controlling of these motor symptoms in animals [273]. In this respect, it is believed that SSRI-mediated increase in the serotonin level leads to stimulation of 5-HT1A auto-receptors and overall inhibition of raphe neuronal firing [275, 276]. These results denote the probable positive response of SSRIs in the management of motor side effects of PD patients [277]. However, despite these reports, the clinical implications of SSRIs as non-dopaminergic treatment in PD patients await further clinical investigations.

Despite the distinct role of 5-HT1Rs the in the modulation of the symptoms of Parkinson's disease, the contribution of 5-HT2Rs in this phenomenon is rather unclear. In various studies, the

response of 5-HT2CRs agonists proved deleterious in animal models of Parkinson's disease [164, 278, 279]. For example, peripheral administration of mCPP, RO 60-0175 or WAY 163909 reportedly aggravated the orofacial bouts in neonates or adult 6-OHDA-treated rats [164, 278, 279]. In addition, non-selective Mianserin, as well as selective antagonists of 5-HT2CRs like SER 082 or SB 243213, were successful in resisting such deterioration in animals [164, 278, 279]. Indeed, antagonists of 5-HT2CRs SB 200646 and SB 206553 potentiated the response of D2 agonist Quinpirole or D1 agonist SKF 82958 and ameliorated the rotating behaviour in animals [280-282]. Mianserin, independently, was also successful in diminishing the tremulous jaw movement induced by cholinergic agents in a model of resting tremor [283]. This positive response of 5-HT2CRs antagonists is supported by some clinical studies. For example, in one study, Mianserin helped to reduce the symptoms in 8 out of 12 patients receiving L-DOPA and dopamine agonists [284]. In relation to this, positive clinical responses of other drugs, like Clozapine and Mirtazapine to lessen the symptoms in Parkinson's patients, have been attributed to their antagonistic activity at 5-HT2A and 5-HT2C receptors [284-286]. However, it is argued that the blockage of the 5-HT2A receptors is responsible for the biggest share of the mentioned positive responses in these treatments [284-286]. Currently, different selective 5-HT2A antagonists/inverse agonists, including Primavanserin, are under various stages of clinical trials for the treatment of movement disorders [287]. Their primary results seemed satisfactory [287].

Overall, though it has been shown that 5-HT2CRs might contribute to some of the therapeutic consequences of non-selective drugs such as Mianserin, Clozapine or Mirtazapine, their involvement might be negligible. Thus presently, drugs acting on 5-HT2C receptors are not envisioned as suitable candidates in the treatment of Parkinson's disease. Nonetheless, to clarify the beneficial response of modulation of 5-HT2C receptors alone or in combination with other drugs to manage the side effects of the treatment in PD, further studies in future are needed. On the other hand, the results of current studies illustrate that the agonists of 5-HT2C receptors may deteriorate motor function and produce harmful consequences in some of the animal models. This calls for cautionary approach in the prescription of 5-HT2C active drugs in patients suffering from PD in the clinic.

1.4. Serotonin and the regulation of various dimensions of the behaviour

Historically, as one of the main neurotransmitters in the brain, serotonin is related to a wide array of complex animal behaviours like cognition, sleep and aggression [151, 288]. Since its discovery, the investigation of the contribution of the serotonin system abnormalities in pathogenesis of psychiatric diseases has been at the center of attention of the psychopharmacology field [289-291]. However, despite these relentless efforts, the role of serotonin and its various receptor subtypes in regulation of different dimensions of behaviours in animals is far from completely understood [291, 292]. Of note, the earlier investigations showed an incredible amount of discrepancies regarding the role of serotonin system in pathophysiology and treatment of mental disorders [17, 151, 293]. Multiple authors have suggested this could be due to various technical limitations that these older studies faced. For example, to establish the role of serotonin in various paradigms, most of these studies employed systemic administration of chemicals like toxins to deplete, or precursors to replenish brain serotonin levels [294]. However, profound and enduring consequences of administration of systemic neurotoxins like pCPA and 5,7-DHT cast doubts on the precision and relevance of these findings and their interpretations [17, 151, 293]. Moreover, the non-selectivity of the majority of the older substances could explain some of the ambiguous and contradictory results obtained in some of these studies [9, 294]. Finally, most of the older studies depended on measurements of serotonin or its metabolites in cerebrospinal fluid or periphery due to lack of better measurement techniques in those days [294]. Presently, we know that these indexes are not always an accurate representation of the central state of serotonin neurotransmission [151, 295].

By contrast, today, we have a fairly complete knowledge of neuroanatomical distribution and the signalling pathways of serotonin receptors in the brain [17]. Furthermore, new cutting-edge techniques like functional neuroimaging and precise methods of direct in-tissue measurements like voltammetry and microdialysis have advanced our understanding of the temporal and local functions of serotonin in the brain [171, 174]. In addition to this, recent genetic innovations helped us to identify many genes involved in the metabolism of serotonin as the potential risk

factors of various psychiatric disorders [32, 60, 296]. The creation of genetic animal models based on the manipulation of such known risk factors facilitated studying the significance of related proteins in physiological and pathological conditions [297, 298]. Overall, the recent scientific advances have allowed us to better comprehend the major role of serotonin in mediation of various behaviours of animals in the context of mental disorders.

In the following section, we review briefly the evidence of the implications of serotonin system in mental disorders by mentioning the results of related human and non-human studies. In addition, we will discuss the contribution as well as the therapeutic value of 5-HT2C receptors in regard to the pathophysiology of mental disorders through examining the available data in various disciplines.

1.4.1. Aggressive and impulsive behaviour

Aggression, defined broadly, is a social behaviour with the intention of inflicting harm, damage or unpleasantness on another [299, 300]. While it is an important component of social interaction in animals that compete for resources, it poses a serious problem in human communities [299, 300]. It is estimated that between 5 to 7 percent of the general population show the clinical criteria for aggressive behaviour disorders in their lifetime [301]. Aggressive social behaviour can be isolated or can accompany other mental diseases such as autistic spectrum, schizophrenia and bipolar disorders as a disabling comorbidity [302]. Regarding the social consequences of aggression and its prevalence in the population, comprehension of its biological basis has been the emphasis of many scientific studies. Of note, various abnormalities in the brain structures and connections as well as the level of different neurotransmitters have been observed in aggressive individuals [294, 303]. For example, changes in brain centers related to cognitive control, emotional regulation and mood have been witnessed in individuals with a higher rate of aggressive social behaviour [304, 305]. Consequently, a probable imbalance of various neurotransmitters especially serotonin in these brain centers has been proposed as the biological basis of aggression in these individuals [304-306]. In fact, the role of serotonin in the pathophysiology of aggression has been at the center of many debates since its discovery [306].

The “serotonin hypothesis of aggression” is one of the earliest theories that tried to explain the underpinning mechanisms of aggressive responses in violent individuals [294]. In this theory, the under-activity of serotonergic neurons and therefore the lack of adequate serotonin in relevant brain structures, disposes the animal or individuals to aggressive actions [303, 307, 308]. This hypothesis was supported by the evidence of low serotonin metabolite levels in cerebrospinal fluid (CSF) of violent human subjects and observed aggressive behaviour following chemical depletion of serotonin in animals [294, 309]. Despite this earlier evidence indicating the role of serotonin as a suppressor of aggression in individuals, today, this model seems much too simplistic [308, 310-312]. In fact, our recent knowledge shaped by genetic and pharmacological research in various animal models points to a far more complex nature of regulation of aggression by serotonin.

For many years, animal models have been used to investigate the contribution of the serotonin system in the regulation of aggressive behaviour. Studying the genetic animal models with altered brain serotonin content revealed the predominant influence of serotonin neurotransmission on the control of aggressive and impulsive behaviours in various paradigms. For example, SERT-KO HO mice, among other central and peripheral responses, showed a decreased amount of aggression in comparison to wild types due to a higher level of serotonin concentration [313]. In fact, reduced aggressiveness in resident-intruder test along with mild depressive and anxious-like phenotype has been described in these mice [314, 315]. These results are in line with the observations in Pet-1-deficient mice model. Evidence supports that the ETS transcription factor Pet-1 (FEV in humans) is essential for the survival of serotonergic cells in the developmental stage [316]. Consequently, in Pet-1-deficient mice, with diminished or no serotonin system development, up to 90 percent of brain serotonin depletion has been registered [316]. Such drastic diminution of brain serotonin in these mice led to an increased anxiety level as well as a higher frequency of aggressive behaviour [316, 317]. Similarly, it has been shown in mice that the complete deletion or mutation in different loci of the TPH2 coding gene can lead to exacerbation of aggression, risk-taking traits and impulsivity due to lack of serotonin [288, 318, 319]. However, conflicting results regarding the actual outcome of the absence of TPH2 protein in TPH2-KO HO mice have been reported by various groups [320, 321]. These mice showed aggressive and depressive-like behaviours in some studies while others were unable to confirm

these findings [322, 323]. Lastly, higher levels of aggression and impulsivity in social interaction along with cognitive abnormalities have been reported in homozygote TPH2 R439H knock-in (KI) mice [210, 324]. These mice lack up to 80 percent of normal brain serotonin level compared to their wild type littermates [13, 324, 325].

Though most of these studies confirm the serotonin hypothesis of aggression, such opposite relationship between serotonin and aggressive behaviour has not always been observed in animal studies. MAO-A is an important enzyme involved in the metabolism of serotonin, norepinephrine and dopamine and its absence leads to an increased level of serotonin in the brains of animals [326]. Consequently, genetic animal models like Tg8 and MAOAA863T-KO mice exhibit a decreased MAO-A activity and an enhanced brain serotonin level [326, 327]. Interestingly, these mice show an increased level of aggression in numerous paradigms including resident-intruder and isolation-induced aggression test, featuring a direct relationship of serotonin to aggression [328]. Remarkably, activation of MAO-A in forebrain was enough to restore the aggressive behaviour of animals in MAO-A deficient mice [329]. Related to this, in adult mice that experienced stressful challenges at a younger age, increased prefrontal MAO-A gene expression and exaggerated aggression was recorded [330]. Altered amygdala and orbitofrontal reactivities have been proposed as an underlying mechanism for observed behaviour in these mice [330]. Moreover, normal behaviour was restored by chronic treatment with the MAO-A inhibitor Clorgyline in these mice [330]. These results emphasize the complex interaction of environmental triggers and biological predispositions that might lead to an aggressive pattern of behaviour in adulthood [330]. Altogether, the direct relationship of serotonin and aggression observed in these investigations points to the complex nature of serotonin control over the behaviour of animals. Comprehension of the exact details of such regulation and the contribution of various brain compartments in this matter demands further investigations.

In parallel to the mentioned results, recent human genetic studies connect the regulation of serotonin system to the control of violent behaviour in the population. For example, the prevalence of different variants of TPH2 gene have been associated with a higher rate of antisocial, borderline and narcissistic personality disorders in human populations especially in

criminal prisoners [318]. In fact, various mutations in the genes coding proteins like MAO-A, TPH-1, SERT and serotonin receptors have also been recognized as potential risk factors in different violent groups [331]. For instance, in carriers of different functional single nucleotide polymorphisms (SNPs) of 5-HT1B and 5-HT2A receptors genes, alterations of serum serotonin predicted high callous-unemotional traits in antisocial males with behavioural problems [332, 333]. Similarly, in other studies, abnormal levels of serum serotonin were observed in inmates compared to non-aggressive individuals [334, 335]. This has been explained by down-regulation of MAO-A enzyme activity due to hypermethylation of the MAO-A gene promoter in these subjects [334, 335]. Similar to the MAO-A gene, SLC6A4 the gene encoding the SERT protein is one of the most studied polymorphisms in regard to aggressive behaviour. The linked polymorphic region, the regulatory region upstream the SLC6A4 gene has two main known alleles. The short (S) variant is associated with reduced SERT protein availability and function, and the long (L) form, with higher levels of SERT expression [336]. The S allele has been related to a wide range of psychiatric disorders from higher anxiety levels to neuroticism in individuals in different populations [337, 338]. Evidence shows that subjects carrying the S allele scored significantly higher in the guilt, irritability, hostility, negativism, resentment, and verbal aggression subscales of the Buss–Durkee Hostility Inventory [339]. Apart from this, the presence of S allele has been connected to the altered response of SSRIs treatment in aggressive and impulsive patients. In this respect, homozygote L allele carriers had a significantly better response to Fluoxetine than patients carrying S allele in various tests [337, 338]. It is noteworthy that a high degree of contradiction regarding the implications of SLC6A4 mutations in modulation of aggressive behaviour in different subgroups of the population has been reported [296, 340, 341].

Pharmacological studies have been one of the principal means of investigation into the contribution of the serotonin system in aggressive behaviour. Years of research in animals has gathered a large body of evidence supporting the implication of serotonin in aggression and impulsivity [342-344]. In the early days, most of the animal studies were based on the observation of an animal's social interaction following a robust alteration of brain serotonin level [343]. However, today different techniques like microdialysis and electrophysiology are employed to understand the temporal and spatial implications of serotonin in control of the

aggressive behaviour of animals [297, 345, 346]. This is especially vital since most of these agonistic episodes would not last more than a couple of seconds in animals. As mentioned, the majority of older pharmacological studies indicated the opposing role of serotonin in the expression of aggression in animals [311]. Remarkably, some of these studies, do not support such linear control of the serotonergic system on aggressive behaviour [347]. There are multiple reports of the inhibitory response of administration of 5-HT1A or 5-HT1B receptors agonists on aggressive behaviour [291, 348, 349]. For instance, agonists of 5-HT1A or 5-HT1B receptors like Ipsapirone, Buspirone and 8-OH-DPAT blocked aggression in several animal models [350]. Moreover, it has been shown that the anti-aggressive effect of these agonists was mediated via the inhibition of serotonin release by acting on presynaptic receptors and not through the postsynaptic neurotransmission [350]. This has been achieved, by the development of compounds like S 15535, which acts as a preferential agonist of the somatodendritic 5-HT1A auto-receptors and as an antagonist at postsynaptic 5-HT1A receptors [348].

Similar to these observations, recent research proposes a multilayered and complex role for serotonin in regulation of hostile behaviour. For instance, inhibition of release of serotonin after induction of lesions in raphe nucleus demonstrated that, at least in some paradigms, reduction of serotonin release leads to less aggression in animals [348, 351]. In another study, inhibition of the synthesis of serotonin by chemicals in rat brains prevented the expression of aggression in resident-intruder test [352]. In the same study, a higher degree of activation of serotonergic neurons was recorded after the confrontation in high-aggressive strain compared to low-aggressive strain rats and controls [352]. In parallel, a positive correlation between the level of aggression and basal CSF concentrations of serotonin and 5-HIAA in an aggressive strain of rats has been reported [294, 353]. However, in one study, damaging serotonin neurotransmission by 5,7-DHT caused a decrease in concentration of serotonin and 5-HIAA in CSF but did not increase the aggressive behaviour of animals [294, 353]. Interestingly, the dramatic decrease of the release of serotonin during and after the confrontation in the PFC but not NA of rats with inherent higher aggression rate has been witnessed [345]. This phenomenon lasted for an hour after such hostile interaction [345]. Another study showed that in animals conditioned to fight for 10 days, serotonin levels decreased by 30 to 35 percent in the NA in anticipation of the next fight [346]. However, the same study reported no changes in the serotonin efflux in NA during and

after a single random aggressive episode [346]. Accumulation of such incongruous results challenged the proposition of linear regulation of aggression by serotonin and urged the development of a more multifaceted hypothesis to explain this interaction [350, 354].

For instance, a distinction between what is called impulsive or "state aggression" versus the instrumental or "trait aggression" in animals have been suggested by some authors [294, 347]. This outlook establishes a bimodal function for serotonergic neurons in the brain depending on the type of aggressive behaviour experienced. As the result "state aggression" has different biological imprints than "aggression as a trait" in animals [294, 347]. For instance, it seems that in state violence, activation of serotonergic neurons is an essential part of the mechanism of the termination of aggression [347]. On the other hand, the low concentration of serotonin and its metabolites in aggressive strains of animals and in human subjects with violent history points to the modulatory role of serotonin in aggression as a constant and permanent predisposition [347]. This model can explain the differences observed in the brain of aggressive strains of animals versus the findings in animals with the history of reactive aggression [312]. In this regard, both forms of aggression are modulated by a rapid and short surge of serotonin during the aggressive act. However, this release happens from a strongly inhibited serotonergic tone as a baseline in violent individuals and animal strains. It is possible that this might not be detectable in the following measurement [312]. This point of view reconciles the original serotonin deficiency hypothesis as observed in pathological forms of aggression with the recordings of serotonin system activation in cases of state aggression. Indeed, the present-day study of models of highly aggressive strains of animals is considered to carry more face and construct validity than traditional pharmacological animal models of aggression [294, 347].

The low concentration of metabolites of serotonin in CSF of the people with a violent history has been a consistent factor in different longitudinal studies [311]. However, in the re-examination of some of these studies, impulsivity scores have been a better predictive of lower CSF 5-HIAA level in these subjects [294, 309, 355]. It appears that this observation can be replicated in heterogeneous impulsive and aggressive cohorts of personality disorders, alcoholism and suicide regardless of the underlying aetiology [344, 356-358]. In fact, it has been insinuated that impulsivity might play an important role in aggressive behaviour in these populations and might

even be independently regulated by serotonin in the brain [359]. To support this, there is recent neurobiological evidence that links impulsivity to aggression and suicidal behaviour in the perspective of the serotonin regulation [360]. By definition, impulsivity is considered the tendency to execute swift actions that are poorly conceived and/or prematurely expressed [11, 361]. Impulsivity, like aggression, is associated with a number of psychopathologies like autism spectrum disorders, schizophrenia and suicide [307]. Besides some phenotypic similarities, the pathophysiology of impulsive behaviour has many comparable features to aggressive behaviour [362, 363]. It has been discussed that it is almost impossible to discern aggression from underlying or comorbid impulsivity conclusively and for this reason, in many studies, they are reported together [360].

Various animal studies, including the observation in primates which share more complex behaviours with humans, confirmed the modulatory role of serotonin in impulsivity. For example, various primate studies showed that the abnormalities in serotonergic system is related to developmental difficulties, lack of social aptitude and impulsive behaviour in adult animals [344, 356-358]. In rhesus macaques, the low-activity allele of rhMAO-A increased the chance of impulsivity, aggression, and alcohol consumption in these animals [362, 363]. Likewise, in humans, a spectrum of genes involved in serotonin metabolism have been identified as risk factors for impulsive behaviour in the background of conduct or personality disorders in humans [362, 363]. Moreover, lower levels of serotonin metabolite 5-HIAA in the CSF of suicidal patients has been reported before [359, 364, 365]. On the other hand, in clinical studies, augmentation of the level of brain serotonin by administration of supplementation lessened the expression of aggression and impulsivity in humans and other species [311, 366]. Along other serotonergic drugs SSRIs have shown a positive response in the regulation of aggressive and impulsive behaviours in various animals and clinical studies [354, 367, 368]. Of note, SSRIs are routinely added to the other group of drugs including anticonvulsants and neuroleptics to achieve the control of difficult aggressive and impulsive behaviour in severe psychiatric disorders [369-371]. Indeed, repeated comprehensive meta-analysis recorded lower levels of aggression and impulsivity in different cohorts of patients receiving SSRIs especially Fluoxetine in comparison to the placebo-treated group [343, 354]. However, the exact mechanism of action of these drugs in control of impulsivity and aggression has not been completely understood yet [354, 367, 368].

It has been debated whether the therapeutic responses of SSRIs originate from the augmentation of serotonin levels following the administration of these drugs [291, 372, 373]. It has been shown that despite the immediate increase of serotonin in the brain following SSRIs, most of these drugs require 2 to 4 weeks to reach their peak efficacy [291, 372]. Therefore, several long-term post and pre-synaptic changes via activation of various serotonin receptors have been proposed as the mechanism of action of these drugs [291, 372, 373].

Concerning this, 5-HT2CRs have been shown to carry some of the therapeutic effects of SSRIs in various behavioural dimensions [34]. The functional involvement of 5-HT2CRs in the modulation of aggression and impulsivity in animals is supported by various genetic and pharmacological studies. For example, there are genetic observations that link impulsive and suicidal behaviour to the altered RNA editing of 5-HT2C receptors in the brain of the patients [374]. Indeed, suicide victims showed a different pattern of 5-HT2C pre-mRNA profiles compared to controls in multiple studies [32, 36, 39]. Until recently, due to the lack of selective drugs, the pharmacological characterization of various subtypes of 5-HT2 receptors was only partially possible [9, 342]. However, there are indications of involvement of this family of receptors in the aggressive and impulsive behaviour of animals even in older studies. For example, DOI, and other non-selective agonists that act on both 5-HT2A and 5-HT2C receptors, also TFMPP, a mixed 5-HT1B/2C receptor agonist, reduced impulsive and aggressive behaviour in rats in different paradigms like aggressive social dominance [375-378]. Some of these results have been confirmed by the employment of novel and selective ligands of 5-HT2CRs. For instance, acute treatment with WAY-163909, a 5-HT2CRs agonist decreased the chance of occurrence of aggression in resident-intruder test and led to more docile behaviour in rats [379]. The aberrant interaction of 5-HT2CRs with other serotonin receptor subtypes has been studied in the context of aggressive behaviour. For instance, the absolute level of availability or sensitivity of 5-HT2C to 5-HT2A receptors has been reported to predict the possibility of aggression in animals [38, 380]. The activation of 5-HT2C receptors by mCPP enhanced the display of submissive and defensive behaviour, whereas activation of 5-HT2A receptors by MDL 11,939 disrupted the development of conditioned defeat response in animals [381]. In contrast to the positive profile of agonism of 5-HT2CRs in aggression and impulsivity paradigms, the final response of 5-HT2CRs antagonism in this regard has been less studied. In five-choice serial

reaction time task, 5-HT2C receptors antagonist SB 242084 increased premature responding in all animal groups [382]. Likewise, various antagonists of 5-HT2CRs deteriorated cognitive indexes in tests like reversal learning by increasing the impulsivity and repetitive behaviours in the animals [383].

Recently, the beneficial characteristic of 5-HT2CRs activation in the context of food and drug addiction has been observed. This is pertinent since stronger chances of relapse in humans and animals with addictive propensities have been attributed to the presence of higher impulsivity index [236]. In most of these studies, evidence shows that 5-HT2CRs agonists, by increasing the inhibitory control, regulated the reward-seeking behaviour in conditioned animals and prevented further relapse [237, 384]. In agreement with this, the positive anti-addictive and anti-impulsive properties of Lorcaserin, a new selective 5-HT2C receptors agonist in both pre-clinical and clinical studies has been observed [212, 236, 239, 255]. Moreover, at least part of the anti-obesity outcome of Lorcaserin is believed to be related to its anti-impulsive response in the patients [212, 385]. Currently, indications of 5-HT2CRs agonists in the context of addiction to various drugs of abuse are under investigation [224, 236]. Preliminary results described a significant positive response for Lorcaserin in the treatment of nicotine addiction in the vulnerable population [141, 237].

Combined, this evidence supports the central role of 5-HT2CRs in mediation of the regulatory response of serotonin system in different dimensions of aggressive and impulsive behaviours in animals. Recent clinical experiments demonstrate the practical implications of positive regulatory function of these receptors in the context of food and drug addiction. However, despite such promising results, more investigations in the future are needed to determine the exact place of 5-HT2C receptors in the management of impulsive and aggressive behaviour in the clinic.

1.4.2. Anxiety-related behaviour

Anxiety is a physiological reaction to an undesirable situation with protective functions in animals [386, 387]. It could manifest itself through a range of responses like avoidance, fight or escape depending on a variety of biological and environmental factors [386, 388]. Anxious emotional state is normally accompanied by activation of the sympathetic nervous system and its physiological bodily responses [386]. However, in humans, exaggerated, unjustified or prolonged anxious responses can cause a great deal of suffering and disturb the everyday life of an individual [387, 388]. Of note, anxiety disorders are the most prevalent groups of mental disorders in present society with the lifetime prevalence between 10 and 30 percent varying on the type of the criteria used [387, 389]. Consequently, in western societies antidepressants as the mainstay in the treatment of both anxiety and depressive disorders are among the most prescribed medications [390]. Indeed, depression with up to 50 percent rate of coexistence is a common finding in anxiety disorder patients and these disorders are often diagnosed and treated together in clinical settings [391, 392]. For a long time, it has been discussed that despite some demographic and symptomatic distinctions, the division between anxiety and depressive disorders as two separate classes of the mental disease is rather arbitrary [392]. Lately, the discovery of many shared genetic mutations and similar underlying pathophysiology fuelled the ongoing critique of the current classification of these disorders [392, 393]. The ensemble of this evidence indicates an emerging new perspective regarding the classification of anxiety and depression in particular and mental disorders in general in scientific milieu [392, 394]. Concerning this, “mixed anxiety and depression” disorder as an independent entity has been recently added to the list of the International Classification of Diseases, 10th edition (ICD-10) [391]. Furthermore, different Genome-Wide studies and brain network analysis have attempted to formulate a new spectrum-based model of mental disorders with common biological explanations in recent years [391, 392]. As the result, many authors, suggested a dimensional approach founded on etiological and molecular basis to understand and classify psychiatric disorders [392, 394]. Such proposition is supported by the result of numerous scientific data gathered via various modalities that shaped our recent understanding of the biological basis of psychiatric disorders [392, 394].

Various genetic animal models been generated in the recent years to investigate the final contribution of serotonin in the pathophysiology of anxiety and depressive disorders. Successively, a vast spectrum of behaviour changes has been reported in these animals after the alteration of serotonin neurotransmission. For example, SERT-KO HO mice showed anxiety-related traits in paradigms like elevated plus maze (EPM) and open-field test [395, 396]. Also, depressive-like features, including increased immobility in forced swimming test (FST) and anhedonia in sucrose preference test have also been observed in these mice [395, 396]. SERT-KO mice exhibited higher social anxiety level and aggressive sexual behaviours in interaction with other animals [395, 396]. At the same time, transgenic mice overexpressing the human SERT protein demonstrated a low-anxiety phenotype in EPM and novelty-induced suppressed feeding tests [397]. Interestingly, acute administration of Paroxetine restored the normal anxiety level in these mice [397]. While these reports point out to the direct connection of serotonin and anxiety, the review of the majority of existing results in the literature suggests a more complex role for serotonin in regulation of mood and emotion in animals. For example, the Pet-1-deficient mouse with up to 90 percent of brain serotonin depletion, showed increased anxiety and frequency of aggressive behaviour in multiple studies [316, 317]. Moreover, in 5-HT1A null mutant mice a complex phenotype, including increased anxiety level, abnormal stress-coping, and decreased responsiveness to antidepressants, has been recorded [398, 399]. Interestingly, solely the absence of 5-HT1A auto-receptors in the brainstem was sufficient to replicate almost all of these results [400, 401]. Furthermore, while in adult mice, suppression of these receptors at raphe level did not affect anxiety, post-natal suppression of 5-HT1A receptors led to an increased anxiety level later in life [402]. This indicates that the control of raphe output on the anxiety depends among others on the availability of different serotonin receptor populations during brain development [403]. Surprisingly, in Tg8 mice, the lack of MAO-A enzyme increased the serotonin and norepinephrine levels but did not change the result of the light-dark test compared to wild type animals [326, 404]. Likewise, another MAO-A deficient mice model MAOAA863T-KO manifested no anxiety-like phenotype in elevated T-maze or EPM test [327, 405]. Moreover, these mice showed no difference to control animals in open-field and tail suspension test (TST) [327, 405]. On the other hand, in humanized TPH2-KI animals, the considerable reduction of the brain serotonin was accompanied by anxious behaviours in various settings [319, 324]. In this respect, overt anxious and depressive-like state of these animals has

been reported in paradigms like dark-light, openfield and TST [319, 324]. In addition, these animals showed abnormal 5-HIAA concentrations in CSF and altered responses to SSRIs compared to their wildtype littermates [319]. Similarly, the complete absence of the TPH2 enzyme in TPH2-KO mice led to a variety of abnormal behaviour in anxiety and depression settings [320, 321]. For instance, the higher amount of immobility in FST was recorded in these mice. They also showed abnormal behaviours in EPM, marble burying and novelty-suppressed feeding tests [320, 321]. However, other groups showed that TPH2-KO mice did not display any obvious anxiety-like behaviours in marble burying tests [406]. Besides direct manipulation of the genes, by careful and selective inbreeding of certain endophenotypes, researchers have created several strains of rodents with higher inherent level of anxiety. The Flinders Sensitive Line (FSL) and Flinders Resistant Line are well-known examples of such models used in research. Remarkably, the typical anxious and depressive-like behaviours in FSL rats like decreased appetite and increased immobility in the FST have been rescued by administration of Escitalopram [407]. Remarkably, these rodents show abnormalities at the level of serotonin receptors binding, serotonin metabolism and responses to SSRIs that are commonly reported in anxious and depressive patients [408-410].

Presently, numerous modalities and animal models exist to study the neurobiological basis of anxiety and depression disorders. Earlier stated results were just a few examples of recent efforts to investigate the intricate modulatory role of serotonin in these paradigms. In most of these models, a complex if not contradictory, relationship between the serotonin level and the emotional state of animals has been witnessed [17]. It has been argued that the behavioural outcome of alteration of brain serotonin level, as achieved in most of these models, could produce contradictory results depending on the variation of several contributing elements. For example, the time course of serotonin release, differential responses of various brain networks and the influence of other neurotransmitters could modify the level of observed anxiety in the animals [19, 411-414]. Moreover, the differential outcome of pre- and post-synaptic various serotonin receptors to control the final behavioural outcome of serotonin neurotransmission has been reported before [19, 411-414].

In the recent studies, the vital involvement of brain serotoninergic pathways in the pathogenesis and treatment responses of depression and anxiety disorders has been a constant finding [415, 416]. For example, changes in the blood flow and volume of structures with heavy serotonin innervation like prefrontal, hippocampus, striatum, amygdala and thalamus have been observed in anxious or depressed subjects [417-419]. Besides structural changes, newer imaging techniques allow us to compare the activity of certain proteins in the brain of patients to the control population. Interestingly, in many of these studies, detecting irregularities in the activity and concentration of various proteins involved in metabolism and neurotransmission of serotonin in the brain has been a recurrent observation. For instance, higher level of SERT binding in the brainstem and frontal cortex of patients suffering from panic attacks has been witnessed [420-423]. Similarly, reduced 5-HT1A receptor binding has been shown in various groups of anxious patients while this abnormality was normalised after treatment with Paroxetine in recovered patients [424, 425]. This is in agreement with the results of other studies that suggest an essential role for 5-HT1A auto-receptors in the regulation of emotional reactions [424, 426]. Related to this, in a case-control study, the G allele of C1019G polymorphism (rs6295) in 5-HT1A receptors was accompanied by a higher probability of general anxiety disorder (GAD) in the population [427]. It is known that through a negative feedback loop, these receptors are involved in the determination of final output of the raphe nuclei. Accordingly, an increased risk of suicide, a higher incidence of depression and decreased antidepressant responsiveness have been linked to the presence of this allele in the population [428].

Other polymorphisms have also been linked to the presence of anxious traits in the population. For example, various mutations of MAO-A gene like T allele of the MAO-A T941G polymorphism as well as mutations upstream this gene in “variable number of tandem repeats” have been related to higher scores of anxiety or the presence of GAD in patients [429, 430]. Interestingly, mutations reported in the serotonin transporter linked polymorphic region (HTTLPR) and the consequent altered levels of SERT have been connected to various personality disorders, abnormal medication response and aberrant stress coping mechanisms [431, 432]. For example, the short (S) allele of HTTLPR predicted neuroticism, harm avoidance, and disagreeable tendencies in the population [433, 434]. Also, it has been shown in other studies that the S allele occurs in much higher frequency in anxious patients than in healthy subjects

[435]. This has also been reported in people who rank higher in neuroticism questionnaires or with anxious personality traits or disorders [436-438]. In individuals exposed to early life traumas, the presence of S allele aggravated the risk of occurrence of depression and anxiety in adults pointing out the intricate dynamic of nurture vs. nature in the pathogenesis of these mental disorders [439]. Related to this, even in healthy carriers of S allele compared to homozygous individuals for the L allele, higher levels of amygdala activity is noted [440]. It is worth mentioning that the hyperactivity of the limbic system is one of the most consistently reported anomalies in the depressive and anxious group of patients across various modalities [441-443]. Respecting this, reductions of functional coupling between different brain circuits like the amygdala, anterior cingulate cortex and striatum, in carriers of various genetic risk factors of mood and anxiety disorders have been demonstrated repeatedly [444]. Though the details of such irregular network communications and their importance in the pathophysiology of anxiety disorders are yet to be determined, the central role of serotonin in this regard is very clear to us [415, 416]. In fact, aberrant serotonin neurotransmission to structures like cortex and amygdala has been proposed as a central part of the neurophysiology of anxiety disorders in patients and their counterpart animal models [441, 444].

Pharmacological studies have formed our perspective about the multifaceted role of serotonin in modulation of behaviours in animals. In fact, since its discovery, serotonin has been at the center of many investigations to explain the pathophysiology of anxiety and depressive disorders. Earlier, a growing body of research supported possible anxiolytic responses of serotonergic modulation both in human and non-human animals through various experiments [445-447]. Subsequently, various serotonergic agents have been tried to relieve the symptoms of anxiety over the years. For example, in different tests, 5-HT1 agonists demonstrated an anxiolytic effect in both rodents and anxious patients [448, 449]. As the result, to this date, Buspirone, a non-selective 5-HT1A agonist, is used as an anxiolytic agent in some of the anxious patients [450]. However, it was the development of SSRIs, and their clinical efficacy that changed the standpoint of serotonin neurotransmission in the treatment of anxiety and depression [10, 451]. Concerning this, the efficacy of several SSRIs in the management of different categories of depression and anxiety disorders has been documented in numerous controlled studies and meta-analyses [10, 451]. In fact, the current list of indications of SSRIs in the treatment of various

aspects of mental disorders continues to expand [393, 452]. While the central role of serotonin in the pathophysiology of anxiety and depression disorders is confirmed, the details of abnormal serotonin function in patients is not understood yet [393, 452]. Moreover, whether the therapeutic outcome of SSRIs is simply the result of an increased level of serotonin in the brain of the patients has been debated [10, 451]. Despite some evidence, the causal relationship between the brain serotonin deficiency and the symptoms of depression and anxiety has not yet been established [10, 451]. Moreover, the immediate increase of serotonin level following acute administration of SSRIs would not corroborate with the long-term responses of these drugs [10, 451]. As a result of these disagreements, other modalities, including inducing neurogenesis, enhancement of pituitary hormonal responses and release of natural antidepressants like BDNF, as alternative mechanisms of action of SSRIs have been proposed [393, 452].

Among such proposals, the contribution of various serotonin receptors in the final outcome of SSRIs has been the subject of extensive studies in recent years. It has been observed that the therapeutic anti-anxiety and antidepressant effect of SSRIs in certain settings was partly mediated by 5-HT2CRs [453, 454]. In fact, some anatomical, genetic and pharmacological evidence supports the involvement of 5-HT2C receptors in the regulation of anxiety and depression in human and non-human animals [455]. Concerning this, the vast central distribution of 5-HT2C receptors at major mood-regulating regions of the brain like amygdala, cortex and hippocampus has been proven [15, 57]. This could explain the pervasive nature of phenotypes observed in 5-HT2C-KO animals in related anxiety and depression paradigms. In fact, the deletion of this gene produced an anxiolytic outcome in these mice in elevated plus maze and open-field tests [57, 93]. In addition, while the baseline level of serotonin is reported normal, Fluoxetine had an exaggerated response and increased dramatically the serotonin level in 5-HT2C mutant mice [453]. Conversely, overexpression of 5-HT2CRs in the cerebral cortex and limbic area had an anxiogenic effect in animals in various paradigms [456].

In the early pharmacological studies, activation of 5-HT2CRs has been tied to the anxiogenic responses in non-human animals as well as humans [457-460]. For example, mCPP, a non-selective 5-HT2CRs agonist produced a significant increase on all subjective ratings of anxiety in panic disorder patients [459]. MK-212, another agonist, also induced anxiogenic behaviour

and a panic state in humans similar to mCPP [293, 460, 461]. While comparable results have been replicated in humans and animals, there are also reports of anxiolytic responses of mCPP in certain tests like elevated plus maze in animals [462]. Furthermore, in humans, mCPP reduced appetite without inducing anxiety in healthy volunteers [463]. Interestingly, in contrast to the older studies, some of the newer 5-HT2CRs agonists were completely devoid of anxiogenic effects [462, 464]. For example, RO 60-0175 showed anti-compulsive and antidepressant effects in tests like FST in some rodent models without affecting the anxiety parameters [462, 464]. In connection with this, WAY 163909, another 5-HT2CRs agonist, decreased food intake in normal rats, Zuker rats and diet-induced obese mice without inducing the anxiety [465, 466]. Remarkably, in chronic mild stress-induced anhedonia and olfactory bulbectomy models of depression, 5-HT2CRs agonists have been reported to even possess anxiolytic and antidepressant characters [203, 379]. While the final response of 5-HT2CRs agonists is still debated, the strong anxiolytic and antidepressant properties of 5-HT2CRs antagonists have been established in multiple studies [457, 467, 468]. Several 5-HT2C antagonists have shown direct anxiolytic responses and resisted the anxiogenic effect of other compounds [29]. For instance, pre-treatment with 5-HT2C receptor antagonist SB 242084 inhibited anxiogenic response of various drugs including mCPP in animals [469]. In rats, a non-selective 5-HT2C receptor antagonist Ketanserin along the more selective agents like SB 242084 and RS 102221 augmented the response of SSRIs in TST and increased hippocampal and cortical serotonin level [453]. Further, SB 200646A, a selective 5-HT2C/5-HT2B receptors antagonist, reduced the social anxiety and aggression in rats providing a convincing anxiolytic profile for this drug [457]. Also, 242084, a 5-HT2CRs antagonist, exhibited anxiolytic response and increased time spent, distance travelled and number of entries into open arms of elevated plus maze test in rats [458]. These results favour the positive response of 5-HT2CRs antagonists in depression and anxiety disorders. In agreement with this, some atypical antidepressants like Mirtazapine and Mianserin are partly 5-HT2CRs antagonists [470]. Finally, Agomelatine a 5-HT2CRs antagonist with additional melatonin 1 and 2 receptor agonistic activity is on the market for the treatment of mood and obsessive disorders in Europe and Australia [29, 471].

While there is a consensus about the beneficial responses of antagonism of 5-HT2C receptors in anxiety and mood disorders, the debate about the final implications of 5-HT2CRs agonists in this

regard is still going on. To explain some of the conflicting results, some authors identified the differential responses of 5-HT2C receptors in various brain pathways involved in regulation of emotions. Based on the latest consensus, raphe nuclei in the brainstem modulate the expression of the emotional state of animals through two different pathways [472, 473]. It is shown that fight or flight "fear responses" are conducted through periaqueductal gray matter neurons [289, 474, 475]. Conversely, serotonin transmission via amygdala pathway is responsible to mediate the "anxiety responses" in the animals [289, 474, 475]. Recent evidence shows that this appears to be the case in both non-human and human animal brains [289, 474, 475]. This explains the wide range of expressed emotions and behaviours in stressful conditions as well as the underpinning mechanism of various anxiety disorders [473, 475]. In this theory, dysregulation of "fear pathway" is responsible for manifestations of the psychopathologies like specific phobias, social anxiety and panic disorder through hyperactivity of periaqueductal gray matter [476, 477]. On the other hand, abnormalities of the amygdala-regulated "anxiety pathway" encompasses the pathologies such as general anxiety disorder and the distress experienced in OCD patients [476, 477].

In support of this idea, CP809.101, a selective compound, was anxiogenic in some tests like learned helplessness and fear-inducing paradigms while remaining inert in other paradigms [204, 478-480]. In fact, it has been suggested that only the activation of the 5-HT2CRs in the basolateral amygdala possess anxiogenic responses [204, 478-480]. On the contrary, it is claimed that 5-HT2CRs activation in the periaqueductal gray matter reduces the level of anxiety and has anxiolytic properties in animals [481, 482]. As mentioned before, this pathway is involved in the innate "fight or flight" reactions of animals [481, 482]. Remarkably, this dual role of serotonin receptors in the modulation of anxiety levels has been shown both in human and non-human animals subjects [412, 476, 477]. In this outlook, depending on the responding brain network, some subsystem of the serotonergic pathway increases the anxiety, while others could mediate an anxiolytic response [483]. In fact, it has been proposed that the variation in the observed behavioural outcome of 5-HT2CRs agonists might be due to the distinctive features of central serotonin responses measured in different paradigms [204, 478-480].

Besides our recent insight into various emotional-regulating networks of the brain, new selective ligands have informed our understanding of the precise involvement of 5-HT2CRs in anxiety disorders [34]. It has been proposed that the input of other receptors like 5-HT2ARs and 5-HT2BRs could explain some of the differences seen between the older and newer more selective 5-HT2CRs agonists [9, 34]. For example, selective agents like Ro 600175 or WAY 163909 were not capable of producing the full extent of anxiogenic effects of older drugs like mCPP in a similar setting [203, 462, 464]. Finally, it is speculated that even with the newer compounds, due to the extreme heterogeneity of 5-HT2CRs editing profile and subsequent assorted isomers, the real estimation of the contribution of these receptors in anxiety responses of the animals is rather difficult [34, 204]. Altogether, while the clinical value of antagonism of 5-HT2CRs as in the case of Agomelatine is already witnessed, additional studies in this field are needed to determine the practical implications of agonism of these receptors in depression and anxiety disorders.

1.4.3. Repetitive and compulsive behaviour

Obsessive-compulsive disorder (OCD) is a complex clinical condition affecting 2 to 3 percent of the population and consists of 1- obsessive intrusive and distressful thoughts and 2- compulsive repetitive rituals or actions to stop this distress [484, 485]. Its psychological and economic suffering has been extensively discussed. The World Health Organization (WHO) considers it one of the top ten most debilitating diseases in the population [416, 485, 486]. OCD has been recently removed from anxiety disorders and classified as “obsessive-compulsive and related disorders” in DSM-5 [487]. There are various forms and degrees of severity of this complex group of disorders and its coincidence with other psychopathologies like anxiety and major depressive disorder (MDD) is very common [76, 484, 488]. The evidence indicates the strong genetic component of the pathophysiology of obsessive-compulsive disorders. Presently, more than 80 positional and functional mutations have been associated with OCD. Some of these mutations occur within genes responsible for the regulation of serotonin neurotransmission in different parts of the brain [484, 488].

Animal studies of repetitive and compulsive behaviour have been proven to be a tremendously valuable tool in identifying risk factors and development of efficient medication in OCD disorders [297, 489]. However, we should mention that due to the subjectivity of OCD symptoms, a heterogenous group of repetitive and compulsive behaviours in animals have been interpreted as pertinent models [488, 490]. Such animal models encompass a wide dimension of the emotional and behavioral components of responses that might be related to various brain functions like cognition, compulsion, anxiety, aggression and impulsivity [488, 490]. However, in most of these investigations, compulsive and repetitive features are measured among others by observing behaviours like ritual-like motor activity, compulsive grooming, marble burying or repetitive patterns of action in animals [297]. While some of these models lack fundamental construct validity, they offer relative face validity and sometimes even possess comparable drug responses to the actual disease [491, 492]. Analyses of the repetitive and compulsive behaviours of the animals following the modification of some of the genes identified in the pathophysiology of OCD has been the basis of some research in this field [297, 489]. For instance, an increased grooming activity among other repetitive behaviours has been reported in SERT-KO in both BL6 mice and Wistar rats [493]. Also, serotonin deficient TPH2-KI mice showed a higher level of perseverance and repetitive wrong choices in H-Maze test indicating cognitive abnormalities in these animals [210]. In parallel to this, abnormal behavioral indexes in tests like marble burying have been observed in these animals [321]. Remarkably, serotonergic drugs rescued these behaviours and improved the performance of the animals in this test [210]. Besides the genetic studies, the pivotal role of serotonin in the pathogenesis of OCD is supported by results of pharmacological animal models. Concerning this, 8-OH-DPAT-induced compulsive behaviour is one of the most popular pharmacological OCD animal models. 8-OH-DPAT is serotonin 5-HT1A and 5-HT7 receptors agonist that has been used traditionally to induce pervasive repetitive behaviour similar to OCD in rodents [494]. Similarly, 5-HT1B and 5-HT1A agonist RU 24969 increases the perseverative circling around the perimeter of an open-field chamber in rodents and is used as an animal model of OCD. Interestingly, in multiple studies, serotonergic agents particularly SSRIs reduced these “ritual-like” activities of the animals in both of these models efficiently [495, 496].

In humans, correlation studies have shown that the genes encoding proteins like SERT and catechol-O-methyltransferase (COMT) and MAO-A play an important role in the genetic make-up of OCD disorders [76]. Multiple meta-analyses confirmed the involvement of various mutations in the serotonin transporter-linked polymorphic region (5-HTTLPR), COMT and MAO-A in the pathogenesis of OCD [497-499]. In this respect, SLC6A4, the gene encoding SERT protein and its flanking regulatory regions is one of the most studied risk factors in occurrence of OCD in the population [151]. Various mutations of this gene particularly the I425V variant of SLC6A4 has been strongly linked to the presence of OCD in different groups [500]. Related to this, studies of polymorphisms in various genes encoding serotonin receptors like 5-HT1A and 5-HT1B also connected the pathology of the OCD to the aberrant serotonin neurotransmission [501]. HTR2A gene encodes the 5-HT2A receptors in human and is located on chromosome 13. This gene has been investigated recurrently in regard to the occurrence of OCD in patients [292]. For instance, in a recent meta-analysis, the presence of OCD in the population was significantly associated with the occurrence of rs6311 variations of HTR2A [499]. Furthermore, 1438A, another allele of HTR2A, was associated with perfectionism and obsessive traits in human subjects [502]. Mutations in HTR1B gene that encodes 5-HT1B receptors, especially 861G/C (rs6296) polymorphism have been attributed to severity of OCD and its early onset in various independent studies [292, 503-505]. Most of these results are the outcome of homogenous or even family group studies and some of these conclusions were not replicated in multi-centered samples or in larger populations with dissimilar age or sex trends [506]. However, the major contribution of the serotonin system and in the pathophysiology of OCD is well-defined in the literature [76, 507].

In line with these results, clinical studies following different protocols denoted the importance of serotonergic tone in the presentation of the OCD symptoms in patients. For instance, worsening of OCD symptoms in patients using anti-migraine 5-HT1B/D agonists and different psychostimulants has been reported [508-510]. In one study, a challenge of 100 mg oral Sumatriptan was enough to cause a significant aggravation of symptoms in a cohort of OCD patients [511]. Further, exacerbation of OCD symptoms has been shown after the administration of mCPP in patients, while pre-treatment with Clomipramine prevented this response [512-514]. The same protective effect has been observed by the use of 5-HT1/5-HT2 receptors antagonist

Metergoline [515]. Related to the central role of serotonin in the pathophysiology of OCD, abnormal concentration of SERT and 5-HT2A receptors in the brainstem and midbrain of the OCD patients compared to healthy subjects has been demonstrated in imaging studies [516]. Remarkably, earlier clinical studies coupled higher 5-HIAA levels in CSF with the presence of the OCD symptoms in patients [517]. Recent similar results following the measurements of serotonin content of platelets in patients have even led some authors to propose the peripheral serotonin content as a potential reliable biomarker in diagnosis of OCD [485, 518]. Related to this, higher blood serotonin levels in OCD patients with a family history of the disease have been shown in comparison to the OCD patients without the family history, or to the healthy controls [519]. Moreover, reduced SERT activity in the platelets showed a strong correlation to the presence of the symptoms in patients with the active OCD in various studies [485, 518]. In agreement with these observations, reduction of 5-HIAA levels in the CSF after Clomipramine treatment has been connected to a better clinical outcome in patients [520]. Finally, higher pre-treatment levels of serotonin indicated the better outcome of therapy while SSRIs and Clomipramine treatment decreased blood serotonin level significantly only in recovered OCD patients [521-523].

Today, SSRIs, serotonin-norepinephrine reuptake inhibitors (SNRIs), and tricyclic antidepressants (TCAs) are the backbone of OCD treatment and their clinical effectiveness has been demonstrated consistently in the different trials [524-526]. Therapeutic efficacy of these drugs, which mainly inhibit serotonin reuptake and increase the level of serotonin in the brain, highlight the presence of an unbalanced serotonergic neurotransmission in the pathology of OCD [524-526]. However, despite the abundance of preclinical and clinical evidence, the actual neurobiology of OCD is still the subject of heated discussions in the literature [488, 490]. Moreover, between 40 and 60 percent of OCD patients remain symptomatic despite the maximum treatment in clinical settings [488]. Indeed, several authors believe that the aberrant serotonin neurotransmission, in isolation, is not able to account for the totality of the clinical features of OCD [488]. Parallel to this, in recent years, various brain pathologies including decreased white matter integrity, frontal lobe degeneration, basal ganglia atrophy, and loss of connectivity within the dorsal medial prefrontal cortex have been proposed as the underlying pathology of OCD [527-530]. Additional studies to uncover the precise role of serotonin and

other neurotransmitters in the neurobiology of OCD disease and involved brain circuits are warranted in the future.

While the important role of 5-HT2C receptors in mediation of anxiety and depressive disorders has been vigorously studied previously, the contribution of these receptors in the pathophysiology of repetitive and compulsive behaviours is relatively a new subject. Nonetheless, there are some recent studies that investigated the genetic and behavioural implications of 5-HT2C receptors in these paradigms. It has been observed that the mice lacking 5-HT2C receptors showed compulsive-like behaviours including chewing non-nutritive clay in repetitive patterns and frequent head dips [89]. Related to this, an abnormal cocaine and food consumption pattern have also been observed in this mouse model [88, 94, 531]. Genomic studies in larger human samples have not been successful in showing any association between the mutations of HTR2C and OCD yet [9]. However, abnormal methylation of 3 probes in the HTR2C domain in patients with OCD versus healthy controls has been reported in one study [532]. An increased 5-HT2C receptor mRNA in the orbitofrontal cortex of animals, which were exposed to Clomipramine just after birth, has been documented [533, 534]. It is noteworthy that exposure to Clomipramine in pups evokes cognitive deficits, abnormal marble burying and "hoarding behaviour" later in animal's life and has been used as a classic model of compulsivity in the past [490].

The involvement of 5-HT2CRs in repetitive and compulsive behaviours of animals has been also observed in pharmacological studies in animals. With the recent development of selective 5-HT2C agonists, exploration of the effects of this class of receptors in the regulation of these behaviours has been the subject of few studies. In this regard, 5-HT2CRs agonists like WAY 161503 have been tried successfully against obsessive-compulsive-like traits like marble burying in the animals [535]. Moreover, the agonism of 5-HT2C receptors facilitated the reversal learning by limiting the perseverant behaviour in mice lacking a normal level of serotonin in the brain [210]. Interestingly, m-CPP, which binds non-selectively to 5-HT2C receptors, produced dose-dependent excessive grooming in animals in lower doses while in higher doses attenuated this behaviour [536]. This response was inhibited by pre-treatment with relatively selective antagonists of 5-HT2C receptors like Mianserin, LY 53857 and Metergoline [536].

Administration 5-HT2C agonists successfully inhibited the obsessive-compulsive-like traits of animals in models like schedule-induced polydipsia [203, 535]. Furthermore, the agonists of 5-HT2C like mCPP and RO 60-0175 decreased the marble burying behaviour in various rodent studies [203, 535]. Similarly, the anti-compulsive response of 5-HT2C agonists have also been demonstrated in paradigms like induced-excessive scratching or induced-excessive eating in rats [203, 379, 458]. Remarkably, the 5-HT2C antagonists also improved the indexes of compulsive and repetitive behaviours in animals. For example, 5-HT2CRs antagonists S 32006 and SB 242084 had a positive effect against marble burying and perseverant behaviour induced by mCPP respectively [537, 538]. Also, antagonism of 5-HT2CRs by SB 242084 showed a favourable profile and enhanced spatial reversal learning in animals by reducing repetitive non-rewarding choices [539]. Interestingly, 5-HT2C-KO mice exhibited a similar profile and demonstrated an enhanced reversal learning capacity in this study [539]. However, other studies reported no changes after antagonism of 5-HT2C receptors in repetitive traits of animals [540, 541].

Altogether, it seems that both agonism and antagonism of 5-HT2C receptors attenuating perseverance and diminishing unrewarded responses of animals in various paradigms. These results led several authors to propose a pro-cognitive and anti-compulsive effects for the 5-HT2C drugs via improving the cognitive flexibility [383, 539]. This has been supported by the positive responses of Agomelatine a new antidepressant in preclinical and clinical studies [383, 539]. In fact, Agomelatine, a potent agonist at melatonin receptors and an antagonist at 5-HT2C receptors, reduced the compulsivity in animals and successfully alleviate the OCD symptoms in patients [383, 539]. On the contrary, the exacerbation of OCD symptoms after administration of some older 5-HT2CRs agonists like mCPP in different cohorts of the patients has been reported [542-544]. Overall, while 5-HT2C receptors seem a valuable pharmacological target to relieve the symptoms of OCD in patients, the details of the involvement of these receptors and their potential therapeutic values warrant further clarification in the future.

1.5. Animal models

Historically, animal studies have played an essential role in the expansion of our knowledge in different domains of scientific research [634]. While various ethical and economic issues confine the research in humans to certain limited areas, animal models continue to provide a precious outlook to the understanding of the principles of mental disorders [635, 636]. Broad choice of behavioural study paradigms, having access to the brain tissues and the possibility of multiple pharmacological interventions are some of the appealing features of animal studies [635, 636]. For a very long time, mental disorder research was limited to the pharmacological trials along with the basic behavioural and biochemical analysis on the animal's brain tissue [637, 638]. However, developing knoc-KOut animal models in the 1990s opened the door to discern more precisely the behavioural and molecular events following the absence of certain proteins or receptors in the brain [83, 634]. By the introduction of genetic engineering techniques and the generation of multiple animal models based on the genetic variations in the human population, the animal studies became yet an indivisible modality in the neuroscience research [634].

In respect to this, a large body of our present comprehension about the various central functions of serotonin in human like hunger, sex and sleep has been established through experiments in the animals [7, 17, 611]. The mapping of serotonergic brain regions and the discovery of the central role of serotonin in the regulations of the mood, anxiety, feeding and motivation laid the foundations for the appreciation of the importance of this molecule in mental disorders [291, 540, 587, 591]. Today, the central psychological effects of serotonin system and its significant involvement in mental pathologies like eating disorders, depression, schizophrenia and anxiety are more revealed to us [9, 572]. Following this, much of our advancements in understanding of the pathophysiology of these diseases as well as treatment improvements are still based on the investigations in the animals [637, 638]. In modern scientific paradigm, the preclinical research in animals is an indispensable and obligatory phase of introducing any given newly designed drug in the population. Concerning this, the preclinical studies are designed to gain insights into the efficiency of suggested treatments and ascertaining the safety of such pharmacological

interventions before the clinical phase. Without this working knowledge our ability to infer the therapeutic value and potential adverse reactions of a new treatment would be compromised.

All the animal models are inherently limited. Among many other measures, three criteria are typically used to describe an ideal animal model that can mirror a human disease: construct validity, face validity, and predictive validity [298, 547]. Construct validity is best understood in the creation of the genetic animal models by the manipulation of a putative gene recognized as a causative factor in the literature [193]. This means an alteration in the genetic repertoire of the animal, hopefully, creates the phenotype and associated biomarkers similar to the considered pathology. This seldom happens since many of the mental disorders are polygenic and one independent gene is not enough to produce the whole characteristics of the disease [298, 547]. Plus, many of these genes are triggered only by certain environmental elements during critical periods of development that are unknown to us or hard to replicate in the laboratory [298, 547]. Face validity is the degree of similarity of the observed behaviour in animals and in studied human subjects [193]. Due to a limited range of animal behaviours compared to humans, subjective interpretation of specific behaviours in animals is inevitable. This has been identified as a source of some confusion. For example, repetitive behaviour in animals has been described as a model of anxiety disorders, OCD or, even cognitive disturbances by various authors [321, 490, 492]. Predictive validity is the capacity of an animal model to predict the clinical value of the proposed treatment in the human [193]. For example, tail suspension and forced swimming tests which model despair-like state in animals possess an excellent predictive validity regarding the antidepressant activity of drugs while possessing poor face and construct validity [547]. To date, no single given model meets these three criteria. Hence, the precise assessment of the limitations and strengths of a model in the interpretation of the results is crucial [298]. In addition, one of the limitations of animal models, particularly in mental disorders, is the subjectivity of many of the symptoms experienced by the patients in the clinic. These are not adaptable to the animal models [297]. For example, in the case of anxiety, obsessive-compulsive and depressive disorders, a strong inner disturbance that is experienced and self-reported in patients is the keystone of the diagnosis [545]. In most cases, there are cultural, religious and social elements that would not be applicable to the animal models. Respectively, the lack of ability to measure the effect of psychotherapy as an efficient modality is a good example of how

limitations of animal models shape our way of treating mental disorders. Despite such restrictions, a variety of animal models with different degrees of the face, construct, and predictive validity exist. They are still essential in our scientific investigations in the field of psychiatric disorders [136]. Since most psychiatric disorders are highly heritable, a vast collection of mutations have been recognized as genetic risk factors for various mental disorders in the population [136]. Many of these candidate genes have been modified or deleted in rodents to clarify the role of the corresponding protein in different brain networks [136]. Currently, in order to study mental disorders, many relevant mice lines carrying different genetic mutations with various degrees of the face and predictive validity are available.

As one of the central neurotransmitters of the brain, it is only natural that many of these models involve the alteration of neurotransmission of serotonin in the brain of the animals. Recognition of a nonsense mutation in the MAO-A gene in an aggressive Dutch family was one of the first genetic studies to connect the human behaviour to the content of the brain serotonin [548]. This led to the creation of one of the earliest genetic animal models to study aggression and impulsivity in animals [548]. Deletion of the gene coding MAO-A in this model understandably led to the higher level of serotonin among other neurotransmitters in pups and induced aggressive behaviour in adult mice, reciprocating the clinical picture [326, 327, 331]. Since then, many gene variants related directly or indirectly to the production of the proteins involved in serotonin neurotransmission like SERT, TPH and Pet-1, were used to create relevant animal models [314, 549]. In this respect, TPH genes, that are responsible for coding TPH enzymes and therefore the exclusive control of the production of the brain serotonin, have been the focus of many studies.

TPH is an L-aromatic amino acid hydroxylase enzyme that uses tetrahydropterin O_2 and Fe^{+2} to hydroxylate amino acid L-tryptophan to 5-HTP, an immediate precursor of serotonin [550, 551]. For a long time, scientists believed that this enzyme was responsible for making serotonin for the whole body. However, the creation of the mouse lacking this enzyme showed that this assumption was not precise [552]. Although, these mice lacked serotonin in the periphery and pineal gland had a minor deficiency at the level of the brain serotonin [14, 552]. Following this surprising discovery, the second gene encoding the TPH enzyme was identified in rats and

humans. It was called TPH2, in contrast to TPH1 [14, 552]. Today, we know that TPH1, which is located on chromosome 11 and 7 in humans and mice respectively, is responsible for the production of serotonin in the periphery and pineal gland [14, 552]. In fact, it was observed that TPH1 mRNA is four to six-fold less abundant than of TPH2 in the adult human brain [553, 554]. This explains why for such a long time it was believed that the contribution of this enzyme in the central responses of serotonin was negligible [14, 406]. However, some authors of recent studies have demonstrated the importance of TPH1 enzyme in early age brain development and coping mechanisms in stressful situations [555-557].

On the other hand, it has been shown that TPH2, located on chromosomes 12 and 10 in humans and mice respectively, regulates the production of serotonin in the brain [14]. As a rate-limiting enzyme, this protein determines the total content of serotonin in the brain. Regarding the essential role of this enzyme in brain serotonin neurotransmission, its involvement in various dimensions of psychiatric disorder is not surprising. Multiple studies link various mutations of TPH2 gene to the pathophysiology of mental disorders. For example, bilateral changes in the volume of amygdala and hippocampus and abnormal functional connectivity between cortex and striatum in human carriers of TPH2 mutations have been observed [418, 558]. In this population, decreased cortical function and reduced cognitive performance during challenging mental processes have been observed [418, 558]. Other studies have revealed the possible association of several TPH2 polymorphisms and major mental diseases like mood disorders, attention deficit disorder, autism, and obsessive-compulsive disorder in various cohorts [559-562]. Similarly, certain alleles of TPH2 gene have been recognized as a risk factor for bipolar disorders and schizophrenia in patients [12, 563]. Increased TPH2 protein concentration and mRNA in the prefrontal cortex of bipolar patients and suicide victims has been discovered in post-mortem studies [564-566]. Based on these findings, the TPH2 enzyme has been at the center of attention for many studies in the field of mental disorders since its discovery. Consequently, various animal models have been designed to study the exclusive control of the brain serotonin neurotransmission by the TPH2 enzyme.

At least 4 different groups created TPH2-KO mice independently [320, 406, 567, 568]. In TPH2-KO mice, brain concentration of serotonin was reported as low as 4 percent of a normal animal,

whereas no change in the peripheral level of serotonin was observed [320, 406]. Also, the level of other neurotransmitters like GABA, glutamate, norepinephrine and dopamine were reported unaffected in these animals [320, 567]. However, these mice showed growth retardation, alteration in some brain structures, and higher postnatal mortality mostly due to maternal neglect [320, 406]. Furthermore, deregulation of physiological responses like body temperature, blood pressure and heart and breath rates has been registered in TPH-2-KO [320, 406]. Surprisingly, the results of behavioural studies in these mice showed a great number of discrepancies among different groups. While one of these groups reported no changes in anxiety level of these animals, decreased level of anxiety was found in tests like EPM, marble burying and novelty-suppressed feeding tests in the others [321, 406]. In another study, reduced anxious and depressive-like behaviour, with increased conditioned fear responses, have been reported in these animals [569]. Other studies reported the increased number of buried marbles and aggressive behaviour as the only abnormal behaviour in TPH2-KO mice [406, 570]. In one study these mice showed reduced immobility time in FST and an increased immobility time in TST suggesting intriguing inconsistent results [406]. However, other studies reported reduced immobility time in both TST and FST in TPH2-KO animals [321, 323]. Generally, in most of these studies, anxiety-related paradigms, locomotion, and explorative activity of these mice were unaltered compared to wild type animals [321, 323]. Overall, the absence of the TPH2 gene had a mixed effect in various dimensions of animal behaviours which warrants further clarification in future studies. These results may suggest the considerable importance of the genetic background on the evaluation of the role of various genes in the behaviour of animals. The significance of such genetic factors in the assessment of behavioural responses like anxiety and aggression has been repeatedly demonstrated [314, 571].

Besides such conflicting results, the major setback in TPH2-KO model is the long-term and profound consequences of the absence of serotonin in the brain. Such disruption of serotonin homeostasis, especially during the developmental period, could result in permanent malformation of the synapses and brain circuits in pups [320, 572]. Such profound alteration of the brain structure makes the interpretation of the results in these animals difficult. While such extreme serotonin depletion might help us to understand the physiological role of serotonin in the brain, the relevance of such condition as a model of mental disorder is questionable. Since no

known similar genotype exists in the population, this model lacks the ability to represent an actual clinical condition in humans.

In response to these critiques, many groups have tried to create animal models based on the polymorphisms observed in the TPH2 gene. These “naturalistic“ models are more “true-to-life” since they represent a mutation that occurs naturally and is present in the population. In addition, since alteration of the serotonin level in most of these models is relatively modest, these mice do not suffer from drastic physiological consequences of the absence of serotonin in the early ages. Currently, more than thousand SNPs in the human TPH2 gene have been discovered (http://www.ncbi.nlm.nh.gov). Nevertheless, most of the studies in this field are presently focused on some missense mutations in the coding parts of the human TPH2 gene like S41Y, P206S, R303W and R441H mutations. This is due to the existing evidence of the clinical relevance of such polymorphism in the population. For example, the P206S and S41Y mutations were recognized in different cohorts of the patients diagnosed with bipolar disorders [573, 574]. On the other hand, the R303W mutation was detected in a family with the history of attention deficit hyperactivity disorder [575]. The R441H mutation was found in a cohort of elderly depressive patients in 9 out of 87 cases and 3 out of 219 controls [325]. Interestingly, in this study, besides affecting the prevalence of the disease, it has been proposed that R441H mutation might alter the prognosis of the treatment in affected population [325]. We should mention that R441H missense mutation in which Arginine is replaced by Histidine is rare and therefore some studies have failed to report it [307, 576-578].

Despite this, since its discovery, due to its important clinical implications, this mutation has been the subject of various studies. The actual consequence of R441H mutation in various biological settings has been evaluated. For example, it was shown that this missense mutation resulted in approximately 80 percent loss of function in the TPH2 enzyme when expressed in PC12 cells [325]. TPH2-KI mice carry mouse allele R439H which is the equivalent of the human R441H mutation in the genome of the mice [324]. This model is known for its relevance and construct validity among other available animal models in the field. In this regard, TPH2-KI mice showed no evidence of developmental delay, increased mortality, or abnormal regulation of autonomic responses in various studies [324, 325]. This is probably due to relative availability of serotonin

in the brain compared to TPH2-KO mice. In homozygote TPH2-KI mice, up to 80 percent reduction in serotonin levels of the striatum, frontal cortex and hippocampus have been reported [324]. It has been shown that this mutation does not influence the concentration of other major neurotransmitters like dopamine and acetylcholine in these brain structures [13]. Remarkably, in heterozygous TPH2-KI mice decreased level of serotonin in the frontal cortex was the only detected abnormality in the brain [324]. The ensemble of these features makes TPH2-KI mice one of the most reliable and pertinent animal models available to study the implication of abnormal serotonin neurotransmission in the context of mental disorders.

Recently, the basic characteristics of TPH2-KI animals in related dimensions of psychiatric disorders have been studied. In general, the higher level of anxiety, aggression and impulsivity in these mice has been registered in most of these investigations. For example, in one study, TPH2-KI mutants stayed shorter and had less activity in the light compartment of the dark-light test [324]. While normal locomotion activity was recorded in these mice, they travelled less in the central zone of the open-field test than wild type animals [324]. Furthermore, a higher level of aggression in social interaction and increased immobility time in the TST has been documented in TPH2-KI homozygotes [321, 324]. Impairments in reversal learning and cognitive flexibility manifesting as tendencies to repeat the unrewarded choices were also observed in these animals [210]. Finally, TPH2-KI mice exhibited an exaggerated preference for ethanol in two-bottle choice tests compared to the wild type littermates [579]. In most of these studies, the altered brain serotonin neurotransmission has been highlighted as an underlying pathology for the observed abnormal behaviour in mutant animals [544]. Evidence of endogenous CNS serotonin deficiency like abnormal CSF 5-HIAA level is reported in these mice [319]. Moreover, indications of irregular activity of different serotonin receptors, especially 5-HT2ARs in the prefrontal cortex, striatum and substantia nigra of these mice, have been documented [319, 580].

Similarities of some of these results to the findings in depressive and anxious patients have increased the validity of this model to reflect the actual pathophysiology of these mental disorders [319, 580]. In agreement, TPH2-KI homozygote mice also exhibited abnormal synchrony between mPFC and basal amygdala [581]. This resonates with the recent evidence indicating the involvement of atypical brain network connectivity in the pathology of depressive

and anxiety disorders [572]. Interestingly, chronic treatment with Fluoxetine, in line with the observed therapeutic effects of SSRIs, restored the regular network connection in the brain of these animals [581]. On the other hand, multiple studies reported a distorted response of these animals to different antidepressants. For example, chronic Fluoxetine led to a drastic decrease of the serotonin level in the frontal cortex of homozygote mice, while administration of 5-HTP prevented this reaction [582]. Similar atypical responses to SSRIs were observed in the context of social defeat stress (SDS) test, while the protective outcome of Fluoxetine against SDS-induced social avoidance was absent in mutant animals [583]. These results emphasize the vital role of normal serotonin neurotransmission in achieving the therapeutic outcome of treatment with SSRIs in patients [581]. Some authors have proposed comparable abnormalities in serotonin system as an explanation for the lack of clinical efficacy of SSRIs in resistant cases of depressive and anxious disorders [581]. A normal serotonin neurotransmission has been accounted an essential factor in differential responses of individuals facing stressful events. Abnormal stress coping mechanism of these mice in a battery of stressful life event models has been demonstrated. For example, female TPH2-KI mice facing chronic mild stress showed an exaggerated response including anhedonia-like behaviour in the sucrose preference test [584]. Similarly, exposure to early life stress provoked the anxiety-like behaviour in the wild type mice while inducing the opposite effect in TPH2-KI homozygous animals [585]. Comparable abnormalities have been reported after the exposure to other stressful challenges like learned helplessness or social defeat test in this model [583, 586]. Such observations are in line with the recent evidence indicating the decisive role of serotonin neurotransmission in resilient character of some individuals facing challenging situations [587]. In this respect, the serotonergic input of mPFC-amygdala network has emerged as a determining factor in mediating adaptive or maladaptive stress responses of individuals [587].

Overall, as a result of these studies, TPH2-KI mice have been regarded as one of the most applicable animal models to study the implications of the serotonergic system in the context of mental disorders [324, 325]. In contrast to TPH2-KO animals, TPH2-KI mice have normal morphology, lifespan and rearing. Moreover, no brain malformation or structural CNS abnormality has been reported in these mice. To ensure the genetic background homogeneity, TPH2-KI mice used in the current thesis have been backcrossed over C57BL/6 line for at least

10 generations in our laboratory. Lastly, as in the case of all naturalistic models, the construct validity of TPH2-KI mice representing an actual biological state of some depressive patients is one of the distinguished features of this animal model. The resemblance of abnormalities in the serotonin level, receptors availability, and networks connectivity to the clinical presentation of patients has added to the pertinence of TPH2-KI model [319, 580, 581]. Furthermore, higher level of anxiety, social aggression, repetitive behaviour as well as irregular responses to various stress tests point to the applicability of these mice model in mental disorder research [321, 324, 583, 586]. Largely, the ensemble of these published results signifies the validity of the TPH2-KI animal model as one the best available animal modalities in the field of mental disorders.

1.6. 5-HT2CRs drugs

Mental disorders are a highly disabling heterogeneous group of disease that primarily has no known organic or neurological aetiology. These disorders lead to impairment of cognition, emotion, judgment and behaviour in afflicted subjects [389]. Recently, the financial burden and significant morbidity of mental disorders have been the subject of several studies [389, 588]. A World Health Organization report shows that mental health is one of the most expensive health care expenditures on a global scale [389]. In a new estimation, it is predicted that as high as 6 trillion US dollars would be spent globally on mental health by the year 2030 [389]. According to recent surveys, the lifetime prevalence of some mental disorders like anxiety disorders can reach as high as 28 percent of the population [389, 485]. Furthermore, it is not uncommon for mental disorders to manifest with other psychiatric ailments. For example, in one survey, the lifetime prevalence of any mental disorder was 46.4 percent, while 27.7 percent of respondents had two or more lifetime psychological disorders [389, 485]. Nearly 14.3 percent of deaths worldwide (around 8 million deaths per year including the suicide) have been attributed to various mental illnesses [588]. In addition to the huge socioeconomic burden caused by a high prevalence of mental disorders, the lack of effective treatment is the most striking feature of this group of diseases [526]. Briefly, there are some particular characteristics that have been acknowledged for psychiatric disorders. Among others high prevalence, high rate of treatment-

resisting patients, early age of attack, and lifelong need of treatment for most of the cases separates this group from diseases in other medical specialities [389, 588].

In modern medicine, treatment of mental disorders is based mainly on pharmacotherapy, while occasional cases show the contributions of psychotherapy. However, despite the existence of a vast body of basic science and clinical evidence, our treatment against mental disorders are still at a very primitive stage and have not changed fundamentally since the 1960s [526]. In another word, even with access to a broader spectrum of drugs, a majority of patients still need multiple medications, experience frequent hospitalization episodes, and suffer from major cognitive incapacity [526]. For instance, only 50 percent of depressed patients respond adequately to the treatment and an effective remission happens only in less than one third of patients [10, 526]. The numbers are even worse in the case of obsessive-compulsive disorders [488]. This absence of effective treatment is mostly due to our limited comprehension of the complex nature of mental disorders [526]. Nonetheless, the predominant setback in the field of psychopharmacology is a lack of creative, or reliable, strategies to translate our current knowledge to an efficient and practical treatment [526]. Consequently, the present state of affairs has led to a pessimistic attitude and conservative approach in both academia, and the pharmaceutical sector, in regard to the pharmacotherapy of mental disorder [10, 161]. Facing such reality, identification of new therapeutic targets has been proposed as an indispensable mental health plans to alleviate the personal and socioeconomic burden of mental disorders [29].

Recently, 5-HT2C receptors have gained remarkable attention as a potential new treatment target for psychiatric disorders [29]. There is an accumulating line of evidence supporting the likely implications of these receptors in the pathophysiology of this group of disorders [9]. For example, the presence of 5-HT2C receptors in the major brain circuits involved in anxiety, depression, eating disorders and schizophrenia has been observed using in vivo imaging techniques [34, 191, 237, 589, 590]. In addition, pharmacological studies have shown that 5-HT2C receptors modulation is an active part of various classes of psychoactive drugs including SSRIs, antipsychotics and anxiolytics [30, 34, 161]. For example, at least part of the antidepressant properties of drugs like Mirtazapine, Agomelatine and Fluoxetine is carried by 5-HT2C receptors [29, 453]. Many clinical studies examined the response of 5-HT2CRs

receptors against various conditions in patients. Recently, the benefits of activation of these receptors in treating obesity, depression and schizophrenia in patients have been demonstrated [9, 34]. Successful examples of these systematic investigations introduced medications like Lorcaserin for the treatment of obesity, and Agomelatine for the treatment of mood disorders. A recent clinical trial of antipsychotic properties of 5- HT2CRs agonists like Vabicaserin shows an ongoing trend in the psychiatric field toward the characterization of promises of these receptors [191]. In addition, there has been a considerable effort to employ the anti-impulsive and anti-addictive quality of 5-HT2C drugs in the food and drug addiction context [237, 591]. In a recent pilot study, Lorcaserin in combination with Varenicline has been proposed as a potential new treatment for smoking cessation [238]. Finally, the medical indication of 5-HT2C receptors in resistant epileptic patients and victims of spinal cord injuries is currently under investigation [65]. These examples show that 5-HT2CRs pharmacology is an active and growing part of experimental and clinical research in the field of mental disorders. In the following section, we briefly review the existing preclinical and clinical data on some of the available 5-HT2CRs drugs.

1.6.1. Agomelatine

Agomelatine (Valdoxan®, Melitor®, Thymanax®) is a selective 5-HT2CRs antagonist and melatonin 1 and 2 receptors agonist [592]. It is the first antidepressant on the market that is not following the simple monoamine reuptake blockade as its mechanism of action [592]. It has been prescribed for years in Europe and other countries, like Australia, while due to the “small relative therapeutic value” and safety concerns, it has not yet been submitted for FDA approval [592]. The treatment dosage of Agomelatine is between 25 to 50 mg per day before sleep. Abdominal pain, diarrhoea, dizziness, and nasopharyngitis symptoms are the most common reported side effects in patients [593-596]. While the majority of the patients tolerate the side effects very well, a small percentage experience the increase of liver enzymes [596]. Though rare, the discontinuation of the drug is recommended in case of significant elevation of liver transaminases. This is due to the pharmacokinetic profile of Agomelatine, its metabolism activates the hepatic CYP1A2 pathway [592]. The prescription of this drug in individuals with liver damage, or patients receiving other drugs that increase transaminases, is not allowed

[592]. Besides depressive disorder, Agomelatine is also indicated in the treatment of GAD, OCD and sleep disorders [596]. This is in line with the results of preclinical studies showing amelioration of depression and anxiety-like responses in animals after administration of Agomelatine [592]. The exact mechanism of action of this drug remains unknown. However, based on the observations in animals, the increase of dopaminergic and adrenergic tone in the frontal cortex following its administration has been proposed to mediate its therapeutic response [597, 598]. Of note, the relative decreased PFC versus limbic activity is responsible for the presentation of circadian rhythm deregulation and anhedonia in depressive patients [417]. Apparently, Agomelatine has no effect on extracellular serotonin levels, as opposed to other antidepressants with blocking action on SERT activity [598]. In addition, though possible heterodimers of melatonin and the 5-HT2C receptors have been suggested, the functional importance of such complexes in carrying the therapeutic effect of Agomelatine is not yet proven [122]. It has been shown that melatonin receptor agonists or 5-HT2C receptor antagonists alone do not produce the physiological responses of Agomelatine [122]. Thus, some authors advocated synergistic responses of serotonin and melatonin system as one of the mechanisms of action of Agomelatine [122]. Lastly, also recognized among the therapeutic responses of Agomelatine are enhanced cell proliferation, maturation, and survival following an increased expression of brain-derived neurotrophic factor (BDNF) in the hippocampus and the cortex [599, 600].

Since its introduction, efficacy and tolerability profile of Agomelatine in the treatment of moderate to severe MDD has been shown in multiple controlled studies [593-596]. In most of these studies, Hamilton Rating Scale for Depression (HAM-D17), Montgomery-Åsberg Depression Scale (MADRS), and Clinical Global Impression-Severity (CGI-S) scores, before and after the completion of treatment with Agomelatine or a standard treatment, have been used [593-596]. In this respect, a better tolerability profile has been described for Agomelatine while possessing a comparable efficacy to Venlafaxine, Sertraline and Fluoxetine in MDD patients, [601-604]. Moreover, Agomelatine had a better effect on sleep quality and improved total sleep time, as well as the number of sleep cycles in patients [604, 605]. In addition, sexual side effects were fewer in the Agomelatine group. Following the treatment, there was no decrease in orgasm, male drive or female libido reported [595, 603]. This is notable since 60

percent of individuals receiving SSRIs reported the sexual dysfunction as one of the main reasons of non-adherence to the treatment [606]. Taken together, while offering similar efficacy, the reduced side effect profile of Agomelatine may represent a viable antidepressant alternative, especially in patients with sexual or sleep complaints [592]. Finally, plausible indications of Agomelatine treatment in other groups of mental disorders are currently under investigation [598, 607].

1.6.2. Lorcaserin

Lorcaserin (Belviq®) is a selective and highly efficient 5-HT2CRs agonist approved by FDA for weight reduction in patients with a body-to-mass index (BMI) of> 30 or with a BMI> 27 and the presence of an accompanying comorbidity, such as diabetes [213, 385, 608]. Lorcaserin is available in 10 and 20 mg tablets, while the recommended dose in the majority of the patients is between 20 and 40 mg per day [385]. With headaches being the most common, side effects have been reported in less than 5 percent of cases [212]. The most mentioned complaints aroused in patients without diabetes dizziness, fatigue, nausea, as well as, in patients with diabetes, hypoglycaemia, back pain, cough [212]. Though serious concern has been raised, the incidence of valvulopathy in the patients receiving Lorcaserin and placebo was comparable in clinical trials [212].

Lorcaserin is the product of a long line of evidence indicating the involvement of 5-HT2C receptors in the metabolism and the satiety reflex of animals [591, 609-611]. It has been shown that a higher 5-HT2CRs binding in antipsychotics was related to metabolic syndrome, and weight gain experienced in animal models, as well as patients [612-614]. Further, it has been firmly established that the agonism of 5-HT2C receptors leads to hypophagia in animals [88, 615, 616]. Experiments with both older (mCPP or MK-212) and newer (Lorcaserin, RO 60-0175 or WAY 163909) 5-HT2C receptors agonists confirmed the anorexic response of activation of these receptors in a variety of settings [213, 224, 248, 466]. The opposite response has been demonstrated with the antagonists of 5-HT2CRs. For instance, in animals, pre-treatment with 5-HT2CRs antagonists blocked the hypophagic response of Fenfluramine and its derivatives [83,

202, 609]. Furthermore, mice lacking 5-HT2CRs were prone to both insulin resistance and late-onset obesity and showed hyperphagia and increased body mass index compared to wild types [83, 616]. These animals were resistant to the weight-losing effects of Fenfluramine and other appetite suppressants [88]. However, contradictory reports in some animal models indicating the decrease of appetite after the administration of antagonists exist [617, 618]. Overall, an agreement about the anorexigenic response of 5-HT2CRs agonists like Lorcaserin in human and non-human animals exists in the literature [213, 385, 608].

Despite such overwhelming evidence, the mechanism by which 5-HT2CRs, in general, and Lorcaserin, in particular, enhance satiety is not yet completely understood. There are indications of the involvement of hypothalamic and midbrain/hindbrain circuits following the activation of 5-HT2C receptors in some studies [619]. Also, heterodimers of ghrelin binding receptors, and 5-HT2CRs, have been suggested to be involved in satiety reflex [111]. It is postulated that Lorcaserin prevented ghrelin-induced food intake in mice, probably through interaction with such heterodimers [111]. Recently, the anti-impulsive outcome of Lorcaserin in the context of regulation of excessive food consumption has been suggested as one of its mechanisms of action [141]. Recent literature shows this favourable anti-impulsive response of Lorcaserin might extend to the setting of addiction and consumption of drugs of abuse. In fact, multiple studies in animals showed Lorcaserin resisted the central responses of psychostimulants such as cocaine, ethanol and opiates [237, 255, 620]. Lorcaserin counteracted the reinstatement of stimulants and reduced the chances of the relapse in the animal models of drug addiction [255, 384]. Along the same lines, the protective role of 5-HT2CRs agonists especially Lorcaserin against nicotine consumption has been shown [236]. Lorcaserin demonstrated a very promising profile in all the indexes of treatment of nicotine addiction in animals [236, 255]. These encouraging outcomes propelled new clinical trials to investigate the indications of Lorcaserin in the treatment of nicotine addiction in patients. Recently, in a clinical trial, Lorcaserin met its primary endpoint as an adjunct therapy in the treatment of smoking addiction [141, 237]. In this study, the dose of 10 mg twice daily in combination with Varenicline increased the chance of abstinence from nicotine in the subjects (http://www.eisai.com/news/news201465.html). Altogether, these promising results prove the therapeutic value of Lorcaserin in the context of mental disorders and open the door for further investigations in the future to probe for new clinical indications of this drug.

1.6.3. Experimental 5-HT2C receptors agonists

Besides Agomelatine and Lorcaserin, there are a variety of synthetic compounds with the activity at the level of 5-HT2C receptors that have been generated in recent years. Some of these drugs are used just for experimental purposes, while others are being tested in clinical studies. Historically, compounds like mCPP and MK-212 were used as non-selective 5-HT2C receptors agonists to study the central response of these receptors in the brain [12]. Both of these drugs are non-selective 5-HT2R agonists with limited selectivity for 5-HT2C over 5-HT2A and 5-HT2B receptors [12]. Interestingly, mCPP was one of the earliest compounds to show appetite suppressant qualities for these groups of drugs by reducing the food intake in human and non-human animals [12]. CP809.101 shares an arylpiperazine core structure with MK-212 and mCPP and is a very similar molecule to mCPP. Notably, CP809.101 is one of the most selective and potent 5-HT2C agonists available for the research, with 600-folds selectivity for 5-HT2CRs over 5-HT2BRs [204, 621]. However, despite encouraging preliminary results due to its genotoxicity in preclinical studies, its clinical development has been stopped [204, 621].

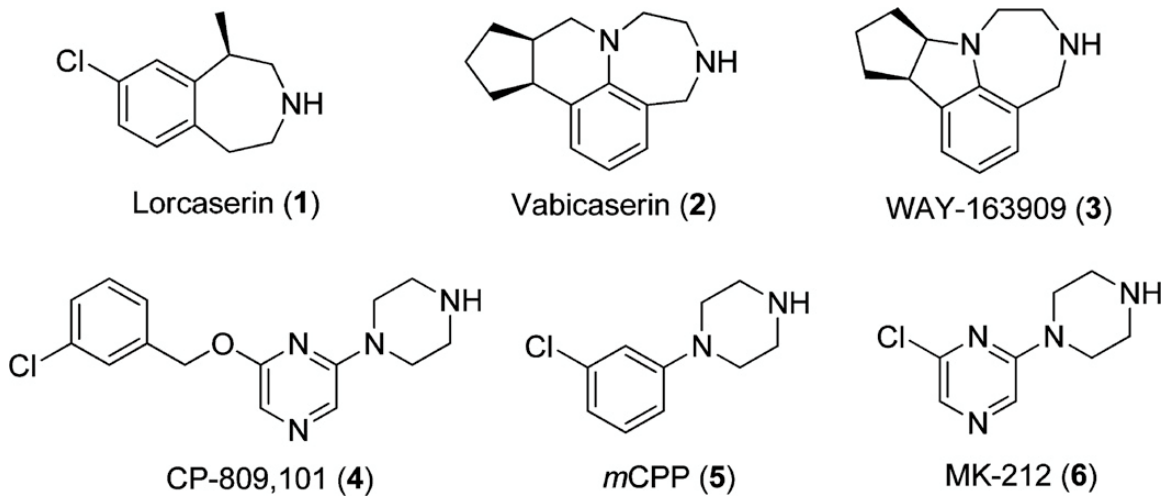

Figure 1.6.3. Molecular structure of various 5-HT2C agonists

Lorcaserin, Vabicaserin and WAY 163909 form other series of 5-HT2C agonists that share a benzodiazepine scaffold [15]. These compounds show great selectivity for 5-HT2CRs over the other two 5-HT2 receptors [15]. In this respect, Vabicaserin (SCA-136), which is a 5-HT2CRs agonist, as well as a 5-HT2A receptors antagonist, showed a promising antipsychotic profile in preclinical studies [214, 622]. This compound caused no weight gain in pilot studies and demonstrated therapeutic effects on both positive and negative symptoms of the patients [15]. However, due to its lack of clinical efficacy, further clinical development of this drug has been stopped [214, 622]. WAY 163909 is a 5-HT2C agonist and a very similar molecule to Vabicaserin. However, it possesses a fair affinity at the level of 5-HT2A and 5-HT2B receptors [198]. Similar antipsychotic-like responses to Vabicaserin have been reported for WAY 163909 in animal models. However, no clinical trial has yet been started for this compound [17,20].

Among the new and potent designed compounds known, JJ-3-42 is one of the most selective 5-HT2CRs agonists [200, 205]. Its optimized 2-phenylcyclopropylmethylamine scaffold is described in the literature. It has been discovered in a recent pharmaceutical campaign using high throughput screening technique [623]. JJ-3-42, previously identified as (+)-16b in the literature, has been published under the chemical name (+)-((1S, 2S)-2-(2-(Allyloxy)-5-fluorophenyl) cyclopropyl)-methanamine Hydrochloride [205]. JJ-3-42 has a similar structure to CP809.101 and similarly shows very little affinity against 5-HT2B and 5-HT2A receptors in binding assays [200, 205]. Such selectivity is important since the activation of the 5-HT2A and 5-HT2B receptors is responsible for the majority of the side effects reported in 5-HT2CRs drugs [624-626]. To understand this, attention to the anatomical and structural characteristics of different members of 5-HT2 receptors family is critical. Primarily, despite structural similarities between these receptors, their anatomical distribution shows a great disparity. For example, 5-HT2ARs are found in both CNS and peripheral tissues especially gastrointestinal and blood vessels [626]. The central activation of these receptors is responsible for hallucinogenic responses of some drugs of abuse and nonspecific 5-HT2 agonists in humans [626]. Alternatively, 5-HT2B receptors are mainly present in vascular and cardiac tissues with a negligible CNS presence [624, 625]. This group of receptors mediate the notorious cardiac side effects including valvulopathy reported following the administration of serotonergic drugs [624, 625]. Finally, 5-HT2C receptors, besides a minimal distribution in cardiac and vascular tissues, are mostly central

receptors [15]. Such pattern of distribution indicates a reduced risk of peripheral side effects of medications targeting these receptors.

Despite the great difference of anatomical distribution of these receptors, 5-HT2 receptors family share a great degree of homology (up to 70 percent) in their amino acid sequence [627]. In fact, due to a high degree of preservation of ligand recognition determinants between these receptors, achieving selectivity in development of the new 5-HT2C compounds is very challenging [627]. This explains the potential harmful side effects following the lack of discrimination against 5-HT2ARs and 5-HT2BRs, as one of the greatest concerns, in the early stages of development of new 5-HT2CRs drugs [628]. While Lorcaserin shows 100-fold selectivity over 5-HT2BRs, in clinical studies, a slightly higher incidence of cardiac valve disorders has been warranted [213]. Meanwhile, binding studies show that JJ-3-42 compound possesses almost no activity at 5-HT2B receptors and 89-fold selectivity against 5-HT2A receptors [200, 205]. In fact, this new 5-HT2CRs agonist shows equivalent potency (EC_{50} = 4.2 nM for JJ-3-42, versus 3.6 nM for Lorcaserin) but improved selectivity (no agonism for JJ-3-42 versus EC_{50} = 478 nM, E_{max} = 92% for Lorcaserin) against 5-HT2BRs compared to Lorcaserin [200, 205]. Recent studies showed that JJ-3-42 also has an excellent brain penetration and favourable toxicological profile [205]. In preclinical tests, JJ-3-42 did not interact with hERG and CYP pathways showing a safe compound toxicological profile. Additionally, in in-vitro cytotoxicity assays, no toxicity, even at higher doses in either HK-2 or SH-SY5Y cells, was detected. These standard assays are intended to rule out the possible nephrotoxicity and neurotoxicity of new drugs [200, 205].

In addition to an excellent biochemical profile, JJ-3-42 has shown promising results in preclinical tests against schizophrenia-like behaviour in both genetic and pharmacological animal models [200, 205]. At 20 mg/kg, this drug successfully suppressed the amphetamine-induced hyperactivity in animals [200, 205]. Administration of this drug alone had almost no effect on the spontaneous locomotor activity of animals and produced very little catalepsy compared to Haloperidol [205]. JJ-3-42 restored amphetamine-disrupted pre-pulse inhibition and enhanced the cognition of the animals in the novel object recognition test in NMDA receptor hypo-functioning NR1-knockdown mice [205]. These results indicate the potential antipsychotic properties of this agent against both positive and negative symptoms, without carrying any motor

side effects. Another study confirmed the similar antipsychotic profile of JJ-3-42 and Lorcaserin in resisting the amphetamine-induced hyperlocomotion and pre-pulse inhibition in rodents [211]. Moreover, the administration of JJ-3-42 improved social behaviour and novel object recognition result in MK-801-treated mice [211]. This observed cognition-enhancing profile of JJ-3-42 is in line with recent clinical trials indicating 5-HT2Rs agonists as potent pro-cognitive agents [191].

In light of present findings, it has been discussed that 5-HT2CRs agonists might be the ideal candidates for the treatment of schizophrenia among other mental disorders. For instance, the anti-obesity and appetite suppressant property of 5-HT2CRs agonists might benefit the patients suffering from metabolic side effects of current neuroleptics [629]. Moreover, superior inhibition of mesolimbic dopamine release over the nigrostriatal pathway by agonists of 5-HT2CRs predicts fewer extrapyramidal side effects for such medications [171]. In line with this, the limited motor side effects of these drugs, even at higher doses, have been observed [197, 204]. The positive profile of 5-HT2CRs agonists in the recent clinical trial indicates the practicality of this current approach [214, 215]. Taken together, excellent selectivity of JJ-3-42 along with the positive behavioural responses in animal studies makes this compound a great candidate for further clinical development in the future [200, 205]. Recent successful introduction of Lorcaserin and Agomelatine in the clinic are outstanding confirmations of the potential therapeutic value of 5-HT2CRs drugs. This favourable perspective urges more comprehensive animal studies in order to elucidate the extent of beneficial responses of JJ-3-42 in various dimensions of mental disorders.

As so, the focus of this thesis is to investigate and discuss the behavioural responses of JJ-3-42, a potent and selective 5-HT2C agonist in a wide range of behavioural tests. In this regard we compare the central responses of this drug with other serotonergic drugs in behavioural tests related to social behaviour, cognition, anxiety and repetitive traits in animals. Moreover, we will study the interaction of the dopamine system and the activation of 5-HT2C receptors following the administration of JJ-3-42 in series of experiments based on the locomotor activity of the animals. The major objectives of this thesis are as follows:

- To characterize the behavioural responses of genetic variation of TPH2-KI animals in basal locomotor activity test, H-Maze test, dyadic social interaction test, marble burying, grooming and digging tests and anxiety related paradigms
- To investigate the behavioural responses of acute injection of JJ-3-42 at 10 mg/kg on the performance of TPH2-KI HO and WT animals in the H-maze test
- To investigate the behavioural responses of acute injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg on the performance of TPH2-KI HO and WT animals in the dyadic social interaction test
- To investigate the behavioural responses of acute injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg on the performance of TPH2-KI HO and WT animals in the marble burying, grooming and digging tests
- To investigate the behavioural responses of acute injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg on the performance of TPH2-KI HO and WT animals in the dark-light, openfield and elevated plus maze tests
- To investigate the responses of acute injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg on the basal locomotor activity of TPH2-KI HO and WT animals
- To investigate the responses of acute injection of JJ-3-42 at 10 and 20 mg/kg on the apomorphine, amphetamine, MK-801 and cocaine-induced hyperlocomotion in TPH2-KI WT animals
- To investigate the responses of acute injection of JJ-3-42 at 10 and 20 mg/kg on the innate locomotor activity of DAT-KO HO mice
- To investigate the responses of acute injection of JJ-3-42 at 10 and 20 mg/kg on the L-DOPA/Carbidopa cocktail-induced locomotor activity in the dopamine deficient DAT-KO HO mice (DDD) model
- To investigate the responses of daily pre-treatment of JJ-3-42 at 10 mg/kg on the locomotive response of a challenge dose of cocaine in sensitized TPH2-KI WT animals

2. Materials and methods

2.1. Outline

As mentioned in the earlier section, a new potent and selective 5-HT2C receptor agonist, JJ-3-42, has demonstrated a promising pharmacological profile in the preliminary tests [630]. However, the complete behavioural responses to this compound are not fully studied yet. In the scope of this thesis for the very first time, we ran JJ-3-42 side-by-side with a non-selective endogenous serotonin ligand, 5-HTP, and a potent selective 5-HT2C receptor agonist, CP809.101, in a cohort of wild type and homozygote TPH2-KI R439H mice in a wide range of behavioural tests.

2.2. Animals

All the experiments were conducted in accordance with the guidelines of Canadian Council on Animal Care and approved by Laval University Animal Care Committee. We used only 2 to 4-month-old mice throughout this study. All the experiments were carried out using the cohorts of animals composed of 50% male and 50% female mice, except for the social interaction test in which, only male mice were used. Nonetheless, throughout our experiments, no behavioural difference between sexes in secondary analysis of various tests was observed. To avoid learning and habituation, each animal was only used once for a specific behavioural test. All mice were housed by the group of 3 to 4 per cage and maintained on a 12-h light/dark cycle (lights on at 7 hours in the morning) in a humidity-controlled room with the adjusted temperature of 23 °C. Mice were kept with food and water available ad libitum throughout their lifetime, except for the habituation or training periods as well as the duration of tests. The condition of water deprivation before the performance of H-Maze test has been described in the related section in detail. To habituate the animals and reduce the anxiety all the animals were manipulated daily for a couple of minutes by the only experimenter for few days before the test. Animals were left undisturbed in the experimental room for acclimatization for an hour before all the tests sessions. All behavioural tests were performed between 9:00 A.M. and 4:00 P.M. All experimental groups

were made of at least 8 to 10 animals. The cohorts of the experiments were of the mix batches of unidentifiable genotype to the examiner in all the experiments. All the experiments were conducted in controlled condition regarding olfactory cues and the main experimenter was the only person with contacts to the animals.

2.2.1. R439H TPH2-KI mice

The detail of the procedure of creation of R439H TPH2-KI mice have been published and described previously [324]. This mouse line has been generated by the introduction of R439H TPH2 allele (GenBank accession no NM173391) into the genome of the mice using the Knock-in technique (figure 2.2.1.1A) [13, 324]. This variant of TPH2 gene is the mouse equivalent of the human R441 HTPH2 allele (GenBank accession no NM173353) that was identified in a cohort of unipolar depressive patients earlier (figure 2.2.1.1.B) [325]. Briefly, using genomic DNA obtained from 129S6/SvEv mice as a template, the corresponding introns and exons of the mouse TPH2 gene have been identified in the laboratory [324]. Following this, the site-directed mutagenesis has been used to replace the guanine 1449 in exon 11 by adenosine to engineer the R439H mutation in the samples (figure 2.2.1.1.A) [324]. Then, this oligonucleotide was sub-cloned into a targeting vector along the insertion of various selection cassettes (figure 2.2.1.1). Next, this construct was transfected into 129S6/SvEv mouse embryonic stem cells (ES) [324]. Using a standard double selection protocol, clones carrying recombinant TPH2 alleles were selected and the presence of homologous recombination was confirmed by the polymerase chain reaction (PCR) technique using the following primers: (5-CACCCAATTTGCCTGCCGTAGGGA-3; 5-GCTGCAAAACATATCACAGAACTCATTCAAGACCA-3) [324]. It is noteworthy that the same primers were also used for routine genotyping of TPH2-KI mice before and after our experiments. Breeding the chimeras generated from these selected ES cells with C57BL6/J mice led to the birth of the first generation of genetically combined mice carrying this mutation [324]. In the following step, by using the PCR analysis, the mutant animals that inherited the desired TPH2 allele combination were identified and selected [324]. Finally, the expression and integrity of the mutant TPH2 construct in the selected progenitor mice was reconfirmed by RT-PCR technique [324]. In our laboratory, in order to have the homogenous genetic background, the

original R439H TPH2-KI mice have been backcrossed for at least 10 generations on the C57BL/6 mouse line. The genotype of all mice has been twice tested and verified by ear biopsy punches at the beginning of the life (2-3 weeks) and at the end of each experiment after the euthanasia. Hereafter, when it is not otherwise specified, by using HO, HET or WT, the corresponding C57Bl/6 R439H TPH2-KI homozygotes, heterozygotes and wild types are meant throughout this manuscript.

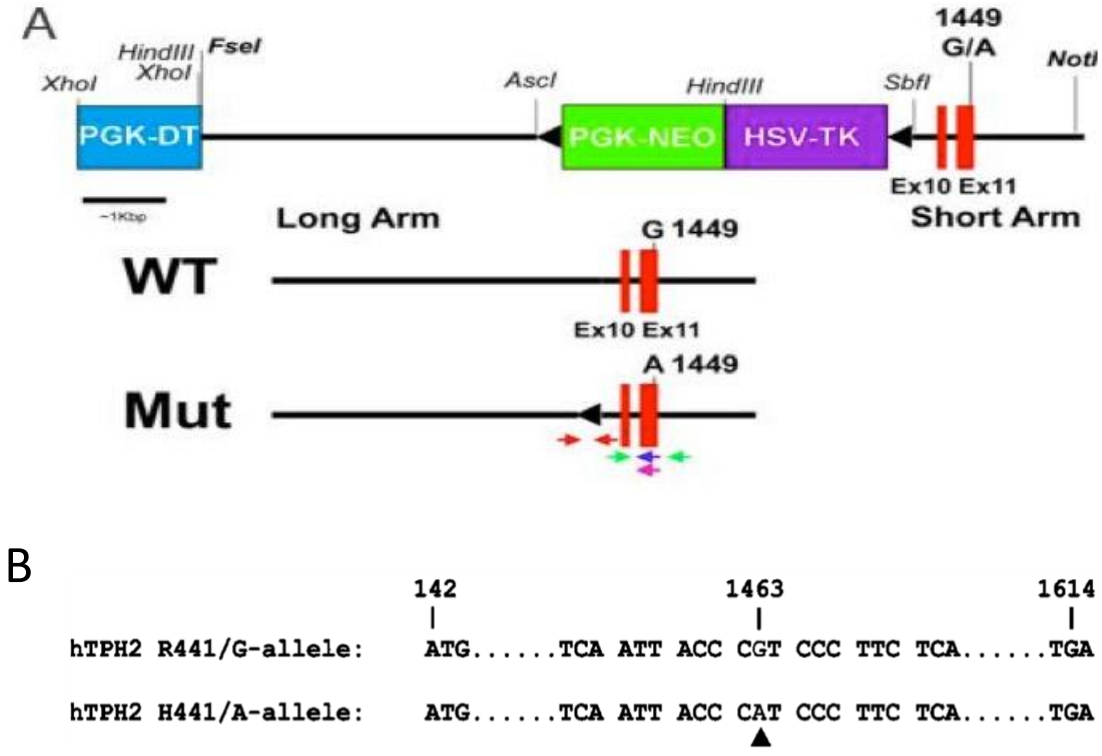

Figure 2.2.1. 1. The creation of TPH2-KI mice

Figure 2.2.1.1.A Shows the diagram of the designed vector and the TPH2 locus. The G1449A coding mutation is located in exon 11 of the mouse TPH2 gene. After homologous recombination and using various PCR techniques, only the ES cell clones carrying the mutated exon remained in the pool [324]. Figure 2.2.1.1.B The (G1463A) polymorphism in hTPH2. The G/A polymorphism is highlighted with an arrowhead. Nucleotide numbers are shown for the start and stop codons of hTPH2 as well as the site of polymorphism [4].

2.2.2. C57DAT-KO mice

We also used C57DAT-KO mice and their wild type littermates in our locomotion experiments in this thesis. The generation of homozygote C57DAT-KO mice and their characteristics have been described and studied in detail before [631, 632]. This mouse is known for its hyperdopaminergic state at the level of synaptic cleft due to the lack of dopamine transporter on the cell membrane. This feature makes these mice an ideal model to study the interaction of various drugs and dopaminergic system in the brain [631, 633]. Lack of dopamine transporter at the cell membrane leaves the De Novo synthesis of dopamine in the cells the only viable pathway of dopamine neurotransmission in adult mice. Blocking the synthesis of dopamine in these animals and the following acute dopamine depletion state is the basis of the model that has been used in this thesis [633]. The further details of this experiment are provided further in the relevant section. This model is widely used in the investigation of properties of potential drugs of interest in the movement disorders [633].

2.3. Drugs

CP809.101 (Tocris Cookson, Ballwin, MI) was administered at 1 mg/kg while 5-hydroxy-L-tryptophan (5-HTP; Sigma-Aldrich, Oakville, Canada) at 20 mg/kg throughout this report. These doses were based on previous published results and a series of dose response pilot studies in our laboratory [204, 210]. Amphetamine (Tocris Cookson, Ballwin, MI) at 3 mg/kg and cocaine (Tocris Cookson, Ballwin, MI) at 20 mg/kg Apomorphine (Tocris Cookson, Ballwin, MI) at 2mg/kg and MK-801 (Tocris Cookson, Ballwin, MI) at 0.2mg/kg were prepared according to the recommendations of the manufacturer in corresponding experiments. L-DOPA/Carbidopa cocktail (both Tocris Cookson, Ballwin, MI) at the dose of 20/20 mg/kg was obtained after dissolving both drugs in saline and bringing the solution to the final volume as described in previous studies [633]. α-Methyl-L-tyrosine (Alfa-MPT), an inhibitor of tyrosine hydroxylase enzyme, (Sigma-Aldrich, Oakville, Canada) was prepared at 250 mg/kg in saline using agitation and temperature as previously described [633]. All drugs were administered 30 minutes before the behavioural tests intra-peritoneally (i.p.) and in a similar volume of 10 ml/kg. These doses

and time intervals were based on previous published results and a series of dose response pilot studies in our laboratory [204, 210]

In our study, JJ-3-42, a selective and potent 5-HT2CRs agonist, provided by our collaborator (Dr.Kozikowski, Drug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago) was injected at doses 10 or 20 mg/kg based on the previous published results [205, 211]. The process of production and optimization of JJ-3-42 (also known as (+)-16b in the literature) with the chemical name of (+)-((1S, 2S)-2-(2-(Allyloxy)-5-fluorophenyl) cyclopropyl)-methanamine Hydrochloride has been discussed in detail in previous publications [200]. In summary, the development of JJ-3-42 has been based on the improvement of 2-phenylcyclopropylmethylamine scaffold through a series of biochemical engineering steps to enhance its properties. This scaffold has been discovered and described initially in a course of a high throughput screening (HTS) campaign in earlier years [623]. In the following years, through the application of rational drug design techniques combined with the principles of structure–activity relationships (SARs), a series of compounds based on 2-phenylcyclopropylmethylamine scaffold with various chemical groups have been developed [200]. In fact, JJ-3-42 is one of these new compounds with some minor modifications at the position 5 and an allyl ether derivative at position 2 on the benzene ring [200]. Such adjustments led to a better potency at the level of 5-HT2C as well as a greater selectivity against the 5-HT2B and 5-HT2A receptors [200]. In the consequential binding essays, JJ-3-42 displayed an EC_{50} value of 4.2 nM at 5-HT2CRs while being 89-fold selective against 5-HT2ARs [200]. More importantly, there was no reported activity at the level 5-HT2BRs for this compound [200]. This is important since the activation of the 5-HT2A and 5-HT2B receptors, has been associated respectively with detrimental side effects like hallucinogenic properties and cardiac valvulopathy in serotonergic drugs [626].

In the same way, investigation of the potential off-target activity of new drugs is an integral part of the pharmacological assays in development of new drugs. Various targets, including GPCRs, ion channels, neurotransmitter transporters, and sigma receptors were studied in binding assay tests of JJ-3-42 compound [200]. As expected, JJ-3-42 displayed high affinity for the 5-HT2B and 5-HT2C receptors and showed the affinity as low as <1 μM K_I values at the 5-HT1A, 5-

HT6, 5-HT7, α2a, α2b, α2c, and dopamine D3 receptors. Taken all these results into consideration, JJ-3-42 is one of the most potent and selective 5-HT2C agonists available to date [200].

Drug	Description	Dose	Affinities (Ki)		
			5-HT2C receptors	5-HT2B receptors	5-HT2A receptors
JJ-3-42 (Kozikowski lab)	*Potent and selective 5-HT2C receptor agonist*	*10 and 20 mg/kg*	*4.2 (nM)*	_	*56 (nM)*
CP809,101 (Tocris)	*Potent and selective 5-HT2C receptor agonist*	*1 mg/kg*	*1.6 (nM)*	*64 (nM)*	*6 (nM)*
5-HTP (Sigma-Aldrich)	*Precursor of endogenous ligand and Non-selective 5-HT receptor agonist*	*20 mg/kg*	*5.7 (nM)*	*13.5 (nM)*	*16 (nM)*

Table 1. the comparison of the affinity of serotonin agonists in this study [200, 205, 211].

Drug	Description	Dose
Cocaine (Tocris)	Inhibiting monoamine reuptake	*20 mg/kg*
Apomorphine (Tocris)	Agonist of dopamine receptors	*2 mg/kg*
Amphetamine (Tocris)	Releaser and inhibitor of monoamine reuptake	*3 mg/kg*
MK-801 (Tocris)	Noncompetitive antagonist of the N-Methyl-D-aspartate (NMDA) receptors	*0.2 mg/kg*
L-DOPA/ Carbidopa Cocktail (Tocris)	Precursor to catecholamines /Inhibitor of aromatic-L-amino-acid decarboxylase	*20/20 mg/kg*
α-Methyl-L-tyrosine (Sigma-Aldrich)	Tyrosine hydroxylase inhibitor	*250 mg/kg*

Table 2. The list of the description and the dosage of the various drugs in this study

2.4. The behavioural studies

We tested a newly improved 5-HT2C agonist in a vast battery of behavioural responses such as cognition, aggression, anxiety and compulsion in the following chapters. To shed light on the behavioural impact of JJ-3-42, we tested this drug side by side of other serotonergic agonists using a cohort of well-known animal models in a series of valid and standard behavioural paradigms. In this regard, the following section describes in detail the principles and the detailed methodology of the animal studies employed in this thesis.

2.4.1. H-Maze automated test

As mentioned before the efforts to find new antipsychotic drugs with more suitable cognitive profile have gained momentum recently [195, 196, 200]. While definition of cognition contains complex brain functions such as learning, memory, language and concentration in humans, measuring cognitive functions could be a daring effort in the animals [634]. In fact the dimensions and the significance of what is measured as cognitive functions in animals have been discussed elaborately [634, 639]. Beside the conceptual difficulties as the primary concern, in most of the behavioural studies, distinguishing the cognition capacity from the confounding component like conditioning, repetitive behaviour, resilience and social interaction is almost implausible [639].

The automated H-Maze is a relatively new “cognitive plasticity” test that measures the ability of animals to forget a pattern and relearn a new set of rules [210, 640]. The automated H-Maze is made of two testing chambers (C1 and C2) built of hamster tubes connected by a straight plastic tube in the middle. All the arms and the middle tube are equipped with the infrared sensors that track the movement of the mice in the maze (please check the figure 2.4.1.A). The superior half tube in the arms can be removed easily to clean and to place the mouse in the maze. There is an automated water delivery system at the extremities of the arms that allows the distribution of water to the animals when it is indicated (please check the figure 2.4.1.A).

As we mentioned H-maze is a fully automated test that collects and registers the gathered data on a computer hard disk though a customized interface. In addition, there is a manual mode in the software that allows the control of the maze by the user during the training sessions. There were three habituation sessions once per day that lasted 10 minutes for each mouse. In these sessions mice are encouraged to explore the maze and get familiar with the water delivery system. On the first day, the mouse could run freely throughout the maze with water ad libitum while on the second day 10 µl of water was delivered only to one of the chambers through manual control of the software. On the third day, 10 µl of water was distributed in only one of the extremities on each chamber but not in the other. After 3 days of habituation, it is assumed that the mouse has associated the presence of the water in the extremities to their exploratory movements.

On the test day, mice are placed in the machine for one single hour-long session. To encourage the exploratory movement of the animals they were kept without the water bottle for 12 hours before the training sessions and the day of the experiment. The mice have to discover the right pattern of navigation between two chambers of the H-Maze to induce the automatic delivery of water. The software tracks and assesses the performance of the mouse in the two consecutive delayed response tasks namely Alternance (ALT) and Non-Alternance (N-ALT) [640]. In the first task (ALT), the animals learn to alternate between two chambers by turning left, then right, in alternation (please check the figure 2.4.1.B). They could move to the second task only after learning the first task (measured by executing 4 consecutive correct sequences). In the second task, starting immediately after the first one, mice have to accomplish an inhibition of the first task pattern in order to learn the second set of rules. The second pattern (N-ALT) is consisted of turning to the same side rather than the alteration at the entrance to the chamber (for example: right, right and right (please check the figure 2.4.1.B).

We used a cohort of HO TPH2-KI animals and their wild type counterparts in this experiment. The effect of treatment with JJ-3-42 at 10 mg/kg vs. saline 30 min before the test on the performance of the mutants and the wild types of TPH2-KI was studied. The TPH2-KI HO and WT littermates were tested in the mixed batches and in an unidentifiable pattern by appearance to the examiner. The variables like the time and the number of trials needed to finish the ALT, N-ALT and the percentage of perseverant behaviour to the total number of trials in H-Maze test

was calculated. The perseveration was defined as episodes of more than five repetitive failed attempts as described before [210]. Such patterns show the cognitive inflexibly in animals to choose an alternative pattern despite the lack of reward following the application of an invalid strategy [210]. The complete description of this machine and the explanation of the training and test sessions are available in previous publications [640].

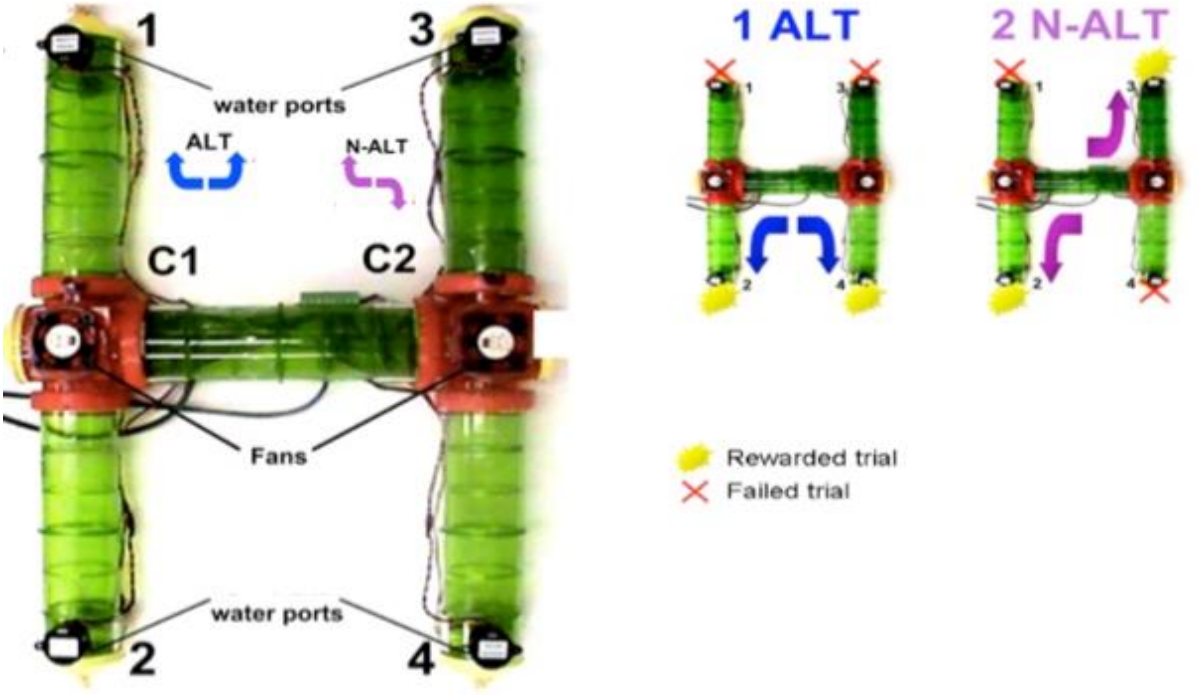

Figure 2.4.1. Schema of H-Maze test

Figure 2.4.1. Shows the pattern of successful ALT and N-ALT trials in the machine have been shown. The sensors are located at all extremities and C1 and C2 to track the movement of mice. The automated water ports deliver water only of the mice succeeded in doing the correct sequence. Adapted images from former publication [210].

2.4.2. Dyadic social interaction test

Social interaction is a complex set of behaviours that embeds all the exchanges between members of species [611, 641]. Depending on various elements including the emotional state of the animal such exchanges could vary from mild and amicable to more aggressive interactions [611, 641]. Disturbed social interaction is a trademark symptom of many psychiatric disorders such as major depression, schizophrenia and autism spectrum disorders [642]. The dyadic social interaction test is a validated and simple test that observes the quality of an ethologically natural exchange of behaviour between two animals as a measure of their emotional state [641, 643]. This simply means that the test does not involve training of the animals neither it requires the use of an external or artificial stimuli to induce certain behaviour. This paradigm has been validated repeatedly and its sensitivity to demonstrate the modulatory response of various pharmacological trials on the level of anxiety or aggression in the animals has been proven previously [642-644].

The dyadic social interaction test sets to measure dependent variables such as mild (e.g. sniffing, following or grooming the partner) or aggressive behaviours (e.g. biting, attacking a partner) in interaction of the animals. Since the behaviour of one mouse influences that of the other and there is an element of familiarity in repeated social interactions, as a general rule, all the animals should meet only once in this test [643]. Normally, a pair of male mice with relatively similar age and weight that have never met are used for this test [643]. Females are avoided since it is speculated that there are some important sex differences in regard to the function of the social interaction under natural or stressful situations [641, 645]. It is important to keep in mind that the couple is normally treated as a unit, and only one score for the pair is recorded [643]. In addition, to encourage more vigorous social exchange, various protocols of a short social isolation period are used before the application of social interaction test. In most of the studies, the animals are normally singly housed from 5 to 14 days depending on the protocols [643]. This reliably increases the time spent in social interaction between the two animals [646]. In support of such isolation period, it has also been argued that removing abruptly an animal from a crowded house for the period of the test could be considered an unwanted acute stressful event, which could be avoided by introducing a buffer isolation period [641, 643].

In our study, mice were singly housed for a period of 14 days before the test. The social interaction test was performed in a transparent 50 × 50 × 20 cm Plexiglas arena with a 5 cm layer of litter at the bottom. Unfamiliar pairs of age and weight-matched male animals were placed at the same time in the arena for the first time and were allowed to interact freely for 10 minutes. A control WT C57BL/6 male was paired with a mouse from the TPH2-KI C57BL/6 line (WT or HO). In each pair of animals, the following parameters were measured: the number of events and time spent by the animals in mild social interactions (including sniffing, following, allogrooming and crawling) and the number of events and time spent in aggressive social events (including escaping and wrestling, fighting and biting). Acute i.p. administration of saline or 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 20 mg/kg took place 30 minutes before the test. The TPH2-KI HO and WT littermates were tested in the mixed groups and in an unidentifiable pattern by appearance to the examiner. As usual, mice were habituated to the room for 1 hour before the test. Due to high aggressively of TPH2-KI HO mice, we had to limit our observation to only 10 minutes per couple in our experiment.

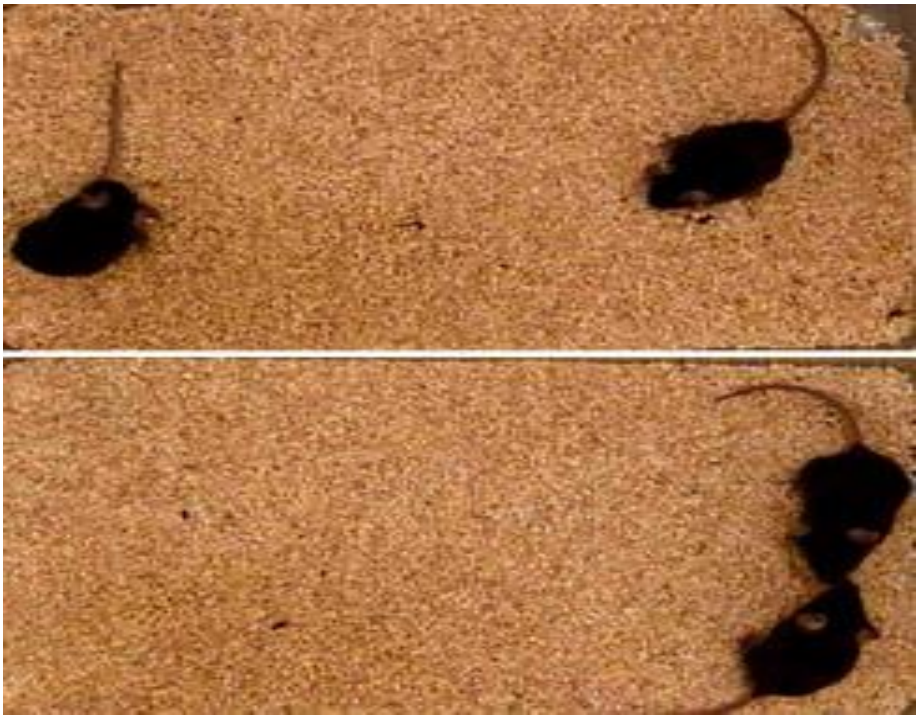

Figure 2.4.2. A picture of dyadic social interaction test

2.4.3. Tests of repetitive and compulsive-like behaviour

2.4.3.1. Marble burying test

Repetitive and ritualistic patterns of behaviour is a common feature of OCD, autism spectrum disorder and profound cognitive disabilities in human [297, 647, 648]. Related to this, many animal models have been designed to recapitulate these phenotypes in the laboratory animals [297, 647, 648]. Marble burying test is one of the most validated tests to study such repetitive and compulsive-like behaviours in animals [649]. This simple test measures an innate compulsive and repetitive behaviour of animals in a new and therefore anxiogenic environment. This test is based on the established fact that burying a foreign object, which occurs spontaneously in mice, would be amplified in stressed animals [492, 535]. Given that obsessive-compulsive disorder and autism spectrum disorders are highlighted by the repetitive and ritualistic behaviours in subjects, marble burying paradigm is a standard test in the development of drugs against such conditions [649, 650].

In our setting, the marble burying test was performed in a washed and cleaned Plexiglas rat cage (26 cm x 48 cm x 20 cm) containing a 5 cm fresh corncob layer at the bottom and a fitted filter cover on top. Twenty glass marbles (15 mm diameter, 5.2 g in weight) were positioned evenly on the surface of the bedding in 5 rows of 4 marbles (please see the picture 2.4.3.1). A single mouse was placed on top of the litter far from the marbles. Acute i.p. administration of saline in the study of genotype and control groups or the i.p injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 20 mg/kg in the study of the responses of the various agonists took place 30 minutes before the test. As usual, mice were habituated to the room for 1 hour before the test. The TPH2-KI HO, HET and WT littermates were tested in the mixed and unidentifiable pattern by appearance to the examiner. The experiment lasted 30 minutes and immediately after, the animals were removed from the box and the number of buried marbles was counted. Only marbles that had at least two thirds of their superficies covered with corncob were considered buried.

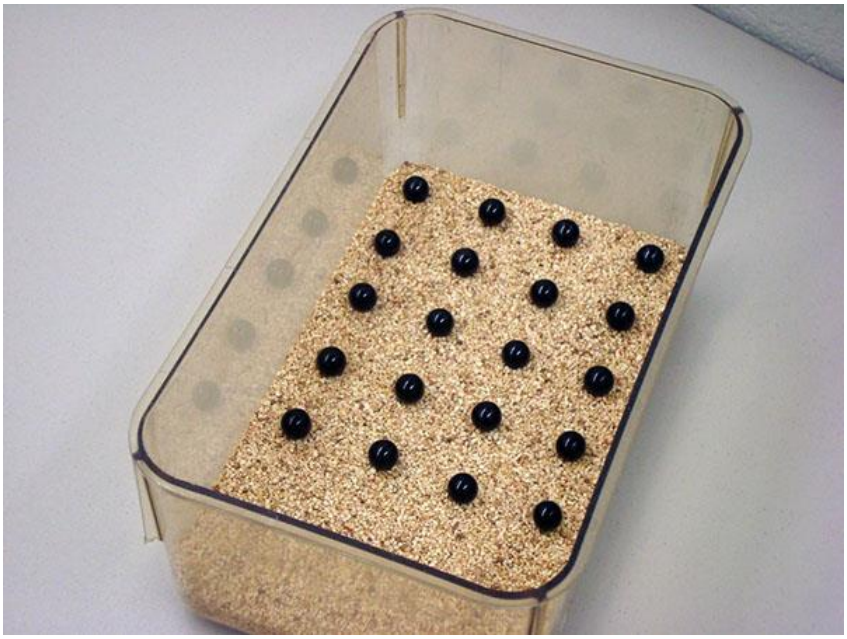

Figure 2.4.3.1. A picture of marble burying test

2.4.3.2. Grooming and digging tests

Similar to the marble burying test, the grooming and the digging tests are both reliable and simple tests that quantify the recurring obsessive-like behaviour of the animals in the context of a stressful and new environment [496, 638]. Grooming is a maintenance behaviour that maintains the comfort and hygiene of the mouse [496, 638]. This behaviour occurs spontaneously but can become more intense in stressed animals [405, 492, 651]. In the same way, digging is a physiological behaviour of mice used for various purposes [405, 492, 650]. However, in stressed and anxious mice, this normal behaviour can take compulsive and ritualistic form. Similar to marble burying test, grooming and digging tests are widely used in development of various drugs for the population with autism, anxiety disorders and OCD [405, 492, 650, 651].

These tests were performed in a washed and cleaned Plexiglas rat cage (26 cm x 48 cm x 20 cm) containing a 5 cm fresh corncob layer at the bottom and a fitted filter-top cover on top. A single mouse was placed in the middle of the cage and the experimenter left the room while a video camera recorded the whole experiment. Acute i.p. administration of saline in the study of

genotype and control groups or the i.p injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 20 mg/kg in the study of the responses of the various agonists took place 30 minutes before the test. The TPH2-KI HO and WT littermates were tested in the mixed groups and in an unidentifiable pattern by appearance to the examiner. As usual, mice were habituated to the room for 1 hour before the test. Later, mice were scored for the delay to start and the time spent in grooming and digging spouts in 30 minutes.

2.4.4. Anxiety-related behavioural tests

Anxiety is an emotional state that has a very similar physiology and presentation in both primates and the other animals [388, 483]. This is understandable since from the point of view of the evolution, those anxiety-related reactions to a stressful or dangerous situation played a major role in the survival of the individual [388, 483]. However, the exaggerated anxious feelings or the excessive anxious responses to neutral situations are common features of psychopathologies like anxiety disorders in humans [387, 547, 652]. As discussed before, the anxiety disorder is one of the most common mental disorders in the human societies as the anxiolytics are one of the most common globally prescribed medications [387, 547, 652]. While development of better anxiolytic medications is an ongoing endeavour, various prescribed drugs can increase anxiety in the population as one of their major side effects [653]. This is why the precise estimation of the anxiety profile of any newly developed drug in an early stage is of at most importance [388, 483].

2.4.4.1. Dark-light test

The dark-light test is a well-known test that estimates the emotional state of the animals facing a new and stressful setting [654]. The test is based on the conflict between the cautious tendency of an animal to avoid an open well-lit space and the curiosity of exploration of a new environment (here entering the light chamber) [654]. In this paradigm, it is believed that indexes such as the number of crossings, the time spent and the travelled distance in the light compartment are the reflections of the emotional state of the animal [655, 656]. Predictive value

of this test has been demonstrated recurrently by displaying the anxiolytic response of various drugs like benzodiazepines and SSRIs in the animals [655, 656].

In our experiment, the dark-light test was performed in an automated machine with black plastic inserts that occupied half of the box (21x42x25 cm) forming the dark chamber with a connecting hole (please see the figure 2.4.4.1). The lit compartment was brightly illuminated by a lamp (600–700 lux), whereas the other chamber kept dark (less than 10 lux). Mice were initially placed at the center of the dark chamber and were allowed to move freely between the two chambers for the following 5 minutes. The TPH2-KI HO, HET and WT littermates were tested in the mixed groups in an unidentifiable pattern by appearance to the examiner. All the cages containing mice were transferred to the behaviour testing room 60 minutes before the first trial.

Acute administration of saline in the study of genotype and control groups or the injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 20 mg/kg in the study of the responses of the various agonists took place 30 minutes before the test. Sensors registered the data regarding the delay to enter the lit chamber, as well as the total number of transitions to the light chamber automatically. In addition, the time spent, and distance travelled on the lit side were automatically recorded as related dependent variable to measure anxiety-like state of the animals.

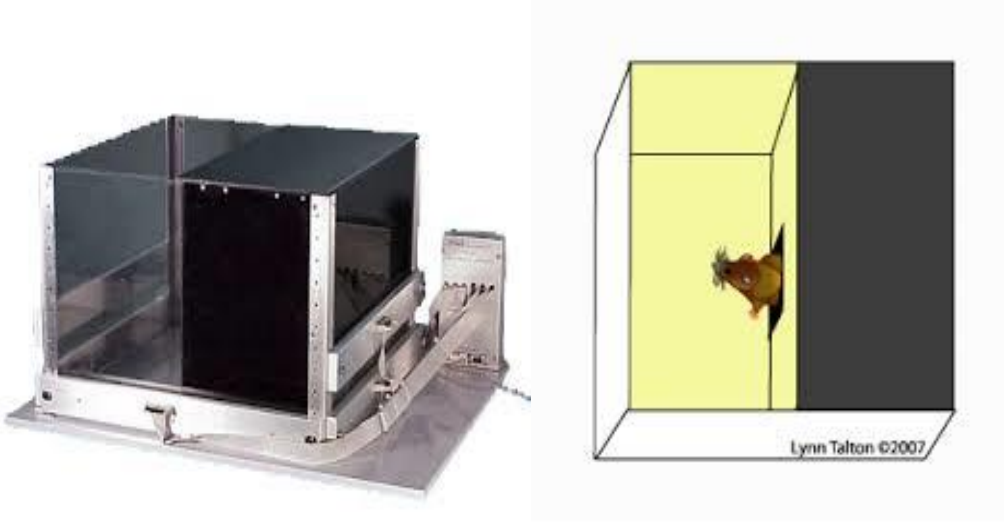

Figure 2.4.4.1. A photo and schema of dark-light test apparatus

2.4.4.2. Openfield test

Like the dark-light test, the openfield test also is a very common paradigm to measure the anxiety-like state of the animals [654]. Openfield test is an easy and fairly rapid assessment of well-defined exploratory behaviour of animals in an open arena [388]. Long years of evolution have selected the animals with more protective reflexes to avoid presumably dangerous locations like the central zone of a large, brightly lit, open and unknown arena [657]. In this way, the concept of openfield test is to measure the display of anxiety-mediated fear or flight responses to such stressful stimulus in the animals [657]. Similar to the dark-light test, this test grades the exploratory drive of the animal in contrast to such protective reflexes [654]. It is considered normal that most of the rodents would spend a significantly greater amount of time moving in the peripheries [388]. It is believed that the pattern of the movement in such arena could be an index of the emotional state of the animal. Number of variables mostly connected to the locomotor activity of the animals like the distance travelled and times spent in the central zone of the arena is normally recorded [388].

In our experiment, the openfield test was performed in an automated Omnitech Digiscan apparatus (Accuscan Instruments, Columbus, Ohio United States) with transparent Plexiglas walls. Each mouse was placed at the corner of the box and allowed to freely roam for the next 30 minutes. The examiner left the room after covering the box with the fitted top to avoid animal escape. Mice in the openfield arena were monitored by an automated grid of infrared photocell beams that registered the movement of the mice for the allotted time. The central 25 percent of the arena was defined as the central zone by the experimenter using the incorporated software. The TPH2-KI HO, HET and WT littermates were tested in the mixed groups in an unidentifiable pattern by appearance to the examiner. All the cages containing mice were transferred to the behaviour testing room 60 minutes before the first trial begins. Acute administration of saline in the study of genotype and the control groups or the injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 20 mg/kg in the study of the responses of the various agonists took place 30 minutes before the test. In our study horizontal activity in the central zone and the number of entries to the center were recorded as desired variables. Moreover, total travelled distance and the delay to visit the central zone for the first time were recorded as well. The

registered data was analyzed by the default software such Animal Activity Monitoring System (AccuScan Instruments, Inc., Columbus, Ohio, USA).

Figure 2.4.4.2. A picture of openfield test machine

2.4.4.3. Elevated plus maze test

The elevated plus maze is another well-known and popular test to measure the anxiety-like behaviour of the animals with a strong predictive value for screening the anxiolytic drugs [658, 659]. Consisted of 2 open and 2 closed arms, which are mounted on a plateau, this test is based on the conflict of avoidance and exploratory reflex of the mice like previous two tests [658, 659]. In other words, more anxious a mouse is, less time it would spend in the open arms. It is believed that while the open and closed arms have a similar attraction for the mice to explore, avoidance of the open arms is driven by the anxiety of the height and exposure [658]. In this regard, anxiolytic drugs specifically reduced the delay to enter and increased the spent time in the open arms in various studies [462, 659]. These indexes are considered a useful indicator of general emotional state of mice facing a new and stressing environment [462, 659].

In our lab, the elevated plus maze apparatus is constructed from white Plexiglas and contains two open and two enclosed arms with the similar dimension (16 × 5 × 10 cm long×wide×high). The four arms are connected by a common central platform (5 × 5 cm) (please see the figure 2.4.4.3). The maze is elevated 50 cm from floor level and lit by the ambience light in the room. Animals were individually placed at the end of a closed arm and were allowed to move freely for 10 minutes while the whole session was filmed for the further analysis. After each trial, all arms and the center area were cleaned, and dried.

The TPH2-KI HO, HET and WT littermates were tested in the mixed groups in random and unrecognizable pattern to the examiner. All the cages containing mice were transferred to the behaviour testing room 60 minutes before the first trial began. Acute i.p. administration of saline or 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 20 mg/kg took place 30 minutes before the test. In our experiment, the amount of spent time on the open arms and latency to visit the open arm was recorded with a stopwatch. All data was transferred to an Excel sheet and processed by mentioned statistical analyses.

Figure 2.4.4.3. A figure of elevated plus maze test

Adapted from Stoelting Co. website

2.4.5. Locomotor activity test

Most of behavioural tests require certain bodily activity and locomotion in their setup [660, 661]. In this regard, any physical or pharmacological intervention that impairs the animal's natural activity might be regarded as a confounding factor. Thus, special attention has been given to the integrity of locomotor activity of the animals before the interpretation of animal studies in the paradigms that require the movement of the animals [657, 660]. One of the easiest ways to answer such challenge would be to assess the basal locomotor activity of the animals after a specific genetic or pharmacological intervention in comparison to their wild type littermates. In our study, openfield machine is used to monitor the locomotor activity of the animals after injection of various 5-HT2CRs agonists in wild type and HO TPH2-KI mice to clarify the validity of the observed behavioural responses [657, 660].

In our experiment, locomotion was assessed under normal light and temperature in small chambers created by using the dividers in an automated Omnitech Digiscan apparatus as described before [662]. For the evaluation of locomotor activity, mice were brought to the room 1 h before the test for acclimatization. Mice received an injection of saline or either 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg or JJ-3-42 at 10 mg/kg intraperitoneally. 30 min after the injection mice were put in the machine and their locomotor activity was recorded for the next 120 min. The TPH2-KI HO and WT littermates were tested in the mixed groups and in random patterns unrecognizable to the examiner by the appearance. Cumulative horizontal activity and travelled distance for the 120 minutes following the drug injection were measured as an index of animals' locomotor activity and presented in the corresponding graphs. The registered data was analyzed by the default automated systems such as the VersaMax Animal Activity Monitoring System and included Analyzer software (AccuScan Instruments, Inc., Columbus, Ohio, USA).

2.4.6. Drug-induced locomotor activity

Dopamine is critically involved in a wide variety of vital functions such as locomotion, emotion, and reward system in the brain [5, 155]. The major dopaminergic pathways of the brain originate

from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) in the brainstem [192]. SNc neurons form nigrostriatal system that is heavily involved in the mediation of locomotion of the animals [192, 663, 664]. On the other hand, VTA neurons form the mesolimbic and mesocortical systems are responsible for relating the emotions, reward system and motivations in the animals [663, 664]. These pathways have been greatly studied in various disciplines of neuroscience from addiction, movement disorders and psychopathologies like schizophrenia, depression and bipolar disorder [30, 155, 192, 267, 665]. This common neurobiological basis might explain why studies of the antipsychotic or anti-addictive responses of potential drugs usually involve the investigation of locomotion activity of the animals [191, 193]. Concerning this, it is believed that the locomotor activity of the animals correlates with the central concentration of dopamine in the brain [94, 193, 633, 666]. This provides a reliable and easy way to investigate the interaction of various drugs and dopaminergic system in the animals [193]. In this regard, potentials of the new drugs in resisting the hyperlocomotion responses of stimulant drugs are commonly used protocols in the literature [181, 191, 235].

2.4.6.1. Amphetamine-induced locomotor activity

Amphetamine-induced locomotor activity is one of the most common animal models to investigate the interaction of the candidate drug and the central dopaminergic tone [231, 633, 667]. In our experiment, the response of saline or JJ-3-42 at 10 and 20 mg/kg on the hyperlocomotion induced by amphetamine at 3 mg/kg in WT animals has been studied. Locomotor activity of WT C57BL/6 R439H TPH2-KI mice was measured in an Omnitech Digiscan (Accuscan Instruments, Columbus, Ohio United States) activity monitor under the same condition described in the previous section. All the cages containing mice were transferred to the behaviour testing room 60 minutes before the first trial began. For the habituation period, mice were placed into the apparatus and their activity was monitored for 30 minutes. Then, mice were removed from the chamber for an injection with agonist or vehicle. After the injection, mice were returned to the monitoring chamber and their locomotor activity was recorded for another 30 minutes. The same procedure was used to inject amphetamine and this time mice were monitored for the following 120 minutes. Horizontal activity as a direct index of the locomotor activity of the animals was measured. The time course of the impact of JJ-3-42 at 10

and 20 mg/kg on the amphetamine-induced locomotor activity has been demonstrated. Consequently, the cumulative basal activities for 30 min after the administration of the agonists and for 120 min following the amphetamine administration in different groups of animals were reported in the corresponding bar graphs.

2.4.6.2. Apomorphine-induced locomotor activity

Apomorphine is a non-selective dopamine agonist, which activates both D2 and D1 receptors and increases the dopamine neurotransmission in the CNS [668, 669]. The apomorphine-induced locomotion is a well-known model to study the central interactions of potential drugs with dopamine system [668-670]. In our experiment, the response of saline or JJ-3-42 at 10 and 20 mg/kg on the hyperlocomotion induced by apomorphine at 2 mg/kg in WT animals has been studied. Locomotor activity of WT C57BL/6 R439H TPH2-KI mice was measured in an Omnitech Digiscan (Accuscan Instruments, Columbus, Ohio United States) activity monitor under the same condition described in the previous section. All the cages containing mice were transferred to the behaviour testing room 60 minutes before the first trial began. For the habituation period, mice were placed into the apparatus and their activity was monitored for 30 minutes. Then, mice were removed from the chamber for an injection with agonist or vehicle. After the injection, mice were returned to the monitoring chamber and their locomotor activity was recorded for another 30 minutes. The same procedure was used to inject apomorphine and this time mice were monitored for the following 90 minutes.

Horizontal activity as a direct index of the locomotor activity of the animals was measured. The time course of the impact of JJ-3-42 at 10 and 20 mg/kg on the apomorphine-induced locomotor activity has been demonstrated. Consequently, the cumulative basal activities for 30 min after the administration of the agonists and for 90 min after the apomorphine administration in different groups of animals were reported in the corresponding bar graphs.

2.4.6.3. MK-801-induced locomotor activity

Non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists such as MK-801 and ketamine have been frequently used as a pharmacological model of schizophrenia [671]. These compounds increase the extracellular glutamate, dopamine, and serotonin level in the brain [671-675]. Therefore the administration of MK-801 mimics the psychotic features, negative symptoms as well as cognitive disturbances in healthy individuals and therefore is the basis of animal models to study the antipsychotic properties of a new compound [673-675]. In this regard, the central anti-psychotic properties of various drugs are evaluated by the inhibition of locomotor activation induced by MK-801 in the animals [673-675]. In our experiment, the response of saline or JJ-3-42 at 10 and 20 mg/kg on the hyperlocomotion induced by MK-801 at 0.2 mg/kg, a dose which produced marked locomotor stimulation in WT animals previously, has been studied [671]. Locomotor activity of WT C57BL/6 R439H TPH2-KI mice was measured in an Omnitech Digiscan (Accuscan Instruments, Columbus, Ohio United States) activity monitor under the same condition described in the previous section. All the cages containing mice were transferred to the behaviour testing room 60 minutes before the first trial began. For the habituation period, mice were placed into the apparatus and their activity was monitored for 30 minutes. Then, mice were removed from the chamber for an injection with agonist or vehicle. After the injection, mice were returned to the monitoring chamber and their locomotor activity was recorded for another 30 minutes. The same procedure was used to inject MK-801 and this time mice were monitored for the following 120 minutes.

Horizontal activity as a direct index of the locomotor activity of the animals was measured. The time course of the impact of JJ-3-42 at 10 and 20 mg/kg on the MK-801-induced locomotor activity has been demonstrated. Consequently, the cumulative basal activities for 30 min after the administration of the agonists and for 120 min after the MK-801 administration in different groups of animals were reported in the corresponding bar graphs.

2.4.6.4. Cocaine-induced locomotor activity

Cocaine is one of the most abused drugs in the western world and its socioeconomic burden has been discussed in the literature in detail [219, 676]. The biochemical and behavioural consequence of administration of cocaine has been studied in various fields from addiction to psychosis for a long time [219, 677]. As mentioned, stimulant-induced hyperlocomotion is a basic behavioural test to study the immediate central responses of various potential drugs in the brain of animals [254].

In our experiment, the response of saline or JJ-3-42 at 10 and 20 mg/kg on the hyperlocomotion induced by cocaine at 20 mg/kg has been studied. Locomotor activity of WT C57BL/6 R439H TPH2-KI mice was measured in an Omnitech Digiscan (Accuscan Instruments, Columbus, Ohio United States) activity monitor under the condition described in the previous section. All the cages containing mice were transferred to the behaviour testing room 60 minutes before the first trial began. For the habituation period, mice were placed into the apparatus and their activity was monitored for 30 minutes. Then, mice were removed from the chamber for an injection with agonist or vehicle. After the injection, mice were returned to the monitoring chamber and their locomotor activity was recorded for another 30 minutes. The same procedure was used to inject cocaine and this time mice were monitored for the following 90 minutes.

Horizontal activity as a direct index of the locomotor activity of the animals was measured. The time course of the impact of JJ-3-42 at 10 and 20 mg/kg on the cocaine-induced locomotor activity has been demonstrated. Consequently, the cumulative basal activities for 30 min after the administration of the agonists and for 90 min after the cocaine administration in different groups of animals were reported in the corresponding bar graphs.

2.4.7. Locomotor activity in DAT-KO HO mice model

DAT-KO mouse is one of the most famous mice models used in the study of the dopaminergic system [633]. The lack of the dopamine transporter in the HO animals leads to a chronic

hyperdopaminergic state in the CNS [633]. The well-described innate hyperactivity of these animals has been the ground to investigate the influence of potential drugs on the dopamine neurotransmission [633]. In our study, DAT-KO HO mice were injected with saline or JJ-3-42 at 10 and 20 mg/kg after 30 minutes of habituation. Then the mice returned to the locomotor boxes and their activity was recorded for the next 120 minutes as described in the previous section. The time course of horizontal activity and cumulative horizontal activity for 120 min following the injection of the agonist was reported.

2.4.8. Locomotor activity in DDD mice model

The advanced loss of dopaminergic neurons and dopamine deregulation leads to the clinical manifestation of Parkinson's disease including resting tremor, rigidity, bradykinesia and akinesia [679, 680]. Some of these symptoms are captured in the DA-depleted DAT-KO mouse (DDD) model. Briefly, in the absence of dopamine transporter, de novo synthesis of dopamine is the only source of the dopamine release in the synaptic cleft in DAT-KO HO animals [667, 681]. In DDD model, acute inhibition of the synthesis of dopamine leads to a state of acute dopamine depletion and therefore induces akinesia in the animals [667, 681]. Consequently, this sudden bradykinetic or akinetic phenomenon can be rescued by the dopaminergic agents like L-DOPA and its derivatives. In this regard, this model is a valuable tool to study the potential of various drugs in the treatment of movement disorders.

In our experiment, we used this model to examine the response of 5-HT2C in the DDD mice. The mice were injected after 30 minutes of habituation in the chamber as described in the previous sections. DAT-KO HO mice became akinetic almost immediately after the treatment with alfa-MPT (250 mg/kg). Then the effect of 5-HT2C agonist or saline alone or in combination with an efficient cocktail of L-DOPA and Carbidopa at 20/20 mg/kg on the locomotor activity of the animal was measured. The time course of horizontal activity and cumulative horizontal activity for 60 min following the injection of L-DOPA and Carbidopa cocktail was reported.

2.4.9. Cocaine sensitization model

Beside the immediate responses, there are more long-term consequences to the consumption of the drugs of abuse including cocaine [682]. It has been proposed that such changes could be due to functional modifications at the level of synapses and the circuits due to chronic exposure to these drugs [682]. To assess the potential of different interventions to inhibit or disrupt such long-term responses in the brain various behavioural protocols are available presently [254, 682]. Behavioural sensitization is arguably one of the most frequently used paradigms for the assessment of long-term responses following repeated treatments with the stimulant drugs of abuse [666, 683]. This test is based on the phenomenon called “reverse tolerance” in which repeated exposures to a psychostimulants leads to an enhancement of the locomotor responses [682]. Various electrophysiological and biochemical essays have shown that the sensitization of the responsible circuits following the synaptic and post-synaptic protein modifications is probably at the core of these exaggerated responses [682]. The first studies of cocaine-induced locomotor sensitization in rodents were developed in 1970s and since then there has been a variety of technic and protocols published in the literature [683, 684]. In our protocol, the sensitized behaviour is measured just after five consecutive doses of the treatment as a convenient and valid method of assessment of anti-addictive properties of the tested drugs. However, we should mention that the duration and the pattern of drug administration vary significantly in different protocols [666, 677]. In some of these studies, the sensitized locomotion would be still detectable even after few months following the trials [666, 677]. The simplicity of the measurement of the changes of the locomotor activity reflecting the long-lasting drug-induced behaviour is one of the favourable features of this test [666, 677, 682].

In our study, to examine the response of JJ-3-42 on cocaine sensitization development, mice were given one daily injection of cocaine at 20 mg/kg 30 minutes after an injection of JJ-3-42 at 10 mg/kg on five consecutive days. Then after a rest day, on the seventh day, mice were challenged by a similar dose of cocaine 30 minutes after the similar pre-treatment with JJ-3-42 (please see the protocol below). The locomotor activities of animals after the injection of cocaine on the first and seventh day have been registered. This protocol has been used and repeatedly described before [682]. Cocaine, saline or agonist were administered daily in a similar context,

pattern and order. The locomotor activity of WT C57BL/6 R439H TPH2-KI mice was measured in an Omnitech Digiscan activity monitor under the same condition described in previous sections. For the habituation period, mice were placed into the apparatus and their activity was monitored for 30 minutes. Then, mice were removed from the chamber for an injection with agonist or saline. After the injection, mice were returned to the monitoring chamber and their locomotor activity was recorded for another 30 minutes. The same procedure was used to inject cocaine and this time mice were monitored for the following 90 minutes. Horizontal activity as a direct parameter related to the locomotor activity of the animals was measured. The time course of the impact of JJ-3-42 at 10 mg/kg on the cocaine-induced locomotor activity on the first day and in sensitized animals on the seventh day has been demonstrated. Consequently, the cumulative horizontal activities for the first 30 min after the administration of the saline or agonist and for the 90 min following the cocaine administration in different groups of animals on the first and seventh days were reported in the corresponding bar graphs.

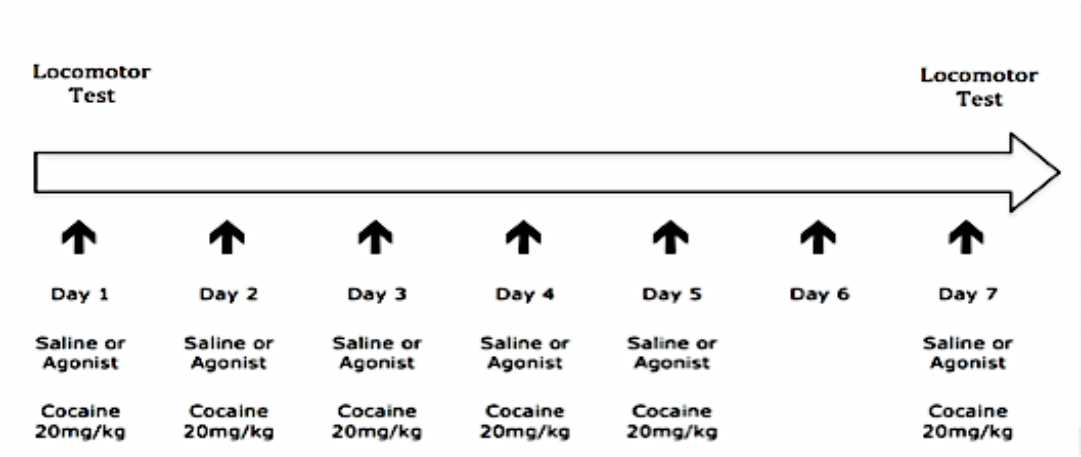

Figure 2.4.9 Protocol of cocaine sensitization

2.5. Statistical Analysis

First, the normal distribution of the data was tested by available statistical tools on Prism statistic software Version 6. Where normal distribution present, data was processed using ANOVA (analysis of variance) followed by Tukey post hoc to compare the performance of 3 or more groups of mice. Two-tailed t-test was used for the analysis of two groups of animals like in the analysis of cocaine sensitization test. Upon the presence of the abnormal distribution of the data like in the case of dyadic social interaction test, non-parametric analysis of Two-tailed t-test with Mann-Whitney for comparison of two groups and Kruskal-Wallis test of ANOVA with Dunn's post hoc for comparison of 3 or more groups were used. All the graphs and the statistical analysis were completed using the Prism statistic software Version 6 (GraphPad Software, La Jolla, CA). Data were presented as means ± SEM (standard error of the mean) and p-levels ($p<0.05$, $p<0.01$, $p<0.001$) in the figures were denoted with asterisks (*, **, and *** in comparison with WT control and #, ##and ### with corresponding HO or agonist-treated control group respectively). Only significant and relevant statistical analyses were presented graphically.

3. Results

3.1. Effects of genotype and acute injection of JJ-3-42 at 10 mg/kg on H-Maze test

H-Maze test is a sophisticated test measuring the capacity to learn, forget and relearn a new pattern as an index of cognitive flexibility in animals. As shown in the figure 3.1A and 3.1B, the number of trials and time needed to finish the first criterion of H-Maze was significantly different between the various groups in this experiment (ANOVA: $F(3, 28) = 4.801$, $p \leqslant 0.01$) and $F(3, 28) = 3.845$, $p \leqslant 0.05$) respectively. Post hoc analysis showed that HO mice required more trials and time than mice from the WT group to finish the task both ($p \leqslant 0.05$). Interestingly the pre-treatment with the JJ-3-42 at 10 mg/kg reduced the number of trials needed to finish the ALT in HO mice.

Result of ANOVA test and the subsequent post hoc showed that there was a significant difference between the number of trials and the needed time between groups to finish N-ALT task [$F(3, 28) = 13.16$, $p \leqslant 0.001$] and [$F(3, 28) = 17.53$, $p \leqslant 0.001$)] respectively (see figure 3.1C and 3.1D). Moreover, acute administration of JJ-3-42 at 10 mg/kg rescued the phenotype of TPH2-KI HO mice and reduced the number of trials ($P \leq 0.01$) or time ($P \leq 0.001$) needed to finish the task (figure 3.1C and 3.1D).

As shown in the figure 3.1E, the inferior performance of the TPH2-KI HO in H-Maze might be attributed at least partly to the higher perseverant tendencies in these animals in comparison to the wild types ($P \leq 0.01$). This phenomenon has been described in detail before [210]. Analysis of the perseverant behaviour in mice by ANOVA test and the subsequent post hoc showed that acute administration of JJ-3-42 at 10 mg/kg improved the performance of the animals by reducing the number of perseverant bouts in TPH2-KI HO mice significantly [$F(3,36) = 9.140$; $P \leq 0.001$] (figure 3.1E).

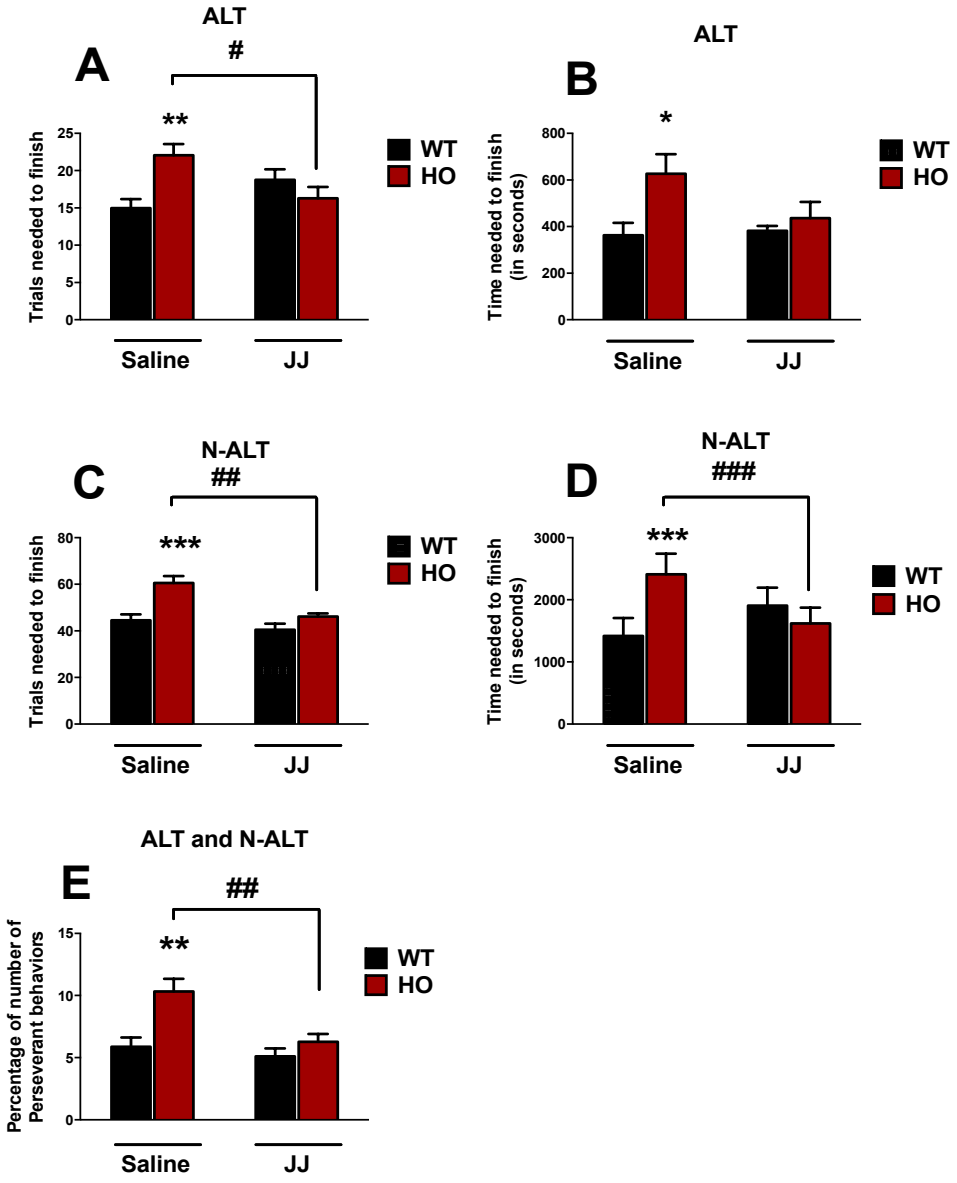

Figure 3. 1. Effects of genotype and acute injection of JJ-3-42 at 10 mg/kg on H-Maze test

Figure 3.1A-E shows the effects of treatment with JJ-3-42 at 10 mg/kg Vs. saline 30 min before the test on the performance of the different genotypes of TPH2-KI in H-Maze test. The number of trials and the time needed to finish the ALT, N-ALT and percentage of perseverant behaviour bouts to the total number of trials was reported. As depicted in figure 3.1A-D, the HO mice needed more trials and time to finish both criteria in comparison to WT and JJ-3-42 at 10 mg/kg, rescued this behaviour. All data is cited as means with SEM, minimum n=8–10 per group. Statistical significance in ANOVA and post hoc compared to WT saline-treated group is shown by * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ or to HO saline-treated mice by #$p<0.05$, ##$p<0.01$, ###and $p<0.001$.

3.2. Effects of genotype and 5-HT2C receptor agonists on dyadic social interaction test

In dyadic social interaction test we measured the time and the number of events of mild social interaction (including sniffing, grooming, following, etc.) and the aggressive behaviour (like biting, scape, attack, etc.) in 10 minutes. As seen in the figure 3.2A and 3.2B there was not a significant difference in the Mann-Whitney test between the WT-WT and HO-WT of TPH2-KI mice regarding the amount of spent time [U= 38.50, P≥0.05] and the number of events [U= 42.50, P≥0.05] in the mild social interaction paradigm. In contrast, there was a significant difference between WT-WT and HO-WT groups interaction as measured by time and the number of events of aggressive behaviours in Mann-Whitney tests [U= 0, P≤0.001] and [U= 0, P≤0.001] respectively (see figure 3.2A and 3.2B).

Figure 3.2C and 3.2D show the response of various 5-HT2CRs agonists on mild social behaviour of animals in the dyadic social interaction test. Interestingly, Kruskal-Wallis test of ANOVA with Dunn's post hoc on the number of events [H (8,80) = 30.15; P≤0.001] and the amount of time spent [H (8,80) = 29.87; P≤0.001] in mild social interaction paradigms showed that while CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg had no effect in both WT and HO animals (P≥0.05), 5-HTP at 20 mg/kg increased the sociability of the HO animals significantly (figure 3.2C and 3.2D respectively). Figure 3.2E and 3.2F show that acute administration of either 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg, was enough to rescue the aggressive behaviour of TPH2-KI HO mice measured in both the number of events [H (8,80) = 37.33; P≤0.001] and time spent [H (8,80) = 30.32; P≤0.001] in aggressive behaviour in dyadic social interaction paradigm.

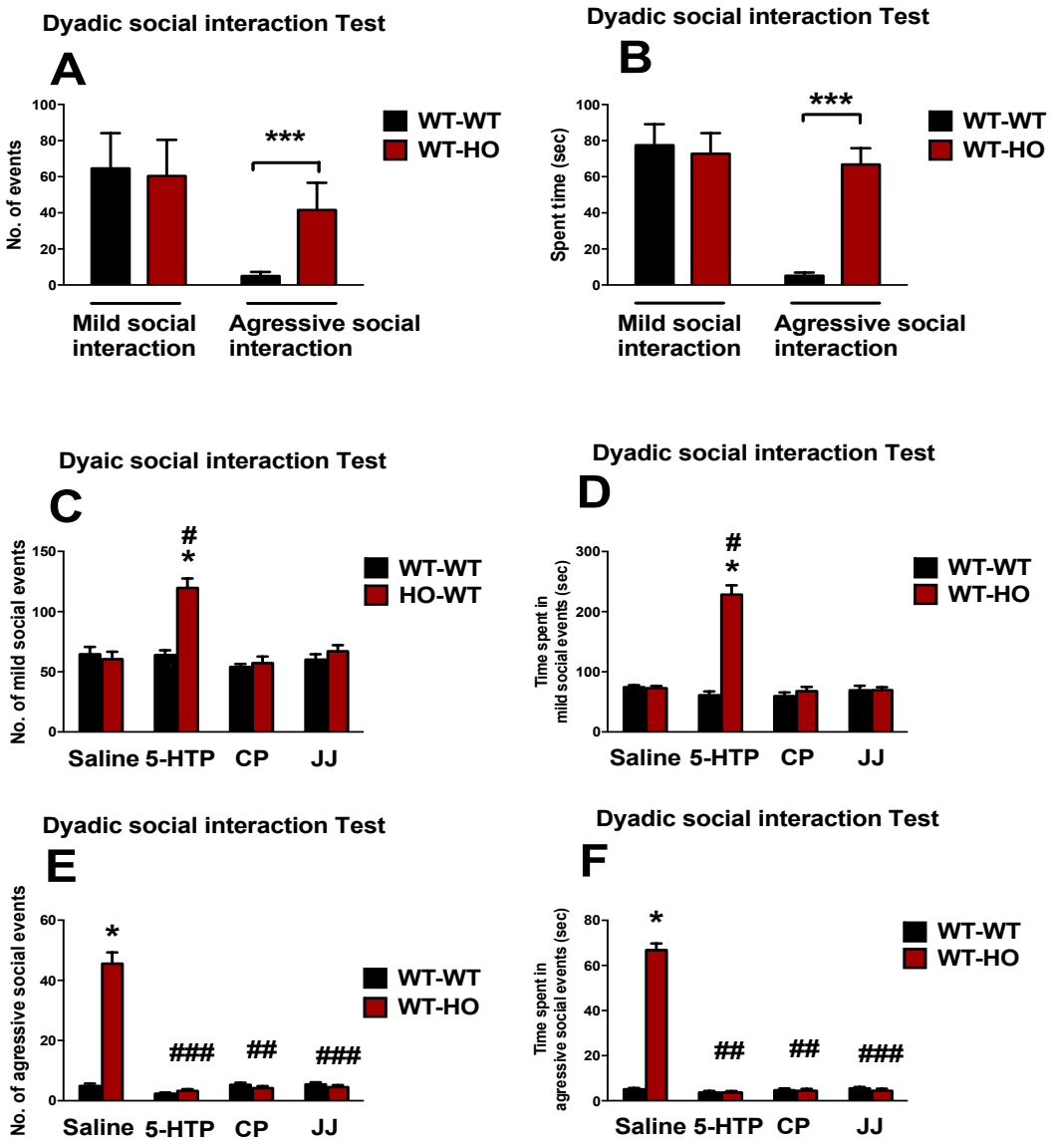

Figure 3.2. Effects of genotype and 5-HT2C receptor agonists on dyadic social interaction test

Figure 3.2A-F shows the performance of the different genotype pair (WT-WT, HO-WT) of TPH2-KI mice in dyadic social interaction test. The time and the number of the aggressive behaviour (measured as biting, scape, attack, etc.) and mild social interaction (including sniffing, grooming, following, etc.) were reported. There was not a significant difference between the WT-WT and HO-WT mice couple interaction regarding the amount of time and number of events in the mild social interaction. In contrast, HO mice showed a significantly higher amount of aggressive behaviour toward their WT counterparts in 10 min test. Acute administration of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg was enough to rescue this aggression in HO mice. All i.p. injections were ware performed 30 minutes before the test. All data cited as means with SEM, n=8–10 per group. Statistical significance compared to WT–WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$.

3.3. Effects of genotype and 5-HT2C receptor agonists on repetitive behaviour paradigms

As shown in the figure 3.3.1A-F we investigated the effects of 3 different genotypes of TPH2-KI (WT, HET, HO) in three different paradigms of compulsive-like behaviour. In marble burying, the results of ANOVA test and subsequent post hoc showed that TPH2-KI HO mice have buried more marbles [$F(2,29) = 20$; $P \leq 0.001$] (figure 3.3.1A) and spent much more time burying [$F(2,26) = 14.95$; $P \leq 0.001$] (figure 3.3.1B) in comparison to WT and HET mice. HO mice also had significantly less delay to start grooming [$F(2,27) = 6.2$; $P \leq 0.01$] (figure 3.3.1C) and digging [$F(2,27) = 13.83$; $P \leq 0.001$] (figure 3.3.1E) while spent more time in compulsive grooming [$F(2,28) = 19.96$; $P \leq 0.001$] (figure 3.3.1D) and digging [$F(2,28) = 4.827$; $P \leq 0.05$] (figure 3.3.1F) in corresponding tests.

Figure 3.3.2A-F shows the result of the acute administration of different 5-HT2CRs agonists on the compulsive/repetitive behaviour of TPH2-KI mice in three different paradigms. In marble burying the results of ANOVA test and the subsequent post hoc showed that acute administration of either 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg, rescued the number of buried marbles TPH2-KI HO mice compared to the controls [$F(7,71) = 23.84$; $P \leq 0.001$] (figure 3.3.2A). Such result was consistent also with the observed effect of these drugs on the time spent in marble burying behaviour in these animals [$F(7,68) = 11.87$; $P \leq 0.001$] (figure 3.3.2B).

In the same way, administration of CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg increased the delay to start grooming [$F(7,67) = 3.807$; $P \leq 0.01$] (figure 3.3.2.C) and digging [$F(7,66) = 8.601$; $P \leq 0.001$] (figure 3.3.2.E) in TPH2-KI HO in comparison to their corresponding controls. However, 5-HTP at 20 mg/kg, while was enough to increase the delay to start digging in the animals failed to have an impact on the grooming test in animals (figure 3.3.2.C and E).Finally, following the administration of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg there was a significant reduction in the amount of spent time in grooming [$F(7,68) = 12.14$; $P \leq 0.001$] (figure 3.3.2.D) and digging [$F(7,68) = 6.541$; $P \leq 0.001$] (figure 3.3.2.F)

behaviour of these mice compared to corresponding behaviour. In general, these results point out to the similarities of the anti-compulsive character of all these 5-HT2CRs agonist.

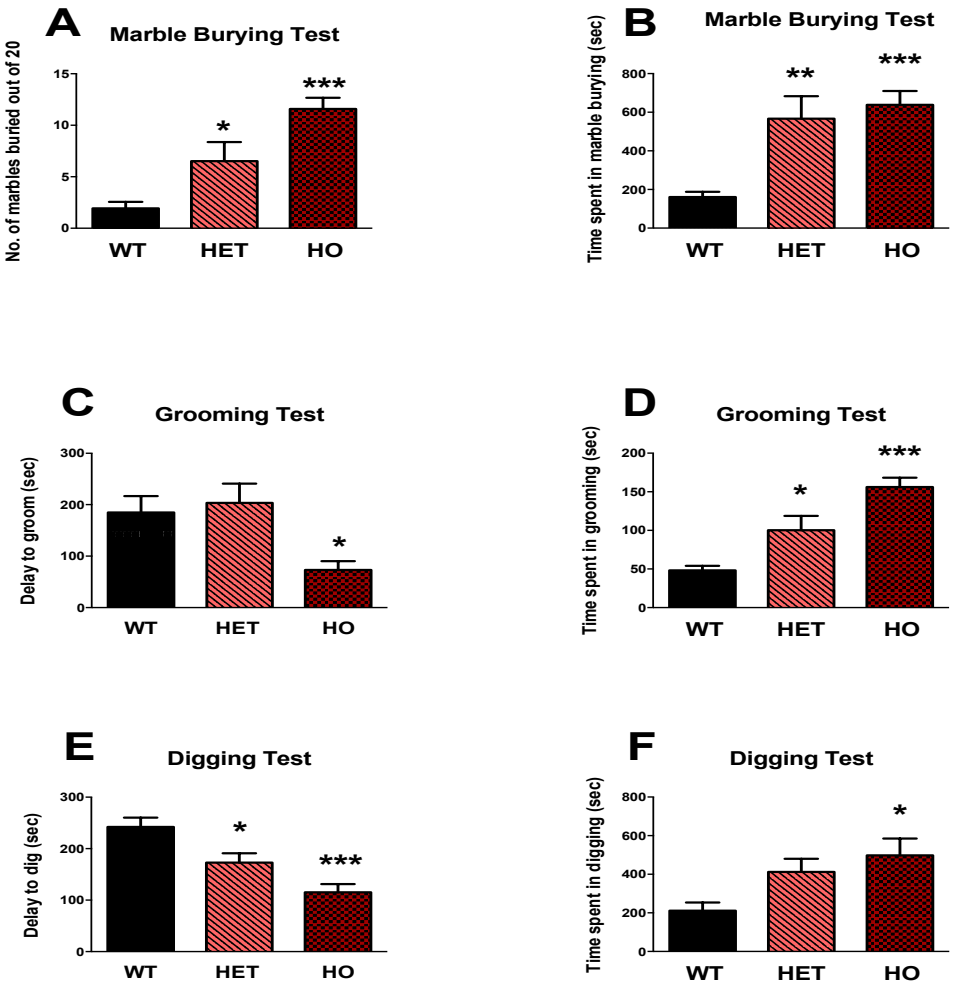

Figure 3.3.1. Effects of genotype on repetitive behaviour paradigms

As shown in the figure 3.3.1A-F, we investigated the effects of 3 different genotypes (WT, HET, HO) of TPH2-KI in three different paradigms of compulsive and repetitive behaviours of mice. In marble burying test TPH2-KI HO mice have buried more marbles and spent more time burying in comparison to WT and HET mice. HO mice also had less delay to start grooming and digging in corresponding tests while spent significantly more amount of time in grooming or digging behaviour. All i.p. injections were performed 30 minutes before the test. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$ or HO saline-treated mice by Tukey test and one-way ANOVA.

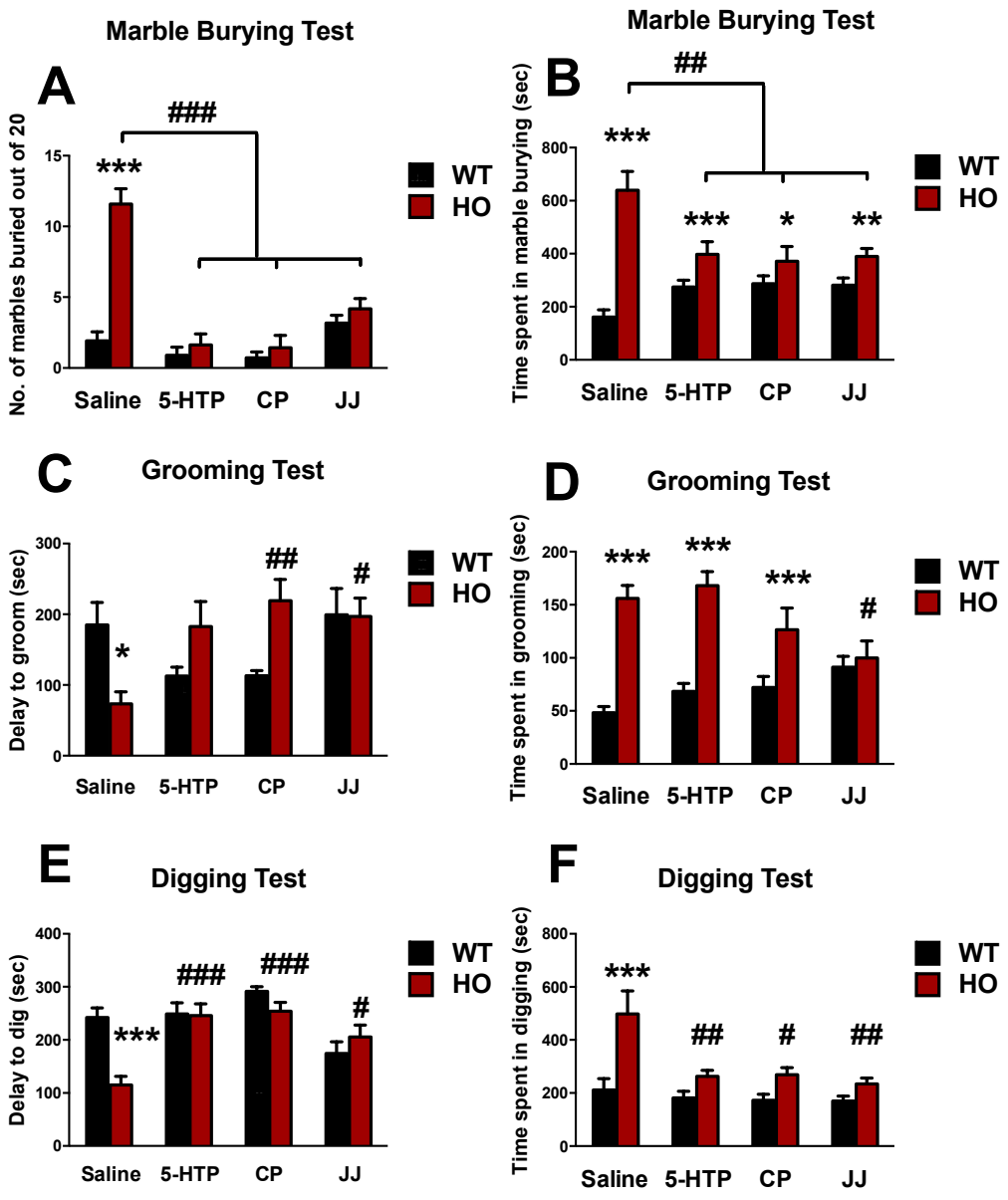

Figure 3.3.2. Effects of 5-HT2C receptor agonists on repetitive behaviour paradigms

Figure 3.3.2A-F shows the result of acute administration of different 5-HT2C agonists on the compulsive and repetitive behaviours of TPH2-KI mice. In marble burying test, acute administration of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg, rescued the compulsive behaviour of TPH2-KI HO mice and reduced the amount of spent time in burying behaviour. In a similar way administration of CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg increased the delay to start and reduced the amount of spent time in grooming or digging behaviour of TPH2-KI HO in comparison to their corresponding controls. 5-HTP at 20 mg/kg, while was enough to increase the delay to start digging in the animals failed to have an impact on the grooming test in animals. All i.p. injections were ware performed 30 minutes before the test. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$ or HO saline treated mice #$p<0.05$. ## $p<0.01$ and ### $p<0.001$, by Tukey post hoc test and one-way ANOVA.

3.4. Effects of genotype and 5-HT2C receptor agonists on dark-light test

Figure 3.4.1A-D shows the result of different genotype (WT, HET, HO) of TPH2-KI animals on the dark-light test. ANOVA test and subsequent post-hoc showed that TPH2-KI HO mice had a higher level of anxiety in 5 minutes dark-light test in comparison to WT mice statistically. This was shown consistently in the following parameters: latency to enter the light zone [$F(2,31) = 4.2$; $P \leq 0.05$] (figure 3.4.1A), travelled distance in the light zone [$F(2,33) = 12$; $P \leq 0.001$] (figure 3.4.1B), amount of time spent in the light compartment [$F(2,37) = 5.1$; $P \leq 0.05$] (figure 3.4.1C) and the number of the entrances to the light zone [$F(2,34) = 18$; $P \leq 0.001$] (figure 3.4.1D). Furthermore, our analysis also demonstrated that in some of these paradigms like the number of the entrances to the light zone and travelled distance in the light zone the HET mice had a similar anxiety profile to the HO mice.

Figure 3.4.2A-D shows the result of acute administration of different 5-HT2C agonists in the dark-light test in TPH2-KI mice. In related paradigms, the results of One-way ANOVA test and subsequent post hoc showed that acute administration of 5-HTP at 20 mg/kg and CP809.101 at 1 mg/kg, did not ameliorate if not worsened the anxiety-like phenotype of the WT and HO animals in most of the following parameters: latency to enter the light zone [$F(7, 66) = 4.875$ $P \leq 0.001$] (figure 3.4.2A), travelled distance in the light zone [$F(7, 64) = 13.41$ $P \leq 0.01$] (figure 3.4.2B), time spent in the light compartment [$F(7, 62) = 6.421$, $P < 0.001$] (figure 3.4.2C), and the number of entrances to the light zone [$F(7, 64) = 8.773$, $P < 0.001$] (figure 3.4.2D). In most of these tests, due to already very anxious profile of HO mice detecting a further anxiogenic response of these drugs were not possible.

Interestingly, post hoc analysis showed that effective dose of JJ-3-42 at 10 mg/kg not only failed to demonstrate such anxiogenic profile in both HO and WT controls but also had a slight anxiolytic effect in HO animals and increased the distance travelled in the light zone ($P < 0.01$ in comparison to HO mice). Overall, it seems that the potent dose of JJ-3-42 is not carrying an anxiogenic profile despite the other 5-HT2CRs agonist in the dark-light profile.

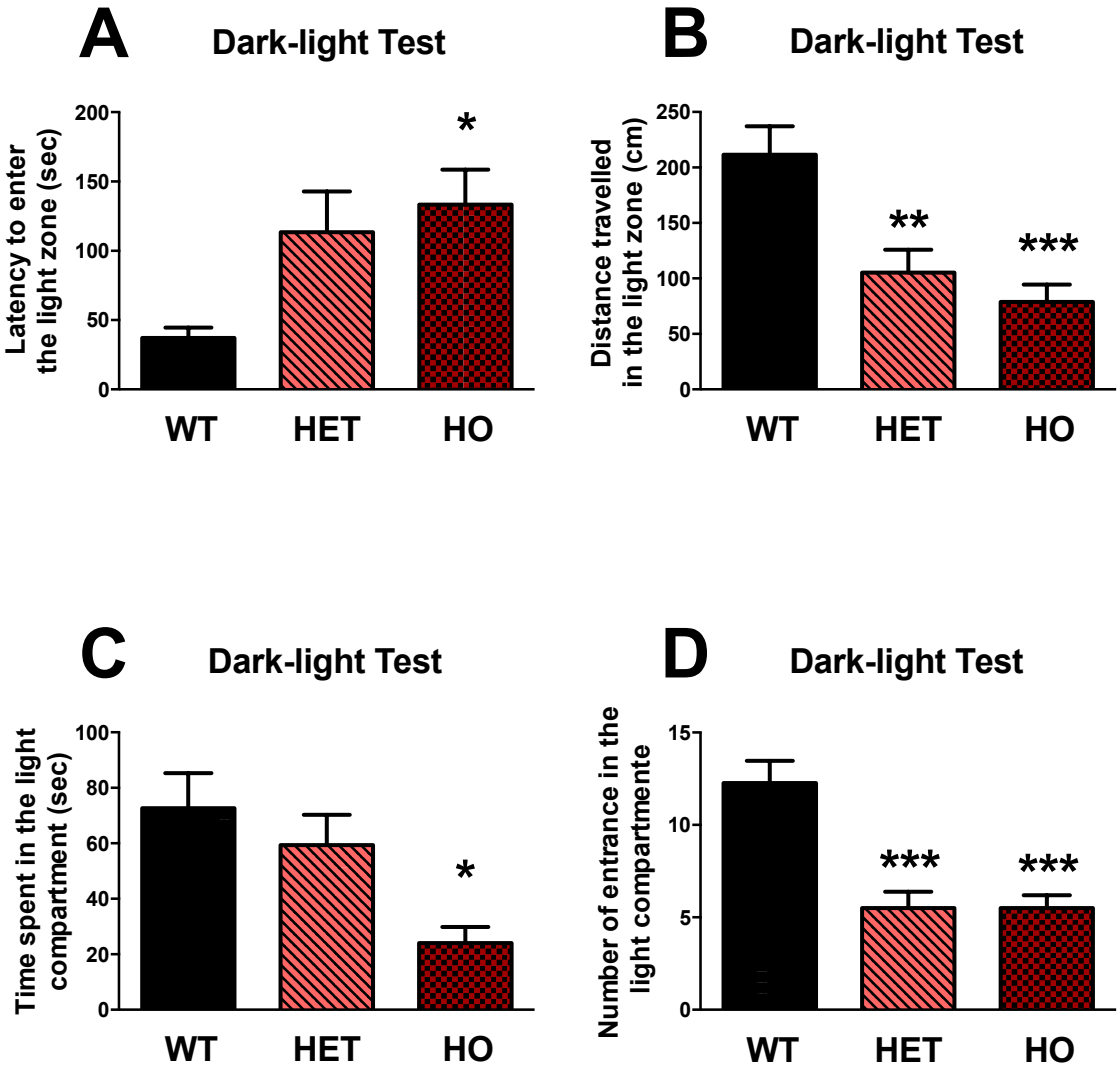

Figure 3.4.1. Effects of genotype on dark-light test

Figure 3.4.1A-D Shows the effect of different genotypes (WT, HET, HO) of TPH2-KI on latency to visit the light chamber, distance travelled in the light zone, time spent in the light zone and number of the entrances to the light zone. In all of these paradigms the HO mice showed higher anxiety profile compared to WT mice. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$.

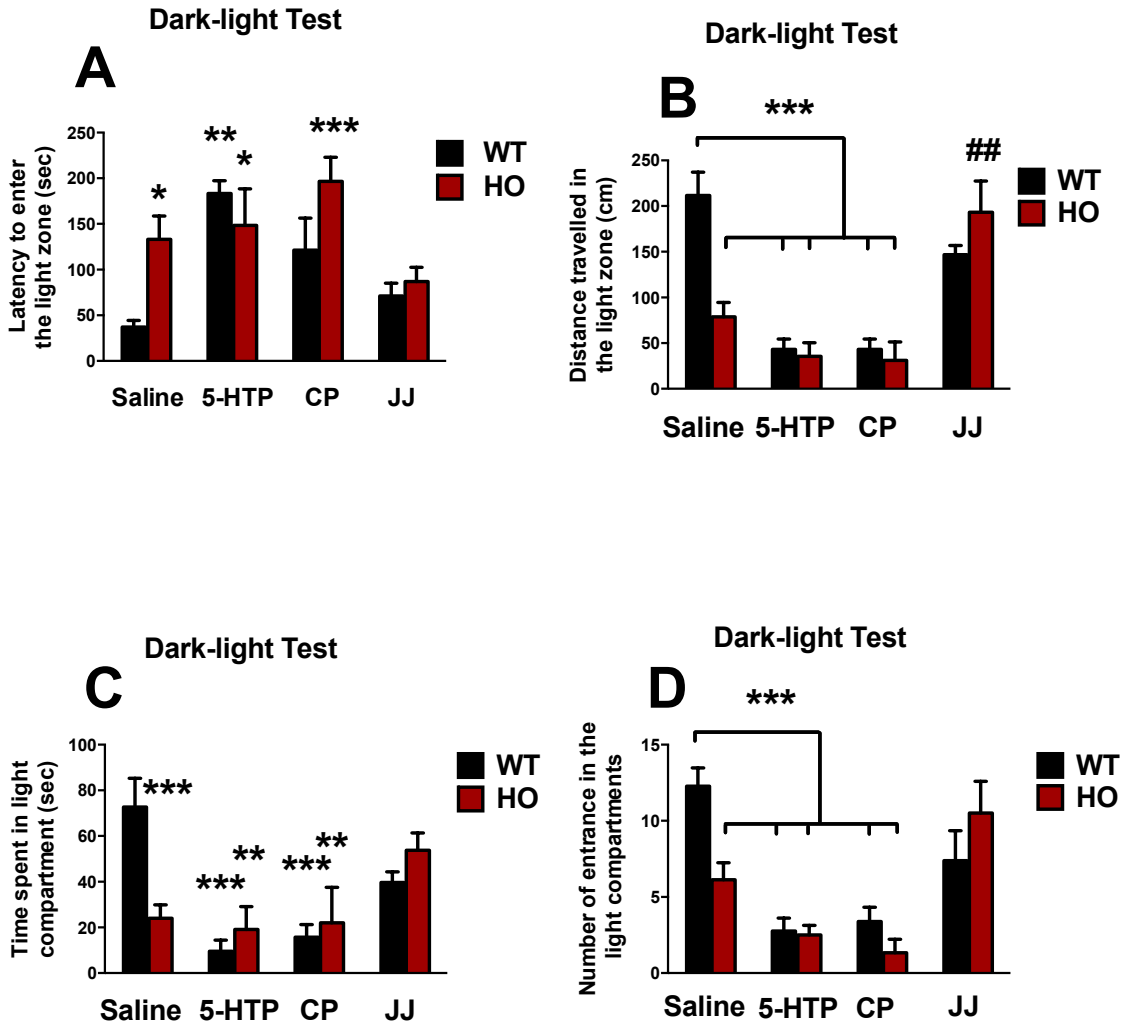

Figure 3.4.2. Effects of 5-HT2C receptor agonists on dark-light test

Figure 3.4.2A-D shows the effect of i.p injection of different serotonergic agonists 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg Vs. saline, 30 min before the test on the behaviour of WT and HO TPH2-KI in dark-light test. The latency to visit the light chamber, distance travelled in the light zone, time spent in the light zone and the numbers of entrances to the light zone were reported. In contrast to the other two agonists, JJ-3-42 at 10 mg/kg did not increase the level of the anxiety in the HO and WT animals in these tests. All i.p. injections were ware performed 30 minutes before the test. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * p <0.05 ** p<0.01 *** p<0.001,or with HO saline-treated mice #p<0.05. ##p<0.01 ###, P <0.001, by one-way ANOVA and Tukey test.

3.5. Effects of genotype and 5-HT2C receptor agonists on openfield test

Figure 3.5.1A-D shows the result of different genotypes WT, HET, HO of TPH2-KI in the openfield test. The results of One-way ANOVA test and the subsequent post hoc showed the higher anxiety level of TPH2-KI HO mice in comparison to WT similar to HET mice. This included the parameters like total distance in the central zone [$F(2,25) = 25.51$; $P \leq 0.001$] (figure 3.5.1A), horizontal activity in the central zone [$F(2,27) = 44.03$; $P \leq 0.001$] (figure 3.5.1B), latency to enter the central zone [$F(2,27) = 42.89$; $P \leq 0.001$] (figure 3.5.1C) and the number of entrances into the central zone [$F(2,28) = 9.296$; $P \leq 0.001$] (figure 3.5.1D).

Figure 3.5.2A-D depicts the effects of acute administration of different 5-HT2C agonists on the openfield test in TPH2-KI mice. In different paradigms the results of ANOVA test and subsequent post hoc showed that the acute administration of 5-HTP at 20 mg/kg and CP809.101 at 1 mg/kg worsened the anxiety-like phenotype of the HO and WT animals. This was consistent for most of the variables like the travelled distance in the central zone [$F(7, 70) = 59.51$, $P < 0.001$] (figure 3.5.2A), horizontal activity in the central zone [$F(7, 71) = 110.1$, $P < 0.001$] (figure 3.5.2B), latency to enter the central zone [$F(7, 72) = 15.06$, $P < 0.05$] (figure 3.5.2C) and the number of entrances to the central zone [$F(7, 71) = 27.53$, $P < 0.001$] (figure 3.5.2D). However, our results show that efficient and potent dose of JJ-3-42 (10 mg/kg) does not possess anxiogenic effect in the paradigms like the travelled distance in the central zone (figure 3.5.2A), horizontal activity in the central zone (figure 3.5.2B) and latency to enter the central zone ($P < 0.001$) (figure 3.5.2C) ($P \geq 0.05$). On the other hand, treatment with JJ-3-42 at 10 mg/kg in WT mice reduced the number of entrances to the central zone (both $P < 0.001$) (figure 3.5.2D).

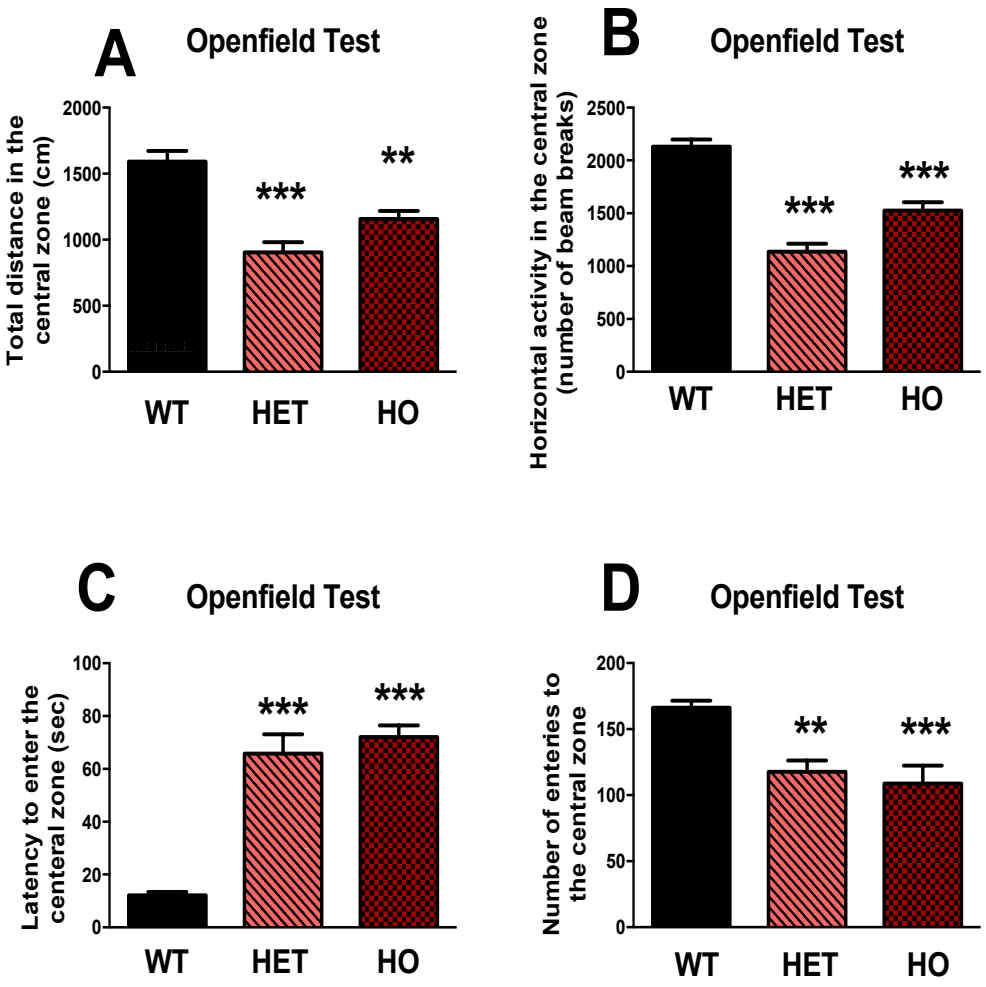

Figure 3.5.1. Effects of genotype on openfield test

Figure 3.5.1A-D shows the result of genotype variation (WT, HET, HO) on the openfield test in TPH2-KI mice. In different parameters related to anxiety-like behaviour of mice, our results showed that TPH2-KI HO mice had a higher level of anxiety in 30 minutes openfield test in comparison to WT but almost similar to HET mice. All i.p. injections were ware performed 30 minutes before the test. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$ or HO saline-treated mice by Tukey test and one way ANOVA.

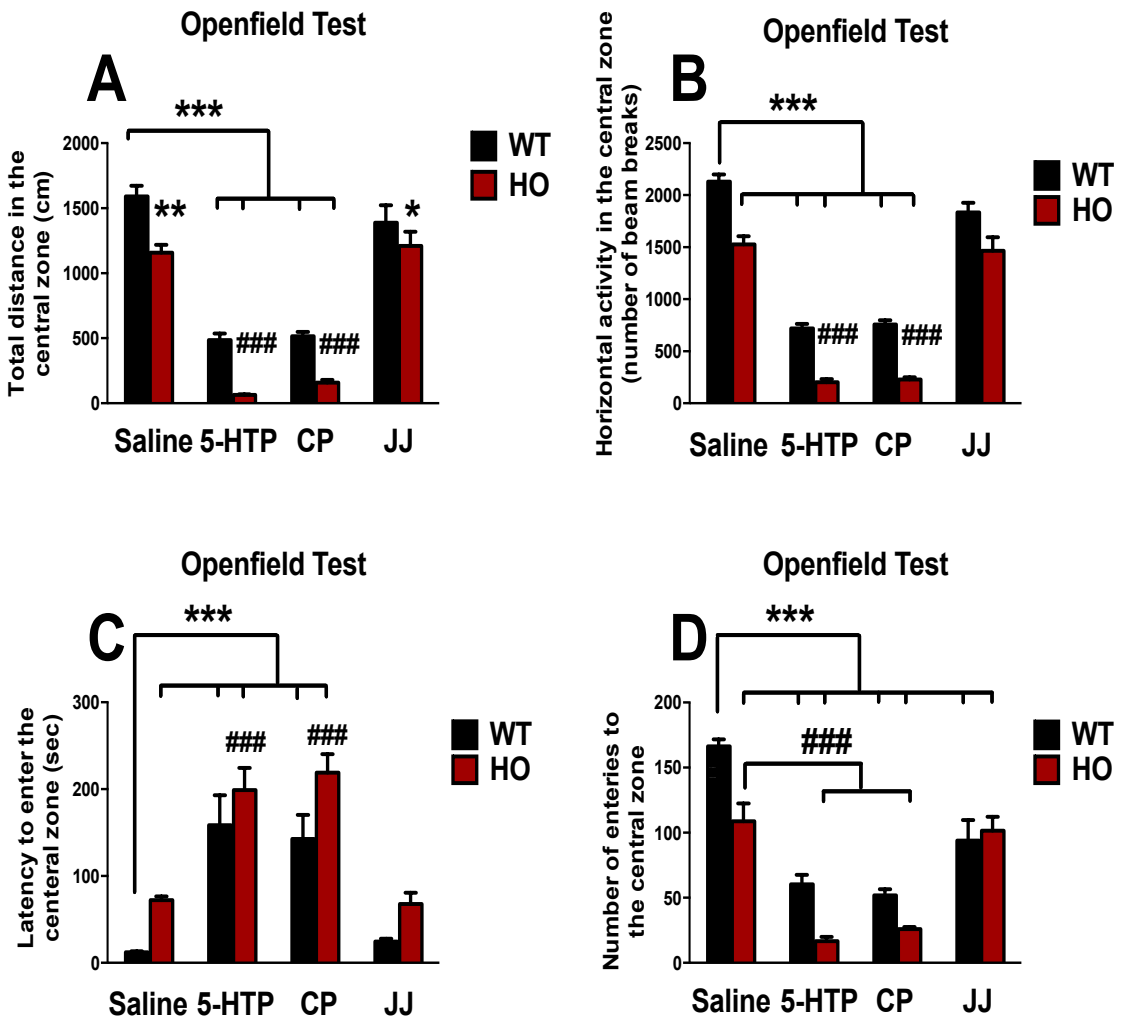

Figure 3.5.2. Effects of 5-HT2C receptor agonists on openfield test

Figure 3.5.2A-D shows the result of acute administration of different 5-HT2C agonists in the test in TPH2-KI mice. Variables like horizontal activity in the central zone, travelled distance in the central zone, latency to enter the central zone and number of entrances to the central zone was reported in this test. The results of One-way ANOVA test and subsequent post-hoc showed that acute administration of 5-HTP at 20 mg/kg and CP809.101 at 1 mg/kg worsens the anxiety-like phenotype of HO and WT animals these parameters. Interestingly it seems that effective and potent dose of JJ-3-42 (10 mg/kg) does not make the animals (WT and HO) more anxious when compared to their corresponding vehicle-treated littermates ($P \geq 0.05$)in most of the depicted paradigms. All i.p. injections were ware performed 30 minutes before the test. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$, or HO saline-treated mice #$p<0.05$. ##$p<0.01$ ###, $P<0.001$ by one-way ANOVA and Tukey test.

3.6. Effects of genotype and 5-HT2C receptor agonists on elevated plus maze test

Figure 3.6.1A-B shows the result of different genotype of TPH2-KI mice (WT, HET, HO) on the EPM test. In different paradigms related to anxiety-like behaviour of mice, the results of One-way ANOVA test and subsequent post hoc showed that HO and HET mice had higher levels of anxiety in 10 minutes EPM test in comparison to WT littermates. This was seen for both the delay to enter the open arm [$F(2,25) = 23.77$; $P \leq 0.001$] (figure 3.6.1A) and in the time spent in the open arm [$F(2,25) = 14.03$; $P \leq 0.001$] (figure 3.6.1B) parameters. Figure 3.6.2A-B shows the result of acute administration of different 5-HT2C agonists in the EPM test of TPH2-KI mice. While 5-HTP at 20 mg/kg increased the delay to enter the open arm in WT animals ($P < 0.001$), CP809.101 at 1 mg/kg ($P \geq 0.05$) and JJ-3-42 at 10 mg/kg ($P \geq 0.05$) did not have any effect [$F(7, 68) = 24.91$, $P < 0.001$] (figure 3.6.2A). Moreover, none of these drugs had changed the behaviour of the animals in the time spent on the open arm parameter ($P \geq 0.05$) [$F(7, 70) = 11.36$, $P < 0.001$] (figure 3.6.2B). In agreement of the results of dark-light test and openfield test the EPM result shows that the potent JJ-3-42 is devoid of central anxiogenic responses in the animals.

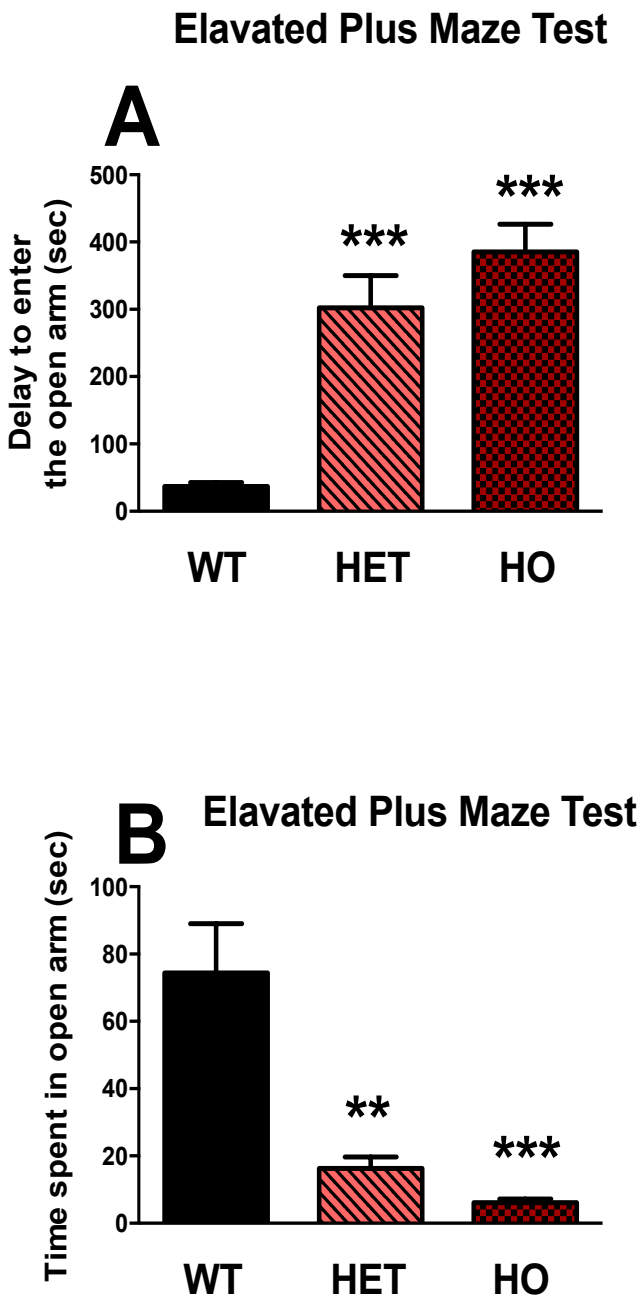

Figure 3.6.1. Effects of genotype on elevated plus maze test

Figure 3.6.1A-B shows the result of different genotype (WT, HET, HO) of TPH2-KI on the EPM test. The results of our test show that TPH2-KI HO and HET mice had a higher level of anxiety in 10 minutes EPM test in comparison to WT in related parameters like delay to enter the open arm and time spent in the open arm. All i.p. injections were ware performed 30 minutes before the test. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$ by one-way ANOVA and Tukey test.

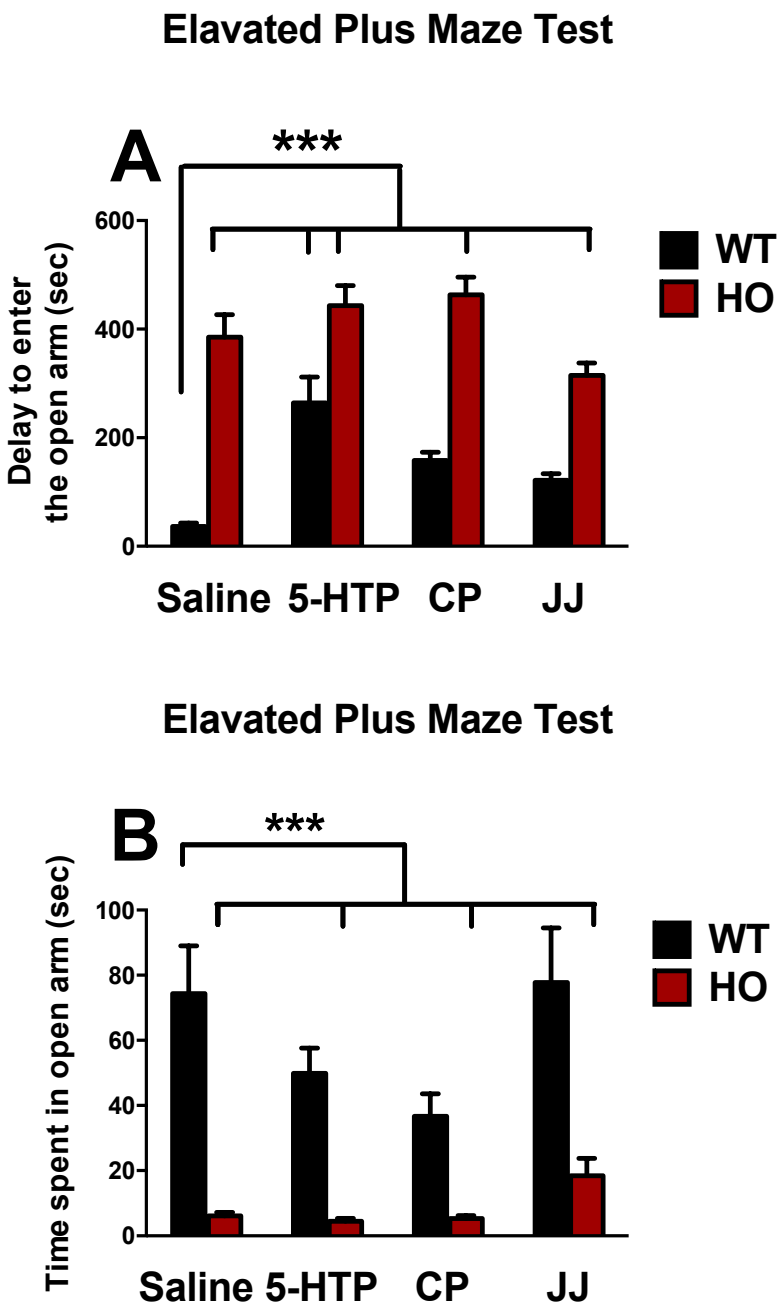

Figure 3.6.2. Effects of 5-HT2C receptor agonists on elevated plus maze test

Figure 3.6.2A-B shows the effect of i.p injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg Vs. saline on the behaviour of WT and HO TPH2-KI in EPM. Acute administration of 5-HTP worsens the anxiety-like phenotype of WT animals, while CP809.101 and JJ-3-42 did not have any effect on the parameters like latency to enter to and amount of spent time on the open arm in WT or HO mice. All i.p. injections were performed 30 minutes before the test. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$ by one-way ANOVA and Tukey test.

3.7. Effects of different 5-HT2C agonists on basal locomotor activity of animals

Figure 3.7A-B shows the result of acute administration of different 5-HT2C agonists on the locomotor activity of HO and WT TPH2-KI mice in the following 120 min. Mice were injected after 30 min of habituation period. The result of ANOVA test and post hoc analysis shows that the administration of 5-HTP at 20 mg/kg CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg did not have any significant effect on the locomotion of these animals in both total horizontal activity [$F(7, 72) = 0.8508$, $(P \geq 0.05)$] and travelled distance parameters [$F(7, 72) = 0.6703$, $(P \geq 0.05)$] (figure 3.7A and B).

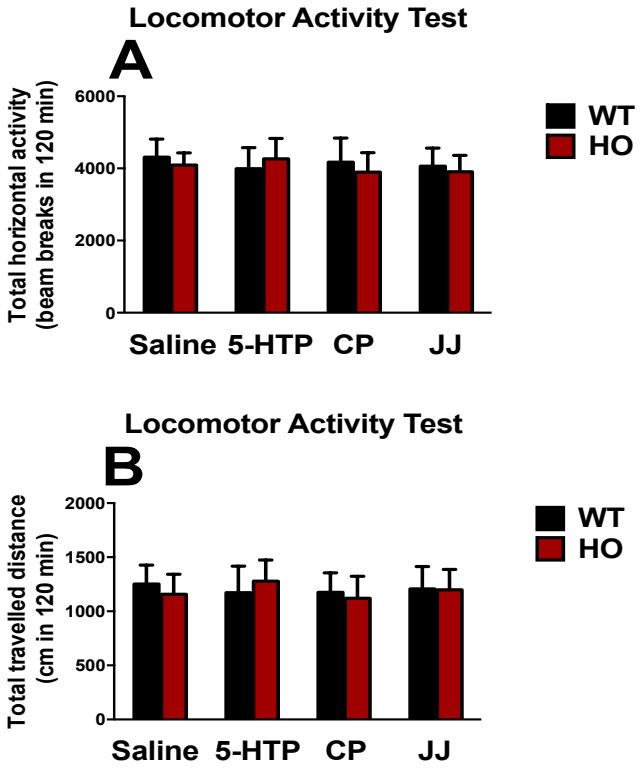

Figure 3.7. Effects of different 5-HT2C agonists on basal locomotor activity of animals

Figure 3.7A-B shows the effect of i.p injection of 5-HTP at 20 mg/kg, CP809.101 at 1 mg/kg and JJ-3-42 at 10 mg/kg Vs. saline on the innate locomotion of WT and HO TPH2-KI mice. Acute administration of none of these 5-HT2C agonists per se did change the locomotion of animals in a significant way compared to the WT and HO control groups. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$ by one-way ANOVA and Tukey test.

3.8. Effects of JJ-3-42 at 10 and 20 mg/kg on various stimulant-induced locomotor activity

3.8.1. Amphetamine-induced locomotor activity

Amphetamine-induced locomotion in animals is a modality that is frequently used to study the potential antipsychotic profile of various compounds. Figure 3.8.1A-C shows the response of 2 different doses of JJ-3-42 (10 and 20 mg/kg) on the locomotion activity of WT animals after the administration of an efficient dose of amphetamine (3 mg/kg).

Figure 3.8.1A shows the time course of the effect of the pre-treatment of saline or agonist on the locomotor activity of animals 120 minutes after the injection of amphetamine. Figure 3.8.1B shows the cumulative horizontal activity of the animals between 30 and 60 minutes in different groups of treatment. Results of One-way ANOVA test and subsequent post hoc showed that JJ-3-42 at 20 mg/kg but not 10 mg/kg tends to reduce the locomotion activity of the animals significantly [$F(2, 15) = 14.25$; $P \leq 0.001$]. Figure 3.8.1C shows the cumulative horizontal activity of different groups between 60 and 180 minutes. Results of One-way ANOVA test and subsequent post hoc showed that JJ-3-42 at 20 mg/kg but not 10 mg/kg reduced the amphetamine-induced activity of the animals compared to the control group [$F(2, 69) = 6.885$; $P \leq 0.001$].

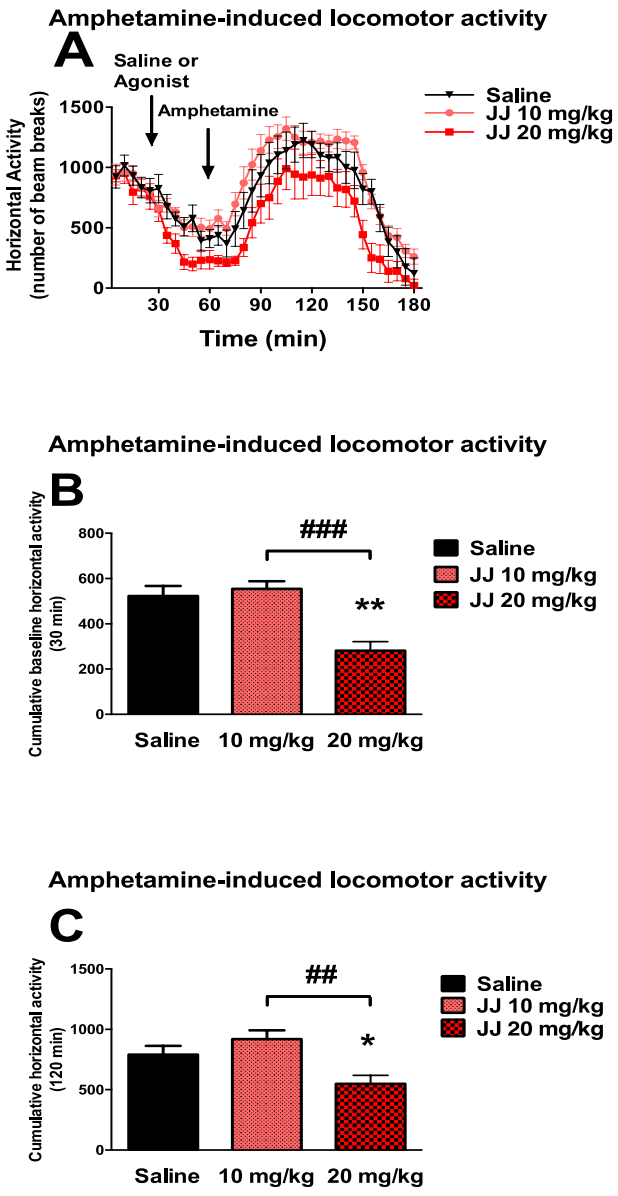

Figure 3.8.1. Effects of acute administration of JJ-3-42 on amphetamine-induced locomotion in animals

Figure 3.8.1A depicts the time course of the response of JJ-3-42 at 10 and 20 mg/kg on amphetamine-induced locomotion in WT animals. Figure 3.8.1B shows the cumulative horizontal activity of animals in 30 minutes following the injection of the agonist or saline. Figure 3.8.1C shows the cumulative horizontal activity in 120 minutes following the injection of 3 mg/kg of amphetamine in WT animals. All groups received the treatment after 30 minutes of habituation. JJ-3-42 at 20 mg/kg attenuated the basal locomotor activity and resisted the hyperlocomotion induced by amphetamine in animals in comparison to control group. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$, or Agonist-treated mice #$p<0.05$. ##$p<0.01$ ###, $P<0.001$ by one-way ANOVA and Tukey test.

3.8.2. Apomorphine-induced locomotor activity

Figure 3.8.2A-C shows the response of 2 different doses of JJ-3-42 (10 and 20 mg/kg) on the locomotion activity of WT animals in response to an efficient dose of apomorphine (2 mg/kg). Figure 3.8.2A shows the time course of the effect of the pre-treatment of saline or agonist on the locomotor activity of animals 90 minutes after the injection of apomorphine. Results of One-way ANOVA test and subsequent post hoc of the cumulative horizontal activity of the animals between 30 and 60 minutes showed that JJ-3-42 at 20 mg/kg but not at 10 mg/kg reduced the locomotion activity of the animals significantly [$F(2, 15) = 6.622$; $P \leq 0.001$] (figure 3.8.1B). Results of One-way ANOVA test and subsequent post hoc on cumulative horizontal activity between 60 and 150 minutes showed that JJ-3-42 at 20 mg/kg but not 10 mg/kg reduced the amphetamine-induced hyperactivity of the animals compared to the control group [$F(2, 69) = 7.430$; $P \leq 0.001$] (Figure 3.8.1C).

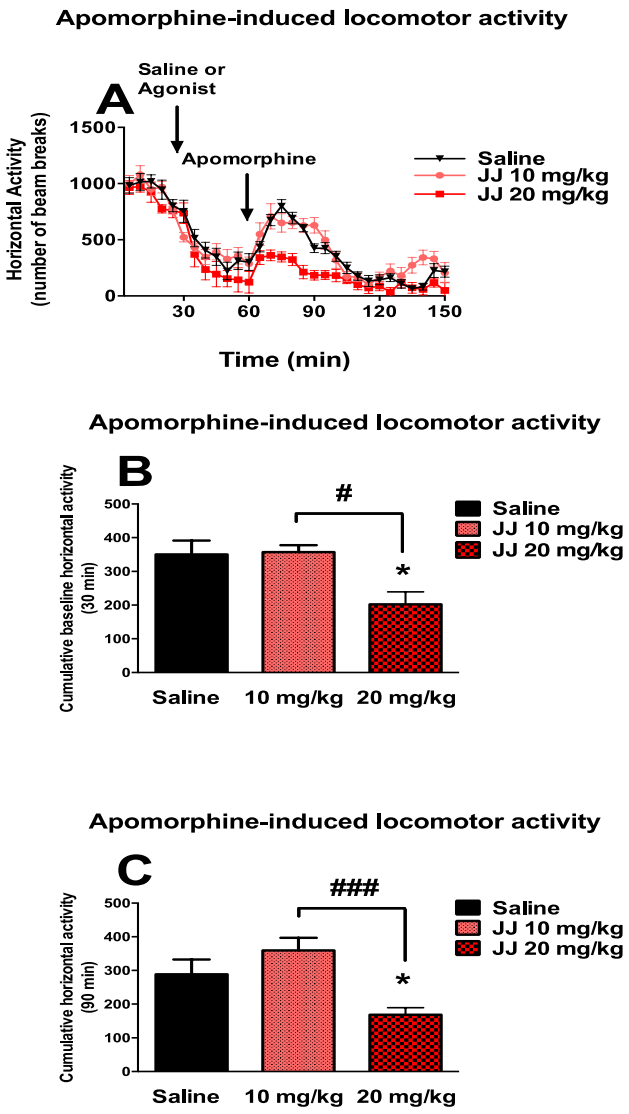

Figure 3.8.2. Effects of acute administration of JJ-3-42 on apomorphine-induced locomotion in animals

Figure 3.8.2A depicts the time course of the response of JJ-3-42 at 10 and 20 mg/kg on apomorphine-induced locomotion in WT animals. Figure 3.8.2B shows the cumulative horizontal activity of animals in 30 minutes following the injection of the agonist or saline. Figure 3.8.2C shows the cumulative horizontal activity in 90 minutes following the injection of 2 mg/kg of apomorphine in WT animals. All groups received the treatment after 30 minutes of habituation. JJ-3-42 at 20 mg/kg attenuated the innate locomotor activity alone and inhibited the hyperlocomotion induced by apomorphine in comparison to control group. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline-treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$, or Agonist-treated mice #$p<0.05$. ##$p<0.01$ ###, $P<0.001$ by one-way ANOVA and Tukey test.

3.8.3. MK-801-induced locomotor activity

Figure 3.8.3A-C depicts the effects of JJ-3-42 at 10 and 20 mg/kg on locomotion activity of WT animals after the exposure to acute dose of MK-801 at 0.2 mg/kg. All animals got injected with saline or different doses of JJ-3-42 after 30 minutes of habituation. Figure 3.8.3A shows the time course of the effect of the pre-treatment of saline or different doses of agonist on the locomotor activity of animals 120 minutes after the injection of MK-801. The results of One-way ANOVA test and the subsequent post hoc of the cumulative horizontal activity of the animals between 30 and 60 minutes showed that JJ-3-42 at 20 mg/kg but not at 10 mg/kg tends to reduce the innate locomotion activity of the animals [$F(2, 15) = 36.34$; $P \leq 0.001$] (figure 3.8.3B). Moreover, the results of One-way ANOVA test and subsequent post-hoc of the cumulative horizontal activity of animals between 60 and 180 minutes showed that treatment with JJ-3-42 at 10 mg/kg leads to a significant increase in the MK-801-induced horizontal movements of animals in comparison to saline-treated group [$F(2, 69) = 27.52$; $P \leq 0.001$] (Figure 3.8.3C).

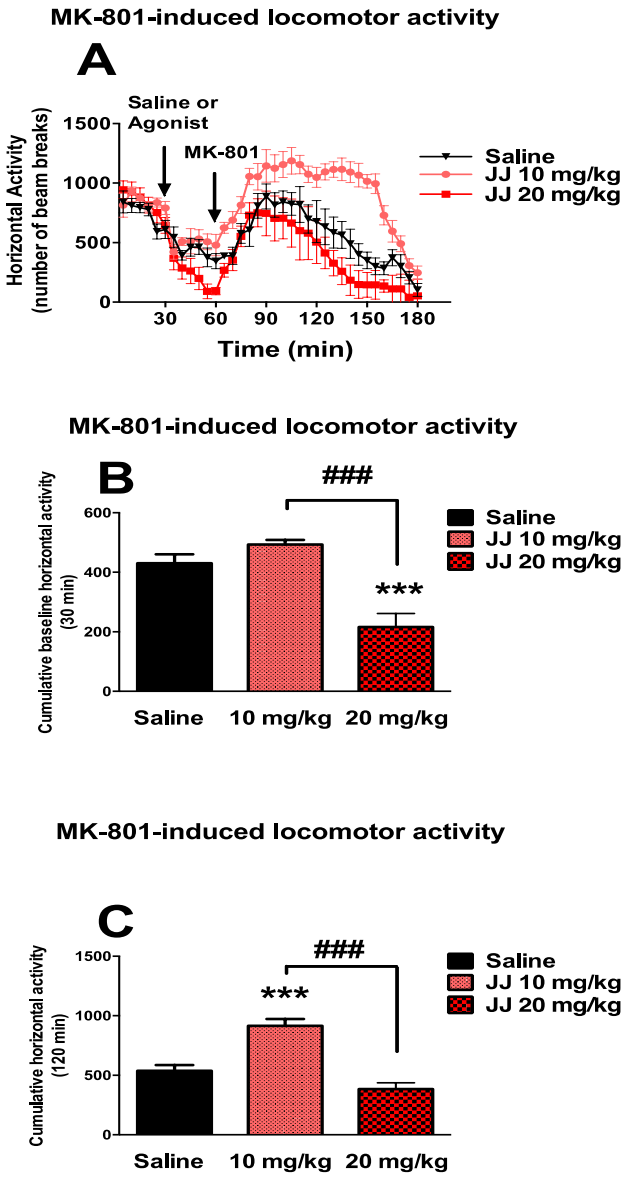

Figure 3.8.3. Effects of acute administration of JJ-3-42 on MK-801-induced locomotion in animals

Figure 3.8.3A depicts the time course of the response of JJ-3-42 at 10 and 20 mg/kg on Mk-801-induced locomotion in WT animals. Figure 3.8.3B shows the cumulative horizontal activity of animals in 30 minutes following the injection of the agonist or saline. Figure 3.8.3C shows the cumulative horizontal activity in 120 minutes following the injection of MK-801 at 0.2 mg/kg in WT animals. All groups received the treatment after 30 minutes of habituation. JJ-3-42 at 20 mg/kg attenuated the locomotor activity alone while at 10 mg/kg potentiated the hyperlocomotion induced by MK-801 in comparison to the control group. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline-treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$, or agonist-treated mice #$p<0.05$. ##$p<0.01$ ###, $P<0.001$ by one-way ANOVA and Tukey test.

3.8.4. Cocaine-induced locomotor activity

Figure 3.8.4A-C depicts the effects of JJ-3-42 at 10 and 20 mg/kg on the locomotion activity of WT animals after the exposure to acute dose of cocaine at 20 mg/kg. All animals got injected with saline or different doses of JJ-3-42 after 30 minutes of habituation. Figure 3.8.4A shows the time course of the effect of the pre-treatment of saline or different doses of agonist on the locomotor activity of animals 90 minutes after the injection of cocaine. The results of One-way ANOVA test and the subsequent post hoc of the cumulative horizontal activity of the animals between 30 and 60 minutes showed that JJ-3-42 at 20 mg/kg but not at 10 mg/kg tends to reduce the innate locomotion activity of the animals [$F(2, 15) = 50.77$; $P \leq 0.001$] (figure 3.8.4B). Moreover, the results of One-way ANOVA test and subsequent post-hoc of the cumulative horizontal activity of animals between 60 and 150 minutes showed that treatment with JJ-3-42 at 10 mg/kg leads to a significant increase in the cocaine-induced horizontal movements of animals in comparison to saline-treated group [$F(2, 51) = 7.704$; $P \leq 0.01$] (Figure 3.8.4C).

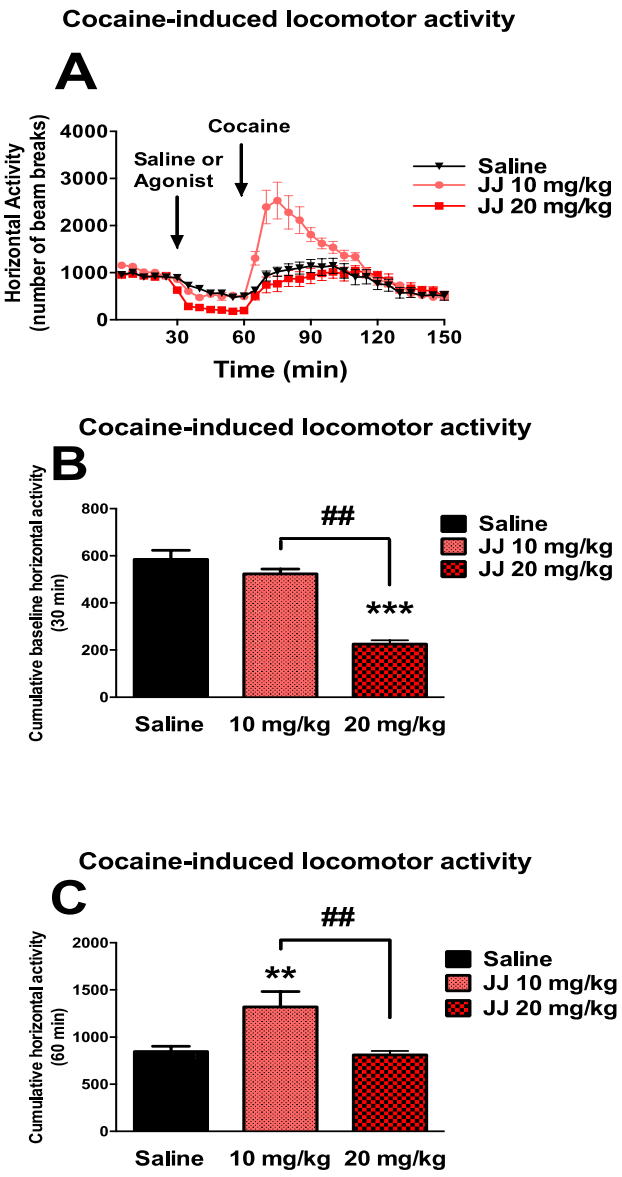

Figure 3.8.4. Effects of acute administration of JJ-3-42 on cocaine-induced locomotion in animals

Figure 3.8.4A depicts the time course of the response of JJ-3-42 at 10 and 20 mg/kg on cocaine-induced locomotion in WT animals. Figure 3.8.4B shows the cumulative horizontal activity of animals in 30 minutes following the injection of the agonist or saline. Figure 3.8.4C shows the cumulative horizontal activity in 90 minutes following the injection of cocaine at 20 mg/kg in WT animals. All groups received the treatment after 30 minutes of habituation. JJ-3-42 at 20 mg/kg attenuated the locomotor activity alone while at 10 mg/kg potentiated the hyperlocomotion induced by cocaine in comparison to the control group. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline-treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$, or agonist-treated mice #$p<0.05$. ##$p<0.01$ ###, $P<0.001$ by one-way ANOVA and Tukey test.

3.9. Effects of JJ-3-42 at 10 and 20 mg/kg on the innate locomotor activity of DAT-KO animals

Hyper-locomotion is a prominent feature of DAT-KO HO mice due to their hyperdopaminergic state [633]. Figure 3.9A shows the time course of the effect of two different doses of JJ-3-42 at 10 and 20 mg/kg on innate locomotion activity of DAT-KO HO animals. Figure 3.9B shows the cumulative horizontal activity of animals between 30 and 150 minutes following the injection in different groups. Results of One-way ANOVA test and subsequent post hoc showed that treatment with 20 mg/kg of JJ-3-42 leads to a significant decrease in the horizontal movements of animals in comparison to WT and HO control groups [$F(2, 36) = 28.80$; $P \leq 0.001$].

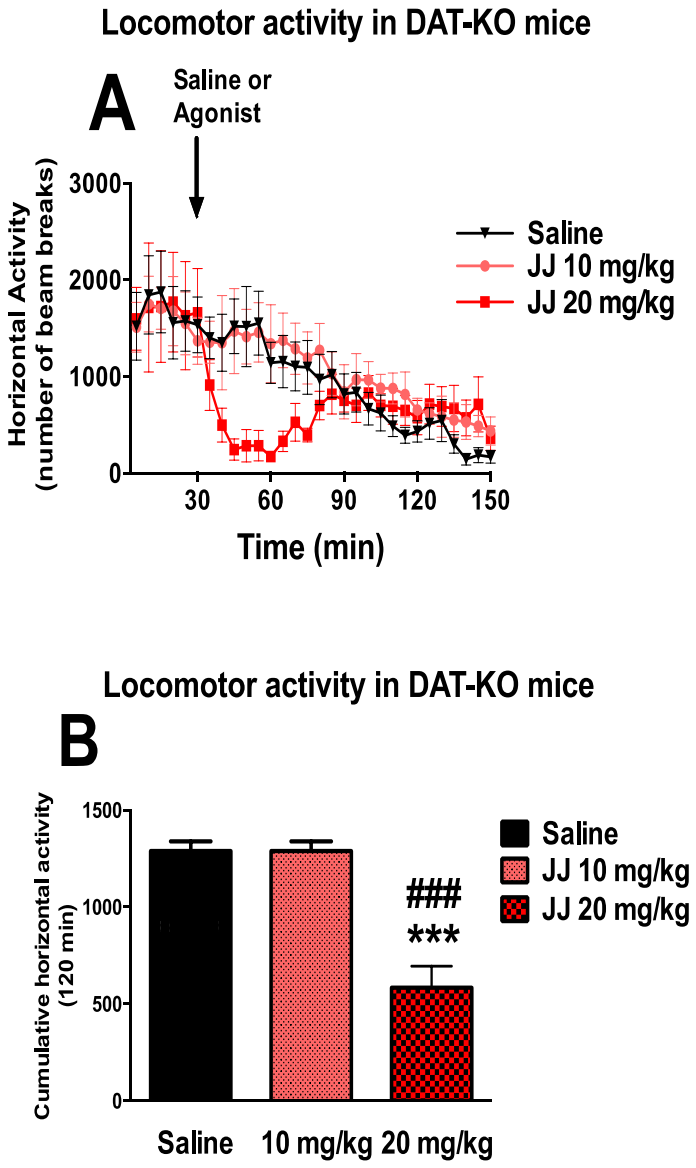

Figure 3.9. Effects of JJ-3-42 at 10 and 20 mg/kg on the innate locomotor activity of DAT-KO animals

Figure 3.9A and 3.9B depict the time course and the cumulative horizontal activity in 120 min following the treatment with saline and JJ-3-42 at 10 and 20 mg/kg in DAT-KO HO animals. All groups received the treatment after 30 min of habituation. JJ-3-42 at 20 mg/kg attenuated the locomotor activity in hyperactive DAT-KO HO significantly in comparison to saline-treated group and JJ at 10 mg/kg. All data cited as means with SEM, n=8–10 per group. Significantly different from saline-treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$, or agonist-treated mice #$p<0.05$. ##$p<0.01$ ###, $P<0.001$ by one-way ANOVA and Tukey test.

3.10. Effects of JJ-3-42 at 10 and 20 mg/kg on the locomotor activity in DDD animals

We used a known pharmacological model of movement disorder in this experiment [633]. Figure 3.10A shows the time course of the effect of various treatments on the locomotor activity of DDD animals. After 30 minutes of habituation, all animals were challenged by 250 mg/kg of Alpha-MPT which rendered DAT-KO animals akinetic. A treatment of saline or different doses of JJ-3-42 (at 10 and 20 mg/kg) was not able to induce any locomotion in the animals per se. However, a cocktail of L-DOPA and Carbidopa at 20/20 mg/kg was enough to restore the locomotor activity of the animals. Figure 3.10B shows the cumulative horizontal activity of animals between 90 and 150 minutes in different groups. Results of ANOVA test and subsequent post hoc showed that treatment with 20 mg/kg of JJ-3-42 leads to significant decrease in the horizontal movements of animals in comparison to the saline group [$F(2, 48) = 5.177$; $P \leq 0.01$].

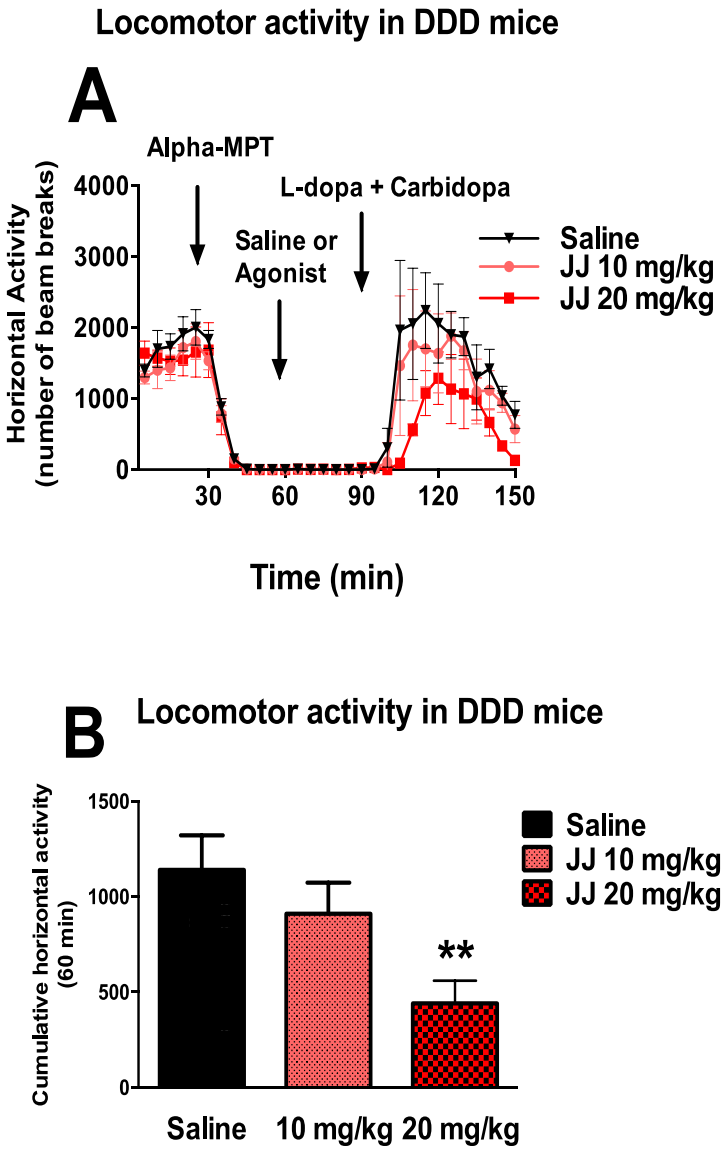

Figure 3.10. Effects of JJ-3-42 at 10 and 20 mg/kg on the locomotor activity in DDD animals

Figure 3.10A Shows the time course and 3.10B the cumulative horizontal activity following the treatment with saline or JJ-3-42 at 10 and 20 mg/kg in DDD mice. An efficient cocktail of L-DOPA and Carbidopa at 20/20 mg/kg restored the locomotor activity in DDD animals. Administration of JJ-3-42 at 20 mg/kg attenuated the locomotor activity restored by this cocktail significantly in comparison to the saline group. Data is represented as mean with SEM, n=8–10 per group. Analyzed by One-way ANOVA with Tukey post hoc analysis. Significantly different from saline-treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$.

3.11. Effects of acute injection of JJ-3-42 at 10 mg/kg on cocaine sensitization test

Figure 3.11A-C shows the effects of JJ-3-42 at 10 mg/kg on the locomotion activity of WT animals after the exposure to acute dose of cocaine at 20 mg/kg on the first day of sensitization protocol. All animals got injected with saline or JJ-3-42 at 10 mg/kg after 30 minutes of habituation. Figure 3.11A shows the time course of the cocaine-induced hyperlocomotion after the pre-treatment of saline or JJ-3-42 at 10 mg/kg. Figure 3.11B shows the cumulative horizontal activity of the animals between 30 and 60 minutes after the treatment with either saline or agonist. The results of t-test of the cumulative horizontal activity between 30 and 60 minutes showed that there was not a significant difference between treatment groups in this test [$t(10) = 1.396$; $P \geq 0.05$] (figure 3.11B).

Figure 3.11C shows the cumulative horizontal activity of different groups between 60 and 150 minutes following the injection of cocaine. Results of t-test showed that JJ-3-42 at 10 mg/kg enhanced significantly the cocaine-induced locomotor activity of the WT animals compared to the control group [$t(34) = 2.748$; $P \leq 0.01$] (figure 3.11C).

Figure 3.11D-F shows the effects of JJ-3-42 at 10 mg/kg or saline on the locomotion activity of TPH2-KI WT animals after the exposure to a challenge dose of cocaine at 20 mg/kg on the seventh day of sensitization protocol. All animals got injected with saline or JJ-3-42 at 10 mg/kg after 30 minutes of habituation. Figure 3.11D shows the time course of the cocaine-induced hyperlocomotion after the pre-treatment of saline or JJ-3-42 at 10 mg/kg. Figure 3.11E shows the cumulative horizontal activity of the animals between 30 and 60 minutes in different genotypes after the treatment with either saline or agonist. T-test of the cumulative horizontal activity between 30 and 60 minutes showed no difference between various treatment groups in this test [$t(10) = 0.5452$; $P \geq 0.05$] (figure 3.11E).Figure 3.11F demonstrates the cumulative horizontal activity of different groups between 60 and 150 minutes following the injection of a challenge dose of cocaine. Results of t-test and showed that JJ-3-42 at 10 mg/kg did not change the cocaine-induced locomotor activity of the animals in WT mice compared to the control group [$t(34) = 0.3084$; $P \geq 0.05$] (figure 3.11F).

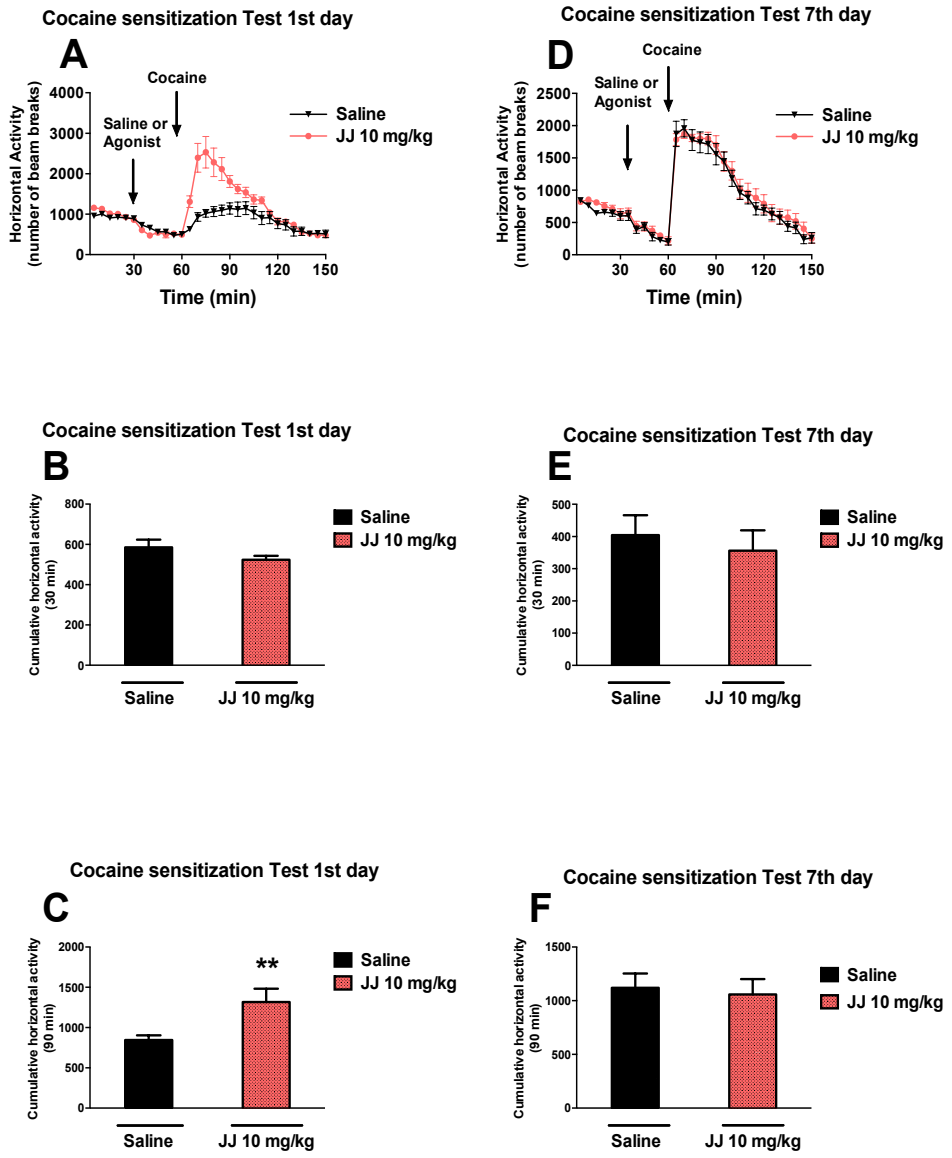

Figure 3.11. Effects of acute injection of JJ-3-42 at 10 mg/kg on cocaine sensitization test

Figure 3.11A depicts the time course of cocaine-induced locomotion following the pre-treatment with either saline or agonist in WT and HO animals. Figure 3.11B shows the cumulative horizontal activity of the animals in 30 minutes following the injection of the agonist or saline. Figure 3.11C shows the cumulative horizontal activity in 90 minutes following the injection of 20 mg/kg of cocaine in WT and HO animals. Figure 3.11D depicts the time course of cocaine-induced locomotion following the pre-treatment with either saline or agonist in WT animals on the seventh day of the sensitization protocol. Figure 3.11E shows the cumulative horizontal activity of the animals in 30 minutes following the injection of the agonist or saline. Figure 3.11F shows the cumulative horizontal activity in 90 minutes following the injection of 20 mg/kg of cocaine in animals. All groups received the treatment after 30 minutes of habituation. All data cited as means with SEM, n=8–10 per group. Significantly different from WT saline treated group * $p<0.05$ ** $p<0.01$ *** $p<0.001$, or corresponding HO control mice #$p<0.05$. ##$p<0.01$ ###, $P<0.001$ by one-way ANOVA and Tukey test.

Tests / Drugs	Basal locomotion	H-maze	Social interaction	Repetitive behaviours			Anxiety tests		
				Digging	Grooming	Marble burying	Dark-light	Openfield	Elevated plus maze
JJ-3-42 (10 mg/kg)	No effect	Enhanced the cognition by reducing the perseverance	Reduced the aggression	Reduced digging	Reduced grooming	Reduced marble burying	No obvious anxiogenic effect	No obvious anxiogenic effect	No obvious anxiogenic effect
Cp809.101 (1mg/kg)	No effect		Reduced the aggression	Reduced digging	Reduced grooming	Reduced marble burying	Increased the anxiety	Increased the anxiety	Increased the anxiety
5-HTP (20mg/kg)	No effect		Reduced the aggression and increased the sociability	Reduced digging	Reduced grooming	Reduced marble burying	Increased the anxiety	Increased the anxiety	Increased the anxiety

Table 3. The summery and the comparison of behavioural responses of different 5-HT2CRs agonists in various behavioural tests

Locomotor based behaviour tests									
Drugs	TPH2-KI WT							DAT-KO HO	
	Innate activity	Stimulant-induced locomotor				Sensitization test		Locomotor	
		Amphetamine	Apomorphine	MK-801	Cocaine	1st day	7th day	Innate activity	DDD model
JJ-3-42 10 mg/kg	No effect	No effect	No effect	Increased	Increased	Increased	No effect	No effect	No effect
JJ-3-42 20 mg/kg	Reduced	Reduced	Reduced	No effect	No effect			Decreased	Decreased

Table 4. The summery of the behavioural responses of different doses of JJ-3-42 in tests related to the interaction with dopamine system

Discussion

Outline

Along with genetic advancements, the development of recent compounds with higher selectivity has allowed a greater appreciation of the degree of involvement of 5-HT2C receptors in the pathophysiology of different psychiatric disorders [9]. Using the new and selective 5-HT2CRs agonists, various groups showed the benefits of activation of 5-HT2C receptors in animal models for mood disorders, addiction and schizophrenia [34]. The regulation of dopamine neurotransmission as one of the primary mechanisms of action of 5-HT2C drugs has been proposed by various authors [9, 34]. The design and production of JJ-3-42 as one of the most potent and selective 5-HT2CRs drugs is among the latest developments in the field of psychopharmacology to harvest the clinical potential of these drugs [200]. With almost no activity at the level of 5-HT2B and 5-HT2A receptors, JJ-3-42 is literally one of the most selective 5-HT2C receptor agonists available presently [200, 205, 211]. In addition to favourable pharmacokinetic and toxicology profiles, strong antipsychotic and pro-cognitive properties have also been attributed to this compound [205, 211]. Despite these preliminary results, the extent of beneficial responses of this new 5-HT2C receptor agonist in various dimensions of animals' behaviour, as well as the detailed features of its interaction with dopaminergic system, remains unclear. In our study, first we compared the final central outcomes of systemic administration of JJ-3-42 to 5-HTP and CP809,101 in a battery of behavioural tests in chronic brain-serotonin deficient TPH2-KI mice model. Secondly, we examined the modulatory properties of JJ-3-42 in interaction with the dopamine system in various behavioural paradigms using indexes of locomotor activity in animals.

In this chapter we will discuss the significance of our findings in each cluster of behaviour paradigms in light of relevant available studies in literature. We will attempt to offer explanations for the incongruent results encountered in our study. Further, we will examine the direction of the future investigations in order to answer some of the outstanding questions.

Finally, we will discuss the new prospects of harvesting the benefits of JJ-3-42 as a selective 5-HT2C agonists in the clinical settings.

JJ-3-42 in cognition, social interaction, repetition and anxiety paradigms

As depicted in figures 3.1A-D, TPH2-KI HO animals showed a significant cognitive deficit compared to wild type animals as they needed more time and number of trials to finish the ALT and N-ALT criteria of automated H-Maze test. A potent dose of JJ-3-42 was able to improve the performance and restore the normal behaviour of mutant animals. As depicted in figures 3.1A-D, while having no apparent effect on WT animals, JJ-3-42 at 10 mg/kg reduced significantly the amount of time and trials needed to finish the corresponding tasks in homozygote TPH2-KI mice. As shown in figures 3.1E, the dramatic reduction in repetitive and perseverant behaviour of mutant animals offers a plausible explanation for better performance of these animals following the administration of the agonist. These findings are in line with the reported favourable outcome of activation of 5-HT2C receptors in different dimensions of cognition in animals [205, 211]. In our study, the positive consequences of administration of JJ-3-42 in H-Maze test was also observed in improvement of the quality of social interaction as well as the diminution of repetitive and compulsive behaviours of mutant animals (figures 3.2A-F and 3.3.2A-F). As demonstrated in figures 3.2A-F and 3.3.1A-F, TPH2-KI HO animals showed a significant increased level of hostility as well as obsessive-compulsive-like traits in dyadic social interaction and compulsive/repetitive behavioural tests respectively, in comparison to WT animals. In both paradigms, as seen in figures 3.2A-F and 3.3.2A-F, the restoration of serotonergic tone via different serotonergic agents rescued the behaviour and improved various indexes of performance of the animals in a similar manner.

Our findings in H-Maze, dyadic social interaction and marble burying tests in brain-serotonin deficient TPH2-KI HO mice reiterate multiple lines of evidence denoting the significance of serotonin homeostasis in regulation of important aspects of behaviours of the animals (figures

3.1A-E, 3.2A-F and 3.3.1A-F) [17, 336]. The importance of an intact serotonin system in different developmental stages and its fundamental role in expression of various dimensions of cognition, complex social interaction and impulse control in human and non-human animals has been extensively discussed [17, 291, 347, 572]. A plethora of behavioural abnormalities in various animal models, following a significant alteration in brain serotonin level, has been documented in the past [321, 685]. For example, TPH2-KI animals demonstrated an exaggerated response to the early life stress as well as higher degree of behavioural disinhibition and impulsivity in adulthood [583, 585]. Moreover, an increased level of anxiety, impulsive hostility and unchecked ethanol consumption tendencies have been previously witnessed in these animals [324, 686]. In parallel to this, abnormal behavioural indexes, in tests like elevated plus maze, marble burying and novelty-suppressed feeding tests, have been observed in TPH2-KO animals [321]. These animals also manifested a higher degree of aggressive behaviour in the resident-intruder test toward an unfamiliar mouse [321]. Altered hippocampal neurogenesis, abnormal plasma corticosterone levels and perverse biochemical responses in the amygdala have been proposed as plausible explanations to justify some of the abnormal behaviours of these animals following chronic brain serotonin deficiency [583, 585].

In our study, and in agreement with previous findings, decreased cognitive capacity, marked perseverant/compulsive tendencies and increased impulsive aggression have been observed in TPH-2 KI HO animals (figures 3.1A-E and 3.2A-F). As demonstrated in 3.3.1A-F, our results show that the presence of TPH2 R439H allele had a significant dose-response effect on the frequency of repetitive and compulsive behaviour of TPH-2 KI animals in related tests. Indeed, homozygote TPH-2 KI animals spent more time, and had shorter delays, to start their bouts of repetitive behaviours in marble burying, grooming and digging tests compared to their HET or WT littermates (figures 3.3.1A-F).

The role of dysregulation of serotonin system in abnormal impulse control as an essential component of cognitive and social function in rodents, as well as humans, has been previously discussed [294, 347, 644]. The role of serotonin homeostasis in modulation of impulsivity as a determining factor in the final presentation of aggression vs. sociability of various species of animals has been established in literature [366, 685, 687]. From the early days of modern

science, the primary evidance linking the serotonin concentration to the control of impulses, in the context of auto and hetero-aggressive behaviours in various population, has been at the core of the monoamine hypothesis of serotonin [356, 688-690]. Despite such early attempts, due to extreme complexity of the intertwined regulatory mechanism of behaviours and emotions in higher animals, the study of isolated and distinct components of serotonin-regulated behaviour has proved impossible [291, 691]. The interpretations of some of the most commonly used behavioural paradigms to investigate complex behavioural features, like cognition or social interactions in animals, has been disputed by various authors [11, 361, 496, 647, 648]. Concerning this, various authors pointed to the usefulness of studying the common underlying contributing factors like impulsivity in the manifestations of a plethora of heterogenous dimensions of abnormal behaviours in animals [11, 690, 692]. Indeed, employing selective drugs in pharmacological interventions, using genetic models when possible and conservative interpretation of behavioural paradigms have been recommended as judicious exercise in studying the serotonin regulated behaviours in animals [11, 17, 690, 692]. Despite such difficulties, the involvement of different components of the serotonin system as a primary regulator of various behaviours in humans and non-human animals has been recognized repeatedly [17, 291, 347, 572]. In line with this, the role of 5-HT2C receptors in regulation of behaviour and emotional state of animals has been appreciated lately [89, 211, 693].

The positive impact of 5-HT2C ligands to improve cognitive flexibility and reduction of perseverant/compulsive behaviour has been previously confirmed [9, 694]. Our results in the H-maze test resonate with previous published studies advocating the pro-cognitive character of activation of 5-HT2C receptors. For instance, in different studies, agonists like CP809,101 and Vabicaserin demonstrated beneficial outcomes in pre-pulse inhibition and H-maze tests in animals [191, 198, 210]. JJ-3-42 improved the performance of animals in tests like novel object recognition in NMDA receptor hypo-functioning NR1-knockdown mice and restored amphetamine-disrupted pre-pulse inhibition while improving the social behaviour of the animals [205, 211]. This conforms with our results in H-maze and dyadic social interaction tests following the administration of JJ-3-42 in HO animals. Remarkably, recent studies identify the decline of impulsivity and perseverant tendencies as an explanation of observed improvement of the performance of the animals in various behavioural dimensions following the administration

of 5-HT2C agonists [695]. Related to this, an anti-impulsive and anti-compulsive propensities have been attributed to the activation of 5-HT2C receptors by various authors [210, 236, 382]. Several 5-HT2CRs agonists including RO 60-0175 and CP809,101 reduced various indexes of impulsivity which resulted in better cognitive performances in animals facing multiple serial choices [695]. Similarly, potent doses of both Lorcaserin and CP809,101 reduced premature responding in the five-choice serial-reaction time test and improved accuracy of rodents in the Go-No Go paradigm [693]. 5-HT2C receptor stimulation by RO 60-0175 prevented the impulsive action and premature responses induced by amphetamine, cocaine and MK-801 in animals [696]. Echoing our results in marble burying, grooming and digging tests, administration 5-HT2C agonists successfully inhibited the obsessive-compulsive-like traits of animals in models like schedule-induced polydipsia [203, 535]. Furthermore, the agonists of 5-HT2C like mCPP and RO 60-0175 decreased the repetitive tendencies in tests like marble burying in rodents [203, 535]. Similar to our results in digging and grooming tests, such anti-compulsive response of 5-HT2C agonists have also been demonstrated in paradigms like induced-excessive scratching or induced-excessive eating in rats [203, 379, 458].

In contrast to these results, the antagonists of 5-HT2CRs deteriorated various cognitive indexes in tests like reversal learning by increasing the impulsivity and repetitive behaviours in animals [383]. 5-HT2CRs antagonist, SB 242084, increased the number of premature responses in five-choice serial-reaction time test [695]. This phenomenon was not present in 5-HT2C receptor null mice emphasizing the selective contribution of 5-HT2C receptors in this phenomenon [695]. Interestingly, the absence of 5-HT2CRs led to a pervasive repetitive and compulsive-like pattern of chewing in 5-HT2C-KO mice model [57, 89]. Overall, these results confirm the pro-cognitive and anti-impulsive potential of agonists of 5-HT2C drugs.

Our findings in dyadic social test endorse the previous studies indicating the implication of 5-HT2C receptors in the regulation of social anxiety and peer interaction in animals [374, 457, 469]. As shown in figures 3.2E-F, the efficient dose of either CP809,101 or JJ-3-42 could readily reduce the chances of an aggressive encounter in mutant TPH2-KI mice to the level of WT animals. The restoration of aggressive behaviours subsequent to re-establishing the serotonergic tone in various models has been previously shown [350, 370, 378]. Recently, the important place

of 5-HT2C receptors in modulation of complex social behaviour in both human and non-human animals has been investigated [9]. Curiously, 5-HT2C receptor null animals exhibit various degree of social behaviour deficits as adults [697]. The ratio of 5-HT2A to 5-HT2C receptors in regions of the brain like cortex may play an important role in the incidence of behaviours like aggression and maternal rearing in animals [300, 381, 698]. In human genome studies, the association between certain 5-HT2CRs polymorphism and quality of social behaviour in both schizophrenic and healthy individuals has been recently shown [699]. SSRIs and some of the antipsychotics seem to exert their anti-aggressive profile through reducing the impulsivity of the animals by acting on 5-HT2C receptors [300, 378, 700]. In agreement with these reports, our study showed that a rescue of aggressive and impulsive pattern of behaviour in TPH2-KI HO following the activation of 5-HT2C receptors supports the central role these receptors in regulation of social behaviours of animals.

As shown in figures 3.2A-B, homozygote TPH2-KO mice exhibited a higher level of aggressive behaviour while their indexes of mild social interaction did not vary from wild type animals. Intriguingly, as seen in figure 3.2C-D, 5-HTP, a non-selective serotonergic agent, at 20 mg/kg augmented significantly the time and the frequency of mild social interactions in TPH2-KI HO mice while selective agonists of 5-HT2C had no effect. It follows that such hyper-socialization sessions might be due to the activation of serotonergic receptors other than 5-HT2CRs since such result was not observed following the administration of both CP809,101 and JJ-3-42 (figure 3.2C-D). The same dose of 5-HTP could not increase the amount of mild social behaviour in WT animals in this experiment. This could indicate the foregoing abnormal serotonin neurotransmission as an indispensable underlying biological component of this peculiar phenomenon in HO animals (figure of 3.2C-D). Similar hyper-sociability bouts in both humans and other animals following manipulations of serotonergic tone, by administration of potent psychotropic drugs like Ecstasy, have been reported [701-704]. It has been shown that the 5-HT2A and 5-HT1B/1D receptors are major contributors of similar central responses of MDMA in animals [705]. One could assume that comparable responses of 5-HTP in HO animals might be due to the hypersensitization of these serotonin receptors as the result of chronic serotonin deficiency in TPH2-KI animals. Regarding the aberrant activity of different serotonin receptors, particularly 5-HT2A receptors, some evidence has been previously reported in TPH-2 KI mice

[319, 580]. However, the precise contribution of these receptors in the reported behaviour of mutant animals is still to be studied. In general, our results imply the critical involvement of normal serotonin neurotransmission through 5-HT2C receptors in the regulation of social behaviours in animals. However, the detailed role of 5-HT2CRs in the development and modulation of various aspects of such complex behaviours of animals is yet to be determined. As social deficit is a prominent feature of schizophrenia and other chronic mental disorders, in the future the investigation of the beneficial impact of 5-HT2C active drugs in the improvement of social interaction and other negative symptoms of the patients would be of clinical interest.

As shown in figures 3.4.1A-D, 3.5.1A-D and 3.6.1A-B, the level of anxiety in animals in dark-light, openfield and EPM tests had a direct and dose-responsive relationship to the presence of TPH2 R439H allele. The results of our experiments showed strong anxiogenic features for acute doses of 5-HTP at 20 mg/kg and CP809,101 at 1mg/kg in both WT and HO animals (figures 3.4.2A-D, 3.5.2A-D and 3.6.2A-B). In fact, the acute administration of both drugs led to higher levels of anxiety of animals in various indexes of dark-light, openfield and EPM tests. Our findings are in accordance with previous studies linking the various mutations of TPH2 gene and anxiety features of various transgenic animal models [557, 585]. The involvement of the serotonin system as a major contributor in the development and severity of psychopathologies like generalized anxiety, depression and bipolar disorders has been previously established [17, 416]. An anxiogenic profile has been attributed to various serotonergic drugs in both human and non-human studies in the past [17, 415, 461]. Following the improvement of the selectivity of available agonists and antagonists in recent years, the investigation of central emotional outcomes of the stimulation of various serotonin receptors especially 5-HT2CRs has regained momentum [9, 57, 481]. However, there is still no consensus on the final impact of 5-HT2C drugs on the emotional state of human and non-human animals as most of the existing reports are contradictory [415, 459, 462, 464]. In the early pharmacological studies, activation of 5-HT2CRs had been linked to a robust anxiogenic response in different experimental models [457-460]. For example, older and non-selective 5-HT2CRs ligands like mCPP and MK-212 have a proved anxiogenic reaction in rodents and also in humans [172, 706]. In fact, some of these drugs have been used in clinical studies to induce anxiety and panic in the subjects [293, 461]. The observed anxiogenic profile following the administration of 5-HTP and CP809,101 in both WT and HO

animals echoes the results of these older studies (figures 3.4.2A-D, 3.5.2A-D and 3.6.2A-B). Conversely, in some clinical studies, mCPP showed appetite suppressant responses without inducing any anxiety or depression in the participants [463]. Newer agents like RO 60-0175 showed anti-compulsive and anti-depressive properties in tests like FST and marble burying without affecting the anxiety parameters in related paradigms [462, 464]. Interestingly, both mCPP and RO 60-0175 have shown a strong antidepressant-like properties in the anhedonia model of depression in rodents [462, 707]. Likewise, various 5-HT2C agonists also improved different indicators of mood and anxiety in bulbectomy-induced model of depression in animals [203, 379].

Despite such ambiguities about the outcome of 5-HT2C agonists, there is more or less an agreement about the anti-anxiety/anti-depressant-like profile of 5-HT2CRs antagonists among the researchers. For instance, the blockage of 5-HT2CRs reduced significantly and consistently the anxiety of the animals in the context of feeding, social interaction and exploratory behaviours [29, 457, 468]. The deletion of 5-HT2C receptors in mice led to a lower level of anxiety and had an anti-depressant effect in tests like tail suspension test in animals [453]. Furthermore, the antagonists of 5-HT2CRs potentialized the effect of various SSRIs in different paradigms including tail suspension test in animals [453]. The atypical antidepressants Mirtazapine and Mianserin as well as newly developed Agomelatine have clear antagonistic 5-HT2CRs activity although their profile is not selective [203, 379, 470, 471, 526].

The confusion regarding the ultimate emotional outcomes of activation of 5-HT2CRs appears to be multifactorial and complex. To explain these inconsistencies, various lines of arguments have been generated by experts in the field. For instance, it has been argued that sedative properties of some of these 5-HT2C compounds, by influencing the locomotor behaviour of the animals, might have confounded some of the older results [203]. However, such sedative properties have not been reported in more recent 5-HT2C drugs [196, 458, 462]. In our study, as shown in figures 3.7A-B, we observed no significant locomotor changes for 2 hours following the administration of any of our drugs in either WT or HO animals. Administration of potent doses of either 5-HTP at 20 mg/kg, CP809,101 at 1mg/kg or JJ-3-42 at 10 mg/kg alone was not enough

to alter the locomotor activity of animals ruling out the possibility of this confounding factor (figures 3.7A-B).

Apart from this, the mentioned contradictory results have been partly connected to the peculiarities of different animal models of anxiety and depression. For example, both agonists and antagonists of 5-HT2CRs have been reported to carry anxiolytic/antidepressant properties in models like chronic mild stress-induced anhedonia and olfactory bulbectomy models of depression in rodents [203, 379, 462, 707]. However, this was not the case with other models of depression or anxiety [203, 379, 462, 707]. Recent 5-HT2C agonists like CP809,101 showed mixed anxiogenic and anxiolytic properties in rodents depending on the employed behavioural paradigm and measured parameters [204, 478-480]. It has been shown that the specific components of anxiety-related behaviours were measured by using different behavioural paradigms in animals [478, 479]. Accordingly, a pathway-dependant emotional responses to 5-HT2CRs have been proposed by some authors [412]. This is based on the recent neuroanatomical and pharmacological evidence that propose two main independent neuronal groups in periaqueducal grey and basolateral amygdala to convey anxiety-related behaviours in mammals [478, 479]. Periaqueducal grey pathway has been recognized as the main pathway involved in the presentations of depression, obsessive-compulsive disorder or panic attacks in clinics, while dysregulation of basolateral amygdala has been documented in generalized anxiety disorder and post-traumatic stress disorder (PTSD)[481, 482]. It is believed that 5-HT2CRs activation of the periaqueducal grey neurons is mostly anxiolytic [482]. Such hypothesis led some authors to the proposal of 5-HT2CRs agonists for the treatment of depression, obsessive-compulsive disorder or panic attacks [481, 482]. Alternatively, the local activation of the 5-HT2CRs in the basolateral amygdala is thought to be anxiogenic [29, 455]. Therefore, the antagonists of 5-HT2CRs have been considered to relieve the symptoms of generalized anxiety disorder or PTSD in inflicted patients [29, 455]. This might account for some of the controversial results of systemic administration of 5-HT2C drugs in previous studies.

Apart from this, it seems that the lack of the selectivity among both agonists and antagonists, especially in the older studies, contributed to the reported inconsistencies [34, 458]. The absence of anxiogenic responses in recent serotonergic compounds compared to the older ones has been

illustrated by some authors [203, 462]. This is in agreement with our results indicating a neutral anxiety profile for JJ-3-42 at a potent dose in contrast to 5-HTP and CP809,101 in the corresponding paradigms (figures 3.4.2A-D, 3.5.2A-D and 3.6.2A-B). Some researchers have attributed the anxiogenic responses of the older studies to the contribution of other 5-HT2 receptors than 5-HT2CRs [708]. In reality, our results are in agreement with the latest evidence suggesting that the newer selective agonists of 5-HT2C receptors might carry a slight anxiolytic effect in rodents (figures 3.4.2A-D, 3.5.2A-D and 3.6.2A-B) [462, 464]. This is possible since JJ-3-42 is one of the most selective 5-HT2C receptor ligands known yet displaying no activity at the level of 5-HT2BRs while being 89-fold selective against 5-HT2ARs [200]. In fact, this new 5-HT2CRs agonist shows equivalent potency (EC_{50} = 4.2 nM for JJ-3-42, versus 3.6 nM for Lorcaserin) but improved selectivity (no agonism for JJ-3-42 versus EC_{50} = 478 nM, E_{max} = 92% for Lorcaserin) against 5-HT2BRs compared to FDA-approved Lorcaserin [200, 205]. This could be an acceptable explanation in our study for dissimilar response of JJ-3-42 to CP809.101.

In our experiments, we recorded a strong pro-cognitive and anti-compulsive character for the new 5-HT2C agonist JJ-3-42 across related behavioural paradigms in TPH2-KI mice. We also observed a significant reduction of repetitive and aggressive behavioural tendencies of these mice following the administration of various serotonergic agents. Moreover, by using two selective 5-HT2C agonists CP809,101 and JJ-3-42 we showed that these beneficial responses were most likely carried via activation of 5-HT2C receptors in the brain. Finally, our results indicate the favourable profile of JJ-3-42 compared to other selective 5-HT2C agonist CP809.10 in anxiety-related tests. Our studies showed that despite other tested serotonergic agents, at a potent and therapeutic dose, JJ-3-42 is empty of any significant anxiogenic response.

The role of 5-HT2C receptors as the major player acting on the intricate interface of cognition, impulsion and compulsion has been recognized before [120, 690, 692]. Various clinical studies tried successfully to harvest the potentials of 5-HT2C drugs to increase the impulse control and reduction of compulsivity in the context of obesity and drug seeking behaviour in human [236, 693]. Recent research showed that the therapeutic effect of Lorcaserin in eating disorders might partly be mediated by its complementary anti-impulsive properties [693]. Lately, similar positive results in the treatment of nicotine addicted population has been registered for Lorcaserin [212,

239, 255]. Plus, the benefits of 5-HT2C activation in the treatment of depression, obsessive-compulsive disorder or panic attacks has been discussed by various authors [481, 482].

Despite such outstanding positive results, the clinical presence of 5-HT2C drugs is minimal and at an early stage. The results of our study with JJ-3-42 help us to better determine the therapeutic character as well as the limitations of activation of 5-HT2C receptors in the clinical scenarios. Our results point to the plausible medical benefits of JJ-3-42 to enhance the cognitive indexes and improve the quality of social interactions in the future. Reduction of impulsivity and decrease in the repetitive and compulsive tendencies following the activation of 5-HT2CRs are likely mechanisms that mediate the positive responses of JJ-3-42. These results might prove to have clinical implications in the treatment of various mental disorders including schizophrenia, OCD and drug addiction. Remarkably, and in contrast to the other serotonergic drugs, the mentioned positive characteristics of JJ-3-42 were not associated with any anxiogenic response. This is important regarding the high prevalence of the anxiety disorders as a comorbidity in various populations of mental disorders patients. However, in the future, the replication of the current results using different animal models and across various behavioural modalities in the future is required. Meanwhile, judicial interpretations of these results and further preclinical toxicologic and biochemical studies to establish the safety of this drug in the future clinical studies seems reasonable.

JJ-3-42 in locomotor paradigms: the interaction with the dopamine system

In our study, as shown in figures 3.10A-B, JJ-3-42 could not induce any locomotor activity in dopamine-depleted DAT-KO (DDD) animals by itself at either 10 mg/kg or 20 mg/kg. These results underscore the manifestation of the regulatory function of 5-HT2C receptors upon the availability of a minimum dopaminergic tone in the brain as previously shown [254]. A cocktail of L-DOPA and Carbidopa restored the innate locomotion in DDD animals. Interestingly, JJ-3-42 at 20 but not at 10 mg/kg was able to inhibit the reinstatement of the locomotion activity following the administration of these dopaminergic drugs in mice (figures 3-10A-B). The similar inhibitory response for JJ-3-42 at 20 mg/kg was also observed in experiments with stimulant-

induced locomotion and in hyperactive DAT-KO mice (figures 3.8.1A-C, 3.8.2A-C and 3.9A-B). Concerning this, while the injection of JJ-3-42 at 10 mg/kg could not change the amphetamine or apomorphine-induced locomotion, at 20 mg/kg it was enough to prevent the induced hyperactivity in WT animals (figures 3.8.1A-C and figures 3.8.2A-C). Similar dose response profiles for JJ-3-42 were obtained in modulation of the innate hyperactivity of DAT-KO HO mice (figures 3.9A-B). The ensemble of these results confirms the inhibitory profile of JJ-3-42 on the behavioural consequences of hyperdopaminergic state in animals. Similar modulatory responses following the activation of 5-HT2C receptors have been previously reported in the literature [254, 709]. In contrast to this, an acute dose of JJ-3-42 at 10 mg/kg enhanced significantly the response of MK-801 and cocaine, while at 20 mg/kg it failed to show any difference to the saline group (figures 3.8.3A-C and 3.8.4A-C). Such results may indicate differential interaction of the 5-HT2C system and various drugs of abuse with distinctive mechanisms of action. The complexity of interaction of these systems, especially following the administration of different drugs of abuse, has been previously mentioned [254, 709]. Finally, our results indicated that repetitive pre-treatment of JJ-3-42 at 10 mg/kg did not inhibit the development of sensitization following the repeated exposure to cocaine in a seven-day sensitization protocol (figures 3.11D-F). In fact, the amplitude of locomotor activity following a challenge dose of cocaine in the agonist-treated group was not different than the saline-treated group in our experiment (figures 3.11D-F).

Evoked locomotor hyperactivity is one of the most widely used behavioural modalities to investigate the beneficial properties of new drugs in regulation of abnormal dopamine neurotransmission [155, 710]. In this test, the acute induction of locomotor responses reflects the hyperactivity of motor centers after a significant dopaminergic surge in the brain of the animals [155, 711]. Resisting the acute central responses of various psychostimulants has been regarded as a valuable screening tool to predict the efficiency of future treatments against schizophrenia and addiction [34, 254]. Concerning this, the therapeutic potentials of 5-HT2C receptors in regulation of the central dopamine system have been previously discussed [140, 189, 712]. In fact, the proposal of the major interaction of 5-HT2C receptors and dopamine system is supported by several lines of scientific evidance. 5-HT2C receptors are heavily present throughout the mammalian brain and specifically across dopaminergic structures like cortex,

substantia nigra, NA and VTA [15, 140, 141]. The mRNA and protein for 5-HT2CRs are densely expressed in both mesolimbic and nigrostriatal pathways in animals [142]. On top of the anatomical evidence, the recent pharmacologic and electrophysiological knowledge point to the importance of the interconnectedness of 5-HT2C and dopaminergic systems in the regulation of various brain functions [85, 154, 183, 346]. In fact, an important neuromodulatory control on both mesocorticolimbic and nigrostriatal dopamine release for 5-HT2C receptors has been previously described [34, 145]. Many authors suggested 5-HT2CRs as a relevant pharmacological target to treat dopamine related neuropsychiatric disorders like drug dependency and schizophrenia [155, 158, 713].

The analysis of our results shows that JJ-3-42 at 20 mg/kg inhibited the spontaneous locomotor activity of WT animals in different tests (figures 3.8.1B,3.8.2B, 3.8.3.B and 3.8.4.B). However, this was not the case for JJ-3-42 at 10 mg/kg (figures 3.8.1B,3.8.2B, 3.8.3.B and 3.8.4.B). Neither of these doses could initiate the locomotor activity in DDD mice, pointing to the necessity of dopamine charge to observe the regulatory responses of 5-HT2C receptors in animals (figures 3.10A-B, JJ-3-42). In agreement with this, various authors have reported the dose-dependent reduction of innate locomotor activity of animals following the administration of different 5-HT2CRs active compounds [195, 200]. Furthermore, JJ-3-42 at 20 mg/kg inhibited the innate hyperactivity of DAT-KO HO and resisted the induced-hyperlocomotion of WT animals after amphetamine and apomorphine (figures 3.8.1B,3.8.2B, 3.8.3.B and 3.8.4.B). Similar neuroprotective profile against pathological hyperdopaminergic states for different 5-HT2C receptor ligands has been witnessed before [141, 152]. For example, WAY 163909, CP809,101 and vibacaserin blocked amphetamine-induced climbing and inhibited PCP and amphetamine-induced hyperlocomotor activity in rodents [171, 198, 204, 714]. Similarly, the activation of 5-HT2C receptors reduced the locomotor responses of methamphetamine, MDMA and marijuana alkaloid Δ9–THC in relevant animal models [196, 200, 231, 235, 715]. Independent of measures of locomotor activity, the antipsychotic profile of 5-HT2C receptor agonists has been documented through other models as well. For Instance, various 5-HT2C agonists, alone or in combination with other drugs, opposed the head twitch and the vertical activity of the animals after the injection of different drugs of abuse [195, 197, 204]. Moreover,

these agonists decreased the induced inhibition deficit following the administration of amphetamine, MK-801, PCP and DOI in PPI test [191, 204, 716].

As a result of these and similar studies, the plausible benefits of activation of 5-HT2C receptors in recovering the sensory-motor gating deficits, resisting the positive symptoms and improving the cognitive functions in schizophrenic patients in the clinic has been acknowledged [717]. In favour of this proposal and in contrast to common neuroleptics, agonists of 5-HT2C receptors were not capable of inducing cataplexy in different animal models alone [171, 198, 204, 714]. A specific regulatory function of 5-HT2C receptors in major brain dopamine pathways has been suggested as an explanation. It has been shown that the agonists of 5-HT2C receptors diminish the spontaneous activity of the dopamine neurons originating from the VTA without influencing the dopaminergic activity of the SNc neurons [156, 171]. For example, both acute and chronic administration of drugs like WAY 163909 and RO 60-0175 selectively modulated the dopamine concentration of nucleus accumbens versus dorsal striatum encouraging lesser chances of extra pyramidal side effects [156, 171, 465]. The earlier mentioned antipsychotic and pro-cognitive responses of 5-HT2CRs ligands along such favourable motor profile led to the proposal by different authors of these ligands as the next generation of antipsychotics [191, 694].

However, the results of the clinical trials in the treatment of the positive and negative symptoms of schizophrenic patients has so far been disappointing. Vabicaserin, a potent agonist of 5-HT2CRs did not reach the primary clinical efficacy target and its development was terminated by Pfizer [65]. Furthermore, Sertindole, a potent 5-HT2CRs inverse agonist, and dopamine D2, α1-adrenergic receptor and 5-HT2AR antagonist was withdrawn from the market, due to its cardiovascular side effects [50, 216, 217]. Despite this, it is important to mention that Vabicaserin was successful in alleviating positive symptoms and showed encouraging metabolic features in clinical settings [65, 214]. Also, Sertindole efficiently reduced the anxiety and enhanced the cognition and memory indexes in the treated population [50, 216]. In light of such results, some researchers proposed the 5-HT2C agonists as an ideal potential adjunct medication in the clinical treatment of schizophrenia. Indeed, some evidence suggests that by potentiation, of typical and atypical antipsychotics, these drugs might reduce the probability of dose-dependent motor side effects in the patients [197]. Moreover, the anorexic profile of 5-HT2C compounds

could potentially protect this population against the notorious metabolic side effects of chronic treatment with neuroleptics [197].

In contrast with the results of the experiments with apomorphine and amphetamine, pre-treatment with JJ-3-42 at 10 mg/kg significantly potentiated the locomotor response of an acute dose of cocaine in mice (Figures 3.8.4A-C and 3.11A-C). Similarly, an acute dose of JJ-3-42 at 10 mg/kg enhanced significantly NMDA antagonist MK-801-induced hyperlocomotion in our study (figures 3.8.3A-C). In both experiments, JJ-3-42 at 20 mg/kg could not alter the locomotion of the animals following an acute and potent dose of either cocaine or MK-801 (Figures 3.8.3A-C and 3.8.4A-C). Multidimensional involvement of serotonin 5-HT2C receptors in regulation of basal and phasic dopamine concentration has been discussed before [246, 254, 718]. These receptors could modulate dopamine neuronal firing, dopamine release and may alter dopamine exocytosis in activated dopamine neurons [229, 244, 245]. Such inhibitory control is generally thought to be mediated via the GABAergic interneurons of the substantia nigra and VTA [145, 168, 172].

However, the results of recent studies show a much more complex and composite portrait of the interaction of these two systems. The response of serotonin 5-HT2C receptors could be different depending on the different receptor population involved or the degree of activity of dopaminergic neurons, [249, 250]. The complexity of involvement of 5-HT2CRs in dopamine concentration changes following the administration of systemic doses of different psychotropic drugs and particularly cocaine has been discussed [190, 244, 712]. For instance, a U-shape and circuit-dependant control of dopamine concentration by 5-HT2CRs following the cocaine administration in animals has been suggested by some authors [246, 718]. Recent studies following the local microinjection of various drugs in different brain compartments have helped us to better understand the contribution of distinctive brain circuits in the complex interaction of these two systems. For instance, it has previously been demonstrated that 5-HT2CRs agonists enhance, whereas their antagonists inhibit, the elevated dopamine efflux in the NA following the cocaine administration [190, 244, 712]. Similar to our results, the intra-NA injection of 5-HT2C agonists like RO 60-0175 or MK-212 enhanced the cocaine-induced locomotor activity of animals in these studies [247, 254]. On the other hand, the intra-PFC or VTA injection of agonist

and antagonist had the opposite effect and decreased and increased the cocaine-induced locomotion behaviour in animals, respectively [241, 248, 251, 718]. Thus, it has been proposed that there is a significant difference between the outcome of the activation of 5-HT2CRs in the NA compared to other dopaminergic regions of the brain like PFC or VTA [241, 247, 248].

These results reveal the difficulty of interpretation of the outcomes of the systemic cocaine administration in animals as it may involve the response of different central populations of 5-HT2C receptors across dopaminergic system [254]. In our study, one could hypothesise that the activation of 5-HT2C receptors in NA following the administration of cocaine might have been responsible for the observed amplified responses of cocaine. It has been proposed that the degree of activity of nigrostriatal dopamine neurons may alter the subsequent serotonin-dopamine interaction in similar conditions [179, 719]. It has been suggested that an exaggerated dopamine synthesis and/or release might be required to permit the occurrence of a 5-HT2C modulatory control [179, 719]. This evidence supports the essential protective role of 5-HT2C receptors against hyper-excitatory events in the brain dopamine circuits [179, 719]. Consequently, in our study, one could imagine that the distinctive degree of dopamine system excitation due to different mechanism of action of various drugs of abuse could have led to the incongruent observed responses of JJ-3-42. The differential interaction of drugs such as cocaine and amphetamine within the serotonin system and the consequent divergent behavioural and biochemical responses have been previously demonstrated [720-722]. However, future studies with precise measures of dopamine concentration changes in NA and other brain compartments by methods like microdialysis following the local microinjections of JJ-3-42 and various drugs of abuse is necessary to confirm this hypothesis.

While the modulatory role of 5-HT2C receptors on cocaine central responses are relatively well studied, this is not the case for MK-801 and other NMDA receptor drugs (3.8.3A-C). The few available studies have shown either no interaction or an inhibitory influence for the agonists of 5-HT2C receptors following the administration of these drugs in animals [196, 197, 211]. While this is the first report of a potentiating influence of JJ-3-42 pre-treatment on the central response of MK-801, this peculiar interaction merits further investigation in the future. The details of the

alteration of dopamine concentration in various neuronal circuit following the administration of MK-801 and other NMDA receptor drugs is yet to be determined.

The involvement of different brain regions to mediate the central effects of psychostimulants like amphetamine versus NMDA antagonists has been previously shown [696]. In fact, the medial prefrontal cortex has been recognized as the likely site of action for NMDA receptor antagonists. This is in contrast with amphetamine-induced dopaminergic responses through ventral striatum [696]. Moreover, it has been observed that, in the case of non-exocytotic impulse-independent dopamine release, the modulatory response of 5-H2CRs is not prominent [162, 179]. It is noteworthy that such non-exocytotic dopamine release mostly occurs after the administration of amphetamine in the brain [162, 179]. In this regard, the availability and distinctive responses of different 5-HT2CRs positive cell populations in these sites might explain the observed opposite response of MK-801 and amphetamine in our study. In the future, the understanding of such interaction of 5-HT2C receptors and dopamine system following the administration of these drugs in the brain of animals might be of clinical significance in the treatment of addiction to such substances [723, 724].

While the interaction of 5-HT2CRs and dopamine system in acute settings is relatively well studied, the implication of these receptors in regulation of dopamine changes in chronic settings remains obscure. It has been shown that repeated and chronic use of drugs of abuse leads to the development of long-term physiological and psychological modifications in the subjects [155, 194, 201]. It is believed the biochemical consequences of chronic exposure to these drugs might be different than the observed changes in the acute settings [155, 194, 201]. Drug sensitization tests are one of the most common behavioural paradigms to study the sub-chronic or chronic influence of potential treatments in the context of repeated exposure to psychostimulants [194]. In contrast to acute induced locomotion studies, drug-induced sensitization test is based on repeated, and sometimes intermittent administration of the drugs of abuse [194]. This model is based on the phenomenon called “reverse tolerance” that represents a long term neuronal adaptation process following the repeated exposure to a particular drug of abuse [194]. Concerning this, it has been shown that repeated administration of cocaine leads to different lasting modifications at the intracellular and synaptic level that are not present following an acute

single exposure [725, 726]. Higher concentration of serotonin in the NA and abnormalities in orbitofrontal cortex have also been observed following the repeated administration of cocaine in different animals [725-727]. Some of these changes have been proposed to explain higher sensitivity of animals to the central responses of a challenge dose of cocaine in sensitized animals [725-727].

In our study, consecutive administrations of JJ-3-42 did not change the locomotor activity of the animals following a challenge dose of cocaine on the seventh day of sensitization protocol (Figures 3.11D-F). In fact, our findings demonstrated that the repetitive pre-treatment with JJ-3-42 at 10 mg/kg, did not alter the development of sensitization to cocaine in the animals compared to saline group (Figures 3.11D-F). Similar to our results, inefficacy of different 5-HT2CRs agonists to inhibit the sensitization to various drugs has been reported in past studies. For instance, concomitant treatment with either 5-HT2C receptor agonists or antagonists did not alter the locomotor results of a challenge dose of cocaine in sensitized rodents [718, 723]. Similar to our results, 5-HT2C receptor agonist MK-212 failed to attenuate the locomotor response of challenge dose of cocaine following a five-day cocaine sensitization protocol in male Wistar rats [718]. However, the reports of favourable impact of 5-HT2C receptor ligands on the addictive behaviours of animals are frequent. Indeed, it has been shown that selective agonists of 5-HT2C like RO 60-0175 and WAY 163909 significantly suppressed the amount of self-administered cocaine in animals [222-225]. Moreover, it has been shown that 5-HT2CRs stimulation diminished the cocaine-evoked and cocaine-associated behavioural responses in a dose-dependent manner in rodents [222-224, 248, 728, 729]. In other studies, the activation of 5-HT2C receptors inhibited the development of cocaine sensitization in different animal models [135, 199]. Selective blockade of these receptors produced the opposite effects on behaviours of rodents in these studies [242, 705, 730]. Interestingly, 5-HT2CRs knockout mice are found to show abnormal response in intravenous cocaine self-administration test [94]. Also, hypersensitivity and an exaggerated response to cocaine has been reported in these mice [718, 723]. 5-HT2C KO mice demonstrated a higher constitutive activity of dopaminergic neurons in NA and dorsal striatum, which was related to hyperactivity of SNc neurons [85]. Subsequently, the level of dopamine concentration in the NA following the administration of cocaine was higher in these mice than the controls [94].

Taken together, these results indicate the implications of activation of 5-HT2C receptors in the treatment of addictive disorders by modulation of the dopamine system activity. As a result, the benefits of different 5-HT2C agonists in the treatment of addiction to nicotine, ethanol and opiates have been lately demonstrated [59, 718, 723, 731, 732]. Remarkably, some of these results were reproduced in experiments with non-human primates [733-735]. Lately, a series of clinical experiments unveiled the practical implications of agonism of 5-HT2CRs by Lorcaserin in nicotine addicted population [236, 238, 255]. The anti-addictive properties of activation of 5-HT2C receptors in the context of the addiction to other drug of abuse is currently under investigation [255, 736].

In our experiment, JJ-3-42 showed a robust and dose-dependent antipsychotic profile by resisting the hyperlocomotion induced by the administration of apomorphine and amphetamine in animals. In fact, while at lower dose of 10 mg/kg this compound had no effect on the innate or induced hyperdopaminergic state of the animals, at 20 mg/kg it reduced the locomotor activity of DAT-KO HO and psychostimulant-treated animals significantly. Interestingly, in our study, JJ-3-42 at 10 mg/kg potentiated the locomotor responses of cocaine and MK-801in mice pointing out to the complexity of the interaction of serotonin 5-HT2C receptors and dopamine system. Finally, pre-treatment of animals with JJ-3-42 at 10 mg/kg, did not change the response of a challenge dose of cocaine in the sensitized mice compared to saline.

These results endorse the outcomes of previous studies indicating the important implications of 5-HT2C receptors in the regulation of dopamine system in various dimensions [222, 227, 736]. Indeed, our findings showed that JJ-3-42 a 5-HT2CRs selective agonist could be a promising potential drug in the treatment of dopamine-related mental disorders like schizophrenia and drug addiction. However, further studies are needed to delineate the details of such interaction in the future. For example, microdyalisis and electrophysiological recordings following the local microinjections of JJ-3-42 and/or selective antagonists would be necessary to clarify the extent of the contribution of various population of 5-HT2CRs positive cells in this regard. Such studies particularly following the local administration of drugs like cocaine and MK-801 would provide further details of the complex interaction of 5-HT2C receptors and dopamine system and might explain some of the existing contradictions. The outcomes of such analyses might bear a

significant clinical importance to dictate our therapeutic approach for addiction to different drug of abuse in the future. Our preliminary result, however, might warn against the concomitant usage of 5-HT2C drugs in certain population of patients with cocaine or hallucinogenic drug abuse profiles. Despite our disappointing results in sensitization test, further behavioural studies to characterize the potential clinical benefits of chronic administration of JJ-3-42 in the treatment of addiction is suggested. For instance, using cocaine and other substances in tests like drug seeking or different self-administration paradigms might indicate new potentials for JJ-3-42 in the treatment of addictive disorders.

In a recent study, JJ-3-42 produced very little catalepsy in comparison to Haloperidol, at a dose that resisted amphetamine-induced hyperactivity [205]. In this regard, while in our study, there was no indication of motor side-effects related to the injection of JJ-3-42, the safety of this drug should be additionally tested in the future. Further measurements of central dopamine concentration in both mesolimbic and nigrostriatal pathways following the administration of JJ-3-42 could be considered. Such investigations would reveal better the mechanism of beneficial responses of JJ-3-42 and confirm the safe profile of this drug in clinics.

Conclusion

According to the WHO report, psychiatric disorders are the leading cause of disability in the human population and carry an outstanding socio-economic burden on the modern society [389]. Today, despite the production of a considerable amount of scientific evidence, our understanding of the pathophysiology of mental disorders is still at the very early stage [161, 737]. This is mostly due to the tremendous complexity of the brain functions and structures [161, 737]. Subsequently, our treatment strategies for mental disorders have not changed fundamentally in decades [389, 737]. Currently, only a small fraction of psychiatric patients responds adequately to the available medications and can return to their former social and individual functions [389, 737]. Moreover, the majority of these treatments retain a variety of undesirable acute and chronic side effects in the target population [389, 737]. A critical change in our approach to close the gap

between the available neuroscientific evidence and the actual clinical setting has been urged by various authors [526, 738]. Concerning this, the relevant animal study of potential new drugs with validated mechanisms of action has been recognized as an essential part of such complex transitions [161, 526, 738]. In this perspective, the introduction of the new and scientifically-endorsed drugs are considered the cornerstone of any durable mental health plan in the future [526, 738]. The evaluation of therapeutic potentials of a new and selective 5-HT2C receptor agonist, JJ-3-42, across different behavioural paradigms is the main purpose of this thesis.

The benefits of activation of 5-HT2C receptors in the treatment of psychiatric disorders have been the subject of various studies recently [9]. 5-HT2CRs are the only member of GPCRs family with the simultaneous expression of multiple variants of the receptor in mammalian brain [41]. This unique feature is due to the complex RNA editing process that results in the modifications of the protein amino acid sequence [43, 44]. Current attention to the therapeutic benefits of 5-HT2C receptors is justified through a series of the arguments put forward by various authors in different fields [694]. For instance, the presence of these receptors in major brain compartments like mPFC, amygdala, hippocampus and striatum has been shown by various methods [15, 145]. It has been argued that mostly central distribution of these receptors would ultimately lessen the chances of any systemic unwanted side effects of drugs acting on these receptors [15, 145]. Moreover, the implications of these receptors in regulation of feeding, mood, cognition as well as addictive behaviour in various animal models have been shown lately [34, 694]. In line with this, the role of 5-HT2CRs in mediation of the beneficial responses of different antipsychotics, antidepressants and anxiolytic drugs has been demonstrated [34]. Finally, genome-wide studies linked the presence of multiple HTR2C variations to higher prevalence of different psychopathologies as well as altered therapeutic responses in various subgroups of the population [23-25].

Furthermore, the exceptional interaction of these receptors with the dopamine system makes the 5-HT2C receptors a valid target in the treatment of psychiatric disorders [694]. It has been suggested that the ability of these receptors to modulate the abnormal dopamine neurotransmission, particularly in mesolimbic pathway, makes them a potential alternative drug in the treatment of psychosis and substance abuse addiction [145, 153, 158]. Such preferential

dopamine modulation has favoured the 5-HT2C drugs as the future generation of antipsychotic agents with less motor side effects [83, 91, 531]. It has also been debated that the anorexic properties of these ligands could possibly protect the patients from the known metabolic syndrome and cardiovascular consequences of current antipsychotics [27, 653]. In recent years, this growing body of evidence led to clinical trials of new drugs like Vabicaserin and Sertindole for the treatment of schizophrenia [50, 216, 217, 694]. Lately, the FDA-approved anti-obesity drug Lorcaserin (Belviq) showed promising clinical results in management of addictive disorder in patients, while Agomelatine (Valdoxan or Thymanaxas) is widely used as an adjunct treatment in depression and sleep disorders. [237, 596]. Multiple new 5-HT2C drugs are currently are under scrupulous investigations for various psychiatric disorders like addiction and psychotic disorders [9, 34]. Finally, among others, implications of these drugs in the treatment of a variety of non-psychiatric diagnosis like spinal cord injury-induced spasms and epilepsy has recently been considered [34].

For years, due to the high degree of homogeny in the sequence of 5-HT2 receptors, the lack of selectivity in available drugs hindered our investigations of implications of 5-HT2C drugs in psychiatric patients. To this day, it is still a challenge to synthesize a 5-HT2CRs ligand empty of 5-HT2A and/or 5-HT2B receptors interactions [200, 205, 211]. This is important because such cross-reaction might potentially cause hallucinations and/or cardiac valvulopathy in the subjects [196, 626, 739]. As one of the most selective known 5-HT2C agonists, JJ-3-42, with a 2-phenylcyclopropylmethylamine scaffold, has recently shown very promising results in pre-clinical studies [200, 205, 211]. The present study is a primary attempt to characterize the beneficial central responses of JJ-3-42 across some of the most-validated available animal paradigms for the very first time. Furthermore, in this study, we sought to establish the advantages of this new selective drug compared to other selective and non-selective serotonergic agents. Finally, we tried to determine the therapeutic limitations as well as the potential side-effects of this drug as an integral part of our investigation.

As discussed, our study has provided some critical and new insights on the beneficial consequences of the activation of 5-HT2C receptors in different aspects of behaviours of animals. JJ-3-42, a selective 5-HT2C agonist, showed a modulatory and dose-dependent

influence on the dopamine-related behaviour of animals. This is in favour of the previously described antipsychotic and anti-addictive outline of 5-HT2C receptors ligands [191, 255]. In fact, the positive outcomes of activation of 5-HT2C receptors in different animal models of psychosis and addiction has been demonstrated before [9]. Moreover, our results point to the desirable consequences of the agonism of 5-HT2C receptors in social interaction, obsession-compulsion and cognitive-related paradigms. It was shown that by reducing the impulsive/compulsive tendencies of the animals, JJ-3-42 enhanced the cognitive and social performance of the brain serotonin-deficient animals. These are very promising results regarding the lack of efficient treatment for social and cognitive impairment of schizophrenic patients in the current clinical settings [191, 717]. In addition, our results demonstrated some of the advantages of JJ-3-42 as a new and selective 5-HT2C agonist in comparison to other tests with other selective and non-selective serotonin agonists. Remarkably, and in contrast to 5-HTP and CP809,101, the mentioned positive characteristics of JJ-3-42 was not associated with any anxiogenic response in our study. In fact, we report that this new 5-HT2C receptors ligand might show a safe and inert, if not beneficial, profile regarding anxiety-related behaviours in animals. In light of high comorbidity of anxiety disorders with other psychopathologies like schizophrenia and drug addiction these findings might encourage further clinical development of this compound in the future [387, 652]. This might be due to higher selectivity of our compound compared to other agonists. Concerning this, JJ-3-42 shows good potency and excellent selectivity for the 5-HT2C receptor (EC50s of 4.2) and with up to100-fold selectivity against 5-HT2A as partial agonists [200, 205, 630]. Most notably, these compound shows no significant activation of 5-HT2B receptors at concentrations up to 10 μM [200, 205, 630].

Despite these promises, we recognize that our understanding of the involvement of the 5-HT2C receptors in the pathophysiology of mental disorders remains minimal. Our results with JJ-3-42 need to be verified in different animal models and across other dimensions of behaviours. Further biochemical and animal studies would be necessary to reassure the safety and confirm the clinical implications of the activation of 5-HT2C receptors. Moreover, it seems sensible in future studies to establish a more comprehensive time-course and dose-response of the JJ-3-42 along validation of the exclusivity of these findings by using the selective 5-HT2C antagonists.

Furthermore, binding essays as well as studying the downstream intracellular reactions following the JJ-3-42/5-HT2C receptor interaction would help us to better understand the specificities of this new compound. Such results determine the exact contribution of canonical vs Arrestin-dependant pathways in the central responses of this drug compared to other 5-HT2C agonists. This might further elucidate the observed differences of central responses of JJ-3-42 vs CP809,101 in our study.

In the future, comprehension of the consequences of activation of various 5-HT2C receptor positive cell populations in the brain in mediation of observed positive responses of JJ-3-42 might be of clinical significance. In this regard, some of the most plausible avenues of investigation will be the behavioural and biomolecular studies following the local administration of JJ-3-42 in different brain compartments in wild type, and/or in partial 5-HT2C KO mice. Such studies could also let us better estimate the contribution of various levels of RNA editing in the beneficial responses of this agonist. Indeed, modification of signaling pathways and trafficking, as well as RNA-editing changes following the administration of 5-HT2C agonists have been associated with their therapeutic properties before [191].

Finally, relevant pharmacokinetics and toxicologic analyses to set up the therapeutic as well as the toxicity indexes of this compound would be a necessary part of further development of this drug before clinical trials in the population. So far, studies showed that JJ-3-42 has an excellent brain penetration and favourable toxicological profile [205]. Moreover, in preclinical tests, JJ-3-42 showed no toxicological index in various in-vitro cytotoxicity assays [200, 205].

In the present study, we demonstrate that the 5-HT2C agonists JJ-3-42 is efficient in ameliorating a broad range of repetitive and compulsive behaviors in mice. Overall, our results suggest compelling antipsychotic and pro-cognitive properties for JJ-3-42 as a potent agonist of 5-HT2C receptors. Moreover, this compound reduces the impulsivity and improves the sociability of the animals with no evidence of anxiogenic response. Taken together, our study implies that JJ-3-42 might possess some therapeutic indications in various dimensions of mental disorders like schizophrenia, drug addiction or obsessive-compulsive disorders. This drug could also lead to fewer side effects and better control of negative and cognitive symptoms in

psychotic patients. Finally, our results indicate that JJ-3-42 might reduce the aggression and impulsivity without inducing the anxiety in target population. This is important since the management of such traits in clinic is often limited by the lack of efficient and safe medication [347, 370]. Overall, considering an urgent need for the development of the new drugs with better efficiency and lower side-effects in the clinics, the results of this thesis are considered a long stride in tantalizing advancement of 5-HT2C psychiatric medication in the future.

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