United Arab Emirates University

College of Medicine and Health Sciences

Department of Pharmacology and Therapeutics

EFFECTS OF ST713 WITH SIMULTANEOUS HISTAMINE H3 AND DOPAMINE D2/D3 RECEPTOR ANTAGONIST PROPERTIES ON COGNITIVE IMPAIRMENTS AND AUTISM-LIKE BEHAVIORS IN BTBR T+TF/J MICE

Mohammed Mustafa Fateh Alawad

This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Medical Sciences (Pharmacology and Toxicology)

Under the Supervision of Professor Bassem Sadek

March 2022

Declaration of Original Work

I, Mohammed Mustafa Fateh Alawad, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Effects of St713 with Simultaneous Histamine H3 and Dopamine D2/D3 Receptor Antagonist Properties on Cognitive Impairments and Autism-Like Behaviors in Btbr T+Tf/J Mice*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Professor Bassem Sadek, in the College of Medicine and Health Sciences at UAEU. This work has not previously formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student's Signature: Mohammed Alawad Date: 28/03/2022

Advisory Committee

1) Advisor: Prof. Bassem Sadek

Title: Professor

Department of Pharmacology and Therapeutics

College of Medicine and Health Sciences

2) Co-advisor: Dr. Shreesh Ojha

Title: Associate Professor

Department of Pharmacology and Therapeutics

College of Medicine and Health Sciences

Approval of the Master Thesis

This Master Thesis is approved by the following Examining Committee Members:

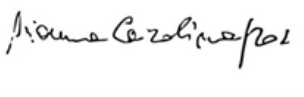
1) Advisor (Committee Chair): Prof. Bassem Sadek
   Title: Professor
   Department of Pharmacology and Therapeutics
   College of Medicine and Health Sciences

   Signature  Date 16/03/2022

2) Member: Dr. Shreesh Ojha
   Title: Associate Professor
   Department of Pharmacology and Therapeutics
   College of Medicine and Health Sciences

   Signature  Date 16/03/2022

3) Member (External Examiner): Dr. Arianna Carolina Rosa
   Title: Associate Professor
   Department of Pharmaceutical Sciences and Technology
   Institution: Faculty of Pharmacy, University of Turin, Italy

   Signature  Date 16/03/2022

This Master Thesis is accepted by:

Acting Dean of the College of Medicine and Health Sciences: Professor Juma Alkaabi

Signature _____ Date 06 April 2022

Dean of the College of Graduate Studies: Professor Ali Al-Marzouqi

Signature Ali Hassan Date 06 April 2022

Copy ____ of ____

Abstract

Autism spectrum disorders (ASD) is a multifactorial neurodevelopmental disorder characterized by two core symptoms which are impairments in social interaction and communication, repetitive and restricted behaviors. Dopamine (DA) and histamine (HA) are two neurotransmitters that are proposed to be involved in several brain disorders including schizophrenia, depression, anxiety, and narcolepsy, all of those disorders are comorbid with ASD. Thus, the palliative effects of the novel multiple-active histamine H3 receptor (H3R) antagonist and dopamine D2/D3 receptor (D2/D3R) antagonist ST-713 with its high H3R antagonist affinity and balanced inhibitory effects on both dopaminergic receptor subtypes D2R and D3R on ASD-like behaviors in male BTBR T+tf/J mice model of ASD were evaluated. Chronic systemic administration of ST-713 (2.5, 5, and 10 mg/kg, i.p.) dose-dependently mitigated social deficits of BTBR mice and significantly reduced the repetitive/compulsive behaviors of tested BTBR mice. Additionally, ST-713 modulated disturbed anxiety levels but failed to balance hyperactivity parameters. Moreover, the ST-713-provided effects on social parameters were entirely reversed by co-administration of the H3R agonist (R)-α-methylhistamine or the anticholinergic drug scopolamine (SCO, 0.3 mg/kg, i.p.). Furthermore, ST-713 (5 mg/kg) attenuated the increased levels of hippocampal and cerebellar protein expressions of Tumor necrosis factor (TNF-α), Interleukins-1β (IL-1β), and IL-6 in treated BTBR mice brains (all $P < 0.01$). The obtained in vivo results demonstrate the effectiveness of a potent multiple-active H3R and D2R/D3R antagonist/inverse agonist against ASD-like phenotype, signifying the potential role of such multiple-active compounds for the therapeutic management of neuropsychiatric disorders, such as ASD.

Keywords: Autistic spectrum disorder, BTBR mice, histamine H3 receptor antagonist, dopamine D2/D3 receptor antagonist, social deficits, stereotyped repetitive behavior, anxiety, neuroinflammation.

Title and Abstract (in Arabic)

تأثيرات ST713مضاد مستقبلات الهيستامين H3R ومستقبلات الدوبامين D2R/D3R على الضعف الإدراكي والسلوكيات المشابهة لاضطراب طيف التوحد في فئران التجارب BTBR T+TF/J

الملخص

تعنى هذه الأطروحة بالتوحد لأنه اضطراب تنموي عصبي متعدد العوامل يتميز بعرضين أساسيين هما ضعف في التفاعل الاجتماعي والتواصل، السلوك المتكرر والمقيد. الدوبامين والهيستامين هما ناقلان عصبيان يُقترح تضمينهما في العديد من اضطرابات الدماغ بما في ذلك الفصام والاكتئاب والقلق والخدار، وكل هذه الاضطرابات تترافق مع اضطراب طيف التوحد. وبالتالي، فإن التأثيرات الملطفة للمركب ST-713 والذي يمتلك صفات كمضاد لملاتقبلات الهيستامين H3R ومضاد لمستقبلات الدوبامين(D2R/D3R) مع تأثيرات مثبطة متوازنة على كلا النوعين الفرعيين لمستقبلات الدوبامين D2R وD3R تم تقييمها على السلوكيات الشبيهة بـ ASD في نموذج الفئران BTBR T + tf / J. جرعات نظامية شبه مزمنة لـ ST-713 (2.5، 5، و10 ملغم / كغم، i.p.) خففت بشكل يعتمد على الجرعة من العجز الاجتماعي لفئران BTBR وقللت بشكل كبير من السلوكيات المتكررة / القهرية للفئران المختبرة. بالإضافة إلى ذلك، فقد وجدت الدراسة أن ST-713 يؤثر على معدل مستويات القلق، ولكنه فشل في موازنة فرط النشاط. علاوة على ذلك، تم عكس التأثيرات المكتسبة من ST-713 على المعلمات الاجتماعية تمامًا عن طريق إضافة المحفز لملاتلالات الهيستامين H3R (*R*) -α-methylhistamine أو عقار مضادات الكولين سكوبولامين (SCO، 0.3 مجم / كجم، i.p.). توضح كل هذه النتائج إمكانيات المركبات متعددة الفعالية للإدارة العلاجية للاضطرابات العصبية والنفسية، على سبيل المثال التوحد. علاوة على ذلك، خفف ST-713 (5 مجم / كجم) من المستويات المتزايدة من تعبيرات البروتين الحُصين والدماغ لعامل نخر الورم (TNF-α) و(IL-1β) Interleukins-1β و-IL 6 في أدمغة الفئران المُعالجة من BTBR (كل $P < 0.01$). علاوة على ذلك، فقد تم عكس التأثيرات الملحوظة لـ ST-713 على السلوكيات العدوانية والاستمالة الذاتية تمامًا عن طريق الجرعة المشتركة لمحفز (*R*)-α-methylhistamine H3R أو عقار سكوبولامين المضاد للكولين. توضح كل هذه النتائج الإمكانيات الدوائية العالية لمركبات متعددة الفعالية في الإدارة العلاجية للاضطرابات العصبية والنفسيةلألألأاحبة لأمراض سلوكيه كثيرة، وعلى رأسها مرض طيف التوحد.

مفاهيم البحث الرئيسية: اضطراب طيف التوحد، الفئران BTBR، مضادات مستقبلات الهيستامين H3، العجز الاجتماعي، السلوك الذ مطي المتكرر، القلق، التهاب الاعصاب.

Acknowledgements

Foremost, I would like to express my sincere gratitude to my advisor Professor Bassem Shaban Sadek for the continuous support of my Master study and researches, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I would like to thank Professor Holger Stark and his research team in the Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Germany for the synthesis and in vitro profiling of the novel multiple-active compound. My sincere thanks also go to Dr. Nermin Eissa, for constantly supporting me throughout this journey. I would like to thank my lab team assistants Dr. Karthikkumar Venkatachalam and Mrs. Petrilla Jayaprakash for their support and assistance throughout my preparation of this project. Special thanks to the Funding Bodies of UAE University for providing the funding for this research. Last but not the least, special thanks go to my parents, wife, sisters who helped me along this way.

Dedication

To my beloved parents and family

Table of Contents

List of Tables

List of Figures

List of Abbreviations

5-HT	Serotonin
ACh	Acetylcholine
AChEI	Acetylcholine Esterase Inhibitor
ADHD	Attention Deficit Hyperactivity Disorder
ASD	Autism Spectrum Disorder
CNS	Central Nervous System
COX	Cyclooxygenase
CPZ	Chlorpromazine
DA	Dopamine
DAT	Dopamine Transporter
DOZ	Donepezil
DR	Dopamine Receptor
DS	Dopaminergic System
ELISA	Enzyme-linked immunosorbent assay
GABA	λ-amino butyric acid
Glu	Glutamate
HA	Histamine
HDC	Histidine Decarboxylase
HNMT	Histamine *N*-methyl Transferase
HS	Histaminergic System
i.p.	Intraperitoneally
IL	Interleukin
iNOS	Inducible nitric oxide synthase
KO	Knockout
LPS	Lipopolysaccharides

MCL	Mesocorticolimbic
NS	Nigrostriatal
PBS	Phosphate buffer Saline
PYR	Pyrilamine
RAM	(*R*)-α-methyl histamine
RIPA	Radioimmunoprecipitation
ROS	Reactive Oxygen Species
SCH	Schizophrenia
SCO	Scopolamine
SI	Sociability index
siRNA	Small interfering RNA
SNI	Social novelty preference index
TNF-α	Tumor necrosis factor-alpha
ZOL	Zolantadine

Chapter 1: Introduction

1.1 Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a multifactorial neurodevelopmental disorder characterized by two core symptoms which are impairments in social interaction, communication, Repetitive and restricted behavior (Guze, 1995). A lot of studies detailed that one in every 160 children have ASD around the world, it may start clearly in first five years of age and after that proceeded to adulthood (Elsabbagh et al., 2012). Individuals with ASD display unusual behaviors in learning, paying consideration, and reacting to different sensations. The etiology of ASD is still unclear. However, ASD is classified as a multisymptomatic disorder produced in a heterogeneous group of patients that share similar behavioral patterns (Delorme et al., 2013). Therefore, there is no standard treatment for all symptoms of ASD, and several research groups have put their immense efforts in developing potential therapeutic compounds, with beneficial pharmacological effects for multifactorial disorders, e.g., ASD (Frantz et al., 2018; Hara et al., 2016; Peñagarikano et al., 2015). Risperidone (Risperdal®) and Aripiprazole (Abilify®) are the only two FDA approved drugs for ASD, and both are antipsychotics drugs that manage irritability and have no clinical effects on sociability impairment, one of the ASD core features (Eissa, Al-Houqani, et al., 2018; Eissa, Azimullah, et al., 2020). Several brain neurotransmitters, e.g., acetylcholine (ACh), 5-hydroxytryptamine (5-HT), dopamine (DA), gamma-aminobutyric acid (GABA), glutamate (Glu), and histamine (HA) play key role in the development of brain, memory, motor activity and behavior regulations (Choudhury et al., 2012). Brain histamine (HA) was found to produce its effects by binding to four known histamine receptor (HR) subtypes having a place in the family of G-protein-coupled receptors

and designated H1 to H4 receptors (H1R-H4R). As mentioned above, at first, the histamine H3 receptor (H3R) described in 1983 was found by negative way to control HA synthesis and release, acting as presynaptic auto-receptors (Pavăl, 2017). In addition, H3Rs functioning as hetero-receptors can also modulate the release of several other brain neurotransmitters like ACh, 5-HT, DA, GABA, and Glu in various brain regions (Croen et al., 2015; Joshi et al., 2013; Lord et al., 2001; Russell et al., 2013). H3Rs are positioned at numerous neurotransmitters crossroads, signifying the importance of targeting H3Rs in number of CNS disorders such as: epilepsy, sleep disorders (narcolepsy), learning and memory and attention-deficit hyperactivity disorder (ADHD), all of which are commonly associated with ASD (Eissa, Al-Houqani, et al., 2018). Moreover, several H3R antagonists were examined for their possible therapeutic utility in neuropsychiatric disorders, and few H3R antagonists were found to mitigate impaired ASD-like features in numerous animal models (Eissa, Azimullah, et al., 2020; Griebel et al., 2012). The brain histaminergic system controls several essential physiological functions e.g., energy and endocrine homeostasis, sensory and motor functions, cognition, and attention, and as such, are all severely affected in neuropsychiatric disorders including ASD (Cangioli et al., 2002; Gemkow et al., 2009; Griebel et al., 2012). Preclinical evidence suggested indirectly that disturbances of brain histaminergic neurotransmission system may be involved in schizophrenia (SCH), proposing that potent and selective H3R antagonists or inverse agonists may potentially lead to therapeutic improvements of cognitive symptoms associated with SCH and ASD (Sadek et al., 2016; Von Coburg et al., 2009; Witkin & Nelson, 2004). The hallmark symptoms of ASD are (i) abnormal reciprocal social interactions (ii) The quality of the communication impaired (iii) stereotyped patterns of behavior, interest and restricted repetitive activities (Losh & Piven, 2007; Volkmar

et al., 2004). One of the different species used in preclinical experiments for discovery of new potential drugs in ASD are the BTBR mice. BTBR mice exhibit all the hallmark-like symptoms of ASD, thus it has been recommended for ASD studies (Silverman et al., 2010). Dopamine (DA) and dopaminergic system (DS) dysfunction play an important role within the phenotypic results of ASD-related behavioral deficits, in both people and animal models (Eissa, Azimullah, et al., 2020) . Also, there is a comprehensive clinical use of numerous antipsychotics that mostly target the D2 receptors (D2Rs) in SCH which shares similarities in several features with ASD (Seeman, 2010). The brain DA is involved in provocation of social interaction in individuals who have low drive for communications (Guze, 1995), and dysfunction of brain dopaminergic system was found to affect ASD-like parameters in preclinical experiments in rodents. Interestingly, BTBR mice that recapitulates ASD-like phenotypes, were found to exhibit significant reductions in both pre- and postsynaptic dopamine D2Rs and adenosine A2ARs function. DA is the neurotransmitter predominately associated with reward processing (Schultz, 2007) . Furthermore, several previous genetic studies have reported that mutations of DA-associated genes, e.g., DA transporter (Hamilton et al., 2013), DA receptors (Reiersen & Todorov, 2011; Staal et al., 2012). and/or enzymes of DA synthesis (Masoud et al., 2015) are connected to ASD. Moreover, accumulated observations showed that mice with increased dopaminergic neurotransmission exhibited significant deficits in ASD-like features, e.g., sociability and repetitive behaviors, while these behavioral changes were switched following administration of several antagonists targeting D1Rs (Lee et al., 2018). Interestingly, D1R agonists induced typical autistic-like features in normal mice or the genetic knockout (KO) of D2Rs (Lee et al., 2018). Moreover, the siRNA-mediated inhibition of D2Rs in the dorsal striatum was shown to replicate ASD-like

phenotypes in D2R KO mice (Lee et al., 2018). BTBR genetic background characterized through multiple genetic and epigenetic aberrations and verified many candidate genes involved in the development of ASD like profile of BTBR (Baan et al., 2016; Meyza & Blanchard, 2017).

Neuroinflammation is one of the significant observations in ASD (Eissa, Al-Houqani, et al., 2018; Eissa, Azimullah, et al., 2020), and BTBR mice were found to display immune cell-mediated inflammation that are also observed in children diagnosed with ASD (Eissa, Sadeq, et al., 2020) . Also, reports from several studies evidenced that autistic child suffered from neuroinflammatory processes in the brain due to abnormally elevated microglial activation (Pardo et al., 2005; Vargas et al., 2005; Zimmerman et al., 2005) , signifying the role of the microglial pathway in ASD. Accordingly, activation of microglia results in the production of reactive oxygen species (ROS) and several proinflammatory cytokines, leading to mitochondrial dysfunction and higher oxidative stress levels in the brain (Al-Haddad et al., 2019; Lull & Block, 2010). Surplus production of ROS and oxidative stress are the primary factors for neurodevelopmental disorders, due to the secretion of inflammatory cytokines like IL-6 and -8, INF-γ and NFκB by microglial cells (Vargas et al., 2005). Interests, in vitro reports, have appeared both pro-and anti-inflammatory influence of histamine on microglial function (Biber et al., 2007). For illustration, histamine can decrease proinflammatory cytokine production such as IL-1b, TNF-α, and IL-6 in response to mediators such as LPS as well modulate overall microglial motility (Ferreira et al., 2012). This may show an anti-inflammatory function of histamine on the microglia. However, in contrast to this, microglial secretion of the pro-inflammatory cytokines, TNF-a and IL-6 is activated by histaminergic stimulation of the H1 and H4 receptors (Dong et al., 2014). Moreover, it has been determined that

there is extensive communication between the immune system and the central nervous system (CNS). Proinflammatory cytokines play a key role in this communication. There is an emerging realization that glia and microglia, in particular, (which are the brain's resident macrophages), are an important source of inflammatory mediators and may have fundamental roles in CNS disorders. Microglia respond also to proinflammatory signals released from other non-neuronal cells, principally those of immune origin, such as mast cells. Mast cells reside in the CNS and are capable of migrating across the blood-brain barrier (BBB) in situations where the barrier is compromised as a result of CNS pathology. Mast cells are both sensors and effectors in communication among nervous, vascular, and immune systems. In the brain, they reside on the brain side of the BBB, and interact with astrocytes, microglia, and blood vessels via their neuroactive stored and newly synthesized chemicals. They are first responders, acting as catalysts and recruiters to initiate, amplify, and prolong other immune and nervous responses upon activation. Mast cells both promote deleterious outcomes in brain function and contribute to normative behavioral functioning, particularly cognition and emotion. Mast cells may play a key role in treating systemic inflammation or blockade of signaling pathways from the periphery to the There is an emerging realization that glia and microglia, in particular, (which are the brain's resident macrophages), are an important source of inflammatory mediators and may have fundamental roles in CNS disorders. Microglia respond also to proinflammatory signals released from other non-neuronal cells, principally those of immune origin, such as mast cells. Mast cells reside in the CNS and are capable of migrating across the blood-brain barrier (BBB) in situations where the barrier is compromised as a result of CNS pathology. Mast cells are both sensors and effectors in communication among nervous, vascular, and immune systems. In the brain, they reside on the brain side of

the BBB, and interact with astrocytes, microglia, and blood vessels via their neuroactive stored and newly synthesized chemicals. They are first responders, acting as catalysts and recruiters to initiate, amplify, and prolong other immune and nervous responses upon activation. Mast cells both promote deleterious outcomes in brain function and contribute to normative behavioral functioning, particularly cognition and emotion. Mast cells may play a key role in treating systemic inflammation or blockade of signaling pathways from the periphery to the brain. Moreover, histamine has a dual roles one of them in a physiological context that will cause the inflammation by triggering the microglia to produce proinflammatory cytokines IL-1b, TNF-α, and IL-6 and another role that related to the inflammatory context in which we had chronic inflammation as in Autism so the release of histamine will regulate the proinflammatory cytokines secretions (Eissa, Sadeq, et al., 2020). Overall, these findings highlight the role of histamine and how it's affecting the secretion of proinflammatory cytokines such as IL-1b, TNF-α, and IL-6. Also, activation of transcription factor, NF-κB may was found to trigger the up-regulations of the expressions of iNOS and COX-2 mediated by lipopolysaccharides (LPS) (Kim et al., 2013). Moreover, several previous studies indicated an imbalance in enzymatic and non-enzymatic antioxidants, e.g., consistent diminished levels of glutathione in the brain and blood of patients with ASD (Nadeem et al., 2019). Considerably, a recent study showed that oxidant-antioxidant balance plays an important part within the severity of ASD-like repetitive behaviors in BTBR mice, illustrating that BTBR mice as an essential model in an investigation of antioxidant intervention procedures that have translational value (Nadeem et al., 2019). Earlier studies from our laboratory proved that H3 antagonists were capable of ameliorating the ASD-like behaviors in various animals' strains (Eissa, Al-Houqani, et al., 2018; Eissa, Azimullah, et al.,

2020). Given the afore-mentioned roles of brain HA and DA, we aimed to evaluate the effects of ST-713, a multiple-active histamine H3R and dopamine D2/D3 receptor antagonist, on the improvements of ASD-like behaviors in BTBR mice. Also, the effects of ST-713 on protein expressions of several cytokine pattern and modulated levels of HA and DA in specific brain regions of treated BTBR mice were assessed.

1.2 Histamine as a Central Neurotransmitter

A Histamine (2-[4-imidazolyl] ethylamine) (Figure 1) was found by Sir Henry Hallet Dale and Sir Patrick Playfair Laidlaw in 1910 (Yoshikawa et al., 2019).

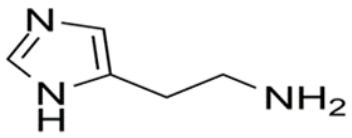


Figure 1: Chemical structure of histamine. Chemical name: 2-(1H-imidazol-4-yl) ethanamine

The brain histaminergic system was found to show an essential role in cognition, sleep, and other neuropsychiatric disorders including schizophrenia (SCH), Hypo – or hyper sensory problems and Tourette disorder that share comorbidity with ASD (Wright et al., 2017). Moreover, the action of neuronal histidine decarboxylase (HDC) histamine a synthesizing enzyme, which was familiar to be available in nerve endings, was reduced in many brain areas after lesions of the lateral hypothalamus (Garbarg et al., 1976). Increasingly, change in gene expression was found for a histamine-*N*-methyltransferase enzyme (HNMT) that's able for the metabolism of central histamine (HA) and for histamine receptor (HR) subtypes H1-, H2-, and H3R (Wright et al., 2017). The breakthrough within the field of neuronal histamine was the production of antibodies against HDC and histamine that were utilized to draw the first map of neuronal histamine pathways utilizing histochemical methods (Watanabe et al.,

1983). The storage area of brain histamine was classified for 2 regions, one in the synaptic vesicles of neuronal endplates and one within the mast cells (Verdiere et al., 1975). Additionally, an important role for the brain, HA has been anticipated, and a range of H3R ligands have been created until now for dual-targeting of both histaminergic and dopaminergic neurotransmissions (Bishara, 2010). In this manner, the essential role of central histamine that impacts behavior in CNS disorder explains why the histaminergic system should be a pharmacological target for therapeutic goals (Haas et al., 2008; Tiligada et al., 2011). Notably, brain H3Rs act as auto-receptors or hetero-receptors that control the biosynthesis and release of HA and a variety of other brain neurotransmitters, e.g. ACh, DA, NA, and 5-HT, therefore positioned at neurotransmitter crossroads influentially involved in numerous cognitive and homeostatic processes (Figure 2; Pavăl, 2017).

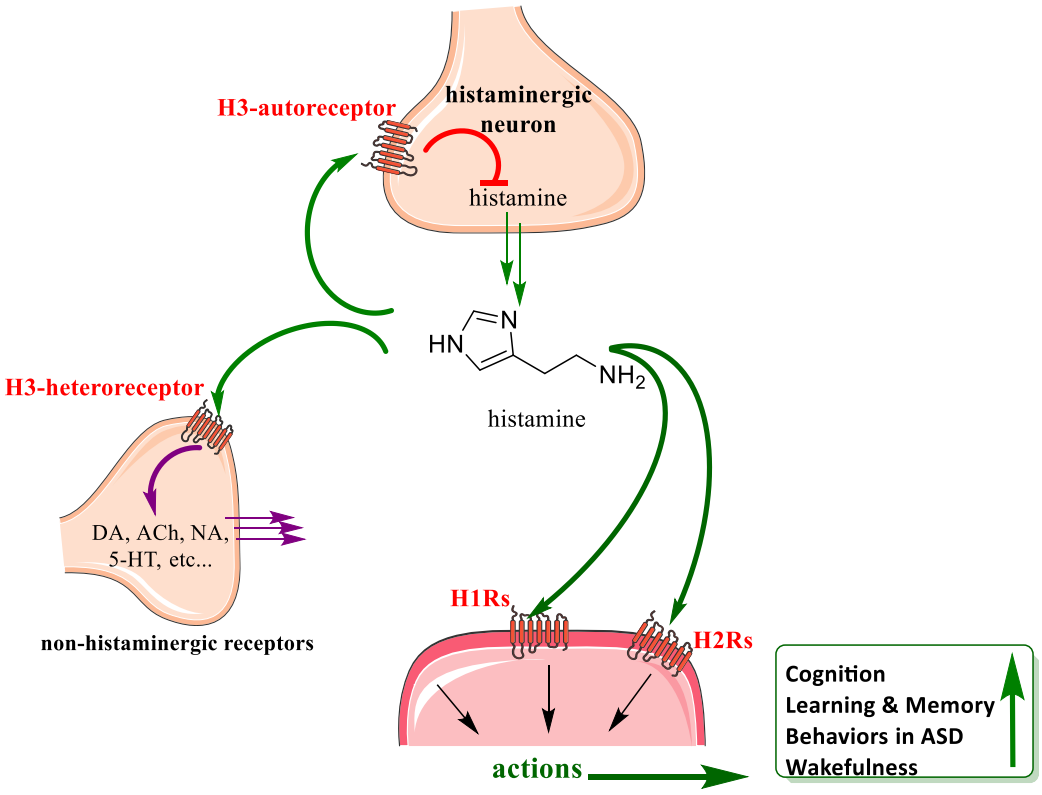


Figure 2: Histamine H3 receptor functioning as auto- and heteroreceptor in modulation several behavioral effects

1.3 Dopamine

Disruption of the dopaminergic system has been noted to affect striatal dopaminergic neurotransmission and DA-dependent behaviors that are involved in different neuropsychiatric disorders and latest in ASD (DiCarlo et al., 2019). Autism is deeply linked to a mutation within the dopamine transporter (DAT) gene SLC6A3, which is a very important code for a protein that assists to the regulation of DA levels within the brain (Hamilton et al., 2013). DAT knockout mice (hyperdopaminergic mutant mice) appeared more prominent invariance in complex fixed action patterns, proposing an association between abnormal DA levels and repetitive (Berridge et al., 2005). As mentioned above, the siRNA-mediated inhibition of D2Rs in the dorsal striatum was shown to replicate ASD-like phenotypes in D2R KO mice (Lee et al., 2018). Like in previous studies, autistic mice show social deficits and stereotyped behaviors as core characteristics. They propose that these concerns arise from alterations of midbrain dopaminergic signaling. Two subpopulations of midbrain dopaminergic neurons are included in controlling the functions traditionally influenced in ASD: the ventral tegmental area and the substantia nigra (Haber, 2014). To begin with, neurons that will come from the ventral tegmental area project to the prefrontal cortex and the ventral striatum's nucleus accumbens, making the mesocorticolimbic (MCL) circuit, which is included in high-order brain capacities such as reward and motivation-related behavior (Chevallier et al., 2012).

Secondly, the neurons that will come from the substantia nigra extend towards the dorsal striatum, making the nigrostriatal (NS) circuit, which regulates the motor aspects of goal-directed behavior in order to create an important activity towards getting a particular outcome (Chevallier et al., 2012; Haber, 2014). To start with, the social deficits noticed in ASD could may indicate an MCL circuit dysfunction, and

that's why to explain the important role in reward and inspiration (Bear et al., 2007). If the previous changes involved social behavior, autistic brains might miss enrolling social experiences as rewarding, further reducing the motivation to seek social interactions and create social abilities. this finding was expressed by the social motivation theory of ASD which explained that autistic subjects are extreme case of reduced social motivation which affects social cognition, ultimately leading to social deficits (Chevallier et al., 2012). A lot of studies support this point of view. At first, autistic subjects show signaling changes within the MCL dopaminergic pathway, Ex: decreasing the release of dopamine within the prefrontal cortex and reduced neural response within the nucleus accumbens (Scott-Van Zeeland et al., 2010).In this manner, studies appear that ASD is characterized by common hypoactivation of the reward system (Dichter et al., 2012). which happens for both social and nonsocial rewards (Dichter et al., 2012; Scott-Van Zeeland et al., 2010). In conclusion, alteration in mesolimbic dopaminergic signaling appeared to change particular reward-related behavior in autistic subjects, such as effort-based decision-making for rewards (Damiano et al., 2012).

1.4 Aims and Objectives

The main aim of this project to assess the effects of chronic systemic administration with the novel multiple-active compound ST-713 with high binding affinities at histamine H3 receptor (H3R), dopamine D2/D3 receptors (D2/D3R), on social deficits stereotyped repetitive behavior of BTBR mice using a battery of standard behavioral tests. Also, the effects of ST-713 on the abnormal anxiety levels and hyperactivity will be evaluated. In addition, the effects of chronic systemic administration of ST-713 on protein expressions of Tumor necrosis factor- α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) of treated BTBR brains will be assessed. through a battery of

standard behavioral tests. Moreover, the modulating effects of ST-713 on brain levels of crucial neurotransmitters, namely histamine and dopamine in specific brain regions of treated BTBR mice will be quantified.

Chapter 2: Materials and Methods

2.1 Animals

BTBR T+ Itpr3tf/J (BTBR) mice (matured 8-12 weeks, weighing 30-32 g) were gotten from Jackson Laboratory (Boulevard, Bethesda, MD 20892-4874, USA), and they were bred in our animal facility and the compound has been tested in BTBR mice because it's mouse model of ASD as it naturally displays core symptoms of ASD which are repetitive behaviors and deficits in sociability and communication and that's why its relevance to the experimental protocols ,and inbred C57BL/6J (C57) mice (aged 8–12 weeks, weighing 20–25 g) were obtained from Animal House, College of Medicine and Health Sciences, UAE College. mice (matured 8-12 weeks, weighing 30-32 g) were obtained from Jackson Laboratory (Boulevard, Bethesda, MD 20892-4874, USA), and they were bred in our animal facility, and inbred C57BL/6J (C57) mice (aged 8–12 weeks, weighing 20–25 g) were gotten from Animal House, College of Medicine and Health Sciences, UAE College. The mice were kept up in an isolated air-conditioned room with controlled temperature and humidity (24 ± 2°C and 55% ± 15%, respectively), 12 h light/dark cycle, and ad libitum to food and water. The whole tests were completed between 9.00 am to 3.00 pm. The methods carried were confirmed by the Institutional Animal Ethics Committee of College of Medicine and Health Sciences/United Arab Emirates University (Approval No: ERA-2019-6013). To reduce the suffering of animals a fewer number of animals were used in this study, though the objectives were not compromised.

2.2 Chemicals

Synthesis and in vitro profiling of ST-713 was carried out in the Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf,

Germany, and according to previously published experimental protocols (Figure 3; Von Coburg et al., 2009). The reference drugs Donepezil (DOZ, 1 mg/kg, i.p.) hydrochloride, Chlorpromazine (CPZ, 1.5 mg/kg, i.p.), the brain-penetrant H1R antagonist pyrilamine (PYR, 10 mg/kg, i.p.), the brain-penetrant H2R antagonist zolantidine (ZOL, 10 mg/kg, i.p.), the muscarinic anticholinergic Scopolamine (SCO, 0.3 mg/kg, i.p.), the CNS-penetrant H3R agonist (*R*)-α-methylhistamine (RAM, 10 mg/kg, i.p.), All the reagents utilized were of analytical grade. All drugs were dissolved in saline and all dosages were expressed in terms of the free base. For estimation of the levels of proinflammatory cytokines (TNF-α, IL-1β, and IL-6), commercially available enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA). For estimation of brain levels of histamine and dopamine in hippocampus and cerebellum, dopamine (BioVision Catalog no: K4219-100) and histamine (Abcam Catalog no: ab213975) were determined using an ELISA kits according to the instructions of the manufacturer.

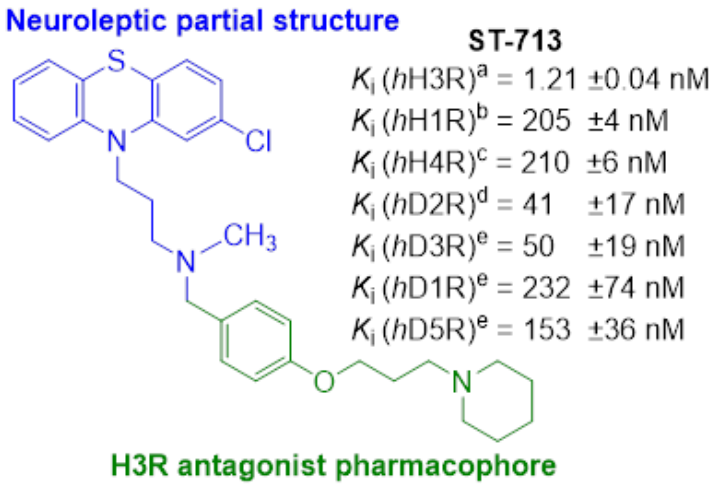


Figure 3: *In-vitro* pharmacological binding profile of ST-713 at selected human histamine and dopamine receptor subtypes

a[3H]Nα -Methylhistamine binding assay performed with cell membrane preparation of HEK cells stably expressing the human H3R (n=4). b[3H]Pyrilamine binding assay performed with cell membrane preparation of CHO cells stably expressing the human H1R (n=2). c[3H]Histamine binding assay

performed with cell membrane preparation of Sf9 cells transiently expressing the human histamine H4R and co-expressed with Gαi2 and β1γ2 subunits (n=2). d,eDisplacement assay was carried out as described previously using membrane suspension of cell lines stably expressing the human dopamine hD1Rs and hD5Rs (HEK) against [3H]SCH23390 and hD2SRs, hD3Rs (CHO) using [3H]spiperone (n=3).

2.3 Experimental Design and Treatments

All the mice were habituated for one week before the beginning of the test, and after that, they were randomly isolated into thirteen groups of seven mice each. The total period of the test was 21 days, the intraperitoneal (i.p.) subchronic treatment began one week before the behavioral tests then proceeded for all the days until the sacrifice. Saline and other drugs were injected 30 minutes before commencement of the behavioral tests each day. The compounds were dissolved in physiological saline before administration and the volume was normalized with body weight (10 ml/kg). The sample size has been chosen according to previous studies in our lab (Venkatachalam et al., 2021). Moreover, all the tests were carried out with seven mice for each group and every three mice were kept in one cage with free access to food and water. Group 1, C57 mice injected with saline served as control. Group 2, BTBR mice treated with saline served as autistic control mice group. Groups 3-5, BTBR mice received i.p. injections of different doses of ST-713, namely 2.5, 5, and 10 mg/kg, respectively. Group 6 and 8, BTBR mice were injected with DOZ (1 mg/kg, i.p.) and the later one co-injected with ST-713 (5 mg, i.p.), Groups 7 and 9, BTBR mice received i.p. injections of CPZ (1.5 mg/kg). In addition, BTBR mice in Group 9 were co-injected with ST-713 (5 mg/kg i.p.), Groups 10-13, BTBR mice were treated with ST-713 (5 mg/kg, i.p.) along with PYR (10 mg/kg, i.p.), ZOL (10 mg/kg, i.p.), SCO (0.3 mg/kg, i.p.), and RAM (10 mg/kg, i.p.), respectively (Figure 4A, B).

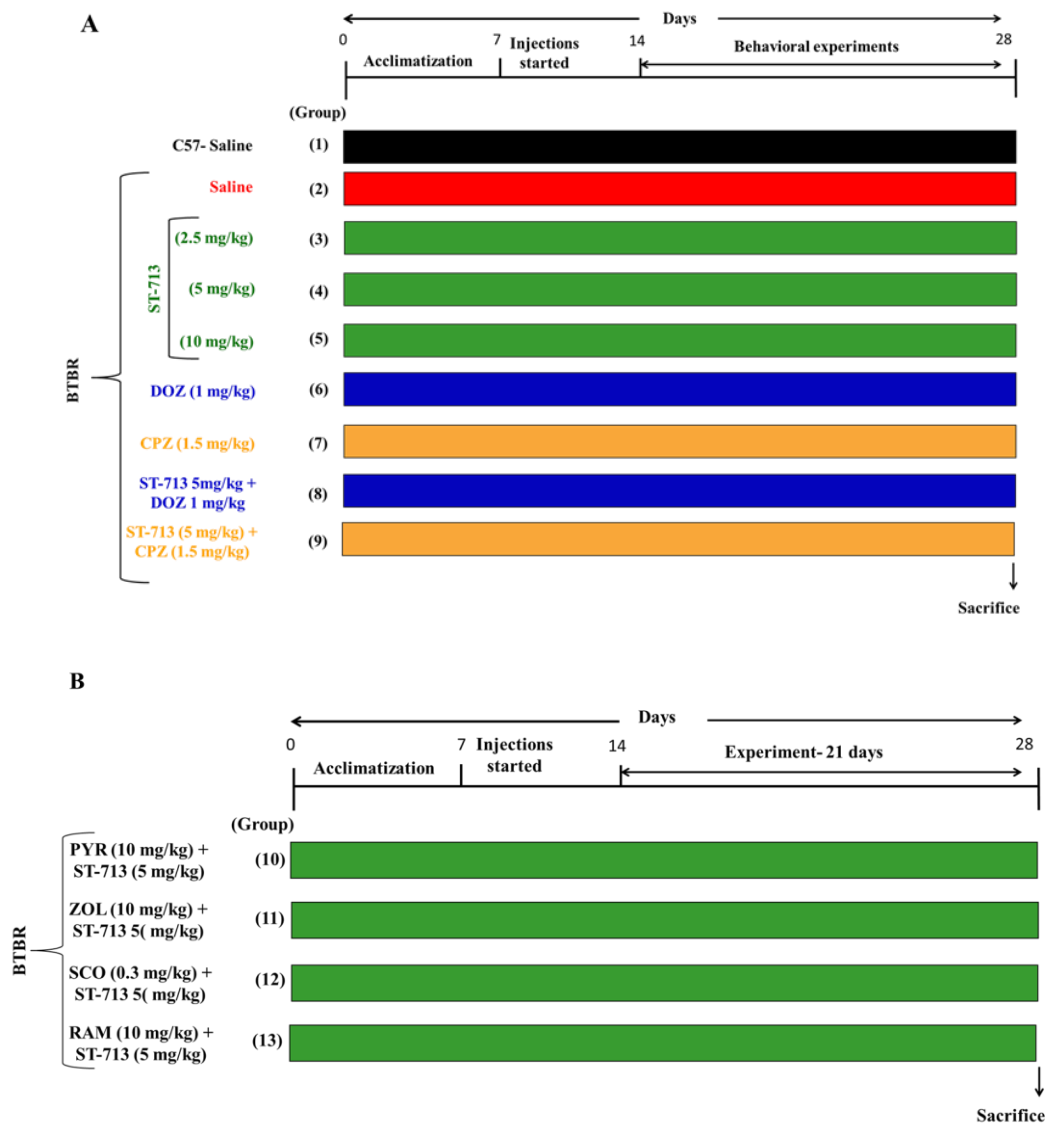


Figure 4: Schematic experimental design

2.4 Behavioral Tests

2.4.1 Three Chamber (TC) Test

TCT was performed accordingly as described previously by (Eissa, Al-Houqani, et al., 2018; Eissa, Azimullah, et al., 2020; Silverman et al., 2010). In detail, the glass equipment consists of three chambers, each chamber has (40 × 20 × 22 cm) equal volume of space. The center chamber has two square shaped openings with doors, which can provide the access to left and right chambers. Two plastic round wired cages were used to separate the stranger mice from experimental mouse. Two stranger mice

(10 to 12 weeks old) were acclimatized in that plastic wire cage for 30 min, one day prior to the commencement of experiments.

In the TCT test, total duration of one test trial was 30 minutes, consisting of two five minutes sessions and two ten minutes sessions. During the first five minutes session, the test mouse was placed in the center chamber with no access to other two chambers, and then it was allowed to access all three chambers by opening the doors for the second five minutes session, for habituation. After habituation and before starting the first 10 minutes test session, a stranger mouse was placed in a wire cage in one side of the chamber (same gender and age as test mouse but with no previous contact) referred to as novel mouse (NM), meanwhile in the opposite chamber an empty wire cage was kept, representing novel object (NO) (Figure 5). The position of the stranger mice was altered regularly to avoid side preferences. In this session test mouse allowed to access all three chambers for 10 minutes. This was followed by the second ten minutes test session immediately by placing a second stranger mouse in the empty wire cage. Now, the first stranger mouse was referred to as familiar mice (FM) and second stranger mouse was referred to as novel mouse (NM) (Figure 5). Similar pattern of time duration was counted as in first ten minutes test session. The whole experiment was recorded and the duration for each session was calculated accordingly using EthoVision® Software (Noldus, Netherlands).

To allow the direct comparison of social behavior of the treated groups the sociability index (SI) and social novelty preference index (SNI) were evaluated. The SI was calculated as [Time exploring NM – Time exploring NO] / [Time exploring NM + Time exploring NO]; while SNI was calculated as [Time exploring NM – Time exploring FM] / [Time exploring NM + Time exploring FM].

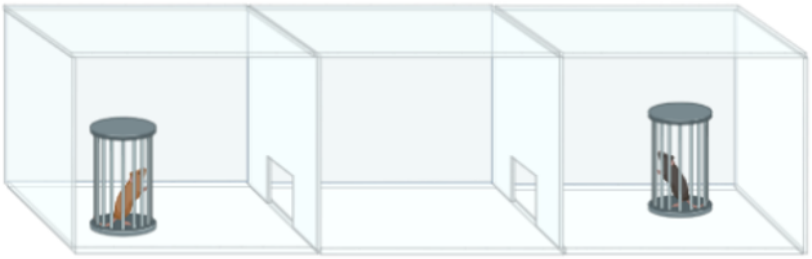


Figure 5: Three chamber test

2.4.2 Marble Burying (MB) Test

To analyze the repetitive behaviors of the animals, MB test was performed, following previously described protocols (Angoa-Pérez et al., 2013; Eissa, Al-Houqani, et al., 2018; Eissa, Azimullah, et al., 2020). Briefly, clean and autoclaved rat cages (26 cm × 48 cm × 20 cm) were utilized for this test. All the cages were filled 5 cm depth with wooden waste as bedding material and leveled by utilizing another flat surfaced cage. Each mouse was kept in an isolated cage for 10 minutes for habituation, at that point they were removed, and 20 black painted glass marbles were kept equally spaced over the bedding (Figure 6). The mice were at that point put back to the center of the respective cages and permitted to explore for 30 minutes. After 30 minutes the mice were returned back in their home cages, and buried marbles were counted physically. Marbles covered by > 50% with bedding were considered as buried marbles (Table 3).

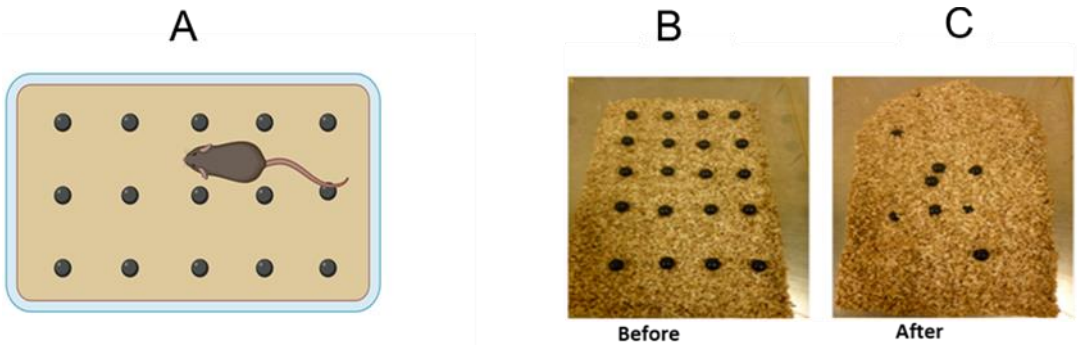


Figure 6: Marble burying test

A cage with equidistant 4 × 5 arrangement of black marbles used for marble burying test (A), the cage when the test started (B), The number of marbles buried was recorded (C)

2.4.3 Nestlet Shredding (NS) Test

To examine compulsive and repetitive behaviors of the animals NS test was performed according to (Angoa-Pérez et al., 2013; Eissa, Al-Houqani, et al., 2018; Eissa, Azimullah, et al., 2020). Mice cages (19 cm × 29 cm × 13 cm) were used to perform this experiment. A clean autoclaved mice cage was included with wooden waste as bedding to form 0.5cm depth. Each mouse was kept in an isolated cage for 10 minutes for habituation, at that point one piece of commercially available, pre-weighed cotton nestlet [5 cm x 5 cm), around 2.5 g] was kept in each cage. All the mice were permitted to explore freely and after 30 minutes, all mice were returned to their home cages and the remaining unshredded intact nestlet was carefully collected with forceps and allowed to dry overnight. Dried nestlets were weighed separately and the percentage of cotton shredded by each mouse was calculated appropriately (Figure 7).

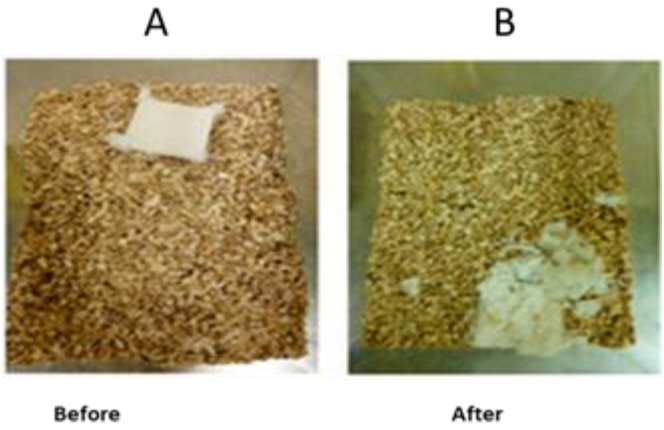


Figure 7: Nestlet Shredding Test

A cage with nestlet was placed on top of the bedding for nestlet shredding test (A), after the test finished, the remaining un-shredded nestlet was weighed (B)

2.4.4 Open Field (OF) Test

To analyze the effect of ST-713 on locomotion and anxiety behaviors in animals, OF experiment was carried out. Beyond locomotor activity, the OF test is usually utilized to measure anxiety-like behaviors in experimental rodents (Eissa, Al-Houqani, et al., 2018; Eissa, Azimullah, et al., 2020; Prut & Belzung, 2003; Sadek et al., 2016b; Seibenhener & Wooten, 2015). The OF box includes a square box (45 ×45 × 30 cm). A 23 cm x 23 cm in the center region was defined as a central arena, the remaining was characterized as a periphery region (Figure 8). The mice with anxiety normally stay closer to the walls of the box and spend timeless within the center, while increased time spent within the central region shows low anxiety levels and high exploratory behaviors (Walsh & Cummins, 1976). First of all, 5 minutes of the test was considered as habituation, following 10 minutes, during the test, total distance moved within the whole arena, time spent within the center and periphery were recorded for 10 minutes utilizing CCD camera-assisted motion tracking apparatus and software (Figure 8) (EthoVision 3.1, Noldus Information Technology,

the Netherlands) (Table 4). After each mouse was tested, the open field chamber was cleaned completely with 70% (v/v) alcohol.

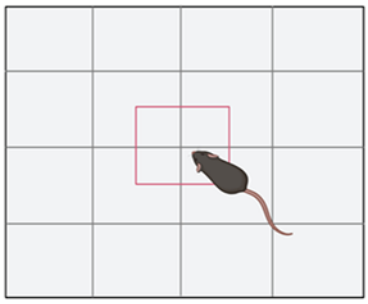


Figure 8: Open field test

At the end of behavioral tests i.e., on day 21 of treatment, all the animals were sacrificed. Prior sacrifice by 2 hours, all mice were injected with lipopolysaccharide (LPS) (from E. coli serotype 0111:B4), (25 µg/kg, i.p.) , then they were anesthetized with pentobarbital (40 mg/kg, i.p.). Cardiac perfusion method was employed to eliminate blood with 0.01 M phosphate-buffered saline at pH 7.4. The skull was removed carefully, brains were removed, and both hemispheres were separated on ice plate. The hippocampus and cerebellum were cut out from brain and allowed to quick freeze immediately with liquid nitrogen for biochemical assays.

2.5 Biochemical Assessments

2.5.1 Brain Collection and Tissue Processing for Stimation of Proinflammatory Markers, Dopamine, and Histamine

After completion of behavioral experiments, animals were sacrificed to analyze proinflammatory markers by ELISA according to published procedures (Eissa, Al-Houqani, et al., 2018; Javed et al., 2016; Tyrtyshnaia et al., 2016). In brief, pentobarbital (40 mg/kg, body weight, i.p.) was used to anesthetize the animals followed by the heart perfusion through intracardial infusion by using 1× PBS (0.01

M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4). The blood was washed out and confirmed by turning of organs brownish red to whitish color (liver, heart and kidney) indicating that they are free from blood. The brain was harvested and placed on an ice plate for further separation. Then carefully, cerebellum and hippocampus parts were separated and flash-frozen in liquid nitrogen flask for biochemical parameters. Before performing biochemical parameters, all the tissue samples were weighed to an equal weight of 40 mg of hippocampus and 100 mg of cerebellum and then homogenized with ice cold RIPA buffer comprised of protease and phosphatase inhibitors to avoid protein degradation. The homogenized samples were centrifuged in a cooling centrifuge (4°C) for 30 minutes at 12,000 rpm. The supernatant from each sample was collected and the levels of dopamine and histamine were determined using ELISA kits according to the instructions of the manufacturer.

2.5.2 Proinflammatory Cytokine Estimations

The ELISA was performed to quantify the levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in the hippocampus and cerebellum of treated mice. The levels of TNF-α, IL-1β, and IL-6 were estimated following the manufacturer's instructions and as described earlier (Eissa, Al-Houqani, et al., 2018; Javed et al., 2016; Venkatachalam et al., 2021). The optical density was determined at 450 nm using a microplate absorbance reader (Sunrise, TECAN). The results were expressed as pg/mg protein.

2.5.3 Estimation of Brain Levels of Histamine and Dopamine

The brain levels of dopamine and histamine were determined using an ELISA kit according to the instructions of the manufacturer. For dopamine, equal volumes of standard and samples were mixed with Biotin-detection antibody in a 96 well plate

and kept 37°C for 45 minutes. The horseradish peroxidase (HRP)-streptavidin conjugate was added to each well after the adequate washes. 3,3',5,5'-Tetramethylbenzidine (TMB) substrate for dopamine was then added and this mixture was incubated for 15-20 minutes at 37°C. The color development was terminated by adding a stop solution containing sulphuric acid, the optical density was then read within 20 minutes at 450 nm. For histamine, in a 96-well plate, equal volumes of the samples and the standard were mixed with histamine tracer and histamine antibody. The mixture was then kept in a plate shaker (≤ 500 rpm) at room temperature for 1 hour. After a sufficient number of washes, the streptavidin- HRP (SA-HRP) conjugate was added to all the wells. It was again incubated at room temperature on a plate shaker for 30 min. TMB substrate was then added before termination of the reaction. The optical density was read at 450 nm as previously described (Alachkar et al., 2021).

2.6 Statistical Analysis

The data observed in behavioral assessments were analyzed for normality by assessing the sample distribution or skewness (-1.5 to +1.5 was considered as normally distributed). After the results had passed the tests for normality, the effects of drug treatments were analyzed by two-way analysis of variance (ANOVA) and post hoc comparisons were performed with Tukey's test in case of a significant main effect. The data observed for estimation of cytokines, HA, and DA data were analyzed by one-way ANOVA followed by post hoc Tukey's multiple comparison test. All data were expressed as mean ± standard error of mean (SEM). Statistical significance was set as $P < 0.05$.

Chapter 3: Results

3.1 Effects of ST-713 on Improving Social Deficits in BTBR Mice

The effects of chronic systemic administration of ST-713 (2.5, 5, and 10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), and CPZ (1.5 mg/kg, i.p.) on autistic-like sociability deficits in TC paradigm are shown in (Figure 9A).The results of two-way ANOVA conclude that there was a critical main effect for strain [$F_{(1,96)} = 123.78$, $P < 0.01$], treatment [$F_{(7,96)} = 7.78$, $P < 0.01$] additionally for the strain × treatment interaction [$F_{(7,96)} = 6.21$, $P < 0.01$] (Table 1). As noticed within the Tukey post hoc analyses, BTBR mice shown significantly lower percentage of SI when compared to C57 mice ($F_{(1,12)} = 38.31$, $P < 0.001$) (Figure 9A). ST-713 (5 and 10 mg/kg) and DOZ (1 mg/kg, i.p.) significantly enhanced sociability of BTBR mice when compared with saline-treated BTBR mice, with ($F_{(1,12)} = 26.71$, $P < 0.001$), ($F_{(1,12)} = 40.85$, $P < 0.001$), and ($F_{(1,12)} = 10.61$, $P < 0.01$), respectively (Figure 9A). However, ST-713 (2.5 mg/kg, i.p.) failed to improve sociability of BTBR mice ($F_{(1,12)} = 0.36$, p = 0.56). Additionally, the reference drug CPZ (1.5 mg/kg, i.p.) worsened the sociability deficits of BTBR mice when compared with saline-treated BTBR mice ($F_{(1,12)} = 6.91$, $P < 0.05$). Moreover, ST-713 (5 mg/kg, i.p.) when co-injected with CPZ (1.5 mg/kg, i.p.), improved the negative effects noticed with CPZ when gave it alone (F(1,12) = 9.87, p < 0.01), but failed to modify the improving effects gotten with DOZ (F(1,12) = 0.04, p = 0.84) (Figure 9A). Additionally, ST-713 (5 mg)-provided effects on sociability were totally reversed by co-administration with the H3R agonist RAM and the anticholinergic SCO, with ($F_{(1,12)} = 6.40$, $P < 0.05$) and ($F_{(1,12)} = 7.12$, $P < 0.05$), respectively (Figure 9B). Notably, H1R antagonist PYR or the H2R antagonist ZOL failed to reverse the ST-713-provided effects with ($F_{(1,12)} = 0.39$, p = 0.54) and ($F_{(1,12)} = 0.23$, p =

0.64), respectively (Figure 9B). In addition, systemic pretreatments with ST-713 (2.5, 5, and 10 mg/kg), DOZ (1 mg/kg), CPZ (1.5 mg/kg), ST-713 (5 mg)+DOZ, and ST-713(5 mg)+CPZ did not alter SI that noticed in control C57 mice in NS test (Figure 9A and Table 1).

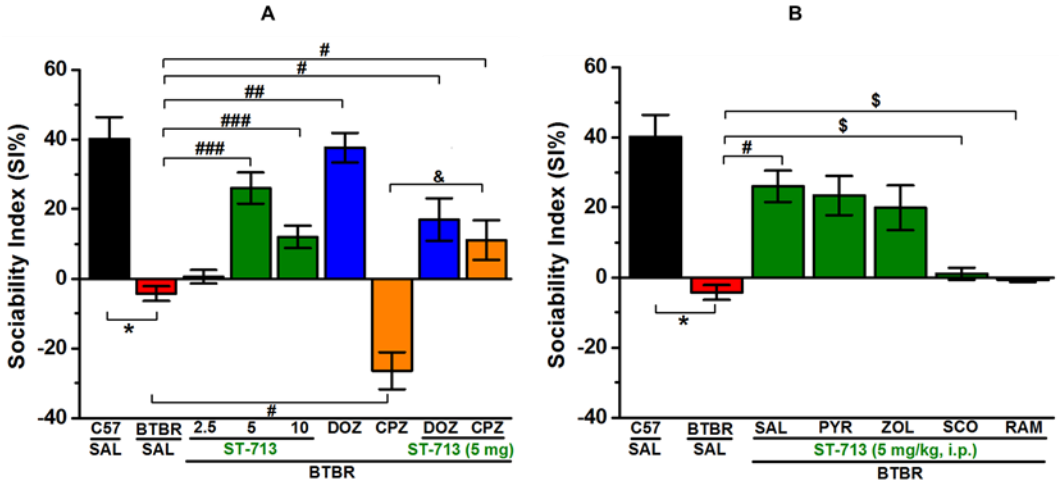


Figure 9: Ameliorated Sociability Deficits of BTBR Mice in TC Test.

After 10 minutes of habituation, male subjects were allowed to explore all chambers for two 10 minutes sessions. C57 and BTBR mice were injected with saline, and BTBR mice were administered with ST-713 (2.5, 5 or 10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), or CPZ (1.5 mg/kg, i.p.) chronically for 21 days. The results obtained were Sociability Index (SI) (Figure 9A), and the effects of chronic (21 days) systemic co-injection of PYR (10 mg/kg, i.p.), ZOL (10 mg/kg, i.p.), SCO (0.3 mg/kg, i.p.), and RAM (10 mg/kg, i.p.) on the ST-713-(5 mg)-provided improvement of sociability (Figure 9B). Figures show mean ± SEM (n=7). $^{*}P < 0.05$ vs. SI of saline-treated C57 mice. $^{\#}P < 0.05$ vs. SI of saline-treated BTBR mice. $^{\$}P < 0.05$ vs. ST-713-(5 mg)-treated BTBR mice. $^{\&}P < 0.05$ vs. SI of DOZ- or CPZ-treated BTBR mice.

Table 1: Results observed following two-way ANOVA analyses of ST-713, DOZ, and CPZ systemic pretreatment on behavioral parameters

Behavioral test	**Effect of strain**	**Effect of treatment**	**Effect of strain × treatment interaction**
Sociability (SI)	$F_{(1,96)}$ = 123.78	$F_{(7,96)} = 7.78$	$F_{(7,96)} = 6.21$
Social novelty preference (SNI)		$F_{(7,96)} = 9.28$	$F_{(7,96)} = 8.25$
MB test	$F_{(1,96)} = 56.23$	$F_{(7,80)} = 3.98$	$F_{(7,80)} = 2.97$
NS test	$F_{(1,80)} = 54.92$	$F_{(7,80)} = 4.65$	$F_{(7,80)} = 4.54$
OF test	$F_{(1,80)} = 23.25$		
(1) Total distance travelled (cm)		$F_{(5,36)} = 0.26^{a}$	$F_{(5,36)} = 0.13^{b}$
	$F_{(1,36)} = 45.58$	$F_{(5,36)} = 0.21^{c}$	$F_{(5,36)} = 0.12^{d}$
(2) Time spent in the periphery (s)	$F_{(1,36)} = 41.46$	$F_{(5,36)} = 1.15^{e}$	$F_{(5,36)} = 1.15^{f}$
(3) Time spent in center of arena (s)	$F_{(1,36)} = 37.26$		

All P values < 0.01, except $^{a} = 0.93$, $^{b} = 0.98$, $^{c} = 0.96$, $^{d} = 0.99$, and $^{e,f} = 0.35$.

3.2 Effects of ST-713 on Enhancing Social Novelty Preference Deficits in BTBR Mice

Furthermore, the effects of chronic systemic administration of ST-713 (2.5, 5, and 10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), and CPZ (1.5 mg/kg, i.p.) on autistic-like social novelty preference were evaluated (Figure 10A). Results of two-way ANOVA appeared that there was a critical main effect for strain [$F_{(1,96)} = 56.23$, $P < 0.01$], treatment [$F_{(7,96)} = 9.28$, $P < 0.01$] , also for the strain × treatment interaction [$F_{(7,96)} = 8.25$, $P < 0.01$] (Figure 10A and Table 1). As noticed within the Tukey post hoc analyses, BTBR mice shown significantly lower percentage of SNI when compared to C57 mice ($F_{(1,12)} = 25.74$, $P < 0.001$) (Figure 10A). As expected, ST-713 (5 and 10 mg/kg) and DOZ (1 mg/kg, i.p.) significantly enhanced percentage of SNI in BTBR mice when compared with saline-treated BTBR mice, with ($F_{(1,12)} = 16.26$, $P < 0.01$), ($F_{(1,12)} = 23.87$, $P < 0.001$), and ($F_{(1,12)} = 23.51$, $P < 0.001$), respectively (Figure10A). Different from the results that showed for SI, the reference drug CPZ (1.5 mg/kg, i.p.) failed to further worsen the social novelty behaviors (SNI) in BTBR mice when compared with saline-treated BTBR mice, with ($F(1,12) = 0.56$, $P = 0.47$). Additionally, ST-713 (5 mg/kg, i. p.) when co-injected with CPZ (1.5 mg/kg, i.p.), failed to enhance the effects watched with CPZ when administered alone ($F(1,12) = 0.06$, $p = 0.80$), or to increase the improving effects gotten with DOZ when administered alone ($F(1,12) = 0.09$, $p = 0.76$) (Figure 10A). Besides, ST-713 (5 mg)-provided effects on percentage of SNI were completely abrogated by co-administration with the H3R agonist RAM and the anticholinergic SCO, with ($F(1,12) = 7.08$, $P < 0.05$) and ($F(1,12) = 9.67$, $P < 0.01$), respectively (Figure 10B). As equal to the results noticed for SI, H1R antagonist PYR or the H2R antagonist ZOL failed to nullify the ST-713-provided effects on percentage of SNI with ($F_{(1,12)} = 0.39$, $p = 0.54$) and ($F_{(1,12)}$

= 0.17, p = 0.68), respectively (Figure10B). Notably, systemic pretreatments with ST-713 (2.5, 5, and 10 mg/kg), DOZ (1 mg/kg), CPZ (1.5 mg/kg), ST-713(5 mg)+DOZ, and ST-713(5 mg)+CPZ did not change SNI assessed in control C57 mice in NS test (Figure 10A, B, Table 1).

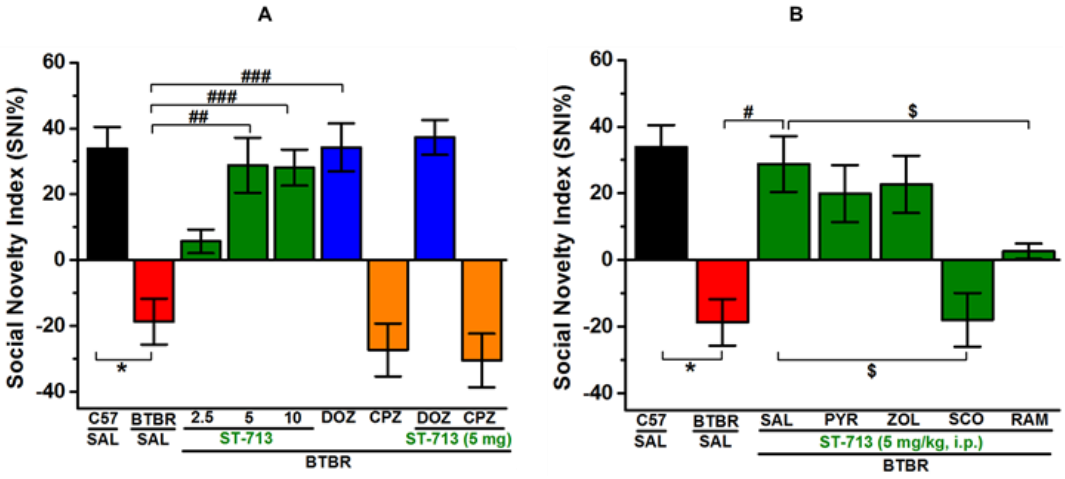


Figure 10: ST-713 alleviated social novelty deficits of BTBR mice in TC test.

After 10 minutes of habituation, male subjects were allowed to explore all chambers for two 10 minutes sessions. C57 and BTBR mice were injected with saline, and BTBR mice were administered with ST-713 (2.5, 5 or 10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), or CPZ (1.5 mg/kg, i.p.) chronically for 21 days. The results obtained were Social Novelty Index (SNI) (Figure 10A), and the effects of chronic (21 days) systemic co-injection of PYR (10 mg/kg, i.p.), ZOL (10 mg/kg, i.p.), SCO (0.3 mg/kg, i.p.), and RAM (10 mg/kg, i.p.) on the ST-713-(5 mg)-provided improvement of SNI (Figure10B). Figures show mean ± SEM (n=7). $^{*}P<0.05$ vs. SI of saline-treated C57 mice. $^{\#}P<0.05$ vs. SI of saline-treated BTBR mice. $^{\$}P<0.05$ vs. ST-713(5 mg)-treated BTBR mice.

3.3 Effects of ST-713 on Mitigating Stereotyped Repetitive Behavior of BTBR Mice in MB Test

The improving effect of chronic ST-713 treatment on repetitive behavior in BTBR mice was evaluated by MB test (Figure11 A, B and Table 2) and NS test (Figure 11C, D). The results of two-way ANOVA appeared that there was a critical main effect for strain [$F_{(1,80)} = 54.92$, $P < 0.01$], treatment [$F_{(7,80)} = 3.98$, $P < 0.01$] and for the strain × treatment interaction [$F_{(7,80)} = 2.97$, $P < 0.01$] (Table 1). The results of the percentage of buried marbles were expected to be increased in saline-treated BTBR mice when comparing with saline-treated C57 mice ($F_{(1,12)} = 14.76$, $P < 0.01$)

(Figure 9A, Table 3). Chronic systemic administration of ST-713 (5 and 10 mg/kg, i.p.) and DOZ (1 mg/kg) significantly counteracted the ameliorated percentage of buried marbles in BTBR mice, with ($F_{(1,12)} = 6.29$, $P < 0.05$), ($F_{(1,12)} = 6.86$, $P < 0.05$), and ($F_{(1,12)} = 11.63$, $P < 0.01$) respectively. However, the BTBR mice administered with lower dosage of ST-713 (2.5 mg/kg) or CPZ did not display any enhancement within the MB test, with ($F_{(1,12)} = 0.01$, p = 0.93) and ($F(1,12) = 0.03$, p = 0.73), respectively (Figure 11A, Table 2). Analyzing of the data characterizing the number of marbles buried yielded essentially the same results (Table 3). In addition, ST-713 (5 mg/kg) associated reduction on the percentage of marbles buried was totally switched by co-administration with the H3R agonist Ram and the anticholinergic SCO, with ($F_{(1,12)} = 13.20$, $P < 0.05$) and ($F_{(1,12)} = 6.18$, $P < 0.05$), respectively (Figure 11B, Table 2). Comparable to the results watched for SI, H1R antagonist PYR or the H2R antagonist ZOL failed to nullify the ST-713-provided decrease on percentage of buried marbles, with ($F_{(1,12)} = 0.00$, p = 0.98) and ($F_{(1,12)} = 0.03$, p = 0.87), respectively (Figure 9B, Table 2). Moreover, systemic pretreatments with ST-713 (2.5, 5, and 10 mg/kg), DOZ (1 mg/kg), CPZ (1.5 mg/kg), ST-713 (5 mg)+DOZ, and ST-713(5 mg)+CPZ did not affect burying behaviors that noticed in control C57 mice in MB test (Table 2).

Table 2: ST-713 treatment mitigates marble burying in BTBR mice

Treatment group	**Number of marbles buried** (mean ± SEM)
SAL (C57)	3.50 ± 0.51
SAL (BTBR)	9.00 ± 1.20**
ST-713 (2.5 mg/kg)/BTBR	8.83 ± 1.14
ST-713 (5 mg/kg)/BTBR	4.83 ± 0.93#
ST-713 (10 mg/kg)/BTBR	5.00 ± 0.70#
DOZ (1 mg/kg)/BTBR	3.83 ± 0.68##
CPZ (1.5 mg/kg)/BTBR	8.33 ± 0.99
ST-713 (5 mg) + DOZ	8.16 ± 0.76
ST-713 (5 mg) + CPZ	7.66 ± 0.65
ST-713 (5 mg) + PYR	4.33 ± 0.38
ST-713 (5 mg) + ZOL	5.17 ± 0.44
ST-713 (5 mg) + SCO	9.17 ± 1.09$
ST-713 (5 mg) + RAM	8.83 ± 1.50$

Mean (± SEM) marbles buried (n = 7). MB test was carried out in BTBR and C57 mice. Each mouse received an i.p. injection ST-713 (2.5, 5, or 10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), or CPZ (1.5 mg/kg, i.p.) chronically for 21 days. BTBR mice buried significantly more marbles compared to that of C57 mice. ST-713 (5 and 10 mg/kg, i.p.) and DOZ (1 mg/kg, i.p.) attenuated the increased number of buried marbles. Effects of chronic (21 days) systemic co-injection of PYR (10 mg/kg, i.p.), ZOL (10 mg/kg, i.p.), SCO (0.3 mg/kg, i.p.), and RAM (10 mg/ kg, i.p.) on the ST-713-(5 mg)-provided attenuation of increased number of marbles buried were evaluated in MBT. $^{**}P < 0.001$ vs. saline-treated C57 mice.

$^{\#}P < 0.05$ vs. saline-treated BTBR mice. $^{\#\#}P < 0.01$ vs. saline-treated BTBR mice. $^{\$}P < 0.05$ vs. ST-713-(5 mg)-treated BTBR mice.

3.4 Effects of ST-713 on Improving Obsessive-Compulsive Features of BTBR Mice in NS Test

The opposing effect of chronic systemic administration of ST-713 on the increased percentage of shredded nestlet in BTBR mice appears in (Figure 11C, D). The outcomes of two-way ANOVA appeared that there was a critical main impact for strain [$F_{(1,80)} = 23.25$, $P < 0.01$], treatment [$F_{(7,80)} = 4.65$, $P < 0.01$] additionally for the strain × treatment interaction [$F_{(7,80)} = 4.54$, $P < 0.01$] (Table 1). Notably, saline-treated BTBR mice have shredded significantly ($F_{(1,12)} = 28.88$, $P < 0.01$), and its more nestlet in comparison with saline-treated C57 mice. Treatment with ST-713 on high doses (5,

10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), and CPZ (1.5 mg/kg, i.p.) significantly ($P < 0.05$) prevent the increase of compulsive behavior in nestlet shredding for BTBR mice in NS test, with ($F_{(1,12)} = 13.20$, $P < 0.05$; $F_{(1,12)} = 13.20$, $P < 0.05$; $F_{(1,12)} = 13.20$, $P < 0.05$; and $F_{(1,12)} = 13.20$, $P < 0.05$), whereas low dose of ST-713 (2.5 mg/kg, i.p.) failed to alter the noticed percentage of nestlet shredded ($F_{(1,12)} = 0.00$, p = 0.92) (Figure 11C). However, ST-713 (5 mg/kg, i.p), co-injected with the H1R antagonist PYR or the H2R antagonist ZOL was not able to prevent the ST-713 (5mg)-provided effects on repetitive/compulsive behavior of tested BTBR mice, (all P values > 0.05) (Figure 11D).

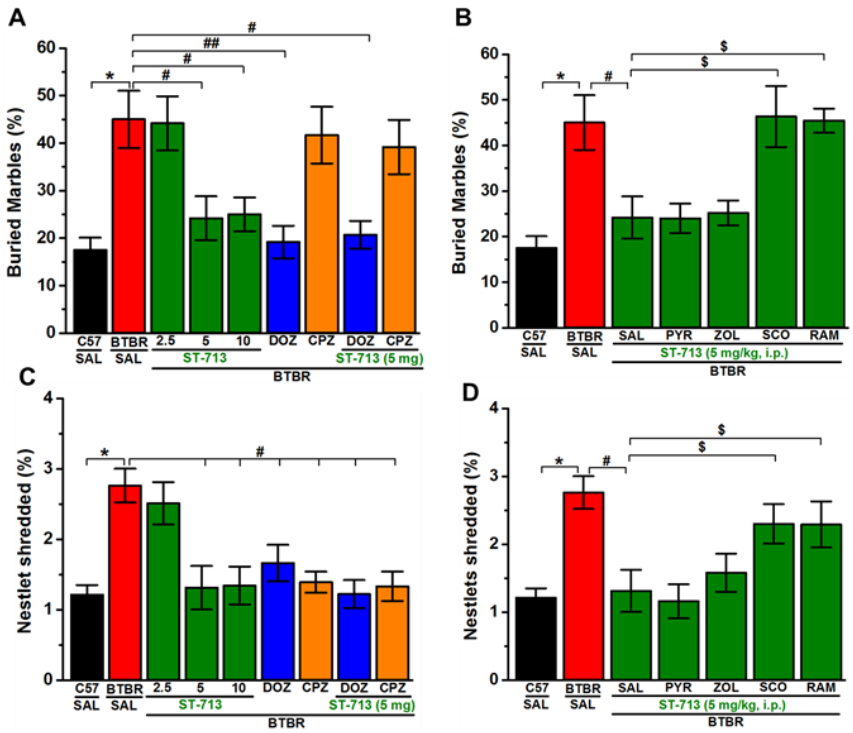


Figure 11: ST-713 attenuated stereotyped repetitive and compulsive behavior of BTBR mice in MB and NS tests.

Stereotyped repetitive paradigms were assessed in MB test (Figure 11 A, B) and NS test (Figure 11 C& 11D) in a 30-minute testing session each. BTBR mice demonstrated elevated stereotyped, repetitive and compulsive behaviors that were significantly increased compared to C57. ST-713 (2.5, 5, or 10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), or CPZ (1.5 mg/kg, i.p.) were administered chronically for 21 days (Figure11A&C). Effects of chronic (21 days) systemic co-injection of PYR (10 mg/kg, i.p.), ZOL (10 mg/kg, i.p.), SCO (0.3 mg/kg, i.p.), and RAM (10 mg/kg, i.p.) on the ST-713-(5 mg)-provided attenuation of stereotyped repetitive and compulsive behaviors of BTBR mice were evaluated in MB test (Figure 11B) and NS test (Figure 11D). Figures show mean ± SEM (n = 6). $^{*}P<0.05$ vs. Saline-treated C57 mice. $^{\#}P<0.05$ vs. Saline-treated BTBR mice. $^{\$}P<0.05$ vs. ST-713-(5 mg)-treated BTBR mice.

Additionally, ST-713 (5 mg/kg, i.p.) co-injected with H3R agonist RAM and the anticholinergic compound SCO, entirely reversed the ST-713 mediated beneficial effects (all *P* values < 0.05) (Figure 11D). However, systemic pretreatments with ST-713 (2.5, 5, and 10 mg/kg), DOZ (1 mg/kg), CPZ (1.5 mg/kg), ST-713(5 mg) +DOZ, and ST-713 (5 mg) +CPZ did not alter shredding behaviors measured in control C57 mice in NS test (Figure 11 C, D, Table 1).

3.5 Effects of ST-713 on Modulating Anxiety and Restoring Locomotor Activity in BTBR Mice in OF Test

Open field test was implemented to evaluate anxiety-associated behavior and locomotor activity of treated mice. The two-way ANOVA presents the total distance travelled and time consumed within the center of arena by the C57 and BTBR mice displayed a significant main effect for strain [$F_{(1,36)} = 45.58$, $P < 0.01$], [$F_{(1,36)} = 37.26$, $P < 0.01$], whereas there was no critical difference between the treatment [$F_{(5,36)} = 0.26$, p = 0.93], [$F_{(5,36)} = 1.15$, p = 0.35] additionally for the strain × treatment interaction [$F_{(5,36)} = 0.13$, p = 0.98], [$F_{(5,36)} = 1.15$, p = 0.35] respectively (Table 3). As noticed in Table 3, BTBR mice appeared significant increase in the distance traveled ($F_{(1,12)} = 20.13$, $P < 0.01$) and time spent within the center of arena ($F_{(1,12)} = 8.67$, $P < 0.05$) when compared with saline-treated C57 mice. In addition to that, BTBR mice shown significant decrease in time spent within the periphery ($F_{(1,12)} = 13.20$, $P < 0.05$) when compared with saline-treated C57 mice. Chronic treatment of ST-713 different dose regimens (2.5, 5 and 10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), or CPZ (1.5 mg/kg, i.p.) showed no improvement in hyperactivity observed in BTBR mice This was represented as BTBR saline-injected mice travelled significantly more distance when compared with the saline-treated C57 control group and ST-713 with all the three doses and DOZ (1 mg/kg, i.p.), or CPZ (1.5 mg/kg, i.p.) failed to decrease the total

distance travelled or time spent in the periphery (all $P > 0.05$). Analysis of variance showed that with ST- 713 (2.5, 5 and 10 mg/kg, i.p.) or DOZ (1 mg/kg, i.p.) treated BTBR mice spent significantly less time within the center of the arena, with ($F_{(1,12)} = 4.89$, $P < 0.05$), ($F_{(1,12)} = 5.89$, $P < 0.05$), ($F_{(1,12)} = 11.20$, $P < 0.05$), and ($F_{(1,12)} = 10.20$, $P < 0.05$), respectively (Table 4). However, CPZ (1.5 mg/kg, i.p.) failed to alter the time spent in the center of the arena ($F_{(1,12)} = 0.05$, p = 0.84). Moreover, systemic pretreatments with ST-713 (2.5, 5, and 10 mg/kg), DOZ (1 mg/kg), CPZ (1.5 mg/kg), ST-713(5 mg)+DOZ, and ST-713(5 mg)+CPZ did not alter the behavioral parameters evaluated in control C57 mice in OF test (Table 3).

Table 3: Effects of ST-713, DOZ, and CPZ systemic pretreatment on anxiety levels and locomotor activity in BTBR mice in OF test

Group	**Distance travelled (cm)**	**Time in center (s)**	**Time in periphery (s)**
SAL (C57)	2433.15 ± 264.23	40.40 ± 4.83	571.05 ± 7.33
SAL (BTBR)	4221.00 ± 221.96**	73.75 ± 7.15**	537.25 ± 3.81*
ST-713 (2.5 mg/kg)/BTBR	3698.26 ± 452.80	54.94 ± 1.42$^{\#}$	536.15 ± 7.52
ST-713 (5 mg/kg)/BTBR	3838.63 ± 440.11	53.64 ± 2.90$^{\#}$	535.84 ± 3.13
ST-713 (10 mg/kg)/BTBR	3863.22 ± 521.57	57.20 ± 3.33$^{\#}$	534.69 ± 2.43
DOZ (1 mg/kg)/BTBR	3632.34 ± 298.16	41.11 ± 3.94$^{\#\#}$	536.02 ± 1.70
CPZ (1.5 mg/kg)/BTBR	3888.22 ± 240.95	71.50 ± 5.40	539.50 ± 2.28

BTBR mice illustrated raised impulsive behavior and reduce cognition as well as locomotor activity behaviors that were significantly increased compared to C57 mice. ST-713 (2.5, 5, or 10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), or CPZ (1.5 mg/kg, i.p.) were administered chronically for 21 days. ST-713 (2.5, 5 and 10 mg/kg, i.p.) and DOZ (1 mg/kg, i.p.) attenuated the expanded time spent in the central field but failed to change the increased total distance traveled as well as the time spent within the periphery in BTBR mice within the OF test. Data are expressed as the means ± SEM (n = 4). $^{*}P < 0.05$ vs. C57 mice. $^{**}P < 0.01$ vs. C57 mice. $^{\#}P < 0.05$ vs. saline-treated BTBR mice. $^{\#\#}P < 0.01$ vs. saline-treated BTBR mice.

The effects observed following chronic systemic administration of ST-713 in all behavioral assessments carried out in the project are summarized in Table 4.

Table 4: Effects of ST-713, DOZ, and CPZ systemic pretreatment on behavioral parameters of control C57 mice

Behavioral test		SAL	ST-713 (mg/kg, i.p.)		
			2.5	5	10
Sociability	SI	40 ± 6.28	39.00 ± 5.90	38.00 ± 5.34	40 ± 6.28
Social novelty	SNI	33.86 ± 6.58	31.00 ± 5.42	32.43 ± 4.24	33.86 ± 6.58
Marble burying	Marble buried (%)	17.50 ± 2.57	16.67 ± 2.26	16.00 ± 2.47	16.17 ± 2.49
Nestlet shredding	Nestlet shredded (%)	1.31 ± 0.12	1.29 ± 0.12	1.25 ± 0.14	1.27 ± 0.13
Open field	Time in Center (s)	30.30 ± 9.70	31.52 ± 7.67	29.50 ± 5.30	28.25 ± 9.41
	Time in periphery (s)	571.05 ± 7.33	567.00 ± 10.03	564.07 ± 14.98	574.25 ± 8.92
	Total distance (cm)	2433.15 ± 264.23	2488.13 ± 234.97	2222.41 ± 238.61	2369.94 ± 294.73
Behavioral test		**DOZ (1 mg/kg, i.p.)**	**CPZ (1.5 mg/kg, i.p.)**	**ST-713 (5 mg/kg)**	
				DOZ	**CPZ**
Sociability	SI	42 ± 5.69	37 ±5.26	44 ± 6.41	36 ± 3.85
Social novelty	SNI	34.57 ± 6.22	31.86 ± 5.84	35.00 ± 5.97	31.86 ± 5.84
Marble burying	Marble buried (%)	15.83 ± 2.27	18.17 ± 2.34	15.67 ± 2.39	17.67 ±2.65
Nestlet shredding	Nestlet shredded (%)	1.25 ± 0.11	1.35 ± 0.12	1.25 ± 0.11	1.35 ± 0.10
Open field	Time in Center (s)	33.06 ± 6.22	26.75 ± 7.14	--	--
	Time in periphery (s)	574.00 ± 10.16	577.75 ± 11.46	--	--
	Total distance (cm)	2220.86 ± 318.27	2481.26 ± 271.26	--	--

Data are expressed as the means ± SEM (n=7). There was no significant difference between saline, ST713 (2.5, 5 and 10 mg), DOZ and CPZ treated C57 control mice.

3.6 Biochemical Assessments

3.6.1 Effects of ST-713 Pretreatment on the Brain Levels of Proinflammatory Cytokines in BTBR Mice

The effects of ST-713 on the levels of proinflammatory cytokines IL-1β, IL-6 and TNF-α in the hippocampal and cerebellar tissues of BTBR mice were assessed (Table 5). The results revealed a significant increase of TNF-α, IL-1β, and IL-6 in hippocampus and cerebellum of BTBR mice compared to control B6 mice (Table 5) (all $P < 0.05$). Chronic systemic administration of ST-713 (2.5, 5, or 10 mg/kg, i.p.) or CPZ (1.5 mg/kg, i.p.) significantly mitigated the increase in the levels of these proinflammatory cytokines in BTBR mice (all $P < 0.01$) (Table 5). Moreover, chronic systemic co-administration of RAM (10 mg/kg, i.p.) reversed the mitigating effects of ST-713 (5 mg/kg, i.p.) against elevation of TNF-α, IL-1β, and IL-6 levels (all $P < 0.05$) in both hippocampal and cerebellar tissues.

3.6.2 Effects of ST-713 Pretreatment on the Brain Levels of Neurotransmitters Histamine and Dopamine in BTBR Mice

The observed results showed a significant decrease of brain levels of HA and DA in hippocampus and cerebellum of BTBR mice as compared to control B6 mice (all $P< 0.05$) (Table 5). However, chronic pretreatment with ST-713 (5 mg/kg) significantly modulated the average of the hippocampal levels of HA and DA with [$F_{(1,10)} = 36.08$; $P < 0.001$], [$F_{(1,10)} = 7.48$; $P < 0.05$], respectively. Also, ST-713 (5 mg/kg) significantly modulated the average of the cerebellar levels of HA in the cerebellum with [$F_{(1,10)} = 21.31$; $P < 0.001$],but failed to alter the levels of DA [$F_{(1,10)} = 0.09$; p=0.76] in cerebellum of treated BTBR mice (Table 5). Contrary, CPZ (1.5 mg/kg) failed to alter the disturbed levels of HA and DA in both assessed brain regions of BTBR mice. Additionally, statistical analyses of observed results showed that the ST-713(5`mg)-

provided enhancements of HA and DA brain levels of BTBR mice were reversed following systemic co-administration with RAM (10 mg/kg, i.p.) (all *P* values <0.05) (Table 5).

Table 5: Brain levels of proinflammatory cytokines and neurotransmitters histamine and dopamine in BTBR mice

Treatment Group	**Proinflammatory cytokines**		**Neurotransmitters**		
	Hippocampus				
B6 (Ctrl) (VEH)	96.98 ± 9.10	21.17 ± 2.41	49.92 ± 9.68	0.49 ± 0.04	54.87 ± 0.87
BTBR (Ctrl) (VEH)	205.87 ± 17.83*	161.21 ± 12.18*	181.45 ± 14.07*	0.37 ± 0.03*	47.75 ± 2.19**
BTBR (ST-713, 2.5 mg/kg)	116.45 ± 15.35$^{\#\#}$	98.36 ± 2.90$^{\#\#}$	69.36 ± 7.08$^{\#\#}$	ND	ND
BTBR (ST-713, 5 mg/kg)	105.26 ± 10.08$^{\#\#}$	81.35 ± 8.26$^{\#\#}$	70.32 ± 14.63$^{\#\#}$	0.78 ± 0.06$^{\#\#\#}$	54.48 ± 0.52$^{\#}$
BTBR (ST-713, 10 mg/kg)	119.20 ± 10.42$^{\#\#}$	82.90 ± 9.72$^{\#\#}$	67.95 ± 13.17$^{\#\#}$	ND	ND
BTBR (CPZ, 1.5 mg/kg)	47.05 ± 4.65$^{\#\#}$	27.61 ± 4.19$^{\#\#}$	56.14 ± 3.60$^{\#\#}$	0.41 ± 0.06	46.56 ± 1.44
BTBR (ST-713, 5 mg)+ RAM	178.88 ± 22.09$^{\$}$	137.30 ± 4.71$^{\$}$	150.04 ± 10.24$^{\$}$	0.40 ± 0.02$^{\$}$	46.58 ± 2.23$^{\$}$
	Cerebellum				
B6 (Ctrl) (VEH)	303.15 ± 7.20	241.69 ± 11.85	48.29 ± 6.03	0.48 ± 0.04	43.47 ± 3.62
BTBR (Ctrl) (VEH)	413.47 ± 12.89*	385.63 ± 31.18*	202.73 ± 10.74*	0.37 ± 0.00*	42.05 ± 0.64
BTBR (ST-713, 2.5 mg/kg)	253.37 ± 3.36$^{\#\#}$	182.64 ± 14.84$^{\#\#}$	92.33 ± 13.81$^{\#\#}$	ND	ND
BTBR (ST-713, 5 mg/kg)	263.90 ± 5.52$^{\#\#}$	205.85 ± 16.74$^{\#\#}$	82.54 ± 4.95$^{\#\#}$	0.47 ± 0.02$^{\#\#\#}$	42.55 ± 1.33
BTBR (ST-713, 10 mg/kg)	268.67 ± 5.25$^{\#\#}$	229.50 ± 19.14$^{\#\#}$	119.30 ± 10.92$^{\#\#}$	ND	ND
BTBR (CPZ, 1.5 mg/kg)	267.70 ± 8.15$^{\#\#}$	233.65 ± 21.62$^{\#\#}$	51.04 ± 9.53$^{\#\#}$	0.39 ± 0.01	42.64 ± 3.02
BTBR (ST-713, 5 mg)+ RAM	336.04 ± 16.57$^{\$}$	378.16 ± 25.34$^{\$}$	142.12 ± 7.95$^{\$}$	0.34 ± 0.01$^{\$}$	42.34 ± 0.53

Modulated Tumor Necrosis Factor (TNF-α, pg/mg protein), interleukine (IL-1β, pg/mg protein), interleukine (IL-6 (pg/mg protein), histamine (HA, ng/mg protein), and dopamine (DA, ng/mg protein) were assessed. BTBR mice showed a significant increase in TNF-α, IL-1β, and IL-6, but significant decrease in HA and DA in hippocampus and cerebellum compared to B6 mice. ST-713 (2.5, 5 or 10 mg/kg, i.p.) or CPZ (1.5 mg/kg, i.p.) were administered subchronically for 21 days in BTBR mice. ST-713 or CPZ significantly decreased TNF-α, IL-1β, and IL-6, and ST-713 (5 mg/kg) significantly modulated disturbed brain levels of histamine (HA) and dopamine (DA). Effects of subchronic (21 days) systemic co-injection RAM (10 mg/kg, i.p.) on ST-713 (5 mg)-provided modulation of proinflammatory cytokines levels were assessed. Data are expressed as the mean ± SEM (n=6). $^{*}P <$ 0.05 vs. VEH-treated B6 mice. $^{**}P < 0.01$ vs. VEH-treated B6 mice. $^{\#\#}P < 0.01$ vs. BTBR mice. $^{\#\#\#}P <$ 0.001 vs. VEH-treated BTBR mice. $^{\$}P < 0.05$ vs. ST-713 (5 mg)-treated BTBR mice. ND; not determined.

Chapter 4: Discussion

Antagonists/inverse agonists of H3R are considered as promising therapy for a number of CNS diseases, including Alzheimer's disease, epilepsy, narcolepsy, and attention deficit hyperactivity disorders (Baronio et al., 2015; Wright et al., 2017).The current investigations aimed to evaluate the in-vivo effects of a multiple-active H3R and D2R/D3R ligand ST-713 on the balancing effects of brain HA, DA, and ACh transmissions in BTBR mice. The intra peritoneal injection of different doses of ST-713 (2.5, 5 and 10 mg/kg) was tolerated well by the BTBR mice, and there was no mortality or macroscopical toxicity observed in this study. H3R antagonists have the ability to cross blood brain barrier to enter in to the neuronal system (Mochizuki et al., 1996). Being a H3R antagonist ST713 may be able to cross the blood brain barrier and block the H3 receptor activities by its antagonistic properties. Male BTBR mice as idiopathic mice display ASD were utilized within the current experiments. Male mice were selected to implement in the tests, as ASD was found to influence females less regularly than males, and several sex-differential genetic and hormonal variables may affect the prevalence of ASD (Werling & Geschwind, 2013; Witkin & Nelson, 2004). Also, involving female mice with the hormonal variation and to the very well-known heterogeneity of ASD will properly influence the severity of the symptoms and thus will affect the level of noticed enhancement provided by test compound ST-713. To avoid all these confounding factors, we chose to test our multiple-active compound on male BTBR mice. The results demonstrated that ST-713 significantly enhanced social deficits of BTBR mice which was shown by the critical improvement in sociability as well as social novelty behaviors given by a dose of 5 mg/kg. However, ST-713 at the dosage level of 2.5 mg/kg failed to counteract the social impairment in BTBR mice.

On the other hand, ST-713 (10 mg/kg) improved the social novelty index in BTBR mice, but with significantly lower effects provided than those noticed with 5 mg/kg of the same compound. The new findings regarding the dose-dependency agree with previous results of a memory-enhancing effect noticed for dual-active compounds targeting H3R and acetylcholine esterase inhibitor (AChEI), where the effect of lower dosage (1.25 mg/kg) was found to be significantly higher when compared to the higher dosages 2.5 and 5 mg/kg (Khan et al., 2016; Sadek et al., 2016b). Interestingly, and in further abrogative studies, the improved social deficit effect of ST-713 (5mg/kg) was reversed following co-administration with the CNS-oenetrant H3R agonist RAM or the antimuscarinic compound SCO. These observations endorsed the hypothesis that the enhancing effects of ST-713 on social parameters were due to the release of several neurotransmitters, such as HA and ACh, which might be based on the inhibition of the H3R-auto- and hetero-receptors, respectively. Notably, the effect of ST-713, at its optimum dose 5 mg/kg was not reversed by co-administration with the centrally acting H1R antagonist PYR nor the H2R antagonist ZOL. Accordingly, these results support the hypothesis that antagonism on H3Rs, but not H1R or H2R, was responsible for the noticed in vivo results. On the other hand, the reference drug DOZ significantly improved sociability as well as social novelty parameters of tested BTBR mice, comprehending our hypothesis that ACh is significantly involved in the effects observed following systemic administration with ST-713.

Recent studies reported that dysfunction of dopaminergic neurotransmitter system and low levels of brain DA are shown in BTBR mice (Eissa, Jayaprakash, et al., 2018; Squillace et al., 2014). Additionally, DA has been linked with a strong relationship to social behavior, attentional abilities, perception, and motor activity, whereas development's abnormalities in these regions have all been connected to ASD as well

(Pavăl, 2017). According to our current observations, the worsening outcomes that were noticed with the antipsychotic CPZ on sociability features were counteracted by co-administration with ST-713, which also chemically incorporates the partial structure of CPZ, demonstrating that ST-713 appear to have balanced the dopaminergic neurotransmission system in a useful way, or its procognitive effect as an H3R antagonist counteracted the negative effects noticed with CPZ when given alone, or it improved the release of neurotransmitters such as ACh, a neurotransmitter of a significant role in the context of witnessed behavioral enhancements. Moreover, ST-713 showed the comparable trend to improve the behavioral deficits of tested BTBR mice within the repetitive and compulsive-like performances. As expected, and in the present series of experiments, ST-713 (5 mg/kg) treated mice in MB and NS tests showed reduced number of marble burying behavior and nestlet shredding than saline alone treated BTBR mice. Also, the improvements witnessed with ST-713 in MB and NS tests were totally abrogated by H3R agonist (RAM) and SCO (muscarinic antagonist), signifying that cholinergic as well as histaminergic neurotransmissions are also included in the observed enhancing effects on repetitive/compulsive-like behaviors of tested animals. The possible mechanism behind these suggestions could be back rooted to its nature of multiple-active H3R and D2/D3R antagonist, which is able to modulate the release of several neurotransmitters including HA, DA, ACh and 5-HT, and of most importance in several particular brain regions. Notably, the reference drug CPZ (either alone or in combination with ST-713 5 mg/kg) reduced the repetitive behavior in NS test (but not in MB test), as comprehended by the observed reduction of shredding behaviors, indicating, suggesting the role of DA in modulating repetitive behaviors in this test model.

The OF test generally allows researchers to assess the behavior levels of animals from general locomotor activity to anxiety-related emotional behaviors (Carola et al., 2002). Therefore, the distance travelled by assessed animals usually reflects the locomotor activity of the tested animal and the time spent in the central provides parameters about anxiety levels of animals. The results observed in our OF test clearly showed that BTBR mice were hyperactive, witnessed with significantly more distance travelled than the control B6 mice. Moreover, time spent in the central for BTBR mice was significantly higher than control mice, reflecting abnormal anxiety levels and, also, impulsive behaviors of tested BTBR mice (Moy et al., 2007; Silverman et al., 2010). Interestingly, ST-713 with all dosages was able to improve the abnormal levels of anxiety in OF test. Thus, our discoveries proposed that a dysregulation between HA, ACh, and DA levels might play a role in irregular levels of anxiety and impulsive behavior noticed in tested BTBR mice. Additionally, DOZ and all doses of ST-713 completely enhanced the abnormal anxiety levels of treated BTBR mice in OF test. However, reference drug CPZ failed to modify the abnormal anxiety-like features of tested animals. Interestingly, ST-713 (2.5, 5, and 10 mg/kg) and reference drugs CPZ, as well as DOZ, failed to restore hyperactivity (increase in total distance travelled or time spent in the periphery) observed by BTBR mice, demonstrating that the compound as well as references drugs failed to have an effect on locomotor activity. This result is essential to avoid any confounding factors, such as alteration of locomotor activity, which may give false-positive findings in regards to the ST-713-provided improvements on sociability, social novelty, repetitive, or compulsive/aggressive features.

Previous preclinical research reports proposed that several proinflammatory cytokines, including TNF-α, IL-1β, and IL-6 are escalated in the autistic brains (Eissa et al., 2019;

Vargas et al., 2005; Venkatachalam et al., 2021). Consistent with these results, our observations showed that the levels of TNF-α, IL-1β, and IL-6 were significantly increased in the hippocampus, as well as the cerebellum of BTBR mice compared with age-matched control B6 mice. Chronic systemic administration of ST-713 at all tested three doses significantly reduced the escalated levels of the assessed proinflammatory cytokines in BTBR mice. Moreover, the reference drug CPZ (1.5 mg/kg, i.p.) showed similar significant modulating effects on the tested proinflammatory cytokines. Interestingly and when co-administered with ST-713, the CNS-penetrant H3R agonist reversed the ST713-induced protective effects against increased levels of proinflammatory cytokines (Table 5), demonstrating the involvement of brain HA in facilitating the neuroprotective effects of ST-713 in BTBR mice with ASD-like features. In another series of experiments, the levels of HA and DA were found to be significantly reduced in hippocampus and cerebellum of BTBR mice and as compared to control B6 mice. However, chronic systemic administration with ST-713 (5 mg, being the dose with most promising enhancing effects on behavioral parameters assessed in BTBR mice) significantly modulated the brain levels of HA and DA in hippocampus and cerebellum of tested BTBR mice, and systemic co-administration with H3R agonist RAM (10 mg/kg) nullified the ST-713-provided effects. Notably, the reference drug CPZ failed to alter the hippocampal or cerebellar levels of measured neurotransmitters. The latter results are crucial and reveal the involvement of H3Rs in the observed effects for ST-713. Also, the results shed light on the multiple-active property of ST-713 in simultaneous modulation of brain neurotransmitters, namely HA, ACh, and DA, which are involved in the pathophysiology of ASD-like features of BTBR mice, and the proposed multiple mechanisms are discussed and illustrated in Figure 12.

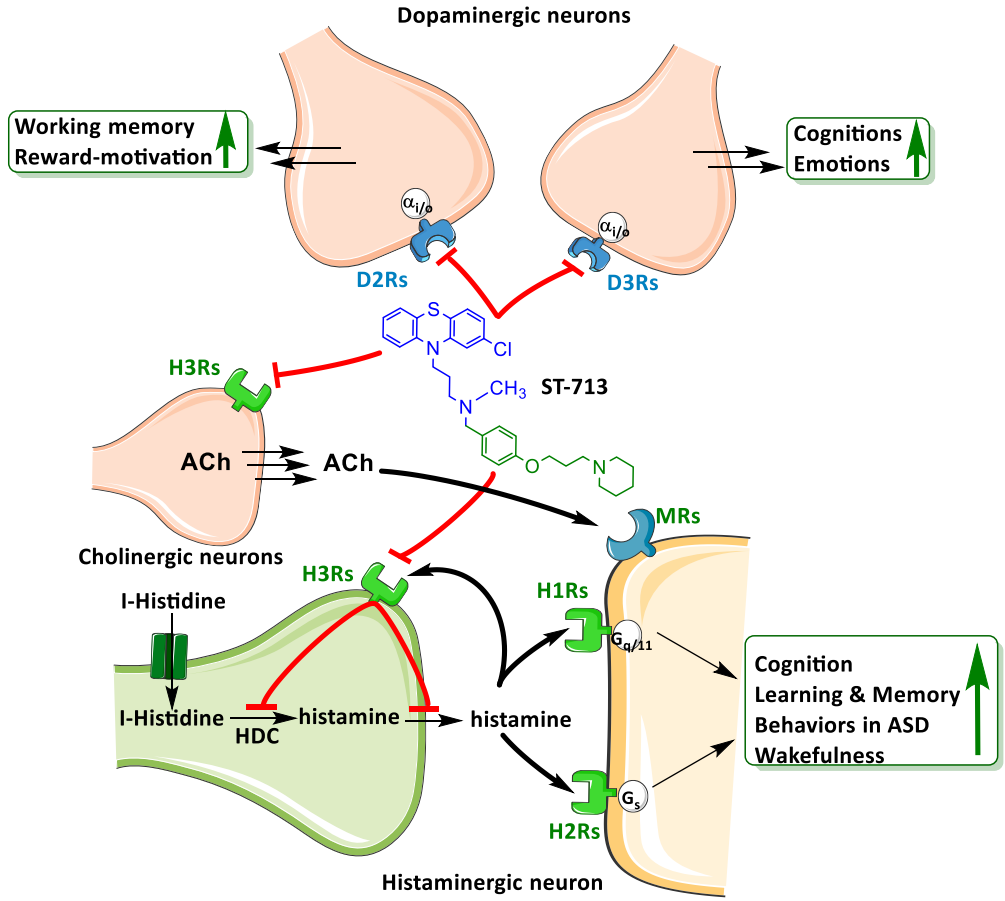


Figure 12: Proposed modes of actions by the multiple-active test compound ST-713 by blocking the histamine H3R acting auto- and heteroreceptor and the dopamine D2/D3Rs.

Chapter 5: Conclusion

The observed results demonstrate that simultaneous targeting of the CNS histaminergic and dopaminergic neurotransmissions by ST-713 is highly beneficial for palliation of several ASD-like features, namely ASD-like social deficits and repetitive/compulsive behaviors. Moreover, the ST-713 mitigated the increased levels of several hippocampal as well as cerebellar proinflammatory cytokines of tested BTBR mice. Also, multiple-targeting approach with ST-713 reduced the increased levels of several proinflammatory cytokines, and brain levels of HA and DA in the hippocampus as well as cerebellum of tested BTBR mice. However, further *in-vivo* assessments in BTBR mice following systemic treatments of ST-713 as well as reference drugs are still obligatory to elucidate whether multiple-targeting compounds, e.g., ST-713, is superior to a standard H3R antagonists/inverse agonists, e.g., pitolisant or ciproxifan, or antipsychotics, such as CPZ, when applied alone. In addition, and to be able to generalize the current conclusions, additional behavioral tests with ST-713 are required in more types of ASD mutant mice, and by extension, in a variety of other neurodevelopmental disease mouse models.

To sum up, ST-713 holds promise for its potential use to mitigate autistic-like behaviors in a genotype mouse model of ASD and the results should lead to additional investigations into the possible applicability of this class in the modulation of autistic features, and to exclude any anticipated off-target effects.

5.1 Future Direction and Limitations of the Study

Future studies are crucial to know which signaling pathways and HRs are included in this histamine-provided neuroprotective role. All of our results opened a possible novel therapeutic window for controlling the core symptoms of ASD.

However, the examination of neural circuits involved with the histaminergic and cholinergic neurotransmission systems is a critical objective for future research to figure out the pharmacological mechanisms underlying the observed effects.

Also, for the limitation of our study, we need to investigate which signaling pathways are involved in this histamine effect. Also, we should know which histamine receptors exactly are included in all of these actions. Moreover, we should try to do our tests in other autistics mouse models.

References

Alachkar, A., Lotfy, M., Adeghate, E., Łażewska, D., Kieć-Kononowicz, K., & Sadek, B. (2021). Ameliorating effects of histamine H3 receptor antagonist E177 on acute pentylenetetrazole-induced memory impairments in rats. *Behavioural Brain Research*, *405*, 113193. https://doi.org/10.1016/j.bbr.2021.113193

Al-Haddad, B. J. S., Jacobsson, B., Chabra, S., Modzelewska, D., Olson, E. M., Bernier, R., Enquobahrie, D. A., Hagberg, H., Östling, S., Rajagopal, L., Adams Waldorf, K. M., & Sengpiel, V. (2019). Long-term Risk of Neuropsychiatric Disease After Exposure to Infection In Utero. *JAMA Psychiatry*, *76*(6), 594–602. https://doi.org/10.1001/jamapsychiatry.2019.0029

Angoa-Pérez, M., Kane, M. J., Briggs, D. I., Francescutti, D. M., & Kuhn, D. M. (2013). Marble Burying and Nestlet Shredding as Tests of Repetitive, Compulsive-like Behaviors in Mice. *JoVE (Journal of Visualized Experiments)*, *82*, e50978. https://doi.org/10.3791/50978

Baan, M., Krentz, K. J., Fontaine, D. A., & Davis, D. B. (2016). Successful in vitro fertilization and generation of transgenics in Black and Tan Brachyury (BTBR) mice. *Transgenic Research*, *25*(6), 847–854. https://doi.org/10.1007/s11248-016-9974-0

Baronio, D., Castro, K., Gonchoroski, T., Melo, G. M. de, Nunes, G. D. F., Bambini-Junior, V., Gottfried, C., & Riesgo, R. (2015). Effects of an H3R Antagonist on the Animal Model of Autism Induced by Prenatal Exposure to Valproic Acid. *PLOS ONE*, *10*(1), e0116363. https://doi.org/10.1371/journal.pone.0116363

Bear, M. F., Connors, B. W., & Paradiso, M. A. (2007). *Neuroscience: Exploring the brain*. Lippincott Williams & Wilkins.

Berridge, K. C., Aldridge, J. W., Houchard, K. R., & Zhuang, X. (2005). Sequential super-stereotypy of an instinctive fixed action pattern in hyper-dopaminergic mutant mice: A model of obsessive compulsive disorder and Tourette's. *BMC Biology*, *3*(1), 4. https://doi.org/10.1186/1741-7007-3-4

Biber, K., Neumann, H., Inoue, K., & Boddeke, H. W. G. M. (2007). Neuronal "On" and "Off" signals control microglia. *Trends in Neurosciences*, *30*(11), 596–602. https://doi.org/10.1016/j.tins.2007.08.007

Bishara, D. (2010). Once-monthly paliperidone injection for the treatment of schizophrenia. *Neuropsychiatric Disease and Treatment*, *6*, 561–572. https://doi.org/10.2147/NDT.S8505

Cangioli, I., Baldi, E., Mannaioni, P. F., Bucherelli, C., Blandina, P., & Passani, M. B. (2002). Activation of histaminergic H $_3$ receptors in the rat basolateral amygdala improves expression of fear memory and enhances acetylcholine release: Facilitation of memory and ACh release by histamine. *European Journal of Neuroscience*, *16*(3), 521–528. https://doi.org/10.1046/j.1460-9568.2002.02092.x

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., & Renzi, P. (2002). Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behavioural Brain Research*, *134*(1–2), 49–57. https://doi.org/10.1016/s0166-4328(01)00452-1

Chevallier, C., Kohls, G., Troiani, V., Brodkin, E. S., & Schultz, R. T. (2012). The Social Motivation Theory of Autism. *Trends in Cognitive Sciences*, *16*(4), 231–239. https://doi.org/10.1016/j.tics.2012.02.007

Choudhury, P. R., Lahiri, S., & Rajamma, U. (2012). Glutamate mediated signaling in the pathophysiology of autism spectrum disorders. *Pharmacology, Biochemistry, and Behavior*, *100*(4), 841–849. https://doi.org/10.1016/j.pbb.2011.06.023

Croen, L. A., Zerbo, O., Qian, Y., Massolo, M. L., Rich, S., Sidney, S., & Kripke, C. (2015). The health status of adults on the autism spectrum. *Autism: The International Journal of Research and Practice*, *19*(7), 814–823. https://doi.org/10.1177/1362361315577517

Damiano, C. R., Aloi, J., Treadway, M., Bodfish, J. W., & Dichter, G. S. (2012). Adults with autism spectrum disorders exhibit decreased sensitivity to reward parameters when making effort-based decisions. *Journal of Neurodevelopmental Disorders*, *4*(1), 13. https://doi.org/10.1186/1866-1955-4-13

Delorme, R., Ey, E., Toro, R., Leboyer, M., Gillberg, C., & Bourgeron, T. (2013). Progress toward treatments for synaptic defects in autism. *Nature Medicine*, *19*(6), 685–694. https://doi.org/10.1038/nm.3193

DiCarlo, G. E., Aguilar, J. I., Matthies, H. J., Harrison, F. E., Bundschuh, K. E., West, A., Hashemi, P., Herborg, F., Rickhag, M., Chen, H., Gether, U., Wallace, M. T., & Galli, A. (2019). Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors. *The Journal of Clinical Investigation*, *129*(8), 3407–3419. https://doi.org/10.1172/JCI127411

Dichter, G. S., Felder, J. N., Green, S. R., Rittenberg, A. M., Sasson, N. J., & Bodfish, J. W. (2012). Reward circuitry function in autism spectrum disorders. *Social Cognitive and Affective Neuroscience*, *7*(2), 160–172. https://doi.org/10.1093/scan/nsq095

Dong, H., Zhang, X., & Qian, Y. (2014). Mast Cells and Neuroinflammation. *Medical Science Monitor Basic Research*, *20*, 200–206. https://doi.org/10.12659/MSMBR.893093

Eissa, N., Al-Houqani, M., Sadeq, A., Ojha, S. K., Sasse, A., & Sadek, B. (2018). Current Enlightenment About Etiology and Pharmacological Treatment of Autism Spectrum Disorder. *Frontiers in Neuroscience*, *12*, 304. https://doi.org/10.3389/fnins.2018.00304

Eissa, N., Azimullah, S., Jayaprakash, P., Jayaraj, R. L., Reiner, D., Ojha, S. K., Beiram, R., Stark, H., Łażewska, D., Kieć-Kononowicz, K., & Sadek, B. (2019). The dual-active histamine H3 receptor antagonist and acetylcholine esterase inhibitor E100 ameliorates stereotyped repetitive behavior and neuroinflammmation in sodium valproate induced autism in mice. *Chemico-Biological Interactions*, *312*, 108775. https://doi.org/10.1016/j.cbi.2019.108775

Eissa, N., Azimullah, S., Jayaprakash, P., Jayaraj, R. L., Reiner, D., Ojha, S. K., Beiram, R., Stark, H., Łażewska, D., Kieć-Kononowicz, K., & Sadek, B. (2020). The Dual-Active Histamine H3 Receptor Antagonist and Acetylcholine Esterase Inhibitor E100 Alleviates Autistic-Like Behaviors and Oxidative Stress in Valproic Acid Induced Autism in Mice. *International Journal of Molecular Sciences*, *21*(11), 3996. https://doi.org/10.3390/ijms21113996

Eissa, N., Jayaprakash, P., Azimullah, S., Ojha, S. K., Al-Houqani, M., Jalal, F. Y., Łażewska, D., Kieć-Kononowicz, K., & Sadek, B. (2018). The histamine H3R antagonist DL77 attenuates autistic behaviors in a prenatal valproic acid-induced mouse model of autism. *Scientific Reports*, *8*(1), 13077. https://doi.org/10.1038/s41598-018-31385-7

Eissa, N., Sadeq, A., Sasse, A., & Sadek, B. (2020). Role of Neuroinflammation in Autism Spectrum Disorder and the Emergence of Brain Histaminergic System. Lessons Also for BPSD? *Frontiers in Pharmacology*, *11*, 886. https://doi.org/10.3389/fphar.2020.00886

Elsabbagh, M., Divan, G., Koh, Y.-J., Kim, Y. S., Kauchali, S., Marcín, C., Montiel-Nava, C., Patel, V., Paula, C. S., Wang, C., Yasamy, M. T., & Fombonne, E. (2012). Global prevalence of autism and other pervasive developmental disorders. *Autism Research: Official Journal of the International Society for Autism Research*, *5*(3), 160–179. https://doi.org/10.1002/aur.239

Ferreira, R., Santos, T., Gonçalves, J., Baltazar, G., Ferreira, L., Agasse, F., & Bernardino, L. (2012). Histamine modulates microglia function. *Journal of Neuroinflammation*, *9*, 90. https://doi.org/10.1186/1742-2094-9-90

Frantz, M.-C., Pellissier, L. P., Pflimlin, E., Loison, S., Gandía, J., Marsol, C., Durroux, T., Mouillac, B., Becker, J. A. J., Le Merrer, J., Valencia, C., Villa, P., Bonnet, D., & Hibert, M. (2018). LIT-001, the First Nonpeptide Oxytocin Receptor Agonist that Improves Social Interaction in a Mouse Model of Autism. *Journal of Medicinal Chemistry*, *61*(19), 8670–8692. https://doi.org/10.1021/acs.jmedchem.8b00697

Garbarg, M., Barbin, G., Bischoff, S., Pollard, H., & Schwartz, J. C. (1976). Dual localization of histamine in an ascending neuronal pathway and in non-neuronal cells evidenced by lesions in the lateral hypothalamic area. *Brain Research*, *106*(2), 333–348. https://doi.org/10.1016/0006-8993(76)91029-5

Gemkow, M. J., Davenport, A. J., Harich, S., Ellenbroek, B. A., Cesura, A., & Hallett, D. (2009). The histamine H3 receptor as a therapeutic drug target for CNS disorders. *Drug Discovery Today*, *14*(9–10), 509–515. https://doi.org/10.1016/j.drudis.2009.02.011

Griebel, G., Pichat, P., Pruniaux, M.-P., Beeské, S., Lopez-Grancha, M., Genet, E., Terranova, J.-P., Castro, A., Sánchez, J. A., Black, M., Varty, G. B., Weiner, I., Arad, M., Barak, S., De Levie, A., & Guillot, E. (2012). SAR110894, a potent histamine H_3-receptor antagonist, displays procognitive effects in rodents. *Pharmacology, Biochemistry, and Behavior*, *102*(2), 203–214. https://doi.org/10.1016/j.pbb.2012.04.004

Guze, S. B. (1995). Diagnostic and statistical manual of mental disorders, (DSM-IV). *American Journal of Psychiatry*, *152*(8), 1228–1228.

Haas, H. L., Sergeeva, O. A., & Selbach, O. (2008). Histamine in the Nervous System. *Physiological Reviews*, *88*(3), 1183–1241. https://doi.org/10.1152/physrev.00043.2007

Haber, S. N. (2014). The place of dopamine in the cortico-basal ganglia circuit. *Neuroscience*, *282*, 248–257. https://doi.org/10.1016/j.neuroscience.2014.10.008

Hamilton, P. J., Campbell, N. G., Sharma, S., Erreger, K., Herborg Hansen, F., Saunders, C., Belovich, A. N., NIH ARRA Autism Sequencing Consortium, Sahai, M. A., Cook, E. H., Gether, U., McHaourab, H. S., Matthies, H. J. G., Sutcliffe, J. S., & Galli, A. (2013). De novo mutation in the dopamine transporter gene associates dopamine dysfunction with autism spectrum disorder. *Molecular Psychiatry*, *18*(12), 1315–1323. https://doi.org/10.1038/mp.2013.102

Hara, Y., Ago, Y., Taruta, A., Katashiba, K., Hasebe, S., Takano, E., Onaka, Y., Hashimoto, H., Matsuda, T., & Takuma, K. (2016). Improvement by methylphenidate and atomoxetine of social interaction deficits and recognition memory impairment in a mouse model of valproic acid-induced autism. *Autism Research: Official Journal of the International Society for Autism Research*, *9*(9), 926–939. https://doi.org/10.1002/aur.1596

Javed, H., Azimullah, S., Abul Khair, S. B., Ojha, S., & Haque, M. E. (2016). Neuroprotective effect of nerolidol against neuroinflammation and oxidative stress induced by rotenone. *BMC Neuroscience*, *17*(1), 58. https://doi.org/10.1186/s12868-016-0293-4

Joshi, G., Wozniak, J., Petty, C., Martelon, M. K., Fried, R., Bolfek, A., Kotte, A., Stevens, J., Furtak, S. L., Bourgeois, M., Caruso, J., Caron, A., & Biederman, J. (2013). Psychiatric comorbidity and functioning in a clinically referred population of adults with autism spectrum disorders: A comparative study. *Journal of Autism and Developmental Disorders*, *43*(6), 1314–1325. https://doi.org/10.1007/s10803-012-1679-5

Khan, N., Saad, A., Nurulain, S. M., Darras, F. H., Decker, M., & Sadek, B. (2016). The dual-acting H3 receptor antagonist and AChE inhibitor UW-MD-71 dose-dependently enhances memory retrieval and reverses dizocilpine-induced memory impairment in rats. *Behavioural Brain Research*, *297*, 155–164. https://doi.org/10.1016/j.bbr.2015.10.022

Kim, D. H., Chung, J. H., Yoon, J. S., Ha, Y. M., Bae, S., Lee, E. K., Jung, K. J., Kim, M. S., Kim, Y. J., Kim, M. K., & Chung, H. Y. (2013). Ginsenoside Rd inhibits the expressions of iNOS and COX-2 by suppressing NF-κB in LPS-stimulated RAW264.7 cells and mouse liver. *Journal of Ginseng Research*, *37*(1), 54–63. https://doi.org/10.5142/jgr.2013.37.54

Lee, Y., Kim, H., Kim, J.-E., Park, J.-Y., Choi, J., Lee, J.-E., Lee, E.-H., & Han, P.-L. (2018). Excessive D1 Dopamine Receptor Activation in the Dorsal Striatum Promotes Autistic-Like Behaviors. *Molecular Neurobiology*, *55*(7), 5658–5671. https://doi.org/10.1007/s12035-017-0770-5

Lord, C., Leventhal, B. L., & Cook, E. H. (2001). Quantifying the phenotype in autism spectrum disorders. *American Journal of Medical Genetics*, *105*(1), 36–38.

Losh, M., & Piven, J. (2007). Social-cognition and the broad autism phenotype: Identifying genetically meaningful phenotypes. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *48*(1), 105–112. https://doi.org/10.1111/j.1469-7610.2006.01594.x

Lull, M. E., & Block, M. L. (2010). Microglial activation and chronic neurodegeneration. *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics*, *7*(4), 354–365. https://doi.org/10.1016/j.nurt.2010.05.014

Masoud, S. T., Vecchio, L. M., Bergeron, Y., Hossain, M. M., Nguyen, L. T., Bermejo, M. K., Kile, B., Sotnikova, T. D., Siesser, W. B., Gainetdinov, R. R., Wightman, R. M., Caron, M. G., Richardson, J. R., Miller, G. W., Ramsey, A. J., Cyr, M., & Salahpour, A. (2015). Increased expression of the dopamine transporter leads to loss of dopamine neurons, oxidative stress and l-DOPA reversible motor deficits. *Neurobiology of Disease*, *74*, 66–75. https://doi.org/10.1016/j.nbd.2014.10.016

Meyza, K. Z., & Blanchard, D. C. (2017). The BTBR mouse model of idiopathic autism—Current view on mechanisms. *Neuroscience and Biobehavioral Reviews*, *76*(Pt A), 99–110. https://doi.org/10.1016/j.neubiorev.2016.12.037

Mochizuki, T., Jansen, F. P., Leurs, R., Windhorst, A. D., Yamatodani, A., Maeyama, K., & Timmerman, H. (1996). Brain penetration of the histamine H3 receptor antagonists thioperamide and clobenpropit in rat and mouse, determined with ex vivo [125I]iodophenpropit binding. *Brain Research*, *743*(1–2), 178–183. https://doi.org/10.1016/s0006-8993(96)01040-2

Moy, S. S., Nadler, J. J., Young, N. B., Perez, A., Holloway, L. P., Barbaro, R. P., Barbaro, J. R., Wilson, L. M., Threadgill, D. W., Lauder, J. M., Magnuson, T. R., & Crawley, J. N. (2007). Mouse behavioral tasks relevant to autism: Phenotypes of 10 inbred strains. *Behavioural Brain Research*, *176*(1), 4–20. https://doi.org/10.1016/j.bbr.2006.07.030

Nadeem, A., Ahmad, S. F., Al-Harbi, N. O., Attia, S. M., Alshammari, M. A., Alzahrani, K. S., & Bakheet, S. A. (2019). Increased oxidative stress in the cerebellum and peripheral immune cells leads to exaggerated autism-like repetitive behavior due to deficiency of antioxidant response in BTBR T + tf/J mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *89*, 245–253. https://doi.org/10.1016/j.pnpbp.2018.09.012

Pardo, C. A., Vargas, D. L., & Zimmerman, A. W. (2005). Immunity, neuroglia and neuroinflammation in autism. *International Review of Psychiatry (Abingdon, England)*, *17*(6), 485–495. https://doi.org/10.1080/02646830500381930

Pavăl, D. (2017). A Dopamine Hypothesis of Autism Spectrum Disorder. *Developmental Neuroscience*, *39*(5), 355–360. https://doi.org/10.1159/000478725

Peñagarikano, O., Lázaro, M. T., Lu, X.-H., Gordon, A., Dong, H., Lam, H. A., Peles, E., Maidment, N. T., Murphy, N. P., Yang, X. W., Golshani, P., & Geschwind, D. H. (2015). Exogenous and evoked oxytocin restores social behavior in the Cntnap2 mouse model of autism. *Science Translational Medicine*, *7*(271), 271ra8. https://doi.org/10.1126/scitranslmed.3010257

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33. https://doi.org/10.1016/s0014-2999(03)01272-x

Reiersen, A. M., & Todorov, A. A. (2011). Association between DRD4 genotype and Autistic Symptoms in DSM-IV ADHD. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *20*(1), 15–21.

Russell, A. J., Jassi, A., Fullana, M. A., Mack, H., Johnston, K., Heyman, I., Murphy, D. G., & Mataix-Cols, D. (2013). Cognitive behavior therapy for comorbid obsessive-compulsive disorder in high-functioning autism spectrum disorders: A randomized controlled trial. *Depression and Anxiety*, *30*(8), 697–708. https://doi.org/10.1002/da.22053

Sadek, B., Saad, A., Sadeq, A., Jalal, F., & Stark, H. (2016a). Histamine H3 receptor as a potential target for cognitive symptoms in neuropsychiatric diseases. *Behavioural Brain Research*, *312*, 415–430. https://doi.org/10.1016/j.bbr.2016.06.051

Sadek, B., Saad, A., Sadeq, A., Jalal, F., & Stark, H. (2016b). Histamine H3 receptor as a potential target for cognitive symptoms in neuropsychiatric diseases. *Behavioural Brain Research*, *312*, 415–430. https://doi.org/10.1016/j.bbr.2016.06.051

Schultz, W. (2007). Multiple dopamine functions at different time courses. *Annual Review of Neuroscience*, *30*, 259–288. https://doi.org/10.1146/annurev.neuro.28.061604.135722

Scott-Van Zeeland, A. A., Dapretto, M., Ghahremani, D. G., Poldrack, R. A., & Bookheimer, S. Y. (2010). Reward processing in autism. *Autism Research: Official Journal of the International Society for Autism Research*, *3*(2), 53–67. https://doi.org/10.1002/aur.122

Seeman, P. (2010). Dopamine D2 receptors as treatment targets in schizophrenia. *Clinical Schizophrenia & Related Psychoses*, *4*(1), 56–73. https://doi.org/10.3371/CSRP.4.1.5

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visualized Experiments: JoVE*, *96*, e52434. https://doi.org/10.3791/52434

Silverman, J. L., Tolu, S. S., Barkan, C. L., & Crawley, J. N. (2010). Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *35*(4), 976–989. https://doi.org/10.1038/npp.2009.201

Squillace, M., Dodero, L., Federici, M., Migliarini, S., Errico, F., Napolitano, F., Krashia, P., Maio, A., Galbusera, A., Bifone, A., Scattoni, M. L., Pasqualetti, M., Mercuri, N., Usiello, A., & Gozzi, A. (2014). Dysfunctional dopaminergic neurotransmission in asocial BTBR mice. *Translational Psychiatry*, *4*, e427. https://doi.org/10.1038/tp.2014.69

Staal, W. G., de Krom, M., & de Jonge, M. V. (2012). Brief Report: The Dopamine-3-Receptor Gene (DRD3) is Associated with Specific Repetitive Behavior in Autism Spectrum Disorder (ASD). *Journal of Autism and Developmental Disorders*, *42*(5), 885–888. https://doi.org/10.1007/s10803-011-1312-z

Tiligada, E., Kyriakidis, K., Chazot, P. L., & Passani, M. B. (2011). Histamine pharmacology and new CNS drug targets. *CNS Neuroscience & Therapeutics*, *17*(6), 620–628. https://doi.org/10.1111/j.1755-5949.2010.00212.x

Tyrtyshnaia, A. A., Lysenko, L. V., Madamba, F., Manzhulo, I. V., Khotimchenko, M. Y., & Kleschevnikov, A. M. (2016). Acute neuroinflammation provokes intracellular acidification in mouse hippocampus. *Journal of Neuroinflammation*, *13*(1), 283. https://doi.org/10.1186/s12974-016-0747-8

Vargas, D. L., Nascimbene, C., Krishnan, C., Zimmerman, A. W., & Pardo, C. A. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology*, *57*(1), 67–81. https://doi.org/10.1002/ana.20315

Venkatachalam, K., Eissa, N., Awad, M. A., Jayaprakash, P., Zhong, S., Stölting, F., Stark, H., & Sadek, B. (2021). The histamine H3R and dopamine D2R/D3R antagonist ST-713 ameliorates autism-like behavioral features in BTBR T+tf/J mice by multiple actions. *Biomedicine & Pharmacotherapy*, *138*, 111517. https://doi.org/10.1016/j.biopha.2021.111517

Verdiere, M., Rose, C., & Schwartz, J.-C. (1975). Synthesis and release of histamine studied on slices from rat hypothalamus. *European Journal of Pharmacology*, *34*(1), 157–168. https://doi.org/10.1016/0014-2999(75)90236-8

Volkmar, F. R., Lord, C., Bailey, A., Schultz, R. T., & Klin, A. (2004). Autism and pervasive developmental disorders. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *45*(1), 135–170. https://doi.org/10.1046/j.0021-9630.2003.00317.x

Von Coburg, Y., Kottke, T., Weizel, L., Ligneau, X., & Stark, H. (2009). Potential utility of histamine H3 receptor antagonist pharmacophore in antipsychotics. *Bioorganic & Medicinal Chemistry Letters*, *19*(2), 538–542. https://doi.org/10.1016/j.bmcl.2008.09.012

Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psychological Bulletin*, *83*(3), 482–504. https://doi.org/10.1037/0033-2909.83.3.482

Watanabe, T., Taguchi, Y., Hayashi, H., Tanaka, J., Shiosaka, S., Tohyama, M., Kubota, H., Terano, Y., & Wada, H. (1983). Evidence for the presence of a histaminergic neuron system in the rat brain: An immunohistochemical analysis. *Neuroscience Letters*, *39*(3), 249–254. https://doi.org/10.1016/0304-3940(83)90308-7

Werling, D. M., & Geschwind, D. H. (2013). Sex differences in autism spectrum disorders. *Current Opinion in Neurology*, *26*(2), 146–153. https://doi.org/10.1097/WCO.0b013e32835ee548

Witkin, J. M., & Nelson, D. L. (2004). Selective histamine H3 receptor antagonists for treatment of cognitive deficiencies and other disorders of the central nervous system. *Pharmacology & Therapeutics*, *103*(1), 1–20. https://doi.org/10.1016/j.pharmthera.2004.05.001

Wright, C., Shin, J. H., Rajpurohit, A., Deep-Soboslay, A., Collado-Torres, L., Brandon, N. J., Hyde, T. M., Kleinman, J. E., Jaffe, A. E., Cross, A. J., & Weinberger, D. R. (2017). Altered expression of histamine signaling genes in autism spectrum disorder. *Translational Psychiatry*, *7*(5), e1126. https://doi.org/10.1038/tp.2017.87

Yoshikawa, T., Nakamura, T., & Yanai, K. (2019). Histamine N-Methyltransferase in the Brain. *International Journal of Molecular Sciences*, *20*(3). https://doi.org/10.3390/ijms20030737

Zimmerman, A. W., Jyonouchi, H., Comi, A. M., Connors, S. L., Milstien, S., Varsou, A., & Heyes, M. P. (2005). Cerebrospinal fluid and serum markers of inflammation in autism. *Pediatric Neurology*, *33*(3), 195–201. https://doi.org/10.1016/j.pediatrneurol.2005.03.014

List of Publications

Venkatachalam, K., Eissa, N., Awad, M. A., Jayaprakash, P., Zhong, S., Stölting, F., Stark, H., & Sadek, B. (2021). The histamine H3R and dopamine D2R/D3R antagonist ST-713 ameliorates autism-like behavioral features in BTBR T+tf/J mice by multiple actions. Biomedicine & Pharmacotherapy, 138, 111517. https://doi.org/10.1016/j.biopha.2021.111517