Unraveling the Compulsive Phenotype through Schedule-Induced Polydipsia: Comorbidity, Glutamate-Modulators, and Genetics Biomarkers

Descifrando el Fenotipo Compulsivo a través de la
Polidipsia Inducida por Programa:
Comorbilidad, Moduladores Glutamatérgicos y Biomarcadores Genéticos

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Abstract

Compulsivity is characterized by persistent, inflexible, and excessive behaviors that either are aimed at avoiding perceived adverse consequences or are dependent on rigid rules, and it is a key factor in the loss of control over behavior. The cardinal symptom of obsessive-compulsive disorder (OCD), which affects 2–3 % of the population, is compulsive behavior, considered a chronic and disabling condition. Moreover, compulsive behavior is observed in several neuropsychiatric disorders such as anxiety, depression, specific phobias and schizophrenia. Therefore, compulsivity has been considered a transdiagnostic trait that should be studied according to a dimensional approach. This is in line with the U.S. National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) and the Roadmap for Mental Health Research in Europe (ROAMER), leading to a reclassification by the DSM-5 (American Psychiatric Association, 2013) and ICD-11 ("WHO | World Health Organization," 2018), which now categorizes OCD as the central disorder in a group of related disorders called Obsessive-Compulsive and Related Disorders (OCRDs). Further investigation is required to understand the neurobehavioral alterations related to compulsivity, and their implications in different disorders.

Compulsivity is not a unitary phenomenon, but the heterogeneity of compulsive symptoms makes treatment efficacy highly variable. Up to 40 % of patients do not respond successfully to classical psychopharmacological treatments, which are mainly based on selective serotonin reuptake inhibitors (SSRIs). However, clinical evidence shows that targeting glutamate mechanisms by psychopharmacotherapy may be of benefit in impaired inhibitory control. Furthermore, clinical evidence by a meta-analysis of genetic association studies, pointed towards the serotonin *Htr2a* and glutamate *Grm2* receptors genetic polymorphism expression in OCD vulnerability. Therefore, the combination of the serotonin 5-HT2A receptor and glutamate mGlu2 receptor might be a possible target to modulate compulsivity in OCDRs.

The present doctoral thesis investigates the neurobehavioral basis of compulsivity through a compulsive phenotype of rats selected by Schedule-Induced Polydipsia (SIP). SIP induces excessive and persistent drinking behavior in rats through intermittent food-reinforcement schedules over 20 sessions, and allows the selection of high compulsive drinkers (HD) and low non-compulsive drinkers (LD) based on their acquisition. The main objectives of the doctoral thesis are: (1) to characterize the compulsive phenotype selected by means of SIP, by identifying comorbid disorders and cognitive constructs in HD rats; (2) to modulate compulsive behavior on SIP by administrating different glutamate-modulators; and (3) to identify new genetic biomarkers in HD rats compared to LD rats.

In order to achieve the objectives exposed, three experimental sets have been designed: behavioral, pharmacological, and genetic studies. The first one was proposed to test Objective 1 and includes two experiments: Experiment 1A assessed the compulsive-like behavior by means of the Marble Burying Test (MBT), depressive-like behavior by means of the Forced Swimming Test (FST), anxious-like behavior by means of the Elevated Plus Maze (EPM), and fear behavior by means of the Fear Condition Test (FCT) in the compulsive phenotype of rats selected by means of the SIP. Experiment 1B investigated the cognitive processing (stimuli processing; reference and working memory; and cognitive flexibility) by means of the Novel Object Recognition Test (NOR), Radial Arm Maze (RAM) and Morris Water Maze (MWM) and reversal MWM in the compulsive phenotype of rats selected by means of SIP. The second experimental set was designed to test Objective 2, exploring the therapeutic potential of different glutamate-modulators in reducing compulsive behavior. For this purpose, the effects of acute administration of N-Acetylcysteine (NAC), an inhibitor of the presynaptic glutamate release; memantine (MEM), an uncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist; and lamotrigine (LAM), an inhibitor of the excitatory amino acids release such as glutamate; were assessed on compulsive drinking on SIP. Finally, the third experimental set was proposed to assess Objective 3. Experiment 3 analyzed the expression of serotonergic, glutamatergic and neuroplasticity-related genes in compulsive HD compared non-compulsive LD rats selected by means of SIP. It was analyzed the expression of *Htr2a* and *Htr2c*; *Grin1, Grin2a, Grin2b, Grin2c* and *Grm2*; and *Bdnf* genes in frontal cortex (FC),

hippocampus (HIP) and amygdala (AMY) in HD and LD rats by means of quantitative reverse transcription polymerase chain reaction (RT-qPCR).

The results from the **first experimental set** revealed that, in Experiment 1A, HD rats presented increased compulsive-like behavior, since a higher number of marbles were partially buried on MBT, and resistance to memory extinction, as an increased percentage of freezing was shown during the retrieval day of FCT. However, HD rats did not differ from LD rats in anxious-like behavior on EPM, nor in depressive-like behavior on FST. They did not either in Experiment 1B, when stimuli processing was assessed on NOR, but did present a reference and working memory deficit (a higher number of errors committed in RAM). Even though HD rats did not exhibit any learning impairment on MWM acquisition, HD rats showed cognitive inflexibility, as demonstrated by a higher latency to reach the platform in the reversal MWM compared to LD rats. In the **second experimental set**, the systemic administration of MEM and LAM reduced, in a dose-dependent manner, compulsive drinking in HD rats on SIP, without any effect in LD rats. With administration of the highest dose of LAM, the differences between both populations disappeared. The administration of NAC did not produce any significant effect on any of the populations. Finally, the **third experimental set** revealed that HD rats presented a down-regulation of *Htr2a*, *Grin1* and *Bdnf* gene expressions in the FC, when compared to LD rats. Also, a significant negative correlation was observed between the amount of water intake and the expression of *Htr2a*, *Grin1* and *Bdnf* genes in the FC. No differences were found in the other genes analyzed (*Htr2c*, *Grin2a*, *Grin2b*, *Grin2c*, *Grm2*) between HD and LD rats. Neither there were differences in HIP and AMY, regarding the assessed genes, between the HD and LD rats.

In summary, the results have allowed us to characterize compulsive HD rats, as selected by means of SIP, at different leves: behavioral, psychopharmachological and genetic. Concerning the behavioral characterization, HD rats presented other forms of compulsive behavior, resistance to memory extinction, memory impairments and cognitive inflexibility if we compare to LD rats. This behavioral profile could be modulating the development and maintenance of compulsivity. Regarding the psychopharmachological characterization, the implication of the glutamatergic signal in the modulation of HD rats' compulsive behavior in SIP have been proved by a dose-dependent reduction in the water intake after the administration of MEM and LAM. No significant effects were found after

the administration of NAC. On the other hand, the genetic characterization evidenced that HD rats presented a downregulated expression of serotonergic, glutamatergic and neuroplasticity-related genes in the FC, suggesting that *Htr2a*, *Grin1* and *Bdnf* genes could constitute vulnerability biomarkers of compulsivity. In conclusion, the present thesis characterized the compulsive phenotype of HD rats through neurobehavioral impairments regarding comorbid disorders with OCD, as well as throuh downregulated genes in FC. Moreover, data pointed to a potential therapeutic role of glutamate-modulators in compulsivity. These results might help to better characterize the compulsive phenotype to improve the detection and treatment of OCRDs.

Resumen

La compulsividad se caracteriza por comportamientos persistentes, inflexibles y excesivos que tienen como objetivo evitar consecuencias adversas y que dependen de reglas rígidas. Es el síntoma principal del trastorno obsesivo-compulsivo (TOC), que afecta al 2-3 % de la población, considerado un trastorno crónico e incapacitante. Además, el comportamiento compulsivo se observa en varios trastornos neuropsiquiátricos como la ansiedad, la depresión, la fobia específica y la esquizofrenia. Por lo tanto, se ha considerado un rasgo transdiagnóstico que debe estudiarse siguiendo un enfoque dimensional, como indican los Criterios de Dominio de Investigación del Instituto Nacional de Salud Mental de Estados Unidos (NIMH RDoC, por sus siglas en inglés) y la Hoja de Ruta para la Investigación en Salud Mental en Europa (ROAMER, por sus siglas en inglés). Estos nuevos criterios diagnósticos han resultado en una reclasificación por parte del DSM-5 (Asociación Americana de Psiquiatría, 2013) y el CIE-11 (Organización Mundial de la Salud, 2018), que ahora clasifican el TOC como el trastorno central de los llamados Trastornos Obsesivo-Compulsivos y Relacionados (OCRD, por sus siglas en inglés). Investigaciones futuras podrían ayudar a comprender las alteraciones neuroconductuales relacionadas con la compulsividad y sus implicaciones en los diferentes trastornos.

La compulsividad no es un fenómeno unitario, por lo que la heterogeneidad de los síntomas compulsivos puede contribuir a que la eficacia del tratamiento sea muy variable. Se ha observado que aproximadamente un 40 % de los pacientes no responden con éxito a los tratamientos psicofarmacológicos clásicos, que se basan principalmente en los inhibidores selectivos de la recaptación de serotonina (ISRS). Sin embargo, estudios clínicos muestran que la modulación de la señal glutamatérgica, mediante psicofarmacoterapia, puede ser beneficiosa para la mejora del control inhibitorio. En este sentido, un metaanálisis sobre estudios genéticos en poblaciones clínicas, señaló que distintos polimorfismos genéticos de los receptores 5-HT2A de la serotonina y mGlu2 del glutamato se asocian con una mayor vulnerabilidad al desarrollo de TOC. Por lo tanto, un posible objetivo

farmacológico para modular la compulsividad en los OCRD sería el heterodímero que forman el receptor de serotonina 5-HT2A y el receptor de glutamato mGlu2.

La presente tesis doctoral investiga las bases neuroconductuales de la compulsividad a través de un fenotipo compulsivo de ratas seleccionadas mediante Polidipsia Inducida por Programa (SIP, por sus siglas en inglés). La SIP induce a las ratas a beber de manera excesiva y persistente, a través de un programa de refuerzo intermitente durante 20 sesiones, permitiendo la selección de ratas compulsivas altas bebedoras (HD, por sus siglas en inglés) y ratas no compulsivas bajas bebedoras (LD, por sus siglas en inglés) en función de su adquisición en la SIP. Los principales objetivos de la tesis doctoral son: (1) caracterizar el fenotipo compulsivo seleccionado a través de SIP, mediante la identificación de conductas alteradas y procesos cognitivos ligados a trastornos comórbidos con TOC en ratas HD; (2) reducir el comportamiento compulsivo en la SIP mediante la administración de diferentes moduladores del glutamato; (3) identificar nuevos biomarcadores genéticos en ratas HD.

Con el fin de alcanzar los objetivos expuestos, se han diseñado tres conjuntos experimentales: estudio conductual, farmacológico y genético. El primero se ha propuesto para lograr el Objetivo 1 e incluye dos experimentos: el Experimento 1A que evalúa el comportamiento compulsivo en el fenotipo compulsivo de ratas, seleccionadas a través de SIP, mediante el Test de Enterramiento de Canicas (MBT, por sus siglas en ingles), el comportamiento depresivo mediante el Test de Natación Forzada (FST, por sus siglas en inglés), el comportamiento ansioso mediante la prueba del Laberinto Elevado en Cruz (EPM, por sus siglas en inglés) y la conducta de miedo mediante la Prueba de Condicionamiento del Miedo (FCT). El Experimento 1B trata sobre el procesamiento cognitivo (procesamiento de estímulos, memoria de referencia y de trabajo, y flexibilidad cognitiva) en el fenotipo compulsivo de ratas, seleccionadas a través de SIP, mediante la prueba de Reconocimiento de Objeto Novedoso (NOR, por sus siglas inglés), el Laberinto Radial de 8 Brazos (RAM, por sus siglas inglés), el laberinto acuático de Morris (MWM, por sus siglas inglés) y el MWM en condiciones inversas. El segundo conjunto experimental se ha diseñado para alcanzar el Objetivo 2: explorar el potencial terapéutico de diferentes moduladores del glutamato en la reducción del comportamiento compulsivo. Con este propósito, se evaluaron los efectos sobre la SIP de la administración aguda de N-Acetilcisteína (NAC), un inhibidor de la liberación presináptica de glutamato; memantina

(MEM), un antagonista no competitivo del receptor N-Metil-D-Aspartato (NMDA); y lamotrigina (LAM), un inhibidor de la liberación de aminoácidos excitatorios como el glutamato. Por último, el tercer conjunto experimental se propuso para cumplimentar el Objetivo 3. El Experimento 3 analiza la expresión de genes serotonérgicos, glutamatérgicos y relacionados con la neuroplasticidad en ratas HD compulsivas en comparación con ratas LD no compulsivas, seleccionadas mediante SIP. Se analizó la expresión de los genes *Htr2a* y *Htr2c*; *Grin1*, *Grin2a*, *Grin2b*, *Grin2c* y *Grm2*; y *Bdnf* en la corteza frontal (FC, por sus siglas en inglés), el hipocampo (HIP, por sus siglas en inglés) y la amígdala (AMY, por sus siglas en inglés) en ratas HD y LD mediante la reacción en cadena de la polimerasa cuantitativa con transcripción inversa (RT-qPCR, por sus siglas en inglés).

Los resultados del **primer conjunto experimental** muestran que, en el Experimento 1A, las ratas HD presentaron un aumento del comportamiento compulsivo, al encontrarse un mayor número de canicas parcialmente enterradas en el MBT, y una mayor resistencia a la extinción, relacionada con un mayor porcentaje de inmovilidad durante el día de supersión del estímulo incondicionado (extinción) del FCT. En comparación con las ratas LD, las ratas HD no mostraron diferencias en el comportamiento ansioso en el EPM, ni en el comportamiento depresivo en el FST. Tampoco lo hicieron en el Experimento 1B en el procesamiento de estímulos en el NOR, pero sí presentaron un déficit en la memoria de referencia y de trabajo, medido mediante un mayor número de errores cometidos en el RAM. Además, aunque las ratas HD no mostraron ningún deterioro en el aprendizaje en el MWM, sí mostraron inflexibilidad cognitiva, cuantificada por una mayor latencia para llegar a la plataforma durante el reversal MWM, en comparación con las ratas LD. En el **segundo conjunto experimental**, las administraciones agudas de MEM y LAM redujeron el consumo compulsivo de agua durante la SIP de forma dosis dependiente en las ratas HD, sin ningún efecto en las ratas LD. Además, con la dosis más alta de LAM, las diferencias entre ambas poblaciones desaparecen. La administración de NAC no produjo ningún efecto significativo en la SIP en ninguna de las poblaciones de ratas. Por último, en el **tercer conjunto experimental** las ratas HD presentaron una regulación a la baja de la expresión de los genes *Htr2a*, *Grin1* y *Bdnf* en el FC en comparación con las ratas LD. Se observó una correlación negativa significativa entre la ingesta de agua y la expresión de los genes *Htr2a*, *Grin1* y *Bdnf* en la FC. No se encontraron diferencias en los otros genes analizados (*Htr2c*, *Grin2a*, *Grin2b*, *Grin2c*, *Grm2*) entre

las ratas HD y LD. Tampoco se hallaron diferencias en el HIP y la AMY a partir de los genes analizados entre las ratas HD y LD seleccionadas mediante SIP.

En resumen, los resultados de la presente Tesis Doctoral han caracterizado a las ratas HD compulsivas seleccionadas a través SIP a nivel conductual, psicofarmacológico y genético. En la caracterización conductual, se han encontrado otras formas de comportamiento compulsivo, resistencia a la extinción de la memoria, déficits de memoria e inflexibilidad cognitiva en comparación con las ratas LD. Este perfil cognitivo podría contribuir al desarrollo y mantenimiento de la compulsividad. En cuanto a la caracterización psicofarmacológica, la implicación de la señal glutamatérgica en la modulación de la compulsividad en las ratas HD en SIP se ha evidenciado por la reducción en la ingesta de agua tras la administración de MEM y LAM, a pesar de no observarse diferencias significativas tras la administración de NAC. Por otro lado, la caracterización genética ha puesto de manifiesto que las ratas HD presentan una expresión regulada a la baja de genes serotonérgicos, glutamatérgicos y relacionados con la neuroplasticidad en el FC. Esto sugiere que los genes *Htr2a*, *Grin1* y *Bdnf* podrían constituir biomarcadores de vulnerabilidad para el desarrollo de compulsividad. En conclusión, la presente tesis ha caracterizado el fenotipo compulsivo de las ratas HD a trevés de conductas alteradas y procesos cognitivos ligados a trastornos comórbidos con TOC, así como de genes regulados a la baja en el FC. Además, los datos de la misma sugirieren un posible papel terapéutico de los moduladores del glutamato en la compulsividad. Estos resultados podrían ayudar a caracterizar mejor el fenotipo compulsivo, para mejorar la detección y el tratamiento de los OCRD.

Contents

Chapter 1. Introduction

1.1. COMPULSIVITY IN MENTAL HEALTH

Compulsive symptoms, such as obsessive thoughts and repetitive behaviors, are present in different psychopathological disorders, such as obsessive-compulsive disorder (OCD), schizophrenia, addiction, hoarding disorder and body dysmorphic disorder (Brock & Hany, 2023). OCD, one of the most characteristic disorders regarding compulsive symptomatology, have a significant impact on individuals worldwide with a prevalence of approximately 2-3 % of the global population, with a similar rate in Spain (Canals et al., 2012; Hirschtritt et al., 2017; "WHO | World Health Organization," 2018). Compulsive symptoms can have a profound impact on individual's mental health and quality of life, individuals tend to avoid uncomfortable situations and to reduce social interactions (Brock & Hany, 2023). Hence, the burden of compulsive symptoms extends beyond the affected individuals, as it can also impact their families, relationships, and daily functioning. Despite advancements in understanding and treating compulsive symptoms, further efforts are needed to raise awareness, reduce stigma, and provide effective interventions to those struggling with these conditions in both global and Spanish contexts. Thus, compulsive symptoms are a global public health concern, posing a considerable burden on healthcare systems and society.

One important issue is that compulsivity is present in different psychopathological disorders. First, according to In the Diagnostic and Statistical Manual of Mental Disorders (5th ed.), the obsessive-compulsive and related disorders family state that the course of OCD is often complicated by the co-occurrence of other disorders, including anxiety, specific phobia, depression, bipolar disorder, schizophrenia, and eating disorders as common comorbid pathologies (American Psychiatric Association, 2013b). In addition, clinical studies show that 90 % of OCD cases meet the criteria for another lifetime disorder. Table 1 summarizes the most common comorbid disorders: anxiety disorders (75.8 %),

followed by mood disorders (63.3 %), impulse-control disorders (55.9 %), and substance use disorders (38.6 %) (Ruscio et al., 2010).

Disorder	Percentage
Anxiety disorders: including panic disorder, agoraphobia, specific phobia, social phobia, generalized anxiety disorder, post-traumatic stress disorder, and separation anxiety disorder.	**75.8 %**
Mood disorder: including major depressive disorder, dysthymic disorder, and bipolar disorder.	**63.3 %**
Impulse-control disorder: including oppositional-defiant disorder, conduct disorder, attention-deficit/ hyperactivity disorder, and intermittent explosive disorder.	**55.9 %**
Substance use disorder: including alcohol abuse/ dependance, alcohol dependance, drugs abuse/ dependance, and drug dependence.	**38.6 %**
Any disorder	**90 %**

Table 1. Percentage of obsessive-compulsive disorders with comorbidity, adapted from Ruscio et al. 2010

Moreover, recent strategies to study mental health based on the dimension of altered behavior, such as The Research Domain Criteria (NIMH » Research Domain Criteria (RDoC), n.d.), have proposed compulsivity as a transdiagnostic trait, which poses challenges for conventional diagnostic systems, prevention, and treatment (Den Ouden et al., 2022). As a result of findings from neuroscience research (Fineberg et al., 2018), the DSM-5 (American Psychiatric Association, 2013a) and ICD-11("WHO | World Health Organization," 2018) have reclassified OCD as the head of a new group of disorders called Obsessive-Compulsive and Related Disorders (OCRDs), which also includes body dysmorphic, hoarding, hair-pulling, skin picking, olfactory reference disorders, and hypochondriasis, all characterized by compulsive behavior (Fineberg et al., 2020). Therefore, more research is needed to understand behavioral alterations associated with compulsivity and its impact in different disorders.

According to these statements above, the introduction of the present thesis will review the behavioral, psychopharmacological and genetic aspects regarding the clinical

and preclinical studies in compulsive spectrum disorders, building a bridge between clinical and preclinical data.

1.2. DECONSTRUCTING COMPULSIVE BEHAVIORS

This section reviews the concept of compulsivity regarding the different constructs implicated in its formation and maintenance, and focuses on the preclinical and clinical evidence supporting the theory.

1.2.1. Compulsive behaviors: Clinical and preclinical evidence

A growing body of evidence indicates that different cognitive mechanisms mediate compulsive behaviors in a wide range of disorders [reviewed in (Fineberg et al., 2014)]. The proposed theories included impaired behavioral inhibition, both in terms of motor and cognitive impulsivity, cognitive inflexibility, and an overemphasis on habit formation at the expense of goal-directed behavior. Additionally, anxiety and stress may exacerbate these mechanisms, as Figure 1 illustrates (Robbins et al., 2019).

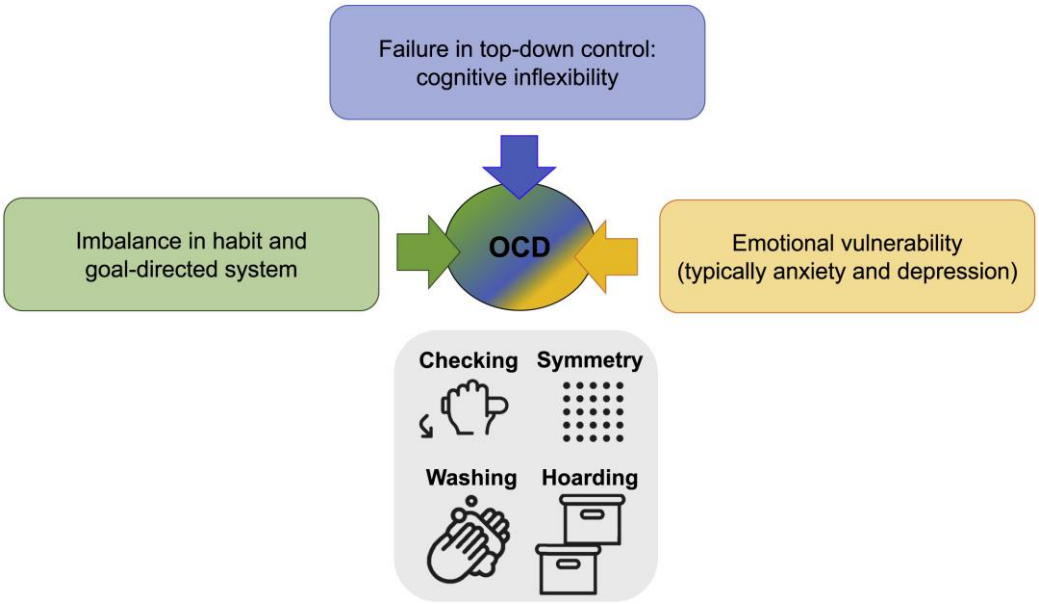

Figure 1. Different constructs resulting in obsessive- compulsive disorders development (From Robbins et al., 2019)

Impulsive behavior, in the context of behavioral inhibition, refers to acting prematurely without considering consequences. This can manifest in two forms: motor impulsivity, which involves excessive behavior, and cognitive impulsivity, which involves making choices for immediate rewards over delayed larger rewards. Research indicates an increased motor impulsivity, typically measured using the Stop-Signal Task, in OCD patients (Boisseau et al., 2012; Chamberlain et al., 2006; Morein-Zamir et al., 2010; Sohn

et al., 2014). According to several clinical studies, cognitive impulsivity, typically measured using the Delay-Discounting Task (DDT), is increased in OCD patients comparing to healthy controls (Benatti et al., 2013; Grassi et al., 2018, 2020; Pinto et al., 2014; Sohn et al., 2014).

Cognitive flexibility refers to the ability to adapt to changes and involves switching between different conceptual representations, particularly in response to alterations in rules and feedback from the environment (Chamberlain et al., 2021). Moreover, recent research suggests that decision-making is also a fundamental construct of OCD (Grassi et al., 2015, 2018, 2020). The impaired ability to execute cognitive flexibility and decision-making tasks in individuals with compulsive behavior may indicate an abnormal processing of the consequences of learning. Therefore, the processing of rewards during compulsions or after avoiding undesired consequences may also play a crucial role in maintaining compulsive behavior. Those with OCDRs may engage in repetitive and inflexible behaviors due to the rewarding effect of performing their compulsions flawlessly or reducing the distress induced by obsessions (Denys, 2011). Compulsive behaviors are driven by rewards (Ferreira et al., 2017) and can be seen as addictive behaviors that arise from faulty processing of natural rewards (Figee et al., 2011). The intradimensional-extradimensional shift tasks evaluate various components of flexibility, such as inhibition, reversal learning, set formation, and the ability to shift attention between stimuli (Fineberg et al., 2018). Impairments in cognitive flexibility have been observed in individuals with OCD and their unaffected relatives (Chamberlain et al., 2006, 2021; Fineberg et al., 2018), as well as in patients with other disorders on the obsessive-compulsive spectrum, including obsessive-compulsive personality disorder and schizophrenia with comorbid OCD (Patel et al., 2010; Vaghi et al., 2017). Referring to decision making, the Iowa Gambling Task is the most commonly used tool to assess this ability (Bechara et al., 1994). Individuals with OCD tend to make risky decisions, preferring options that offer high initial rewards but ultimately result in unfavorable outcomes (Cavedini et al., 2002, 2012; da Rocha et al., 2011; Grassi et al., 2015, 2018, 2020; Kodaira et al., 2012). Moreover, a dysfunctional reward circuit has been suggested in patients with OCD and gambling disorders (Grassi et al., 2020).

Finally, alterations in **motivation and emotion** seem to be involved in compulsive spectrum disorders. Social defeat and subordination may contribute to the development

of emotional disorders such as depression (Gardner & Wilson, 2004). Avoidance behavior is a common pattern associated with disorders exhibiting compulsive symptoms, such as post-traumatic stress disorder, avoidant personality disorder, anxiety disorders, alcohol use disorder, and avoidant/restrictive food intake disorder (American Psychiatric Association, 2013a). Studies suggest that OCD patients exhibit impaired goal-directed behavior and maladaptive habit learning, as well as altered processing of motivational incentives and rewards (Gillan & Robbins, 2014; Jung et al., 2011). Furthemore, disrupted affective processing of feedback from social and environmental contexts is associated with OCD symptoms, and social deficits are common in these populations (Baribeau et al., 2019; O'Kearney, 2007). Harm avoidance is also a significant motivational factor underlying compulsive behavior in OCD (Bejerot et al., 1998). Moreover, individuals with a behavioral compulsive pattern in OCD and addiction disorders may exhibit higher experiential avoidance due to the distress caused by the situation (Den Ouden et al., 2022; Gillan et al., 2020).

1.2.2. A preclinical model to study compulsivity

In the Laboratory of Neuroscience at the University of Almeria we work with Schedule-induced polydipsia (SIP), a preclinical model for the study of compulsivity that meets the validity criteria for studying the compulsive phenotype and modeling different psychopathologies related to compulsive spectrum disorders, such as OCD, schizophrenia and alcohol abuse [for a review (Martín-González et al., 2022; Moreno & Flores, 2012; Moreno-Montoya et al., 2022)]. SIP fulfills the following criteria: 1) Face validity, SIP induces persistent and excessive drinking behavior that is not driven by physiological needs but rather situational distress created by intermittent reinforcement (Falk, 1961; Falk, 1971). These behavioral characteristics are similar to those observed in compulsive behavior according to DSM-V criteria. A similar behavior, psychogenic polydipsia, is also present in 6-20 % of patients with schizophrenia and compulsive spectrum disorders (de Leon et al., 1994; Iftene et al., 2013). 2) Construct validity, the neurobiological basis of SIP behavior is related to compulsivity involving areas such as the prefrontal cortex, hippocampus, and nucleus accumbens (Mittleman et al., 1990, 1992; Robbins & Koob, 1980). The neuroendocrine function of the hypothalamus-pituitary-adrenal (HPA) axis also plays an important role in SIP behavior (Brett & Levine, 1979; Mittleman et al.,

1992). There is a strong neurochemical basis for SIP involving serotonin, 5-HT receptors, and dopamine signaling (Cardona et al., 2006; Mittleman et al., 1990; Mora et al., 2018; Moreno et al., 2010; Robbins & Koob, 1980). 3) Predictive validity, has been demonstrated by the reduction of compulsive drinking in SIP, without affecting regulatory drinking, using drugs used to treat OCD symptomatology, such as antipsychotics and selective serotonin reuptake inhibitors (SSRIs) (Didriksen et al., 1993; Mittleman et al., 1994; Platt et al., 2008; Woods-Kettelberger et al., 1997). 4) Reliability, where the compulsive behavior in SIP is easily measurable, stable in all individuals, and reproducible in any laboratory under specific conditions. The reliability of the model allows for comparisons and progress in research on this behavior. These criteria have been extensively reviewed (Martín-González et al., 2022; Moreno & Flores, 2012; Moreno-Montoya et al., 2022).

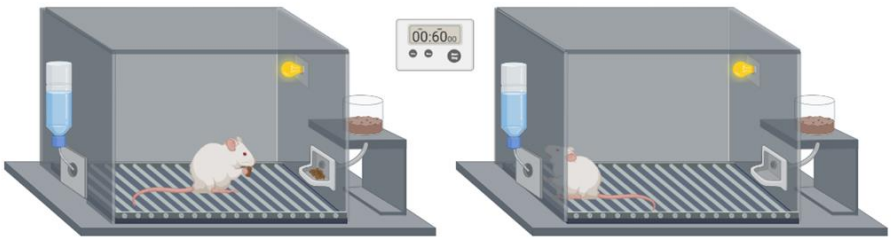

Figure 2. Representation of the Schedule-induced polydipsia procedure, illustrating the disposition of the Skinner box, the dispensation of the pellet each 60-second and the rat behaviors measured during the process

SIP was discovered by John Falk in 1961. Falk conducted a study on fluid regulation in rats subjected to intermittent food reinforcement schedules while having access to a bottle of water during the experimental session as illustrated in Figure 2 (Falk, 1961). The rats were rewarded for lever presses, and Falk observed that they consumed a significant amount of water, sometimes up to half their body weight (Falk, 1966). It is worth mentioning that the drinking behavior observed was not for regulatory purposes, as the rats were not water-deprived. Thus, SIP is characterized by the development of an adjunctive behavior of repetitive drinking in food-deprived animals, which are exposed to intermittent food-reinforcement schedules.

Nevertheless, not all animals develop adjunctive behavior on SIP, individual differences in drinking behavior have been observed after 15-20 SIP sessions (Moreno & Flores, 2012). In our laboratory, we assess compulsivity-related individual differences by categorizing rats into two groups based on their drinking behavior: low drinkers (LD) vs. high drinkers (HD). The categorization is based on whether their drinking rates (averaged for each rat on the last 5 SIP sessions) fall below or above the group median, respectively. The differences in drinking behavior between LD and HD are not due to regulatory water consumption, as there is no difference in water intake between the two groups in their home cages during a 24-hour period or after 23 hours of water deprivation followed by a one-hour access to water (Flores et al., 2014).

In the Laboratory of Neuroscience, we have found consistent differences between these two populations in different constructs related to compulsive behavior:

1. Motor impulsivity measured by premature responses on the sustained attentional 5-Choice Serial Reaction Time Task (5-CSRTT) (Moreno et al., 2010, 2012);
2. Cognitive impulsivity in the DDT and Variable Delay to Signal Task (VDS), expressed by impulsive choices and premature responses (Cardona et al., 2006, 2011; Martín-González et al., 2023; Moreno et al., 2010);
3. Cognitive inflexibility evidenced by an increase in the trials to criterion, number of perseverative responses, or errors in different versions of the Reversal Learning Task (RLT) (Martín-González et al., 2023.; Merchán et al., 2019; Navarro et al., 2015). Indeed, this behavioral inflexibility has also been evidenced by resistant to extinction behavior in HD rats, characterized by an increased compulsivity assessed by perseverative responses under an extinction condition on the 5-CSRTT (Moreno et al., 2012) and insensitivity to outcome devaluation during extinction under selective satiation (Merchán et al., 2019).
4. Decision making showed by increased perseverative responses during the punishment period on Rodent Gambling Task (rGT) (Martín-González et al., 2023). 5) Resistant emotional memory on Passive Avoidance Task (PA), expressed by a sustained higher latency to enter the dark compartment at the last extinction session (Martín-González et al., 2022).

In Table 2, we summarized the most relevant data to characterize the profile of HD compulsive rats.

Behavioral Construct	Task	Result	Reference
Motor impulsivity	5-CSRTT	Premature responses	Moreno et al. 2010
Cognitive impulsivity	DDT and VDS	Impulsive choices and premature responses	Cardona et al. 2006, 2011; Martín-González et al. 2023
Behavioral inflexibility	RLT, PRLT and 5-CSRTT	Increased number of trials to criterion and increased number of perseverative responses	Martín-González et al. 2023; Merchán et al. 2019; Moreno et al., 2012; Navarro et al. 2015.
Decision making	rGT	Perseverative responses during the punishment period	Martín-González et al. 2023
Resistance to emotional memory extinction	PA	Higher latency to enter the dark compartment	Martín-González et al., 2022

Table 2. Data of behavioral alterations of compulsive behavioral constructs in HD rats selected by SIP (5-CSRTT: 5-Choice Serial Reaction Time Task; DDT: Delay-Discounting Task; VDS: Variable Delay to Signal Task; RLT: Reversal Learning Task; PRLT: Probabilistic Reversal Learning Task; rGT: Rodent Gambling Task; PA: Passive Avoidance Task)

Moreover, HD rats have shown selective attentional deficits in an animal model of schizophrenia, by reduced latent inhibition in the preexposed condition to an stimuli, compared to low drinker LD rats (Navarro et al., 2017), and social dominance impairment on the Social Dominance Tube Test (Martín-González et al., 2022).

Despite these studies, there are few experimental approaches in animals that have characterized the comorbidity with other altered pathological behaviors in preclinical models of compulsivity. Previously, no differences were found in anxiety-like behavior measured by EPM in compulsive HD rats (López-Grancha et al., 2008). Besides, there is

little research in the study of cognitive processing, such as stimuli processing, memory and cognitive flexibility in aversive contexts in preclinical models of compulsive behavior. Thus, the present thesis proposes a behavioral characterization focusing on the measurement of different comorbid disorders to OCD and the assessment of cognitive processing in a preclinical model of compulsivity.

	SSRI	**TCAs**	**Benzo-diazepines**	**Others**
Acute efficacy	Citalopram Fluoxetine Fluvoxamine Paroxetine Sertraline Escitalopram	Clomipramine Imipramine	Clonazepam	
Long-term efficacy	Fluvoxamine Sertraline	Clomipramine		
Relapse prevention	Fluvoxamine Paroxetine Sertraline Escitalopram			
Enhances psychological treatment	Fluvoxamine	Clomipramine		
After non-response				Add haloperidol, risperidone, quetiapine, olanzapine or aripiprazole to SSRI

Table 3. The classical psychopharmacology for compulsive spectrum disorders (Fineberg & Gale, 2005; SSRI: selective serotonin reuptake inhibitors; TCAs: tricyclic antidepressants)

1.3. PSYCHOPHARMACOLOGY FOR COMPULSIVE BEHAVIORS

This section offers an overview of the classical treatments for compulsive disorders and focuses on the preclinical and clinical evidence to explain that glutamatergic drugs could be a promising potential benefit in compulsive disorders.

1.3.1. Classical treatments for compulsivity

The clinical psychopharmacology to treat compulsivity in different disorders is focused on selective serotonin reuptake inhibitors (SSRIs) (Nezgovorova et al., 2022), such as fluvoxamine, fluoxetine, sertraline, paroxetine and citalopram (Fineberg & Gale, 2005). Moreover, neuroleptics, particularly haloperidol, risperidone, and aripiprazole, are also used as an augmentation of stable SRI treatment when symptom response is incomplete (Thamby & Jaisoorya, 2019). Table 3 presents the classical psychopharmacology in the treatment of OCD.

Nevertheless, up to 40-60 % of OCD patients do not respond successfully to these treatments (Pallanti et al., 2002). Novel treatments based on glutamate-modulating drugs have been proposed as a potential treatment for OCD (Marinova et al., 2017a).

1.3.2. New treatments for compulsivity: Preclinical and clinical evidence on glutamate modulation

Clinical studies suggest that glutamate-modulating drugs seem to have a beneficial effect in reducing compulsive symptoms in humans (Marinova et al., 2017a) maybe because of its fundamental role in neuronal plasticity, learning, and memory (Javitt et al., 2011). Glutamate, the major excitatory neurotransmitter in the brain, is highly implicated in the cortico-striatal-thalamic circuit (Ting & Feng, 2011), the proposed neuroanatomical basis in compulsive deficit (Fineberg et al., 2010; Menzies et al., 2008) which present a rich glutamatergic receptor density (Monaghan et al., 1985). A dysregulation of glutamatergic signaling in the corticostriatal circuitry has been suggested in OCD, with reduced glutamatergic concentrations in the anterior cingulate cortex, as well as overactivity of glutamatergic signaling in the striatum and orbitofrontal cortex (Milad & Rauch, 2012; Pittenger et al., 2011; Ting & Feng, 2011). Moreover, glutamatergic genes have been implicated in the risk for OCD (Pauls et al., 2014). Clinical studies using a meta-analysis revealed that memantine (MEM), a glutamatergic modulator currently employed

in the treatment of Alzheimer disease, had positive effects in OCD patients (Modarresi et al., 2019). Additionally, glutamatergic anticonvulsant drugs like lamotrigine (LAM) and topiramate, and riluzole, may also provide therapeutic benefits in OCD (Marinova et al., 2017a). In the following paragraphs, we will try to disentangle the clinical and preclinical evidence using glutamatergic drugs in compulsivity.

The N-Acetylcysteine (NAC), glutathione precursor and a cell-permeable antioxidant, decrease the synaptic glutamate release (Moran et al., 2005). In clinical studies, NAC treatment has been shown to be effective in SRI-resistant OCD patients (Lafleur et al., 2006). Chronic treatment of NAC in OCD patients, 10–12 weeks, reduced the Yale-Brown Obsessive-Compulsive Scale [Y-BOCS; (Afshar et al., 2012; Paydary et al., 2016)]. Moreover, it has also shown to improve symptomatology in other psychiatric syndromes, including depression, bipolar disorder, suicidality, and self-injurious behavior (Niciu et al., 2014; Pittenger et al., 2005; Price et al., 2009). In a preclinical study, using an acute administration of 100 mg/kg of NAC reduced ethanol self-administration and ethanol-seeking behavior (Lebourgeois et al., 2017).

MEM, an uncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist (Reisberg et al., 2003) has also shown a beneficial effect in compulsivity. MEM reduces glutamate release through inhibition of voltage-dependent calcium channel and protein kinase C (Lu et al., 2010). In OCD patients, MEM reduced the Y-BOCS scores after chronic treatment with MEM (Ghaleiha et al., 2013; Haghighi et al., 2013). Preclinical studies showed that acute administration of 25 mg/kg MEM suppressed ethanol self-administration in non-dependent rats and decreased by half the one of post-dependent rats during acute withdrawal (Alaux-Cantin et al., 2015). Besides, the administration of MEM (10 mg/kg) and amantadine, another uncompetitive NMDA receptor antagonists (30 mg/kg), significantly inhibited compulsive marble burying in mice (Egashira et al., 2008) Moreover, the combination of MEM and fluoxetine reduced scratching behavior, considered as an effective model for studying compulsive behavior (Wald et al., 2009).

LAM has an antiepileptic activity due to the inhibition of the voltage-sensitive neuronal membrane sodium channels, the inhibition of the excitatory amino acids release such as glutamate and aspartate, and the blockade of the calcium-channel (Cheung et al., 1992; Cunningham & Jones, 2000; Prabhavalkar et al., 2015; Xie et al., 1995). A clinical

study with chronic treatment with LAM evidenced a decrease in Y-BOCS scores in OCD patients, in addition to the Hamilton Rating Scale for Depression scores, the Clinical Global Impression-Improvement scores and the obsession and compulsion subscales (Bruno et al., 2012; Khalkhali et al., 2016). Besides, preclinical research showed that 15 and 30 mg/kg acute treatment of LAM significantly reduced immobility in the Forced Swimming Test (FST) (Li et al., 2010). However, there is insufficient preclinical research on the therapeutic role of these glutamate release modulators on reducing compulsive behaviors.

Thus, the preclinical studies might be an approach to study the neurobehavioral mechanisms associated with compulsive disorders and its possible treatments. However, there are no studies that have previously used the compulsive model of SIP for researching the glutamatergic psychopharmacology in the compulsive phenotype (Moreno & Flores, 2012; Platt et al., 2008; Rodriguez et al., 2017). Previous studies on SIP, revealed the efficacy of antipsychotic (haloperidol, clozapine, and pimozide) and SRI (fluoxetine) drugs in reducing compulsive water intake on SIP (Didriksen et al., 1993; Dwyer et al., 2010; Hogg & Dalvi, 2004; Mittleman et al., 1994; Snodgrass & Allen, 1989). In the compulsive phenotype of HD rats selected by SIP, citalopram and the serotonin 5-HT2A/C receptor agonist DOI reduced compulsive drinking (Navarro et al., 2015). Moreover, a recent study has revealed that HD rats showed cortical reduced serotonin 5-HT2A receptor binding and increased serotonin and reduced glutamate efflux compared to LD rats (Mora et al., 2018). In Table 4 we summarized the most relevant data to describe the data obtained in our compulsive model.

To summarize, recent research points towards the need for new psychopharmacological targets in the treatment of compulsivity, and glutamate-modulating drugs seem to be a new possible candidate as it has beneficial effect in reducing compulsive symptoms in humans (Marinova et al., 2017). Moreover, previous data support the hypothesis of a dysregulation in glutamate levels on the SIP compulsive phenotype: 1) Dysregulation in 5-HT2A and glutamatergic neurochemical signal, as HD rats showed increased serotonin and decreased glutamate efflux in basal conditions (Mora et al., 2018). 2) Increased glutamate tone found in HD blood samples (Abreu et al., 2022). 3) Reduced binding levels of 5-HT2A receptors in FC and BLA (Mora et al., 2018, 2020) that might also indicate a reduction in the glutamatergic metabotropic receptor 2, as the 5-HT2A receptor can form

a heterocomplex with the metabotropic glutamate receptor type II (mGlu2) (González-Maeso et al., 2008). Also, in a GWAS meta-analysis, OCD vulnerability was associated with genetic polymorphism expression of glutamate and serotonin receptors (Taylor, 2013). This might result in additional regulatory effects on the excitation of pyramidal cells (Delille et al., 2013). Previous in vivo preclinical investigations have found that the 5-HT2A and mGlu2 receptors are linked to differences in impulsive behavior (Fink et al. 2015; Fomsgaard et al. 2018; Klein et al. 2014). Based on this, we may hypothesize that dysregulations in prefrontal 5-HT2A receptor signaling among compulsive and vulnerable individuals are related to variations in mGlu2 receptor and glutamate signaling. Thus, the present doctoral thesis proposes a pharmacological study to assess the effect of different glutamate-modulators in an animal model of compulsive behavior.

5-HT2A hallmark	Neuroplasticity changes
SSRI and DOI systemic administration reduced compulsive drinking on SIP (Navarro et al., 2015).	Reduced myelination in corpus callosum, striatum, and BLA (Navarro et al., 2017).
DOI administration into mPFC reduced compulsive drinking through restoration of glutamatergic tone on SIP (Mora et al., 2018). Increased glutamate tone in blood samples (Abreu et al., 2022).	Stereology: Reduced volume of dorsal Hippocampus and Increased volume of BLA (Mora et al., 2020).
Reduction of 5-HT2A receptor binding in FC and in BLA (Mora et al., 2018; 2020).	MRI: Decreased volume in mPFC and Increased volume of white matter structures including Striatum, DG, Amygdala, (Martín-González et al., 2023).

Table 4. Principal data on neuronal mechanism underlying Schedule-induced polydipsia (SIP) compulsive model (Reviewed in Martin-Gonzalez et al. 2022; SSRI: selective serotonin reuptake inhibitors; mPFC: medial prefrontal cortex; FC: frontal cortex; BLA: basolateral amygdala; MRI: magnetic resonance image).

1.4. GENE EXPRESSION IN COMPULSIVE BEHAVIORS

Genes do not solely determine our behavior, they can play a significant role in shaping our personality traits, tendencies, and vulnerabilities. Our genetics can influence our behavior in several ways. Firstly, genes can control the production and functioning of specific neurotransmitters, such as dopamine and serotonin, which are essential for regulating mood, emotion, and behavior. Besides, genes can affect the structure and function of the brain circuits, influencing the way we behave and respond to the environment. For example, some genes may influence the development of specific brain regions, such as the prefrontal cortex, which is crucial for decision-making, planning, and impulse control. Moreover, genes interact with environmental factors, such as stress, nutrition, and social experience, to shape our behavior over time. Hence, genes are an important factor in the research of the underlying neurobehavioral mechanisms of compulsivity.

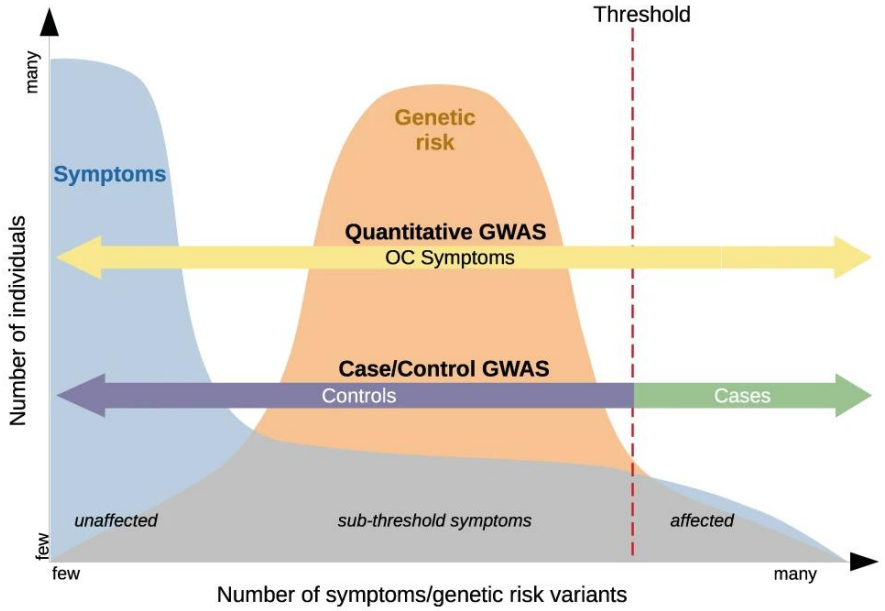

Figure 3. The distribution of genetic-risk variants for OCD in the population, shown in orange from low to high. The distribution of symptoms for OCD in the population is shown in blue, while a red dashed line represents the diagnostic threshold. In a case/control genome-wide association study (GWAS) using a diagnostic framework, individuals to the left of the threshold (indicated by a purple arrow) would be classified as controls, while those to the right (indicated by a green arrow) and diagnosed with OCD would be considered cases. In a quantitative GWAS using a dimensional framework, all individuals across the spectrum of symptom severity and genetic risk (indicated by a yellow arrow) would be included, with subthreshold symptoms also being taken into account (Strom et al., 2021)

Clinical studies have shown genetic alterations in patients with OCD (Figure 3), with multiple genes implicated in its etiology (Johnson et al. 2021). Recent clinical genetic studies on OCD have also considered its comorbidity with other disorders. Genome-wide association studies have elucidated the role of genetic polymorphisms in serotonin transporter (Sert) and the gene that encode serotonin receptor 2A (*Htr2a*) in the vulnerability to OCD (Taylor, 2013). Additionally, emerging evidence has implicated variations in glutamatergic genes, such as the *Sapap* (DLGAP) family, *Slc1a1*, and *Grin*/*Grik* families, in dysfunctional glutamate signaling in OCD (Rajendram et al., 2017). Furthermore, studies have also shown an association between genetic polymorphisms in the Brain Derived Neurotrophic Factor (*Bdnf*) gene and OCD, with lower BDNF serum levels observed in OCD patients (Katerberg et al., 2010; Şimşek et al., 2016; Taj M J et al., 2018).

Recent clinical studies focused on the role of genetic factors in variable treatment responses have examined associations between drug response in OCD patients and candidate genes, including genes related to serotonin regulation, *Htr2a* and *Slc6a4* and; glutamate signaling, *Slc1a1* and *Dlgap2*; and neurodevelopment, *Bdnf* and *Ntrk3*; in individuals with OCD (Fineberg et al., 2020). Moreover, a study conducted on 4645 OCD patients found that comorbidity between OCD with another impulse control disorder (attention deficit hyperactivity disorder) was linked to *Htr2c* genotypes (Nezgovorova et al., 2018).

Recent clinical studies focused on the role of genetic factors in variable treatment responses have examined associations between drug response in OCD patients and candidate genes, including genes related to serotonin regulation, *Htr2a* and *Slc6a4* and; glutamate signaling, *Slc1a1* and *Dlgap2*; and neurodevelopment, *Bdnf* and *Ntrk3*; in individuals with OCD (Fineberg et al., 2020). Moreover, a study conducted on 4645 OCD patients found that comorbidity between OCD with another impulse control disorder (attention deficit hyperactivity disorder) was linked to *Htr2c* genotypes (Nezgovorova et al., 2018). These findings suggest that genetic factors contribute to the pathophysiology and treatment response of OCD, providing valuable insights into the underlying mechanisms of the disorder. Further research in this area could potentially pave the way for personalized treatment approaches for individuals with OCD based on their genetic profiles, lead-

ing to more effective interventions and improved patient outcomes. These findings highlight the complex interplay between genetic factors and OCD, providing valuable insights into the molecular mechanisms underlying the disorder.

To date there are no studies on the genetic basis of the compulsive phenotype of HD rats selected by SIP. However, there is evidence that point towards the implication of the genetic expression of *Htr2a*, the gene that encode 5-HT2A receptor. The compulsive HD rats selected by SIP, have shown reduced cortical 5-HT2A binding levels compared to LD rats (Mora et al., 2018). Indeed, the systemic and prefrontal administration of the serotonin 5-HT2A/C receptor agonist DOI has been shown to reduce in a dose-dependent manner compulsive water intake in HD rats on SIP (Mora et al., 2018; Navarro et al., 2015). Moreover, BDNF might also be implicated, as 5-HT2A receptor agonist DOI has shown to regulate neuroplasticity agents as BDNF (Malkova et al., 2014). Furthermore, glutamatergic genes might as well be playing an important role in compulsive behaviors, as the 5-HT2A receptor can form the heterocomplex with the metabotropic glutamate mGlu2 receptor, as illustrates Figure 4 (González-Maeso et al., 2008). Moreover, in a meta-analysis of GWAS, OCD vulnerability was associated with genetic polymorphism expression of glutamate and serotonin receptors (Taylor, 2013).

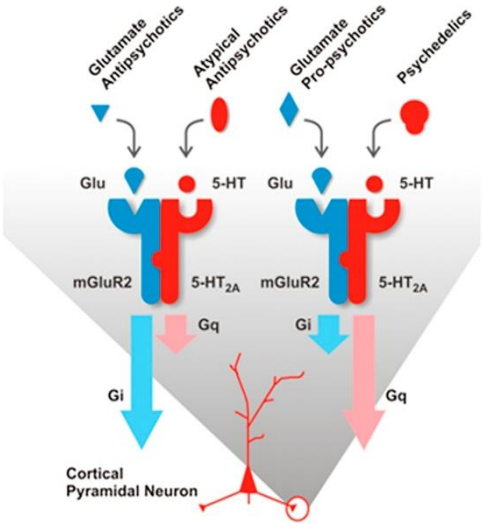

Figure 4. Heteromeric assembly of mGlu2 and 5-HT2A. The assembly enhances glutamate-elicited Gi signaling and reduces 5-HT-elicited Gq Signaling (Fribourg et al., 2011).

Further research in this area has the potential to advance our understanding of OCD and facilitate the development of personalized treatment strategies for individuals affected by this condition. The present doctoral thesis will explore the possible differences in the genetic expression of serotonergic genes (*Htr2a* and *Htr2c*), glutamatergic genes (*Grin1*, *Grin2a*, *Grin2b*, *Grin2c* and *Grm2*) and the neuroplasticity *Bdnf* gene in an animal model of compulsive behavior.

Chapter 2. Approach and objectives

2.1. APPROACH

2.1.1. Background

As exposed in the previous section (general introduction), clinical data demonstrated that OCD patients presented a lifetime prevalence of other comorbid disorders: 56.4 % major depression, 34.6 % social phobia, 34.3 % generalized anxiety disorder, 31.4 % specific phobia (Torres et al., 2016), and 15.3 % panic disorder (Torres et al., 2014).

Moreover, meta-analyses identify at least 5 different endophenotypes of OCD due to the symptomatic heterogeneity of these groups of patients, suggesting that OCD could be a consequence of dysfunctional circuits regulating: response inhibition, cognitive flexibility, planning (and goal-directed behavior), working memory, and error monitoring (Robbins et al. 2019). According to the clinical data, OCD patients present behavioral inflexibility in the Wisconsin Card Sorting Test and persistent skin conductance response in the extinction phase of the FCT (Benzina et al., 2016; Geller et al., 2017). However, the assessment of memory deficits in OCD has not been fully explored (Palit et al. 2022).

Regarding pharmacological clinical data, the treatment of compulsivity in OCD patients has been focused on serotonin reuptake inhibitors (SRIs), such as fluvoxamine, fluoxetine, sertraline, paroxetine and citalopram (reviewed in Fineberg and Gale, 2005). However, recent studies point out that up to 40 % of patients do not respond successfully to SRIs treatment (Marinova et al., 2017). Recent studies suggest that glutamate-modulating drugs seem to have a beneficial effect in reducing compulsive symptoms in humans (Marinova et al., 2017).

Genome-wide association studies have shown that OCD vulnerability is associated with genetic polymorphisms in the *Sert* (serotonin transporter) and *Htr2a* (Taylor et al., 2013). Moreover, recent studies have evidence that variations in glutamatergic genes such as the *Slc1a1*, and *Grin*/*Grink* families that can lead to dysfunctional glutamate signaling

in OCD (reviewed in Rajendram et al., 2017). Many studies support that there is an association between *Bdnf* (Brain Develop Neurotrophic Factor) gene polymorphism and OCD (Katerberg et al., 2009; Taj M J et al., 2018), reporting a lower BDNF serum level in OCD patients (Şimşek et al., 2016; Wang et al., 2011).

2.1.2. Justification

According to the studies mentioned above and the profile of compulsive phenotype of HD rats, the present doctoral thesis aimed to further investigate the compulsive model of SIP in order to better understand the underlying mechanisms of this specific trait by different approaches: behavioral, pharmacological and genetic.

Thus, first we would further explore the compulsive behavior phenotype of HD rats selected by SIP, including comorbid behaviors associated with compulsive disorders, such as compulsive behavior, anxiety, depression and phobia; as well as cognitive processing, including stimuli processing, spatial and working memory and cognitive flexibility; that could be altered in the compulsive population. Then we would try new pharmacological targets related to glutamatergic modulators, such as NAC, MEM and LAM, that could reduce compulsivity on SIP. Finally, we would investigate the specific alterations in the gene expression that might contribute as underlying mechanisms of this specific trait, such as serotonergic (*Htr2a* and *Htr2c*), glutamatergic (*Grin1*, *Grin2a*, *Grin2b*, *Grin2c* and *Grm2*) and neuroplasticity (*Bdnf*) genes in the the neuroanatomical areas related to the cortico-limbic circuit and compulsive drinking on SIP.

In this sense we could better understand compulsive spectrum disorders and try to contribute to the development of new therapeutic and diagnose targets, such as possible biomarkers of vulnerability to compulsive spectrum disorders.

2.2. OBJECTIVES

According to the evidence in the introduction section and justification, the general objectives of the present thesis are:

1. **Behavioral characterization** of the compulsive phenotype of HD rats selected by SIP:

   1A. To characterize the comorbid disorders for obsessive compulsive spectrum disorders in a compulsive phenotype of rats, such as compulsive behavior, anxiety, depression and fear.

   1B. To assess the cognitive function related with stimuli processing, spatial and working memory and cognitive flexibility.

2. **Pharmacological study** to modulate the glutamatergic signal to reduce the compulsive behavior in a compulsive phenotype of rats.
3. **Gene expression analysis** to investigate the gene expression of the serotonin, glutamate and neuroplasticity in the brain circuit related to compulsivity.

Therefore, in the present Doctoral Thesis, we selected two populations with differences in compulsive behavior, high drinkers (HD) and low drinkers (LD) rats by SIP procedure to addresses the following specific objectives:

1) Behavioral characterization

1. To explore the possible presence of other altered behaviors considered as comorbid symptoms for compulsivity, such as depressive, anxiety and fear symptoms, in high compulsive animals selected by SIP using Marble Burying Test (MBT), Forced Swimming Test (FST), Elevated Plus Maze (EPM) and Fear Conditioning Test (FCT) (Experiment 1A).
2. To examine spatial memory and cognitive flexibility, considered as possibly altered processes in compulsive populations, in HD and LD rats using Morris Water Maze (MWM) and Reversed MWM (Experiment 1B).
3. To evaluate different kinds of memory, including reference and working memory, as possible altered processes in compulsive rats selected by SIP by Radial Arm Maze (RAM) (Experiment 1B).

4. To explore stimulus processing and novelty reaction in HD rats selected by SIP by Novel Object Recognition Test (NOR) (Experiment 1B).

2) Pharmacological study

5. To assess the therapeutic potential of the inhibition of the presynaptic glutamate release, by the acute administration of N-Acetylcysteine (NAC), in a compulsive phenotype of rats (Experiment 2).
6. To investigate the therapeutic effect of an uncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist, by the acute administration of memantine (MEM), in a compulsive phenotype of rats (Experiment 2).
7. To examine the effect of the inhibition of the glutamate release by the blockage of the sodium channels, by the acute administration of lamotrigine (LAM), in a compulsive phenotype of rats (Experiment 2).

3) Gene expression analysis

8. To explore the expression of the serotonergic genes, including *Htr2a* and *Htr2c;* in relevant brain areas, such as frontal cortex, hippocampus, and amygdala in compulsive populations of rats (Experiment 3).
9. To screen the expression of glutamatergic genes, including *Grin1*, *Grin2a*, *Grin2b*, *Grin2c* and *Grm2*; in relevant brain areas, such as frontal cortex, hippocampus, and amygdala in compulsive rats (Experiment 3).
10. To explore the expression of *Bdnf* gene in relevant brain areas, such as frontal cortex, hippocampus, and amygdala in compulsive HD rats selected by SIP (Experiment 3).

2.3. HYPOTHESES

Regarding the information presented in the introduction section, we proposed a experimental schedule (see Table 5) and the following hypotheses:

1. Compulsive HD rats will present other altered behaviors, related to OCRDs psychopathologies that have comorbidity with compulsivity symptoms. HD rats might show compulsivity in other paradigms, depressive and anxiety-like behavior, increased fear learning acquisition and resistance to fear extinction (1st experimental set).
2. Compulsive animals will express learning and memory deficits, cognitive inflexibility and altered stimuli processing by a higher novelty reactivation (1st experimental set).
4. The acute administration of the glutamatergic modulators N-Acetylcysteine, memantine and lamotrigine, that modulate glutamatergic signaling, will decrease the compulsive behavior of HD rats on SIP (2nd experimental set).
5. HD rats will present a downregulation in the genetic expression of serotonergic genes *Htr2a* and *Htr2c*, in frontal cortex, hippocampus and amygdala (3rd experimental set).
6. HD rats will show an upregulation in the genetic expression of glutamatergic genes *Grin1*, *Grin2a*, *Grin2b*, *Grin2c* and *Grm2*, in frontal cortex, hippocampus and amygdala (3rd experimental set).
7. HD rats will show a downregulation in the genetic expression of *Bdnf* gene, in frontal cortex, hippocampus and amygdala (3rd experimental set).

1st experimental set: behavioral characterization.
Experiment 1A: comorbid behaviors in compulsive rats selected by SIP.
To screen compulsivity by SIP: HD vs LD.
To test the differences between LD and HD animals in compulsive-like behavior by Marble Burying Test.
To explore the possible differences between LD and HD animals in depressive-like behavior using the Forced Swimming Test.
To assess the differences in anxiety-like behavior on the Elevated Plus Maze Test.
To explore the possible differences between LD and HD groups in fear acquisition, expression, and extinction on Fear Conditioning Test.

Table 5. Experimental schedule

Experiment 1B: cognitive processing in compulsive rats selected by SIP.
To screen compulsivity by SIP: HD vs LD.
To test the differences between LD and HD rats in spatial learning by Morris Water Maze.
To explore the differences between LD and HD animals in behavioral flexibility by reversing the conditions in Morris Water Maze.
To study the differences between HD and LD groups in reference and working memory using the Radial Arm Maze.
To assess the differences between HD and LD rats in stimuli processing and novelty salience by the Novel Object Recognition Test.
2nd experimental set: pharmacological study.
Experiment 2: glutamatergic modulation on SIP.
To screen compulsivity by SIP: HD vs LD.
To assess the potential effect of the glutathione precursor (N-Acetylcysteine) administration in compulsive rats selected by SIP: HD vs LD.
To explore the potential effect of the administration of the uncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist (memantine) administration in compulsivity selected by SIP: HD vs LD.
To assess the potential effect of the inhibitior of the membrane sodium channels (lamotrigine) administration in compulsive rats selected by SIP: HD vs LD.
3rd experimental set: gene expression analysis.
Experiment 3: gene expression analysis of serotoninergic, glutamatergic and neuroplasticity related genes in rats selected by SIP.
To screen compulsivity by SIP: HD vs LD.
To measure the genetic expression of the *Htr2a* gene in frontal cortex, hippocampus, and amygdala in compulsive rats selected by SIP.
To explore the genetic expression of the *Htr2c* gene in frontal cortex, hippocampus, and amygdala in compulsive rats selected by SIP.
To study the genetic expression of the *Grin1* gene in frontal cortex, hippocampus, and amygdala in compulsive rats selected by SIP.
To measure the genetic expression of the *Grin2a* gene in frontal cortex, hippocampus, and amygdala in compulsive rats selected by SIP.

Table 5 (continued). Experimental schedule

Chapter 3. Experiment 1: Behavioral characterization

3.1. EXPERIMENT 1A: COMORBID BEHAVIORS

The present experiment explores the presence of other altered behaviors, including other forms of compulsivity and typical comorbid symptoms, such as depression, general anxiety and pathological fear disorder in the high compulsive drinker rats HD selected by SIP.

3.1.1. Experimental design

First of all, we select HD and LD rats by SIP. After the selection, animals have been assessed by different paradigms related to compulsive comorbidity. The behavioral tasks selected to achieve this goal have been: the Marble Burying Test (MBT) as an assay of compulsive-like behavior (Egashira et al., 2008; de Brouwer and Wolmarans, 2018); the FST developed by Porsolt et al. (1977) as an animal model of depression that assess learned helplessness; the Elevated Plus Maze (EPM) as a behavioral measure of anxiety for rodents (Pellow et al., 1985); and finally, the Fear Conditioning Test (FCT) to assess aversive learning considered as a behavioral paradigm that models specific phobias (Berardi et al., 2012). The experimental events are summarized in Figure 5.

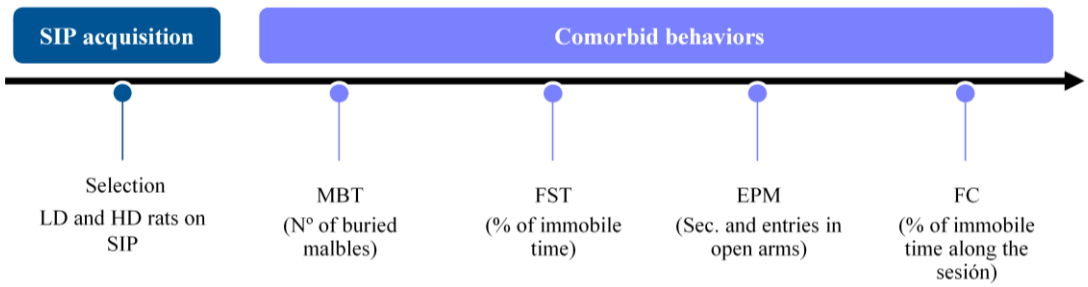

Figure 5. Experimental procedure illustrated in a timetable. SIP: Schedule-induced polydipsia; HD: High drinker; LD: Low drinker; MBT: Marble Burying Test; FST: Forced Swimming Test; EPM: Elevated Plus Maze; FCT: Fear Conditioning Test.

3.1.2. Materials and methods

Subjects

A total of 16 Long Evans male rats (Janvier Labs, France) weighing between 250–350 g at the start of the experiments were used in the present study. The animals were housed in four rats/cages (50 × 35 × 20 cm) kept in a temperature-controlled environment at 22 ± 2 °C, humidity (50 ± 10 %), with a 12:12-h light-dark cycle (light off at 08:00 h am) and food and water provided ad libitum. After ten days of habituation and before behavioral tasks, the rats were gradually reduced to 85 % of their free-feeding body weight through controlled feeding, and their body weights were maintained at this level of deprivation throughout the experiment. Food was provided by daily feedings of lab chow at approximately 30 min after each experimental session. All testing was performed between 9:00 h am and 2:00 h pm. Animals were around 3 months of age when the experiment started and finished it with 5 months of age. All the procedures were conducted following the Spanish Royal Decree 53/ 2013 on the protection of experimental animals, the European Community Directive 2010/63/EU for animal experiments and complies with the ARRIVE guidelines for animal research. The Animal Research Committee of the University of Almeria approved the experiments described here and the authors declare that the research shows commitment to the 3Rs principle (replacement, reduction, refinement).

SIP procedure: HD and LD selection

Initially, over two consecutive days, water consumption in rats was assessed for 60 minutes (baseline). Rats had access to a 100 ml bottle of water and 60 dustless food pellets (45 mg each; catalog number 259901-PE-45/50T TSE Systems, Germany). Following an habituation session to the SIP chambers, the rats were exposed to a fixed-time 60-s (FT-60s) schedule of food reward pellet presentation during 60-min sessions. During each SIP session, a 100 ml bottle of water was placed opposite to the food-magazine in the SIP chamber, and water consumption was recorded at the end of the test session. Licking behavior to the water bottle was detected by the animal touching the metal drinking tube (spout) of the bottle, which is connected to the metal grid of the SIP chamber. An electronic circuit with a low current, less than ten microAmp, that is imperceptible to the animal, connects the spout to the SIP chamber. When the rat touches the water spout, the

circuit is closed, producing a 50 msec pulse that registers a lick. The scheduling and recording of the experimental events were controlled using a computer and the commercial software Med PC (Cibertec SA, Spain). Each rat's total water consumption (in milliliters) from the bottle, the total number of licks to the bottle, and the total entries to the food magazine were recorded. After 20 daily sessions, the rats were classified into two groups, HD and LD, based on whether their drinking rates (averaged over the last five sessions) were above or below the group median, respectively (the number of animals in each group of LD and HD rats was n = 8 for experiment 1 and n= 10 for experiment 2).

Marble Burying Test

MBT began placing the rat into a corner of the cage containing 9 marbles (Figure 6), being careful to place the rat on bedding as far from marbles as possible. Animals were allowed to remain in the cage undisturbed for 30 min. Rats were returned to its home cage after test completion, taking extreme care not to move or dislodge the marbles in the process of removing the subject from the cage. The number of marbles partially and completely buried was counted by two observers blinded to the experimental groups. We found a great concordance between observers. A marble was scored as partially buried if two-thirds of its surface area is covered by bedding and completely buried if all the surface area is covered by bedding (Angoa-Pérez et al., 2013).

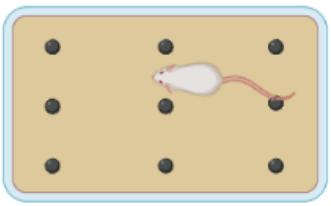

Figure 6. Representation of the Marble Burying Test procedure, illustrating the disposition of the marbles in the cage

Forced Swimming Test

FST was performed in a plastic cylinder containing 20 cm in diameter and 40 cm in height (Figure 7), water temperature was 23-25 °C, and the depth of water was set to prevent animals from touching the bottom. Rats swam in the cylinder for 2 min. The time each animal spent immobile during the last min of the test was counted by two observers blinded to the experimental groups. We found a great concordance between observers.

Immobility was defined as floating or absolute lack of motion (i.e., the absence of all movements except those required to maintain balance; Dong et al., 2018).

Figure 7. Representation of the Forced Swimming Test procedure.

Elevated Plus Maze

For EPM rats were placed at the junction of the four arms of the maze, facing an open arm, and entries/duration in each arm was recorded by a video-tracking system and observer simultaneously for 10 min (Figure 8). We found a good concordance in data collected with both methods. An increase in open arm activity (duration and/or entries) reflects anti-anxiety behavior (Walf and Frye 2007).

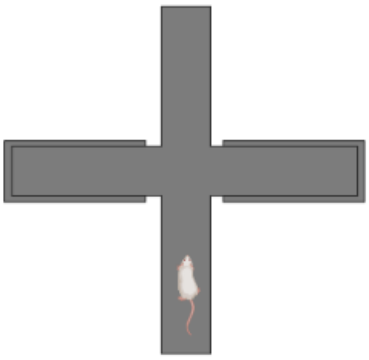

Figure 8. Representation of the Elevated Plus Maze paradigm

Fear Conditioning Test

FCT started placing the rat into a novel cage with a shock grid floor capable of delivering foot-shock where, after 3 min exploration period, they received three pairing of a 10s light (82 lx) with a shock (0.5 mA during 1 sec). The light-shock trials were delivered after a three-minute acclimation time, the inter-lights intervals were 1 min, and the rats remained in the chambers for an additional minute after the last shock (Figure 9). Next day rats were allowed a 3 min exploration period after which they were presented

with 22 lights (10s, 82lx, 1 min inter-lights interval) in the absence of a foot shock (Simone et al., 2017). The freezing time was counted by the Video Freeze Software (Med PC) which detected changes at the pixel level from one video frame to the next. Hence, data can reflect the total time animals spent motionless during the session, the percentage of time motionless and the number of freezing episodes.

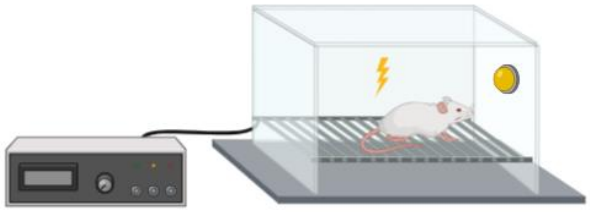

Figure 9. Representation of the Fear Conditioning Test, illustrating the pairing of the light and the shock

Data analyses

Behavioral data on SIP acquisition were analyzed using two-way repeated-measure analysis of variance (ANOVA), with "group" (LD and HD) as the between-subject factor and "sessions" (20 sessions) as the within-subject factor.

The differences on the MBT, FST, EPM, and FCT of the behavioral assessment in LD and HD were studied using Student's t-test (T-test). When appropriate, the effect size of the group differences was calculated using Cohen's d or η^2. Partial eta-squared values of 0.01, 0.06, and 0.14 and Cohen's d values of 0.2, 0.5, and 0.8 are considered to reflect small, medium, and large effects, respectively. The differences on FCT blocks and the effects of the different drugs in LD and HD on SIP were analyzed using two-way repeated-measure ANOVA, with group (LD and HD) as the between-subject factor and "percentage of freezing" (percentage of time spent on freezing during the different blocks of the retrieval day). *Post hoc* comparisons were performed using the Newman-Keuls test. Statistical significance was set at $p < 0.05$. All analyses were computed using Statistica software (version 6.0).

3.1.3. Results

LD and HD Selected by SIP

The mean water intake and licks in LD and HD during the acquisition and maintenance of SIP are shown in Figure 10.

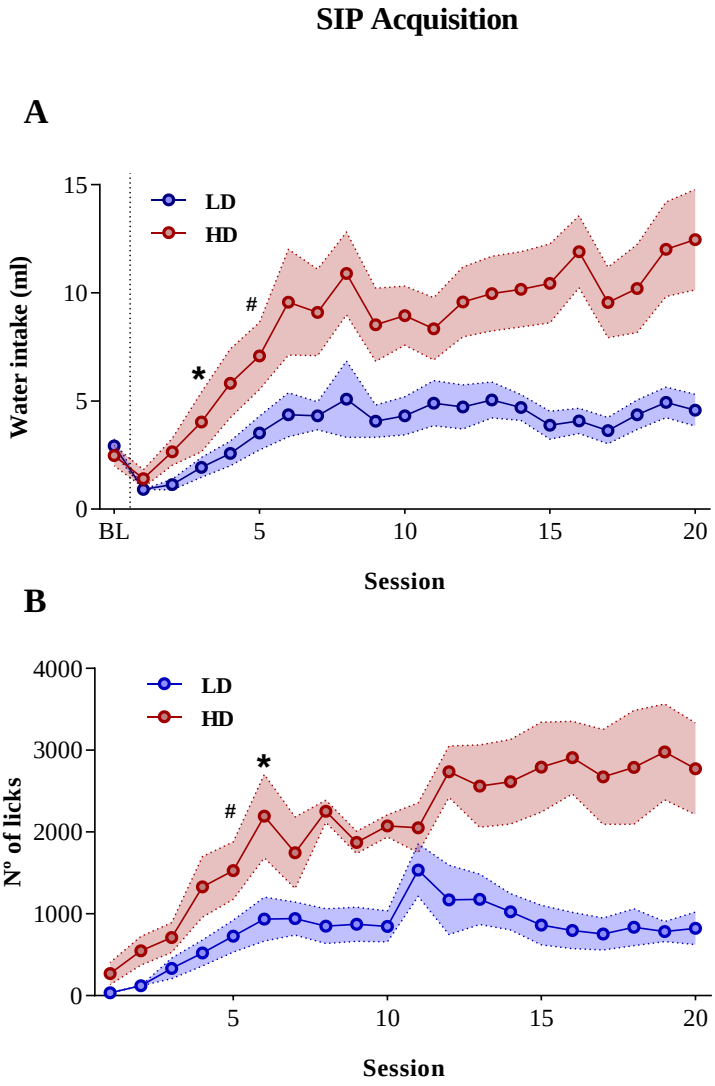

Figure 10. The mean (± SEM) water intake (A) and number of licks (B) in FT-60s across 20 sessions of experiment 1 SIP. Statistical analyses indicate significant differences between low drinkers (LD, n = 8) and high drinkers (HD, n = 8; *p < 0.05). Significant differences between sessions were found from session 5 (#p < 0.05).

The mean water intake over the last 5 days of SIP was 4.3 ± 0.6 and 11.2 ± 1.9 ml for LD and HD, respectively. The number of licks also showed SIP acquisition. The mean total licks averaged across the last 5 days of SIP were 885.1 ± 202.9 and 2742.9 ± 536.9 for LD and HD, respectively. ANOVA revealed significant differences in water intake according to the interaction between SIP acquisition sessions and LD vs. HD (SIP session effect: $F(19,266) = 11.759$, $p < 0.001$; group effect: $F(1,14) = 10.332$, $p < 0.01$; interaction SIP session × group effect: $F(19,266) = 2.58$, $p < 0.001$). This difference was also confirmed by the significant interaction observed in the total number of licks (SIP session effect: $F(19,266) = 11.890$, $p < 0.001$; group effect: $F(1,14) = 13.647$, $p < 0.01$; interaction SIP session × group effect: $F(19,266) = 3.38$, $p < 0.001$). *Post hoc* analysis indicated significant differences between the LD and HD animals in the water intake at session 3 ($p < 0.01$) onwards. Furthermore, animals in the HD group significantly increased their consumption of water from session 5 ($p < 0.05$) compared to session 1. Differences between the LD and HD groups in the number of total licks at session 6 ($p < 0.05$) were also observed, and HD rats increased their number of licks from session 5 ($p < 0.001$) compared to session 1. We also found significant differences in the number of magazine entries according to the interaction between SIP acquisition sessions and LD vs. HD (session × group effect: $F(19,266) = 2.124$; $p < 0.01$; session effect: $F(19,266) = 4.515$, $p < 0.001$; group effect: $F(1,14) = 5.577$, $p < 0.05$). Differences between the LD and HD groups in the number magazine entries at session 11 ($p < 0.001$) were also observed, and HD rats increased their number of magazine entries from session 6 ($p < 0.05$) compared to session 1.

Marble Burying Test

The number of marbles partially (2/3) and completely buried by LD and HD rats on MBT are shown in Figure 11. T-test and the effect sizes by Cohen's d showed that HD rats had a significantly increased number of marbles partially (2/3) buried compared to LD rats ($df = 14$; T-test = −2.22; $p < 0.05$; $d = 1.186$). There was no significant effect on the number of marbles completely buried between LD and HD rats ($df = 14$; T-test = 1.14; $p = 0.27$).

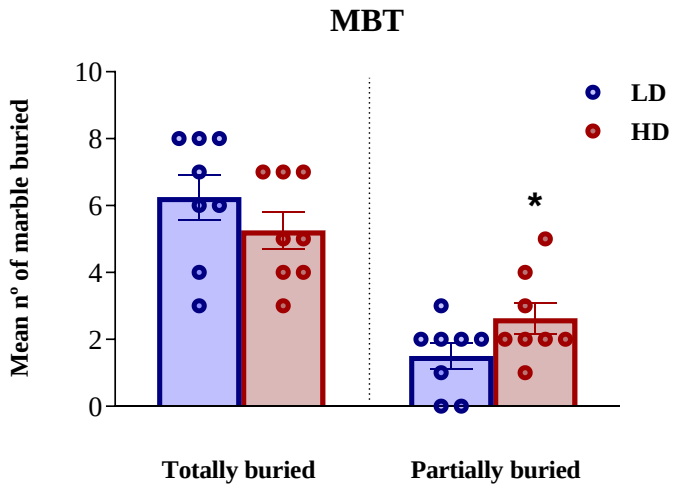

Figure 11. Marble Burying Test (MBT) scores of low drinkers (LD, n = 8) and high drinkers (HD, n = 8) rats. Data are expressed as the means ± SEM. *p < 0.05 to indicate differences between groups.

Forced Swimming Test

The percentage of immobile time of LD and HD rats on FST are shown in Figure 12. T-test showed no significant difference in the percentage of immobile time between LD and HD rats (df = 14; T-test = 0.35; p = 0.72).

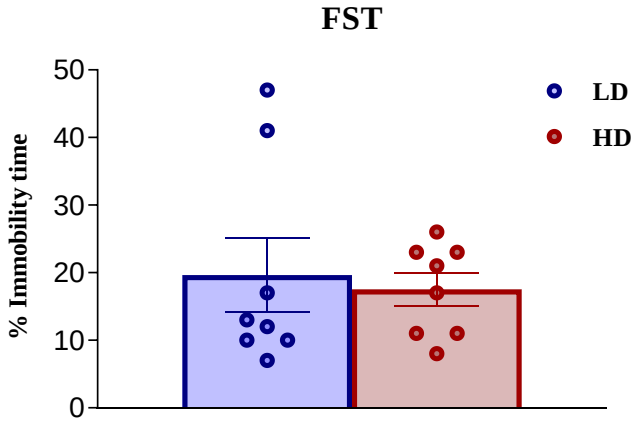

Figure 12. Percentage of immobile time low drinkers (LD, n = 8) and high drinkers (HD, n = 8) rats spent on Forced Swimming Test (FST). Data are expressed as the means ± SEM

Elevated Plus Maze Test

The time LD and HD rats spent on the open arm before changing to the other, and the number of entries in the open arm on EPM are shown in Figures 13 A and B respectively.

The time LD and HD rats spent on the open arm before changing to the other, and the number of entries in the open arm on EPM are shown in Figures 13 A and B respectively. T-test showed that there was no significant difference in the average time and the number of entries in the open arms between LD and HD rats (df = 14; T-test = −0.09; p = 0.92; df = 14; T-test = 0.86; p = 0.40). The mean time LD and HD rats spent on the closed arm before changing to the other was 1.53 ± 0.35 and 1.83 ± 0.39, respectively. The mean number of entries in the closed arm on EPM was 9.38 ± 0.67 for LD rats and 8.88 ± 1.24 for HD rats. T-test showed that there was no significant difference in the average time and the number of entries in the closed arms between LD and HD rats (df = 14; T-test = −0.60; p = 0.56; df = 14; T-test = 0.38; p = 0.71). The mean time LD and HD rats spent on one arm before changing to another one was 1.53 ± 0.06 and 1.70 ± 0.08, respectively. The mean number of entries in open and closed arms was 18.50 ± 1.14 for LD rats and 16.38 ± 1.23 for HD rats. T-test showed that there was no significant difference in the average time and the number of entries in open and closed arms between LD and HD rats (df = 14; T-test = −1.85; p = 0.08; df = 14; T-test = 1.35; p = 0.20).

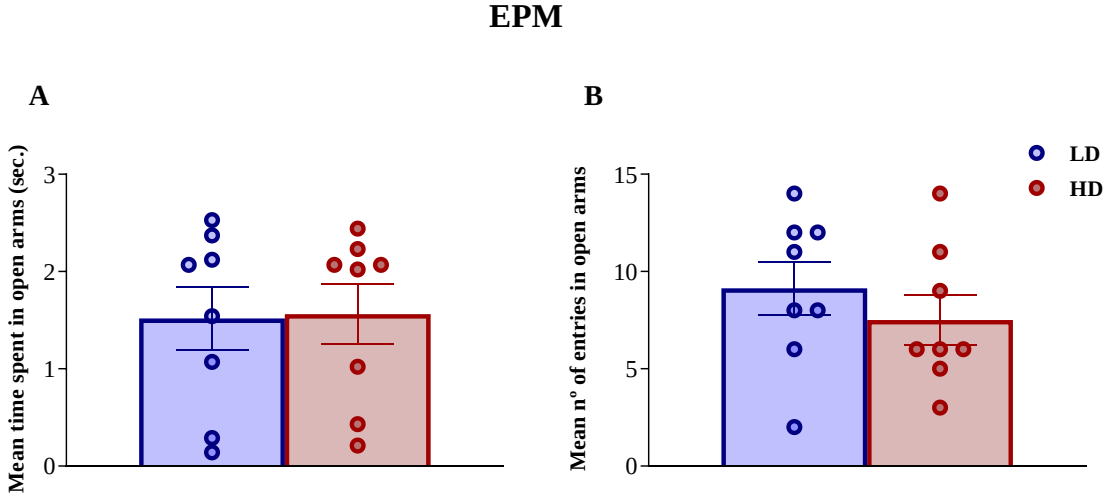

Figure 13. (A) Mean number of entries by low drinkers (LD, n = 8) and high drinker rats (HD, n = 8) on the open arms in the Elevated Plus Maze (EPM), (B) seconds spent by LD and HD rats on the open arms in EPM. Data are expressed as the means ± SEM.

Fear Conditioning Test

The percentages of freezing time of LD and HD rats on FCT during the acquisition day, the percentage of freezing time during the contextual fear test and the cued fear test at the retrieval day, as well as the percentage of freezing during the different blocks of trials on the retrieval day, is shown in Figures 14 A-D. No significant differences were found in the percentage of freezing time spent by LD and HD rats during the acquisition day ($df = 14$; T-test $= -0.45$; $p = 0.65$), nor in the contextual fear test on the retrieval day ($df = 14$; T-test $= -1.51$; $p = 0.15$). However, T-test and effect sizes by Cohen's revealed a significant increase in the percentage of freezing time spent by HD compared to LD rats during the cue presentation on retrieval day ($df = 14$; T-test $= -3.12$; $p < 0.01$; $d = 1.67$). The analyses of the 4 blocks of trials on the retrieval day by ANOVA and $\eta 2$ revealed that both, LD and HD rats, significantly reduced the percentage of freezing time in the different blocks of the retrieval day (Trial effect: $F(3,42) = 36.64$; $p < 0.001$; $\eta 2 = 0.931$); whether the significant increased percentage of freezing time spent by HD compared to LD rats was maintained through the four blocks of trials on the retrieval day (group effect: $F(1,14) = 9.73$; $p < 0.01$; $\eta 2 = 0.933$). No significant differences were observed by group × trial interaction ($F(3,42) = 0.27$; $p = 0.84$).

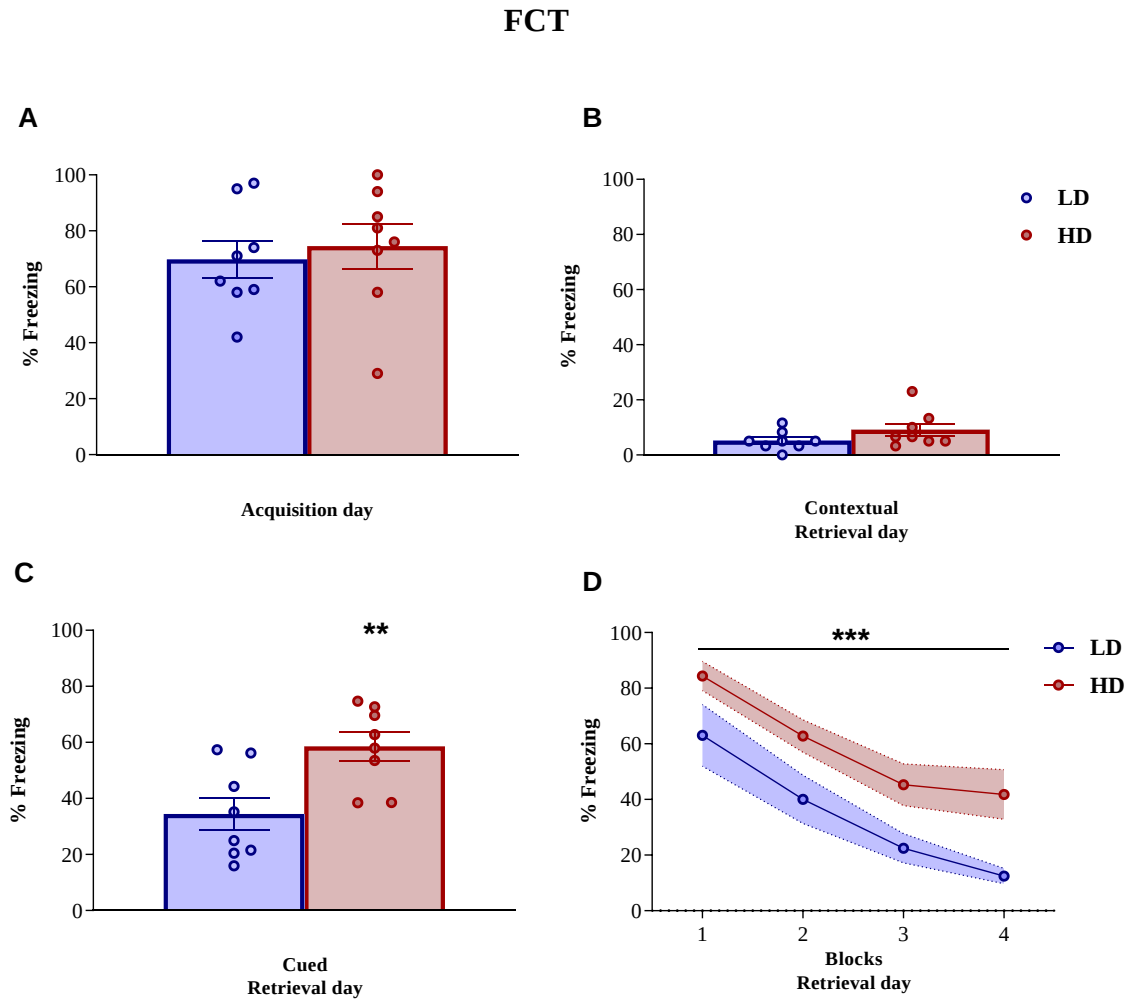

Figure 14. (A) Percentage of freezing low drinkers (LD, n = 8) and high drinkers rats (HD, n = 8) exhibited during fear acquisition day, (B) percentage of freezing LD and HD rats exhibited during contextual fear test on retrieval day, (C) percentage of freezing LD and HD rats exhibited during cued fear test on retrieval day, and (D) percentage of freezing LD and HD rats exhibited during the four blocks of time (6 min per block) at cued fear test on retrieval day of Fear Conditioning Test procedure (FCT). Data are expressed as the means ± SEM. **p < 0.01 and ***p < 0.001 to indicate differences between groups.

3.1.4. Discussion

HD rats selected by SIP showed comorbidity with compulsive behavior on MBT, by a significantly increased number of marbles partially buried compared to LD rats. Previous studies have found that HD rats selected by SIP showed other behavioral compulsivity forms such as compulsive lever pressing, during the pre-training phase to assess latent inhibition (Navarro et al., 2017), proposed as an OCD model (Joel and Avisar, 2001); and behavioral inflexibility in a spatial reversal task (Navarro et al., 2017). In contrast, other studies on rats with high levels of grooming, considered as a compulsive-like behavior, have shown a reduced number of marbles buried in MBT, showing a negative correlation between these factors (Reimer et al., 2015). The reason for these contradictory results could be due to the fact that compulsivity is not a unitary phenomenon and can be expressed by different forms (Fineberg et al., 2018).

The assessment of depressive behavior revealed that LD and HD rats selected by SIP did not exhibit any differences in depressive-like behavior measured on FST. The compulsive HD rats might not have depression signs as a comorbid behavior. Nevertheless, other preclinical studies have shown associations between depressive and compulsive behavior in the same individuals. For example, the administration of 8-OH-DPAT, a 5-HT1A agonist, proposed as an OCD model (Yadin et al., 1991), increased the immobility time on FST (Sela et al., 2010). Moreover, the administration of the purinergic receptor P2R antagonist (pyridoxalphosphate-6-azophenyl-2', 4'-disulfonic acid tetrasodium salt) in Swiss mice, reduced depressive-like behavior in the FST, as well as compulsive-like behavior in MBT (Pereira et al., 2013). The effect of antidepressants on addictions, considered as compulsive disorders, has created some controversy. On the one hand, some preclinical studies have demonstrated reductions in alcohol addiction subsequently to the administration of different 5-HT receptors agonists (Naranjo et al., 1986; Higley et al., 1998; Martijena et al., 2005). On the other hand, the possibility that antidepressant treatment might increase susceptibility to alcoholism has been overlooked (Alén et al., 2013, 2014). Moreover, several clinical studies have shown that pathological gambling, associated with elevated compulsivity, frequently co-occurs with major depression (Cunningham-Williams and Cottler, 2001; Baer et al., 2015; Redden et al., 2015; Agarwal et al., 2016; Grant et al., 2016; Rickelt et al., 2016). More research is needed to clarify the relation between depressive and compulsive behavior.

Anxiety behavior measured by EPM did not show any significant differences between HD and LD rats selected by SIP. Nevertheless, we have replicated the results published in 2008 by López-Grancha, in which there were no differences in the EPM between LD and HD rats selected by SIP (López-Grancha et al., 2008). Moreover, animals with distinct levels of self-grooming emission, considered as a compulsive-like behavior, did not differ in the exploration of the EPM (Reimer et al., 2015). In contrast, a previous study has shown that an increased compulsive behavior in the MBT has also been accompanied by increased anxiety response in the EPM and Open-Field Test in the same animals (Mitra et al., 2016). These contradictory results posit the relevance of the study on individual differences, using populations more prone to a behavioral deficit. Self-grooming and MBT might be evaluating different kinds of compulsivity, as well as anxiety is also a neuropsychological domain that could be expressed by different symptoms (reviewed in Ströhle et al., 2018). For instance, compulsive drinkers HD rats selected by SIP did not differ in anxiety-like behavior assessed using EPM to LD rats, while they differed in anxiety-like behavior measured by freezing time on the retrieval day in FCT.

The assessment of fear behavior by FCT revealed that HD rats selected by SIP showed a significantly augmented percentage of freezing time compared to LD rats during cued-fear memory on the retrieval day. Thus, HD and LD rats had no differences in the percentage of freezing time on the acquisition day, nor in the exploration period when exposed to the fear context on the retrieval day. Previous findings in our laboratory, have shown that under extinction conditions, HD rats had a greater increase in perseverative responses, considered as compulsive behavior, compared to LD rats on 5-CSRT (Moreno et al., 2012). Moreover, HD rats have shown increased c-Fos activity in the basolateral amygdala compared with LD rats (Merchán et al., 2019). The basolateral amygdala, as an essential structure in the neural system for FCT (Phillips and LeDoux, 1992; Vazdarjanova and McGaugh, 1998), is highly implicated in cued-related fear memories and not essential for contextual FCT (reviewed in Curzon et al., 2009). HD animals selected by SIP might be a convenient phenotype to study the neuronal basis of individual differences in habit formation under extinction conditions. Thus, in HD rats, a possible alteration in the basolateral amygdala might underlie the observed increased cued-fear memory on FCT that possibly also affect the vulnerability to develop compulsive behaviors. In this sense, clinical studies demonstrated that OCD patients continued to exhibit a differential

skin conductance response to the conditioned stimuli in the extinction phase of fear conditioned computer task, while control participants extinguished fear (Geller et al., 2017). Translational neuroscience studying fear could help us to better understand brain circuitry underlying fear behavior, although the translation of animal model results into the clinic is limited and more research is needed (Flores et al., 2018).

3.2. EXPERIMENT 1B: COGNITIVE PROCESSING

3.2.1. Experimental design

The present experiment investigates the possible deficits in cognitive processing using different memory-related tasks in compulsive rats selected by SIP. Once we had our two populations divided by the SIP procedure, we explored spatial memory and cognitive inflexibility by Morris Water Maze (MWM) and MWM in reversal conditions. We evaluated reference and working memory by Radial Arm Maze (RAM). Finally, we assessed stimulus processing and novelty reaction using the Novel Object Recognition Test (NOR). The experimental events are summarized in Figure 15.

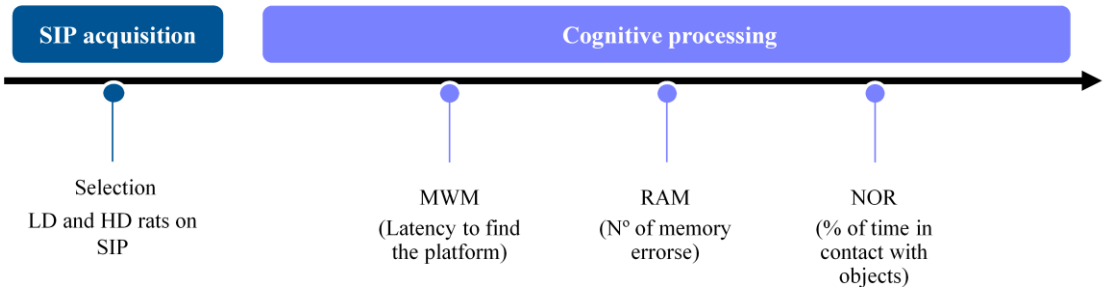

Figure 15. Experimental procedure illustrated in a timetable. SIP: Schedule-induced polydipsia; HD: High drinker; LD: Low drinker; MWM: Morris Water Maze; RAM: Radial Arm Maze; NOR: Novel Object Recognition Test.

3.2.2. Materials and methods

Subjects

A total of 20 male Wistar rats (Envigo, Spain) weighing between 250–350 g at the start of the experiments were used in the present study. The animals were housed in four rats/cages (50 × 35 × 20 cm) kept in a temperature-controlled environment at 22 ± 2 °C, humidity (50 ± 10 %), with a 12:12-h light-dark cycle (light off at 08:00 h am) and food and water provided ad libitum. After ten days of habituation and before behavioral tasks, the rats were gradually reduced to 85 % of their free-feeding body weight through controlled feeding, and their body weights were maintained at this level of deprivation throughout the experiment. Food was provided by daily feedings of lab chow at approximately 30 min after each experimental session. All testing was performed between 9:00 h am and 2:00 h pm. Animals were around 3 months of age when the experiment started

and finished it with 5 months of age. All of the procedures were conducted following the Spanish Royal Decree 53/ 2013 on the protection of experimental animals, the European Community Directive 2010/63/EU for animal experiments and complies with the ARRIVE guidelines for animal research. The Animal Research Committee of the University of Almeria approved the experiments described here and the authors declare that the research shows commitment to the 3Rs principle (replacement, reduction, refinement).

SIP procedure: HD and LD selection

LD and HD rats were selected by SIP following the same protocol described in the first experimental set (see page 40).

Morris Water Maze

The MWM protocol used follows the guidelines defined by De Bruin in 1994, with minor changes (Figure 16). The test was carried out in a black, circular pool with an inner diameter of 150 cm and walls 34 cm high. It was filled with tap water to a depth of 30 cm. The water was at room temperature (22 ± 2 °C). The pool was divided into four quadrants of equal size: A, B, C, and D; with A opposite to D and B opposite to C. A removable circular escape platform (diameter: 10 cm) could be positioned at only one location in each of the four quadrants (in the middle of a quadrant, with the center 30 cm away from the wall). Two types of platforms were used: an invisible one painted black and always 1.5 cm below the water surface and a visible, grey one, always 1.5 cm above the surface. Both platforms had a rough surface providing sufficient grip for the animal to climb on top of it. Release sites were marked on the outside of the pool, each one directly opposite to either one of the four possible platform positions. The walls of the room were equipped with a variety of spatial cues which remained unchanged during the whole experiment. A video camera were used to record behavioral activities during the transfer tests by using Ethovision 3.1. (Noldus).

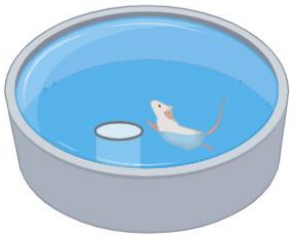

Figure 16. Representation of the Morris Water Maze procedure

Behavioral procedures. During a total of 9 days the animals were trained and tested using the following five procedures.

Spatial training (days 1-4). The invisible white platform was placed for half of the animals of each group in quadrant B, and for the other half in the opposite quadrant (C). The training was conducted in 4-trial sessions with each animal being released into the pool from one of the four release sites. The sequence of the four release sites varied from session to session but was identical for all animals within one session. The animal was released into the pool with its head facing the wall and the time to reach the hidden platform was recorded with a stopwatch (escape latency). If the platform was not located within the maximum trial duration of 90 s, the animal was taken out of the water and placed on the platform. In either case, the animal was left on top of the platform for 30 secs. In between the successive trials of one session, the animal was put in a black plastic bucket for a 30 s intertrial interval period. During this period fecal boluses (if present) were removed from the pool, and the transparent wall was wiped clean. Following the last trial of a session the animal was dried with a cloth towel and placed in a clean cage. There were two sessions a day with an interval of approximately 3 h.

Reinstating memory (day 5). On the afternoon of the 5th day, the animals were again subjected to a spatial training session with the platform in its original position.

Reversal test (days 6-7). Following 2 days without behavioral training or testing, reversal test began. The platform was now placed in the quadrant opposite to the one used during spatial training for two test sessions (each consisting of two reversal sessions, four trials per session); otherwise, all training procedures were identical to the ones described for spatial training.

Visually-cued task (days 8-9). One day after the completion of reversal training animals were subjected to the visually-cued task. Instead of a white invisible platform, a grey visible platform, extending 1.5 cm above the surface, was used. While the release site of the animal remained the same (always opposite to the quadrant where the platform was during spatial training), the platform position varied from trial to trial. The sequence of these positions was the same for all animals. Otherwise, procedures were as described for the spatial training phase.

Radial Arm Maze

The RAM protocol consists of three consecutive phases: habituation (2 days), the learning task (4 days maximal), and the test task (4 days), as described Fole in 2017 (Fole et al. 2017). Rats were trained every day, twice per day. Each rat was placed on the central platform, and the maze could be visited for 10 min (Figure 17). Each rat was placed in the radial maze in a random order that changed every day. The radial maze was cleaned between each animal with diluted ethanol (70 %) and absorbing paper to minimize olfactory intra-maze cues.

The animals' performance was recorded on a computer. Data considered were arm entries; total trial time; first entry latency. With those data, the number of working memory (WM) and reference memory (RM) errors were counted. Every entry in an already visited arm was considered a WM error. Entries in a non-rewarded arm were considered as RM errors.

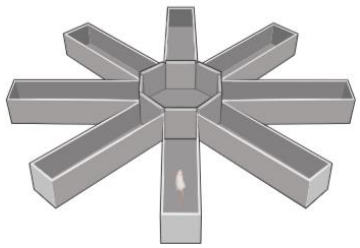

Figure 17. Representation of the Radial Arm Maze paradigm

Habituation. This habituation allowed accustoming the rats to the maze and to collect pellets at the end of their arms. All arms were maintained baited during the trial time. On the first day, some pellets were placed along the arms to invite animals to go to the end of the arms.

Acquisition. Consisted in 4 trials every session (2 sessions per day) until animals reached the minimum values during three consecutive sessions. A trial was finished when: 10 min past or the animal visited the 8 arms at least one time. Criterion consisted in doing either no error for 8 entries or at maximum 1 error for 9 entries.

Test. This protocol aimed at testing the memorization of the task. The rats' performances were evaluated by the number of errors and the rank of the first error. An error

was defined as the rat going back into a previously visited arm, i.e., crossing the first beam of the arm.

Novel Object Recognition Test

The NOR protocol (Cohen et al. 2015) consists of three consecutive phases: habituation (day 1), acquisition task (day 2), and test task (day 2). Each rat was placed in the center of the arena, and it could be explored for 5 min. The arena was cleaned between each animal with water and absorbing paper to minimize olfactory intra-cues (Figure 18).

The animal course was recorded on a computer using Ethovision 3.1. (Noldus). Data considered were speed, mobility, percentage of time in contact with the objects (time in contact with the new or the old object / total time), percentage of time near the objects (time near the new or the old object / total time), and percentage of time in the neutral zone (time in the neutral zone / total time).

Habituation. This habituation allowed accustoming the rats to the maze by free exploration of the arena for 5 min. No objects were placed in the arena in this phase.

Acquisition. 24 h after habituation, two identical objects were placed in opposite quadrants of the arena. The rats were placed in the center of the arena, equidistant from the 2 identical objects, and were allowed to freely explore for 5 min. All sessions were recorded and analyzed.

Test. Two hours after the acquisition phase, one object used during acquisition (the familiar object) and one novel object were placed in opposite quadrants of the arena. Animals were allowed to explore for 10 min. Sessions were recorded and we analyzed the same variables as in the acquisition phase.

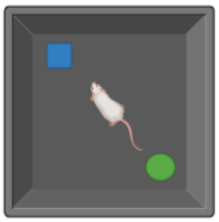

Figure 18. Representation of the Novel Object Recognition Test procedure

Data analyses

Behavioral data on SIP acquisition were analyzed using two-way repeated-measure ANOVA, with "group" (LD and HD) as the between-subject factor and "sessions" (20 sessions) as the within-subject factor. The mean latency and speed in MWM, the speed and number of errors in RAM, and the percentage of time spent in each zone in NOR by LD and HD rats were compared using two-way repeated-measures ANOVA, with between-subject factor (group: HD and LD) and within-subject factor (sessions). The effect size of the group differences was calculated using Cohen's d or η^2. Partial eta-squared values of 0.01, 0.06, and 0.14 and Cohen's d values of 0.2, 0.5, and 0.8 are considered to reflect small, medium, and large effects, respectively. *Post hoc* comparisons were carried out using the Bonferroni test. Statistical significance was set at $p < 0.05$. All analyses were computed using Statistica software (version 6.0).

3.2.3. Results

LD and HD Selected by SIP

The mean water intake and licks in LD and HD during the acquisition and maintenance of SIP is shown in Figure 19.

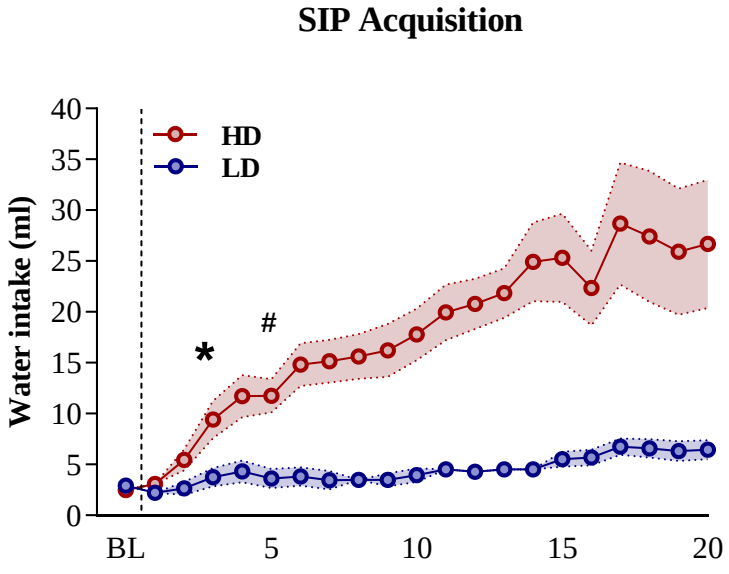

Figure 19. The mean (± SEM) water intake in FT-60s across 20 sessions of experiment 2 SIP. Significant differences between low drinkers (LD, n = 10) and high drinkers (HD, n = 10; *p < 0.05) were found from session 3. Significant differences between sessions were found from session 5 (#p < 0.05).

The mean of water intake over the last 5 days of SIP was 25.9 ± 5.1 mL for HD and 6.1 ± 0.8 for LD, respectively. The number of licks also showed SIP acquisition (data not shown). The mean total licks averaged across the last 5 days of SIP were 3987.4 ± 820.7 and 1441.1 ± 243 for HD and LD, respectively. ANOVA revealed significant differences in water intake according to the interaction between SIP acquisition sessions and group ($F(1,18) = 34.26$, $p < 0.001$, $\eta^2p = 0.16$). Also, differences in water consumption were observed for the session effect ($F(19, 342) = 10.38$, $p < 0.001$) and group effect ($F(19, 342) = 5.47$, $p < 0.001$). ANOVA also showed a significant interaction in the total number of licks (interaction SIP session x group effect: $F(1, 18) = 12.18$, $p < 0.001$, $d = 0.92$; session effect: $F(19,342) = 8.66$, $p < 0.001$; group effect: $F(19, 342) = 3.27$, $p < 0.001$). *Post hoc* comparisons indicated that SIP induced differences in drinking rates across the 20 sessions in both groups. Differences between LD and HD were evident in the water intake at session 3 ($p < 0.01$) onwards. Furthermore, when compared to session 1, animals in the HD group significantly increased their water consumption from session 6 ($p < 0.01$). Differences between the HD and LD groups in the number of total licks at session 8 ($p < 0.01$) were also observed, and HD rats increased their number of licks from session 8 ($p < 0.01$) compared to session 1. There were significant differences between LD and HD animals in the total magazine entries according to session effect ($F(19, 342) = 5.431$, $p < 0.001$). However, there were no significant differences according to the interaction between SIP acquisition sessions and LD vs. HD (interaction SIP session x group effect: $F(19, 342) = 0.933$, $p = 0.54$) and group effect ($F(1,18) = 0.039$, $p = 0.84$).

Morris Water Maze

Figure 20 A shows the latency to the platform during the spatial training and reinstating session spent by LD and HD rats. No significant differences were observed between groups ($F(8,624) = 1,09$, $p = 0,37$). Figure 20 B shows the latency to the platform during the reversal test by LD and HD rats. Significant differences between LD and HD rats were found in test 1 of MWM. ANOVA revealed that HD rats spent more time finding the platform compared to LD rats ($F(18,1) = 5.90$, $p < 0.05$, $\eta^2p = 0.25$). No significant differences were found between LD and HD rats in the latency in test 2 of MWM ($F(1, 18)= 0,13$, $p = 0,7$). LD and HD rats exhibit no significant differences in swimming speed during the reversal (neither in test 1 nor in test 2), as shown in Figure 20 C.

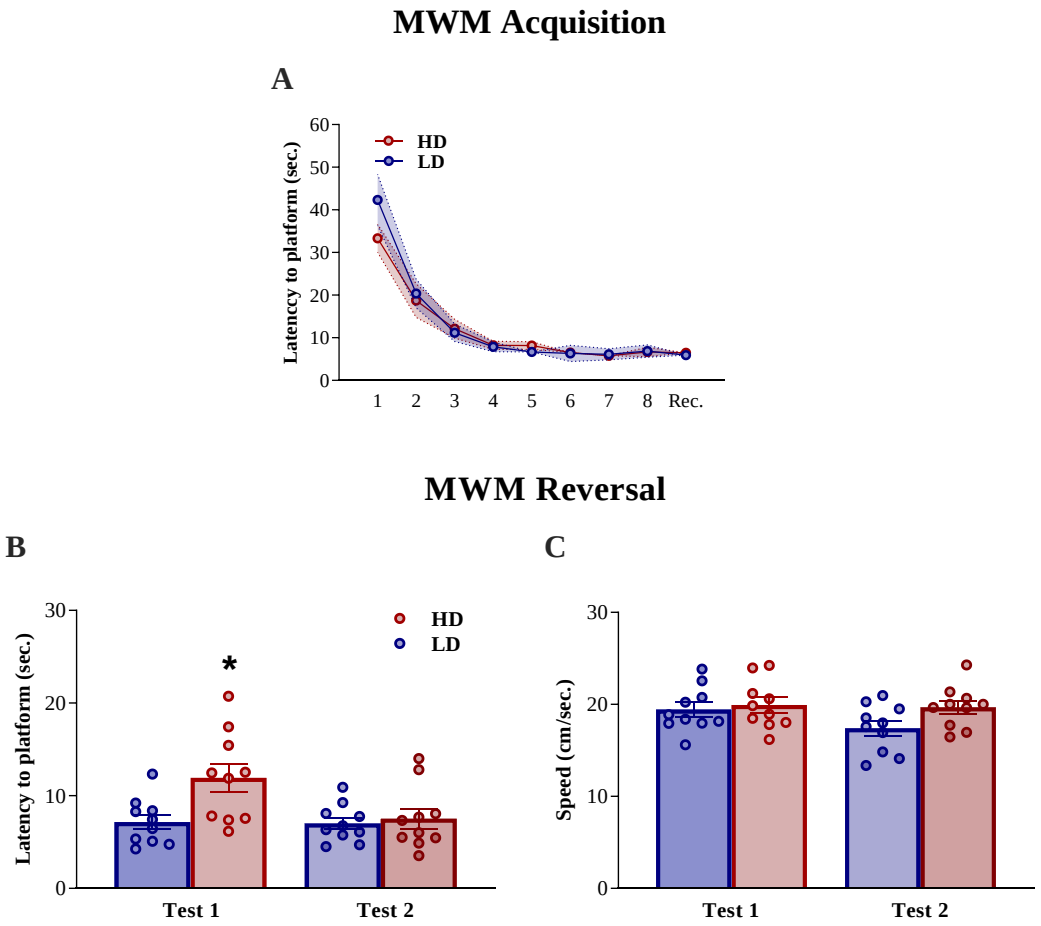

Figure 20. Latency to the platform in seconds (± SEM) in Morris Water Maze (MWM) spent by high drinkers (HD; n = 10) and low drinkers rats (LD; n = 10) in the acquisition (A), the latency in the reversal phase (B) and the swimming speed in the reversal phase (C). Statistical analyses indicate significant differences between LD and HD (*p < 0.05) in the latency to platform in test 1.

Radial Arm Maze

Significant differences were observed in the number of accumulated memory errors committed in RAM by LD and HD rats (Figures 21 A-C). ANOVA revealed that HD rats committed a higher number of accumulated working memory errors (Figure 21 A) compared to LD rats ($F(126, 7) = 3.64$, $p < 0.01$, $\eta^2p = 0.17$). Also, HD rats committed a higher number of accumulated reference memory errors (Figure 21 B) compared to LD rats ($F(126, 7) = 4.41$, $p < 0.001$, $\eta^2p = 0.20$). Moreover, HD rats committed a higher number of accumulated total errors (Figure 21 C) compared to LD rats ($F(126, 7) = 4.08$, $p < 0.001$, $\eta^2p = 0.19$).

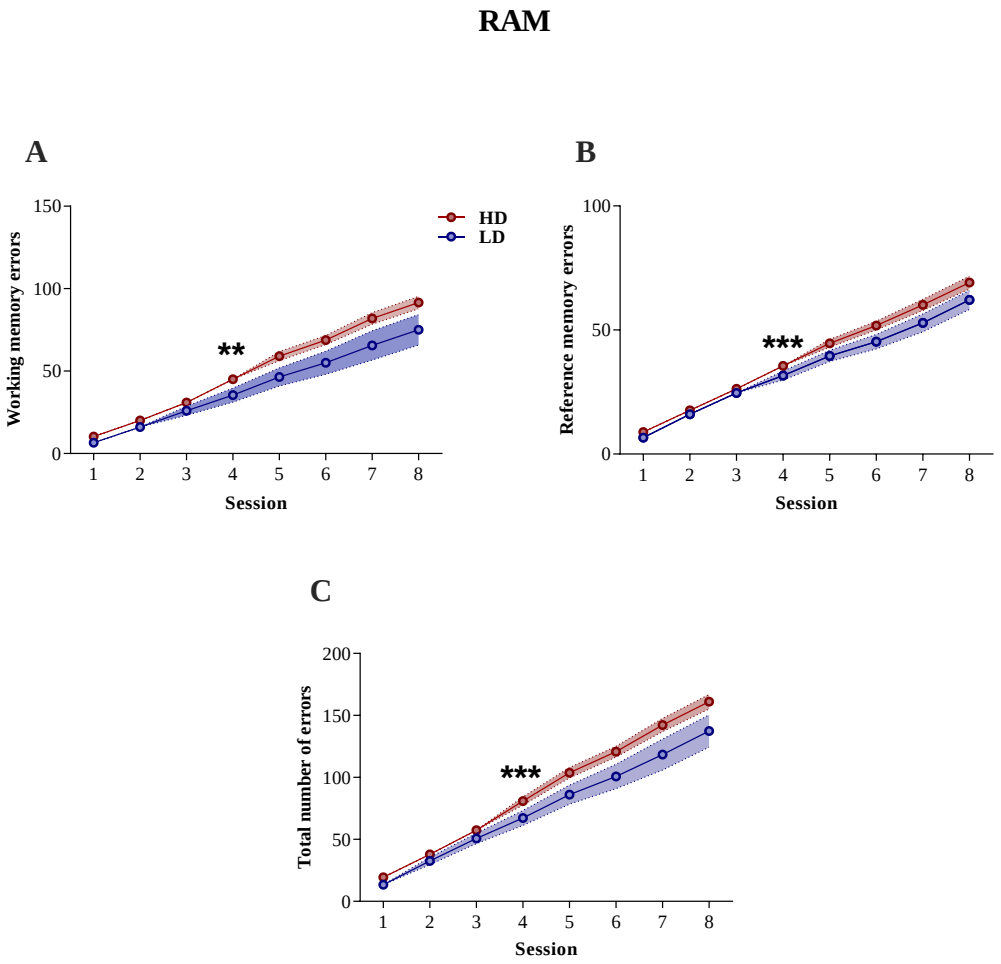

Figure 21. The cumulative number of memory errors (± SEM) committed by high drinkers (HD; n = 10) and low drinkers rats (LD; n = 10) in Radial Arm Maze (RAM). (A) Working memory errors, (B) reference memory errors and (C) the total number of memory errors committed across the 8 sessions of RAM. Statistical analyses indicate significant differences between LD and HD (**p < 0.01; *** p < 0.001) in the number of working memory errors, reference memory errors and the total number of errors from session 4.

Novel Object Recognition Test

Figure 22 shows the performance of LD and HD rats in the NOR test. No significant differences were found between LD and HD rats in the percentage of time in contact with the object, near the object, or in the neutral zone ($F(4,15) = 0.223$, $p = 0.92$).

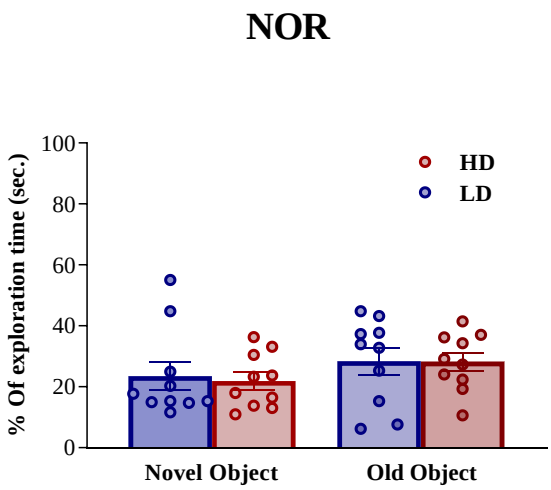

Figure 22. Percentage of time spent by high drinkers (HD; n = 10) and low drinkers rats (LD; n = 10) exploring the novel and the old object in Novel Object Recognition Test (NOR).

3.2.4. Discussion

HD rats and LD rats did not exhibit differences in learning at the acquisition phase of MWM. However, we observed an inflexible behavior by HD rats as they spent more time finding the platform in the reversal phase of MWM. Previous findings have strongly demonstrated that HD rats present an inflexible and perseverative profile. In the Reversal Learning Task HD rats needed more trials to reach the criterion compared to LD rats (Navarro et al., 2017), as well as they performed more incorrect perseverative responses (Navarro et al., 2017; Merchán et al., 2019). Furthermore, Moreno reported an increase in perseverative responses on 5-choice serial reaction time task (5-CSRTT) under extinction conditions compared to LD rats (Moreno et al. 2012).

Also, HD rats expressed an increased habitual-like behavior in the reinforcer devaluation paradigm by a higher number of lever presses during the devaluation test day (Merchán et al., 2019). Moreover, HD rats exhibited a sustained higher latency to enter the dark compartment during the last extinction session of the PA (Martín-González et al. 2022). According to our preclinical compulsive model, OCD patients have diminished behavioral flexibility as they committed more perseverative errors with a pronounced trend towards poorer performance in the Wisconsin Card Sorting Test (reviewed in Ben-

zina et al., 2016). Moreover, OCD patients have shown a deficit in fear renewal and extinction recall in FCT paradigms (Fyer et al., 2020), with a different skin conductance response in the extinction phase (Geller et al., 2017).

The persevering behavior profile shown by HD rats might be related to memory deficits, as revealed by the increased number of working memory errors and reference memory errors compared to LD rats. Few preclinical studies have investigated the role of memory in compulsive behavior. However, in 2010 Andersen observed that rats exposed to clomipramine in early life, considered as an OCD model, had an impaired working memory in a win-shift task, shown by an increased number of errors and longer time to enter each arm than control rats (Andersen et al. 2010). Curiously, the use of enriched environments reduced spatial memory impairments in MWM and compulsive grooming behavior induced by methamphetamine (Hajheidari et al. 2012). The systemic administration of d-cycloserine and d-serine, NMDA modulators that enhance memory, and reduced compulsive aversion-resistant alcohol drinking (Seif et al. 2015). Clinical studies evidenced that OCD patients present a deficit in verbal episodic memory, by an impaired performance to recall short prose passages in Wechsler Memory Scale-Revised relative to controls and non-verbal memory (Exner et al., 2009). Similarly, OCD patients seem to have poorer performance than control subjects when evaluating verbal and visual memory using the Wechsler Adult Intelligence Scale-Revised (Martin et al. 2008). Other researchers assessing neuropsychological skills in OCD patients also found that verbal memory was impaired in these patients measured by California Verbal Learning Test (Tükel et al., 2012).

Chapter 4. Experiment 2: Pharmacological study

4.1. EXPERIMENTAL DESIGN

In the present experiment, we assessed the efficacy of different glutamatergic drugs in reducing compulsive drinking on SIP. Therefore, after the selection of compulsive HD and non-compulsive LD rats on SIP, we explored the dose-response effects of acute administration of three different glutamatergic modulators that reduce glutamate release by different action mechanisms in reducing compulsive drinking on SIP, and its possible implication as a new pharmacological strategy for compulsive neuropsychiatric disorders. The behavioral effects of acute systemic administration of NAC (N-Acetylcisteine), MEM (memantine), and LAM (lamotrigine) were tested in both groups as illustrated in Figure 23.

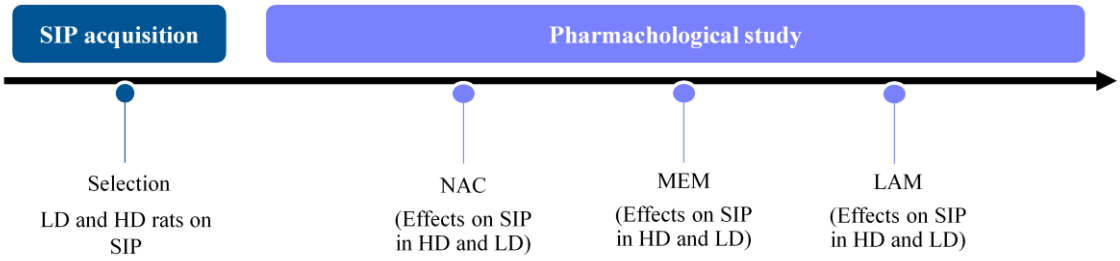

Figure 23. Experimental procedure illustrated in a timetable. SIP: Schedule-induced polydipsia; HD: High drinker; LD: Low drinker; NAC N-Acetylcisteine; MEM: memantine; LAM: lamotrigine.

4.2. MATERIALS AND METHODS

4.2.1. Subjects

A total of 16 male Long Evans rats (Janvier Labs, France) weighing between 250–350 g at the start of the experiments were used in the present study. The animals were housed in four rats/cages (50 × 35 × 20 cm) kept in a temperature-controlled environment at 22 ± 2 °C, humidity (50 ± 10 %), with a 12:12-h light-dark cycle (light off at 08:00 h am) and food and water provided ad libitum. After ten days of habituation and before behavioral tasks, the rats were gradually reduced to 85 % of their free-feeding body weight through controlled feeding, and their body weights were maintained at this level of deprivation throughout the experiment. Food was provided by daily feedings of lab chow at approximately 30 min after each experimental session. All testing was performed between 9:00 h am and 2:00 h pm. Animals were around 3 months of age when the experiment started and finished with 5 months of age. All of the procedures were conducted following the Spanish Royal Decree 53/ 2013 on the protection of experimental animals, the European Community Directive 2010/63/EU for animal experiments and comply with the ARRIVE guidelines for animal research. The Animal Research Committee of the University of Almeria approved the experiments described here and the authors declare that the research shows commitment to the 3Rs principle (replacement, reduction, refinement).

4.2.2. SIP procedure: HD and LD selection

LD and HD rats were selected by SIP following the same protocol described in the first experimental set (see page 40).

4.2.3. Drugs

After the last session of SIP (protocol described in Chapter III.II: SIP procedure), we explored the effects of acute intraperitoneal injections (i.p.) of NAC (25, 50, 100 and 200 mg/kg) (Lebourgeois et al., 2017), MEM (3.1 and 6.2 mg/kg) (Li et al., 2010) and LAM (15 and 30 mg/kg) (Réus et al., 2010) in LD and HD rats in SIP. NAC ((2R)-2-(Acetylamino)-3-mercapto propanamide) and MEM (3,5-Dimethyl-tricyclo [3.3.1.13,7] decan-1-amine hydrochloride) were dissolved in 0.9 % saline. LAM (6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine) was suspended in 1 % Tween-80 in 0.9 % saline. All

drugs were purchased from Sigma-Aldrich (Madrid, Spain). The injection volumes were 1 ml/kg for all drugs. For all drug solutions, the final pH was adjusted to approximately 6.4 using 0.1 M NaOH, and they were aliquoted after preparation and frozen at −80 °C before use.

The drug doses, and the injection time of 60 min before behavioral testing, were selected based on previous experiments (Lebourgeois et al., 2017; Li et al., 2010; Réus et al., 2010). All animals received drugs according to a fully randomized Latin-square design, separated by a minimum of 72 h between drug test sessions. There was a wash-out period of one week between each drug tested (animals continued performing SIP sessions during this week). The experimental sessions were led on Tuesdays and Fridays, and baseline testing was accomplished on Mondays and Thursdays. On Wednesdays, animals performed SIP procedures, but the results were not analyzed.

4.2.4. Data analyses

The differences on FC blocks and the effects of the different drugs in LD and HD on SIP were analyzed using two-way repeated-measure ANOVA, with group (LD and HD) as the between-subject factor "drug" (different doses of drug and vehicle) as the repeated within-subject factor. The effect size of the group differences was calculated using η^2. Partial eta-squared values of 0.01, 0.06, are considered to reflect small, medium, and large effects, respectively. *Post hoc* comparisons were performed using the Newman-Keuls test. Statistical significance was set at $p < 0.05$. All analyses were computed using Statistica software (version 6.0).

4.3. RESULTS

4.3.1. LD and HD Selected by SIP

HD and LD rats were selected as previously described in Experiment 1A (see page 44).

4.3.2. N-Acetylcysteine

The effects of NAC on water intake and licks in SIP are shown in Figures 24 A and B, and the number of magazine entries after NAC administration is shown in Table 6. ANOVA showed that NAC did not induce significant differences in water intake (group × drug interaction, $F(4,56) = 0.63$, $p = 0.64$; group effect, $F(1,14) = 109.15$, $p < 0.001$; drug effect, $F(4,56) = 0.38$, $p = 0.82$), total licks (group × drug interaction, $F(4,56) = 0.57$, $p = 0.68$; group effect, $F(1,14) = 111.89$, $p < 0.001$ drug effect, $F(4,56) = 0.90$, $p = 0.47$), and magazine entries (group × drug interaction, $F(4,56) = 0.28$, $p = 0.89$; group effect, $F(1,14) = 8.41$, $p < 0.05$; drug effect, $F(4,56) = 0.51$, $p = 0.73$).

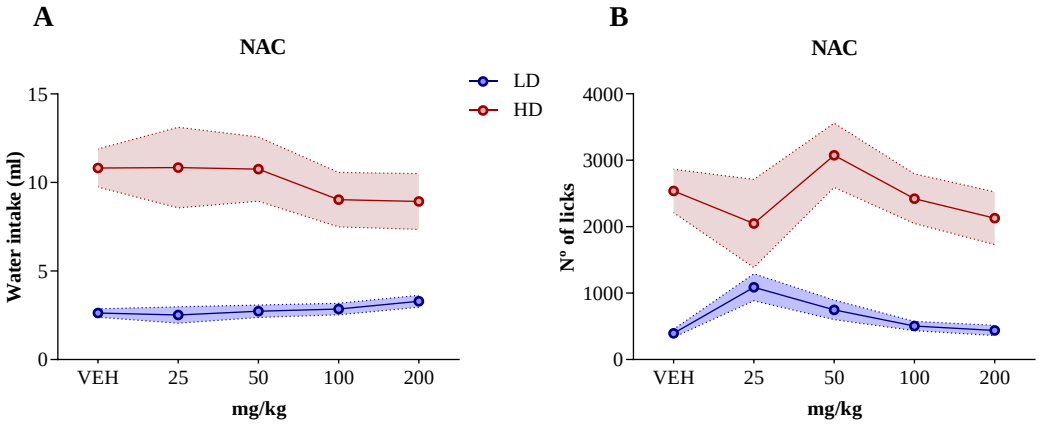

Figure 24. Effects of N-Acetylcysteine (NAC) on SIP. Effects of NAC administration on water intake (A) and number of licks (B) in low drinkers (LD, n = 8) and high drinkers (HD, n = 8) rats on SIP. Data are expressed as the means ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 indicates significant differences vs. vehicle administration in the same group of rats.

4.3.3. Memantine

The effects of MEM on water intake and total licks in SIP are shown in Figures 25 A and B. Effects of MEM on magazine entries are depicted in Table 6. MEM significantly reduced compulsive water intake in HD rats compared to LD rats (group × drug interaction, $F(2,28) = 4.51$, $p < 0.05$; group effect, $F(1,14) = 24.05$, $p < 0.001$; drug effect, $F(2,28) = 8.42$, $p < 0.01$; $\eta 2 = 0.930$). *Post hoc* analyses revealed that MEM reduced dose-dependent water intake in HD rats at both doses: 3.1 ($p < 0.05$) and 6.2 mg/kg ($p < 0.001$) compared with vehicle in the same group. MEM did not affect water intake in LD rats. The comparison between LD and HD revealed a dose dependent reduction of the significant differences in water intake disappearing at the highest dose (vehicle, $p = 0.0001$; 3.1 mg/kg, $p = 0.041$; 6.2, $p = 0.572$). Moreover, MEM also significantly reduced the total licks in HD rats compared with the LD group (group × drug interaction, $F(2,28) = 6.04$, $p < 0.01$; group effect, $F(1,14) = 16.96$, $p < 0.05$; drug effect, $F(2,28) = 5.50$, $p < 0.01$; $\eta 2 = 0.730$). *Post hoc* comparison confirmed a decrease in the total licks in the HD group at the highest dose used 6.2 mg/kg ($p < 0.001$) compared with vehicle in the same group. Differences between LD and HD remained significant at all doses tested. MEM administration did not affect the number of magazine entries in both groups of rats (group × drug interaction: $F(2,28) = 2.663$; $p = 0.087$; drug effect: $F(2,28) = 2.507$; $p = 0.099$; group effect: $F(1,14) = 1.569$; $p = 0.23$).

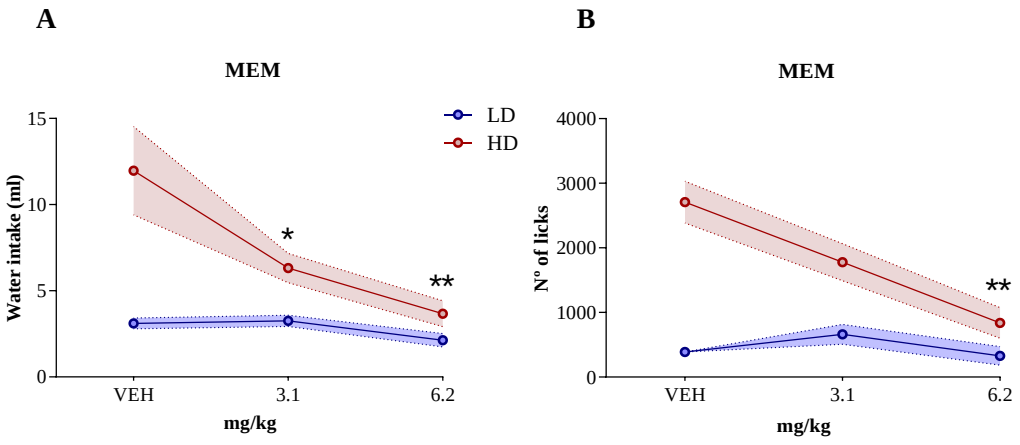

Figure 25. Effects of memantine (MEM) on SIP. Effects of MEM administration on water intake (A) and number of licks (B) in low drinkers (LD, n = 8) and high drinkers (HD, n = 8) rats on SIP. Data are expressed as the means ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 indicates significant differences vs. vehicle administration in the same group of rats.

4.3.4. Lamotrigine

The effects of LAM on water intake and total licks in SIP are shown in Figures 26 A and B. The effects of LAM on magazine entries in SIP are shown in Table 6. LAM significantly reduced compulsive water intake in HD rats compared to LD rats (group × drug interaction: $F(2,28) = 11.396$, $p < 0.0002$; group effect: $F(1,14) = 5.187$, $p < 0.05$; drug effect: $F(2,28) = 3.532$, $p < 0.05$; $\eta 2 = 0.882$). *Post hoc* analyses revealed that LAM reduced dose-dependent water intake in HD rats at both doses: 15 ($p < 0.05$) and 30 mg/kg ($p < 0.01$) compared with vehicle in the same group. LAM reversed the significant differences on water intake between LD and HD rats on SIP (vehicle, $p = 0.008$; 15 mg/kg $p = 0.16$; 30 mg/kg, $p = 0.914$). LAM did not affect water intake in LD rats. Moreover, LAM also significantly reduced the total licks in HD rats compared with the LD group (group × drug interaction, $F(2,28) = 11.40$, $p < 0.001$; group effect, $F(1,14) = 5.18$, $p < 0.05$; drug effect, $F(2,28) = 3.53$, $p < 0.05$; $\eta 2 = 0.870$). *Post hoc* comparison showed a dose dependent decrease in the total licks in the HD group at both doses used 15 mg/kg ($p < 0.05$) and 30 mg/kg ($p < 0.001$) compared with vehicle in the same group. The comparison between LD and HD revealed a dose dependent reduction of the significant differences in the number of licks disappearing at the highest dose (vehicle, $p = 0.0001$; 15 mg/kg $p = 0.005$; 30 mg/kg, $p = 0.86$). LAM administration reduced magazine entries in both groups of rats (group × drug interaction: $F(2,28) = 3.61$, $p < 0.05$; group effect: $F(1,14) = 0.19$, $p = 0.67$; drug effect: $F(2,28) = 4.65$, $p < 0.05$; 0.931). *Post hoc* analyses revealed a decrease in magazine entries in HD rats only at the highest dose tested 30 mg/kg ($p < 0.05$) compared with vehicle and with the LD group.

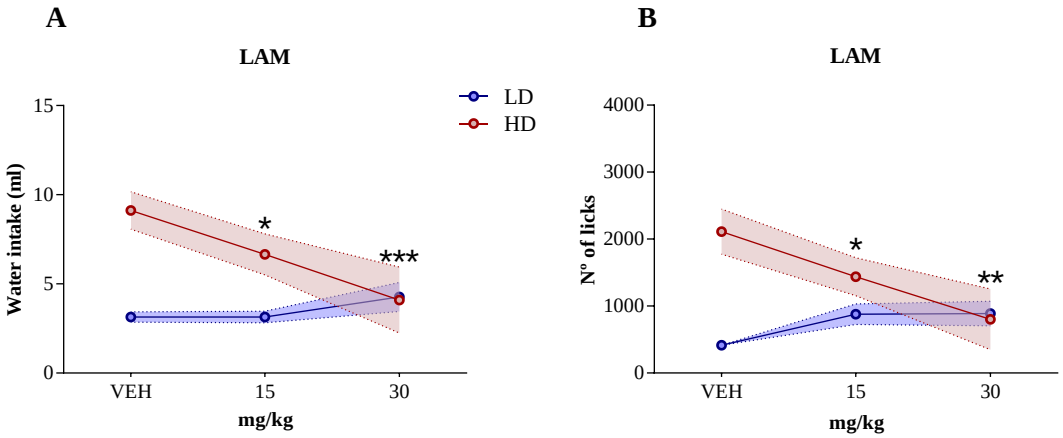

Figure 26. Effects of lamotrigine (LAM) on SIP. Effects of LAM administration on water intake (A) and number of licks (B) in low drinkers (LD, n = 8) and high drinkers (HD, n = 8) rats on SIP. Data are expressed as the means ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 indicates significant differences vs. vehicle administration in the same group of rats.

	Nº of Magazine entries	
N-Acetylscysteine	**LD**	**HD**
Vehicle	996.59 ± 126.34	2052.68 ± 314.41
25 mg/kg	1087.19 ± 205.43	2047.67 ± 664.21
50 mg/kg	1011.57 ± 162.58	2041.77 ± 488.66
100 mg/kg	987.75 ± 199.97	2095.57 ± 676.96
200 mg/kg	1006.29 ± 183.00	1428.29 ± 247.77
Memantine		
Vehicle	961.13 ± 144.41	1584.11 ± 206.60
3.1 mg/kg	992.71 ± 197.17	1173.43 ± 116.98
6.2 mg/kg	930.00 ± 222.24	811.67 ± 182.63
Lamotrigine		
Vehicle	1058.71 ± 134.80	1192.29 ± 111.46
15 mg/kg	1093.57 ± 152.87	1286.14 ± 145.31
30 mg/kg	1135.69 ± 233.17	554.27 ± 162.55 *

Table 6. Effects of N-Acetylcysteine (NAC), memantine (MEM) and lamotrigine (LAM) on total magazine entries in low drinkers (LD, n = 8) and high drinkers (HD, n = 8) rats on schedule-induced polydipsia (SIP).

4.4. DISCUSSION

The administration of NAC (25, 50, 100 and 200 mg/kg) revealed no significant differences in the water intake nor LD, nor in HD rats on SIP. Conversely, previous research has demonstrated that NAC (90 mg/kg), chronically and systemically administered, resulted in significant reductions of compulsive binge eating in a rodent model (Hurley et al., 2016). NAC systemically administrated has been demonstrated to abolish the recovery of compulsive cocaine-seeking behavior in a rodent model through augmenting the glutamate/cystine antiporter activity and reestablishing the concentration of extracellular glutamate in the nucleus accumbens (Baker et al., 2003a,b). Moreover, the acute administration of NAC at 100 mg/kg reduced motivation, seeking and relapsing to self-administration of ethanol in rats (Lebourgeois et al., 2018). However, acute injections of NAC (0, 30, 60, or 120 mg/kg) did not have any result on self-administration of methamphetamine in rats (Charntikov et al., 2018). Some clinical studies have suggested the possible therapeutic role of NAC in OCD patients, showing a reduction in the scores of the Y-BOCS after treatment with NAC during 10 and 12 weeks respectively (Afshar et al., 2012; Paydary et al., 2016).

The acute systemic administration of MEM, 3.1 and 6.2 mg/kg, decreased compulsive drinking in HD rats on SIP, compared to LD rats that remain unaffected. Hence, these results could not be considered as a general effect on rats exposed to SIP, pointing towards the neuropsychopharmacological effects of MEM might be involved in the vulnerability to compulsive non-regulatory drinking on SIP. In contrast, previous studies have found that acute administration of MEM at 5 and 25 mg/kg in mice, did not affect water intake on SIP, but revealed a reduction in regulatory drinking (Escher et al., 2006). Although in this study, mice were not selected according to the rate of compulsive drinking. However, in the same study MEM has been found as a useful treatment for reducing compulsive alcohol intake, the administration of MEM 10 and 25 mg/kg significantly reduced alcohol drinking in mice on SIP (Escher et al., 2006). Moreover, findings revealed that acute administration of 10 mg/kg MEM significantly inhibited compulsive behavior in MBT without affecting locomotor activity in mice (Egashira et al., 2008). Furthermore, acute administration of 25 mg/kg MEM blocked ethanol self-administration in non-dependent rats, as well as it decreased by half the one of post-dependent rats during acute withdrawal (Alaux-Cantin et al., 2015). Otherwise, compulsive lever pressing, proposed as an OCD

model (Joel and Avisar, 2001), was not affected by an NMDA antagonist (MK 801), while an NMDA partial agonist (D-cycloserine) decreased this behavior (Albelda et al., 2010). In this sense, the present results also contrast with the no effect found after ketamine administration in HD and LD rats on SIP (Martín-González et al., 2018). Though both ketamine and MEM typify the same kind of drugs, they diverge in voltage dependence and blocking kinetics (Danysz and Parsons, 1998). In human studies, MEM showed a therapeutic role in obsessive-compulsive patients, by reducing the Y-BOCS scores after chronic treatment with MEM for 8 weeks (Ghaleiha et al., 2013) and 12 weeks (Stewart et al., 2010; Haghighi et al., 2013). Other study investigating MEM augmentation of risperidone treatment in children with autism spectrum disorders revealed that the group receiving MEM showed significant improvements in the subscales: irritability, stereotypic behavior, and hyperactivity of the Aberrant Behavior Checklist-Community (Ghaleiha et al., 2013).

Our data showed that the administration of LAM, 15 and 30 mg/kg, significantly decreased compulsive water drinking in HD rats, compared to LD rats, on SIP. There are few preclinical studies on the behavioral effects of LAM, most of them related to as an anti-depressant like effect. The acute administration of LAM at 16 and 32 mg/kg of LAM induced a reduction in immobility time in the FST (Prica et al., 2008). Similarly, LAM at 15 and 30 mg/kg significantly reduced immobility in the FST (Li et al., 2010). In human studies, have evidenced that 16 weeks of treatment with LAM in obsessive-compulsive patients significantly reduced the Y-BOCS scores, as well as the Hamilton Rating Scale for Depression scores and the Clinical Global Impression-Severity scores (Bruno et al., 2012). More recently, two other studies using adjunctive treatment of LAM in addition to SRIs treatment led in treatment-resistant OCD patients during 8 and 12 weeks respectively, revealed a greater reduction in total YBOCS scores in LAM group (Hussain et al., 2015; Khalkhali et al., 2016).

Collectively, the beneficial effects of MEM and LAM administration in reducing compulsive drinking in HD rats on SIP suggest a therapeutic role for glutamate inhibition, antagonizing NMDA receptor or blocking calcium and sodium channels in presynaptic terminals. In contrast, the lack of effect of NAC in compulsive intake in HD rats on SIP posits the idea of the possible relevance of the differential effect by the specific stimula-

tion of the presynaptic terminal. These results support the possible dysregulation in glutamatergic signal previously observed, in which HD rats selected by SIP showed a decreased basal level of glutamate in the medial prefrontal cortex (mPFC), restored by serotonin 5-HT2A/C agonist DOI (Mora et al., 2018). Moreover, the effects of glutamatergic drugs MEM and LAM suggest a possible modulatory role in the neuroanatomic and neurochemical alterations observed in dopamine D2 receptors and 5-HT2A receptors in HD rats selected by SIP (Pellón et al., 2011; Moreno et al., 2012; Mora et al., 2018).

Preclinical studies on compulsivity, using the dopamine D2 and D3 receptor agonist quinpirole (QNP) in rats (Szechtman et al., 1998), have also evidenced a dysregulation by an increased glutamate release in the substantia nigra and a lower extracellular concentration in the nucleus accumbens (Abarca et al., 1995; Krügel et al., 2004; Escobar et al., 2015). Therefore, the proposed underlying mechanism in compulsivity of the QNP-OCD model was associated with decreased dopaminergic and glutamatergic neurotransmission in the mPFC to the nucleus accumbens, pointing toward a loss of executive control (Escobar et al., 2015). Furthermore, NMDA dependent glutamate neurotransmission in the cortico-striatal circuitry seems to play a central role by the functional interaction with serotonin and dopamine receptors in executive response control and compulsivity measured by the 5-CSRT (reviewed in Carli and Invernizzi, 2014). In example, the local infusions of NMDA receptor antagonist 3-((R)-2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid ((R)-CPP) in the mPFC and also in the infralimbic cortex impaired accuracy and increased premature and perseverative responding, raising glutamate, dopamine, and GABA release in the dorsomedial striatum (Pozzi et al., 2011; Murphy et al., 2011; Agnoli et al., 2013). Similarly, in OCD patients, a dysregulation of glutamatergic signaling in the cortico-striatal circuitry has been suggested, with decreased concentrations of glutamate in the anterior cingulate cortex, accompanied by overactivity of the glutamate signaling in the striatum and orbitofrontal cortex (Pittenger et al., 2011; Ting and Feng, 2011; Milad and Rauch, 2012). Other authors proposed that the beneficial effect of MEM in OCD patients could be mediated by functional disconnection of the hippocampus with critical frontal regions (Vlček et al., 2018), by its effect on decreasing glutamate level in the hippocampus (Glodzik et al., 2009). Finally, we could hypothesize that according to these results, a possible explanation under the differences in compulsive HD rats selected by SIP might be an altered function of glutamatergic NMDA receptors that affect firing

in cortical neurons in mPFC and affect glutamatergic, as well as dopaminergic and serotoninergic signal in the striatum.

Chapter 5. Experiment 3: Gene expression analysis

5.1. EXPERIMENTAL DESIGN

The present study aimed to investigate the possible differences in gene expression of serotonergic, glutamatergic and neuroplasticity related genes in rat populations selected by SIP. Therefore, after the selection of compulsive HD and non-compulsive LD rats on SIP, we analyzed the expression of serotonergic *Htr2a* and *Htr2c*, glutamatergic *Grin1*, *Grin2a*, *Grin2b*, *Grin2c* and *Grm2*, and *Bdnf* genes in the following neuroanatomical areas: frontal cortex (FC), hippocampus (HIP), and amygdala (AMY), related to the cortico-limbic circuit and compulsive drinking on SIP (Mora et al., 2020). Figure 27 summarizes experimental events.

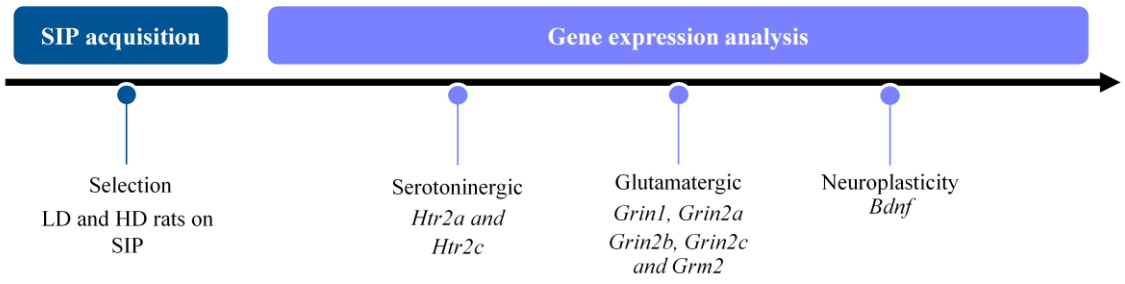

Figure 27. Experimental procedure illustrated in a timetable. SIP: Schedule-induced polydipsia; HD: High drinker; LD: Low drinker.

5.2. MATERIALS AND METHODS

5.2.1. Subjects

A total of 20 male Wistar rats (Envigo, Spain) weighing between 250–350 g at the start of the experiments were used in the present study. The animals were housed in four rats/cages (50 × 35 × 20 cm) kept in a temperature-controlled environment at 22 ± 2 °C, humidity (50 ± 10 %), with a 12:12-h light-dark cycle (light off at 08:00 h am) and food and water provided ad libitum. After ten days of habituation and before behavioral tasks, the rats were gradually reduced to 85 % of their free-feeding body weight through controlled feeding, and their body weights were maintained at this level of deprivation throughout the experiment. Food was provided by daily feedings of lab chow at approximately 30 min after each experimental session. All testing was performed between 9:00 h am and 2:00 h pm. Animals were around 3 months of age when the experiment started and finished it at 5 months of age. All of the procedures were conducted following the Spanish Royal Decree 53/ 2013 on the protection of experimental animals, the European Community Directive 2010/63/EU for animal experiments and comply with the ARRIVE guidelines for animal research. The Animal Research Committee of the University of Almeria approved the experiments described here and the authors declare that the research shows commitment to the 3Rs principle (replacement, reduction, refinement).

After the last session of SIP (protocol described in Chapter III.II: SIP procedure), all rats were sacrificed by rapid decapitation after anesthesia induction by inhalation of 4 % isoflurane, to extract the brain and obtain the samples of structures, by fresh dissection, which would later be subject to analysis: the FC, the HIP and the AMY. The collected samples were immediately frozen on dry ice to prevent the degradation of the ribonucleic acid (RNA). All the samples were then stored at −80 °C until use. All the material used in this procedure was autoclaved (Class-B P Selecta) and treated with ZAP RNAse (Sigma Aldrich) to avoid contamination and degradation of the genetic material. Then, samples were isolated, quantified and diluted to 100 ng/uL. This concentration was used for the complementary DNA (cDNA) synthesis (20uL). Twenty microliters of that cDNA were then diluted (1:4 factor), and this dilution was finally used for the quantitative reverse transcription polymerase chain reaction (RT-qPCR) reaction.

5.2.2. SIP procedure: HD and LD selection

LD and HD rats were selected by SIP following the same protocol described in the first experimental set (see page 40).

5.2.3. Real-time quantitative polymerase chain reaction

By using this technique, a multitude of copies of a particular nucleotide sequence can be generated in vitro from a small amount of genetic material from structures that have been carefully extracted beforehand. Thus, before the exponential replication of the sequences of interest a series of processes had to be developed to enable the polymerase chain reaction itself, as well as its reliability. First, RNA from the samples of the three structures (FC, HIP, and AMY) was extracted and purified using Trizol reagent (Invitrogen) according to the manufacturer's instructions. Trizol reagent reliably extracts and purifies RNA from samples by maintaining RNA integration through inhibition of RNAase activity and destroying cellular components in the homogenization of samples. Thus, a separation of different layers was obtained by adding chloroform, being in the aqueous layer where the RNA is located, so it was collected and, by employing isopropanol and suitable centrifugation, the RNA pellet was precipitated. The supernatant was then removed, and the RNA pellet was washed with 70 % ethanol. Finally, the remaining ethanol was removed and allowed to dry. Once the process was completed, the final number of samples to be analyzed was obtained: 18 frontal cortex, 18 amygdala, and 18 hippocampus. Secondly, RNA quality assessment was carried out by electrophoresis. Electrophoresis is a technique that uses the polar character of the genetic material (negative charge given by the phosphate groups) to consequently move it from the negative to the positive pole on a solid matrix (agarose gel) when a certain voltage is applied. First, the agarose gel was prepared by dissolving agarose in Milli-Q water. The resulting mixture was heated until the agarose was perfectly dissolved. The solution was then poured into a gel holder. A comb installed in the gel holder shaped the wells where the samples were placed once the loading buffer was added to the samples, and the gel was perfectly solidified and placed in the electrophoresis cuvette. The next step was the application of voltage that would cause the desired electrophoretic shift. To visualize the fragments, the gel had to be stained with an intercalating agent that binds to the genetic material. The marker used

was ethidium bromide, which was handled with special caution due to its mutagenic nature. The last step was fluorescence spectrometry, whereby ultraviolet light is applied to cause the ethidium bromide to emit fluorescence, thus seeing the fragments separated according to molecular weight. This process was repeated for the samples of each structure. Also, RNA was quantified by fluorescence signaling with a Qubit® fluorometer (Life Technologies). This specific and sensitive process allowed us to know the concentration and quality, and to discard degradation and/or contamination of the samples. This process was repeated for the samples of each structure. Then, the samples were subjected to a Turbo DNA-free treatment whereby the DNAases remove contaminating genomic desoxyribonucleic Acid (DNA) from the preparations that will then be retro-transcribed. An inactivating reagent was used to stop the effect of the DNAases. The removal of these contaminants allows for a PCR with less interference. Finally, cDNA was obtained from messenger RNA. Retrotranscription allows RNA to be used to obtain cDNA from a reverse transcriptase enzyme. In our case, cDNA was synthesized from DNA-free total RNA using the Maxima First Strand® cDNA synthesis kit (Thermo Scientific), using a mixture of random hexamers and 18-mer oligo (dT) as primers. This process was repeated for samples of each structure. The cDNA samples were stored at −80 °C until qPCR analysis.

5.2.4. Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Gene expression analysis was performed by RT-qPCR using the SYBR Green PCR Master Mix kit on a Step-One real-time PCR system (Applied Biosystems) and a pair of specific primers for each gene analyzed (Table 7). The appropriate efficiency of the primers was controlled by serial dilutions (dilution factor 1:10). The housekeeping gene Gapdh was used as an internal reference for gene expression analyses. The absence of gDNA contamination in the RNA sample analyzed by RT-qPCR was demonstrated using a specific amplicon of an intron section of the Gapdh gene as a control. The melting curves were analyzed to ensure the specificity of the amplification. This process was repeated for samples of each structure.

Gene	Forward	Reverse	Reference
Gadph	ACAACTTTGGCATTGTGGAA	GATGCAGGGATGATGTTCTG	Own design
Htr2a	AACGGTCCATCCACAGAG	AACAGGAAGAACACGATGC	(Kindlundh-Hög-bergetal et al. 2006)
Htr2c	TTGGACTGAGGGACGAAAGC	GGATGAAGAATGCCACGAAGG	(Kindlundh-Hög-bergetalet al. 2006)
Grin1	ATGGCTTCTGCATAGACC	GTTGTTTACCCGCTCCTG	(Lau et al. 2013)
Grin2a	AGTTCACCTATGACCTCTACC	GTTGATAGACCACTTCACCT	(Lau et al. 2013)
Grin2b	AAGTTCACCTATGACCTTTACC	CATGACCACCTCACCGAT	(Lau et al. 2013)
Grin2c	GGCCCAGCTTTTGACCTTAGT	CCTGTGACCACCGCAAGAG	(Lau et al. 2013)
Grm2	CTATGCCACCCACAGTGATG	GCACAGTGCGAGCAAAGTAATC	(Pershina et al. 2018)
Bdnf	GGTCACAGCGGCAGATAA	CCGAACATACGATTGGGTAG	Own design

Table 7. Primers selected for the RT-qPCR study. From left to right, the name of the gene, forward primer, reverse primer, and source. Gapdh: Glyceraldehyde 3-phosphate dehydrogenase. *Htr2a* and *c*: Serotonergic receptor 2 a & b. *Grin1*, *2a*, *2b*, and *2c*: Glutamatergic NMDA subunit ionotropic receptor 1, 2a, 2b, and 2c, respectively. *Grm2*: Glutamatergic metabotropic receptor 2. *Bdnf*: Brain-derived neurotrophic factor.

5.2.5. Data analyses

The study of the differences in gene expression between HD and LD, was analyzed using Student's t-test (T-test) for each of the genes in the three different structures. Post hoc comparisons were carried out using the Bonferroni test. To assess the relation between levels of water intake on SIP and relative gene expression, correlations were calculated using Pearson correlation analysis. The effect size of the group differences was calculated using Cohen's d. Statistical significance was set at $p < 0.05$. All analyses were

computed using the Statistica software package (version 8.0). Graphpad Prism (version 9.0) was used for the graphs presented in the results section.

5.3. RESULTS

5.3.1. LD and HD Selected by SIP

The mean water intake and licks in LD and HD during the acquisition and maintenance of SIP are shown in Figure 28.

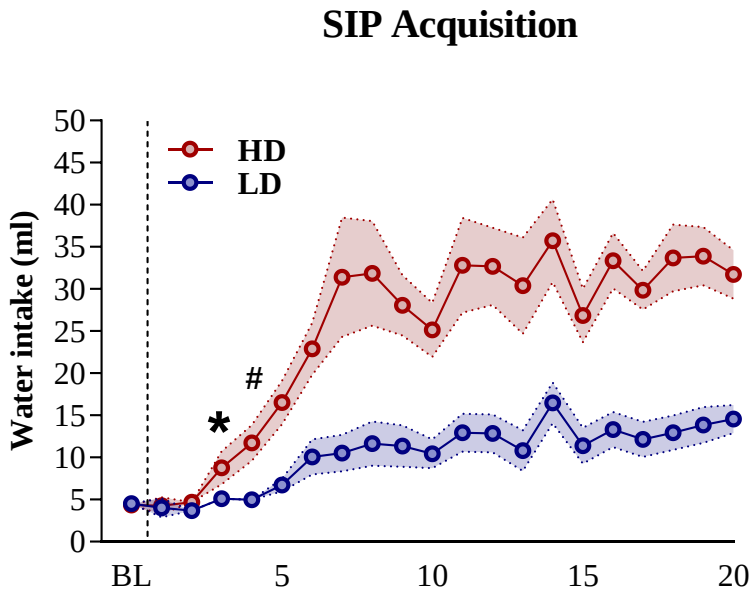

Figure 28. The mean (± SEM) water intake in ml in fixed time FT-60s across 20 sessions of schedule-induced polydipsia (SIP). Statistical analyses indicate significant differences between low drinkers (LD, n = 10) and high drinkers (HD, n = 10; *p < 0.05) from session 3. Significant differences between sessions were found from session 5 (#p < 0.05).

The mean water intake over the last 5 days of SIP was 32,51 ± 2.42 mL for HD and 13.36 ± 1.61 for LD, respectively. The number of licks also showed SIP acquisition (data not shown). The mean total licks averaged across the last 5 days of SIP were 1014.8 ± 320.92 and 765.89 ± 242,2 for HD and LD, respectively (data not shown). ANOVA revealed significant differences in water intake according to the interaction between SIP acquisition sessions and group (interaction SIP session x group effect: $F(19,342) = 4.48$, $p < 0.001$, $\eta^2p = 0.17$). Also, differences in water consumption were observed for the session effect ($F(19, 342) = 18.46$, $p < 0.001$) and group effect ($F(1,18) = 29.31$, $p <$

0.001). ANOVA also showed a significant interaction in the total number of licks (interaction SIP session x group effect: $F(19,342) = 23.95$, $p < 0.001$; session effect: $F(19,342) = 38.56$, $p < 0.001$; group effect: $F(1,18) = 85.42$, $p < 0.001$).

Post hoc comparisons indicated that SIP induced differences in drinking rates across the 20 sessions in both groups. Differences between LD and HD were evident in the water intake at session 3 ($p < 0.05$) onwards. Furthermore, when compared to session 1, animals in the HD group significantly increased their water consumption from session 7 ($p < 0.01$). Differences between the HD and LD groups in the number of total licks at session 7 ($p < 0.01$) were also observed, and HD rats increased their number of licks from session 7 ($p < 0.05$) compared to session 1. There were significant differences between LD and HD animals in the total magazine entries according to session effect ($F(19,342) = 4.76$, $p < 0.001$). However, there were no significant differences according to the interaction between SIP acquisition sessions and LD vs. HD (interaction SIP session x group effect: $F(19,342) = 0.33$, $p = 0.42$) and group effect ($F(1,18) = 0.40$, $p = 0.61$).

5.3.2. Serotonergic genes relative expression in LD and HD rats

Figure 29 shows the messenger RNA (mRNA) expression levels of *Htr2a* and *Htr2c* genes in different brain structures in HD and LD rats selected by SIP. HD rats showed significantly decreased mRNA expression levels of *Htr2a* in the FC ($t = 2.23$, $df = 16$, $p < 0.05$; $d = 1.06$) compared to LD rats. No differences were found in the mRNA gene expression level of *Htr2a* nor HIP ($t = -0.61$, $df = 16$, $p = 0.72$), nor in AMY ($t = -0.91$, $df = 16$, $p = 0.81$) between LD and HD rats (Figure 29 A). A significant negative correlation between water intake on SIP and FC mRNA expression levels of *Htr2a* ($R2 = -0.57$, $p = 0.01$) was found (Figure 29 B). However, no significant correlations were found between water intake and mRNA expression levels of *Htr2a* in HIP ($R2 = -0.01$, $p = 0.96$) and AMY ($R2 = 0.25$, $p = 0.30$). No differences were found in the mRNA expression levels of *Htr2c* in FC ($t = 0.71$, $df = 16$, $p = 0.25$), nor in HIP ($t = 1.08$, $df = 16$, $p = 0.15$) nor in AMY ($t = -0.49$, $df = 16$, $p = 0.68$) between HD and LD rats (Figure 29 C). No significant correlations were found between water intake and mRNA expression levels of *Htr2a* in HIP ($R2 = -0.01$, $p = 0.96$) and AMY ($R2 = 0.25$, $p = 0.30$). Also, there

were no significant correlations between water intake on SIP and mRNA expression levels of *Htr2c* in FC (R2 = -0.21, p = 0.41) (Figure 29 D) nor in HIP (R2 = 0.36, p = 0.14) nor in AMY (R2 = -0.09, p = 0.72) (Table 8).

Gene	Brain structure	R^2	P Value
Htr2a	FC	-0.57	0.01*
	HIP	-0.01	0.96
	AMY	0.25	0.30
Htr2c	FC	-0.21	0.41
	HIP	0.36	0.14
	AMY	-0.09	0.72
Bdnf	FC	-0,67	0.002**
	HIP	-0.19	0.44
	AMY	-0.13	0.60
Grin1	FC	-0.52	0.03*
	HIP	-0.13	0.61
	AMY	-0.09	0.70
Grin2a	FC	-0.06	0.69
	HIP	0.25	0.37
	AMY	0.03	0.93
Grin2b	FC	-0.34	0.17
	HIP	-0.34	0.17
	AMY	0.22	0.40
Grin2c	FC	-0.13	0.70
	HIP	-0.05	0.81
	AMY	-0.36	0.15
Grm2	FC	-0.13	0.62
	HIP	0.02	0.94
	AMY	0.06	0.82

Table 8. Correlations between water intake on SIP and relative gene expression. From left to right, the name of the gene, the brain structure, the R2 value, and P value. Statistical analyses indicate significant negative correlation between *Htr2a*, *Grin1* and *Bdnf* relative expression in the frontal cortex and the water intake. (*p < 0.05; ** p < 0.01.)

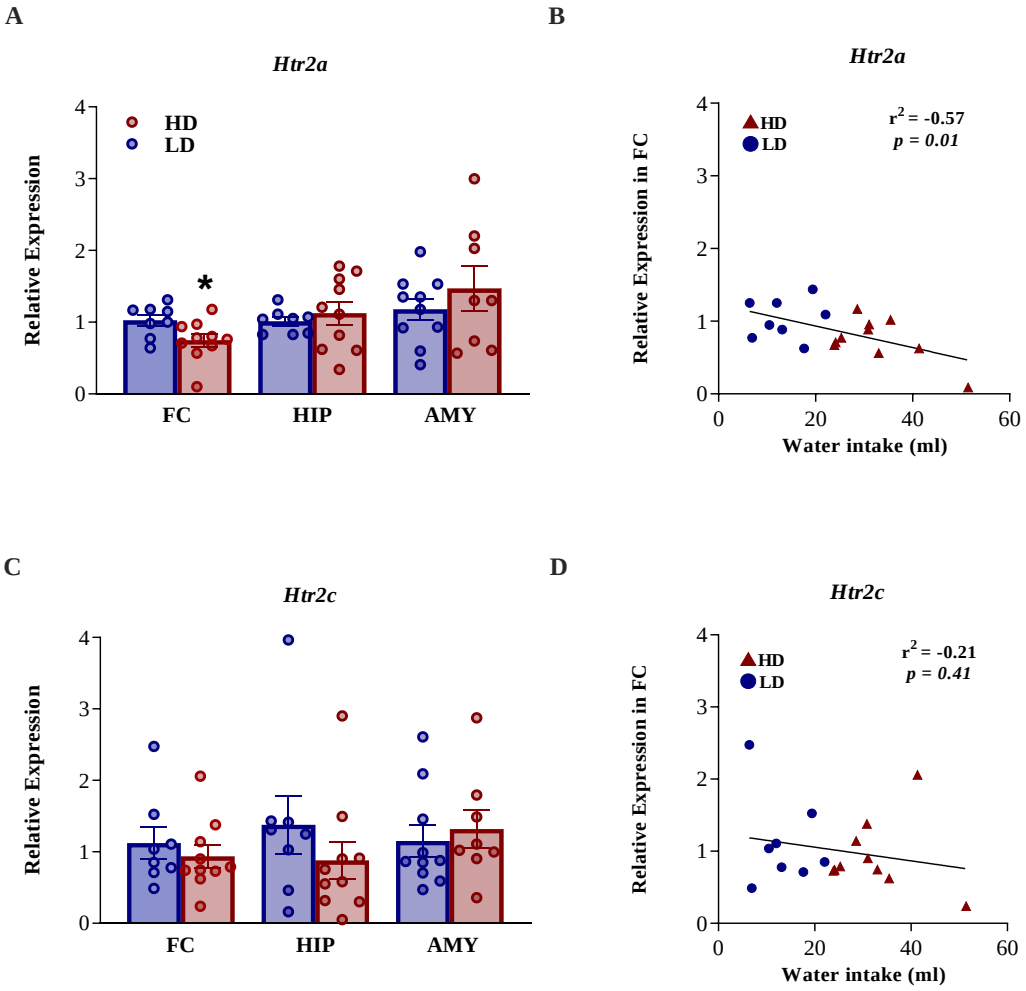

Figure 29. Serotonergic relative expression (± SEM) of *Htr2a* (A) and *Htr2c* (C) of high drinker (HD; n = 10) and low drinker rats (LD; n = 10) in the frontal cortex, the hippocampus and the amygdala. Negative correlation between *Htr2a* (B) and *Htr2c* (D) relative expression in the frontal cortex and water intake on SIP. Statistical analyses indicate significant differences between LD and HD (*p < 0.05) in the relative expression of *Htr2a* in the frontal cortex and significant negative correlation between *Htr2a* relative expression in the frontal cortex and the water intake.

5.3.3. Glutamatergic genes relative expression on LD and HD rats

Figure 30 shows the mRNA expression levels of different glutamatergic genes in different brain structures in HD and LD rats selected by SIP. HD rats showed a significant reduction of *Grin1* mRNA expression levels in FC ($t = 1.95$, $df = 16$, $p < 0.05$; $d = 0.93$) compared to LD rats. A significant negative correlation was also observed between the water intake on SIP and the relative expression of *Grin1* in the FC ($R2 = -0.52$, $p = 0.03$)

(Figure 30 B). No significant differences were found in *Grin1* mRNA expression levels in HIP (t = 0.36, df = 16, p = 0.36) nor in AMY (t = - 0.28, df = 14, p = 0.61) between HD and LD rats (Figure 30 A). No differences were found in the expression levels of *Grin2a* in FC (t = - 1.20, df = 14, p = 0.88), in HIP (t = 0.17, df = 14, p = 0.43) and in AMY (t = - 0.19, df = 14, p = 0.57) between HD and LD rats (Figure 30 C). Moreover, no significant differences were found in the expression levels of *Grin2b* in FC (t = 0.70, df = 16, p = 0.25), in HIP (t = 1,24, df = 16, p = 0.12) and in AMY (t = - 1.22, df = 16, p = 0.88) between HD and LD rats (Figure 30 D). Also, we did not find any significant difference in the expression levels of *Grin2c* in FC (t = - 0.32, df = 13, p = 0.63), in HIP (t = - 0.44, df = 14, p = 0.67) and in AMY (t = 0.01, df = 12, p = 0.50) between HD and LD rats (Figure 30 E). There was a non-significant trend to decreased mRNA expression of *Grm2* levels in FC (t = 1.68, df = 16, p = 0.05), and in the HIP (t = 1.38, df = 16, p = 0.09) between HD and LD rats. No significant differences were found in AMY (t = 0.31, df = 16, p = 0.38) between HD and LD rats (Figure 30 F). The comparison between drinking levels on SIP and mRNA expression levels of the rest of the glutamatergic genes analyzed revealed no significant correlations. The correlation between the water intake on SIP and mRNA expression levels of *Grin1*: in HIP (R2 = -0.13, p = 0.61) and AMY (R2 = -0.09, p = 0.70) are shown in Table 8. No significant differences between the water intake on SIP and the mRNA expression levels of *Grin2a*: in FC (R2 = -0.34, p = 0.20), in HIP (R2 = -0.33, p = 0.97) and in AMY (R2 = 0.92, p = 0.40); *Grin2b*: in FC (R2 = -0.48, p = 0.17), in HIP (R2 = -0.45, p = 0.17) and in AMY (R2 = 0.32, p = 0.90); and *Grin2a*: in FC (R2 = -0.74, p = 0.47), in HIP (R2 = -0.36, p = 0.17) and AMY (R2 = 0.22, p = 0.40) (Table 8). There were no significant correlations between water intake on SIP and mRNA expression levels of *Grm2* in FC (R2 = -0.13, p = 0.62), in HIP (R2 = 0.02, p = 0.94), and in AMY (R2 = -0.06, p = 0.82) (Table 8).

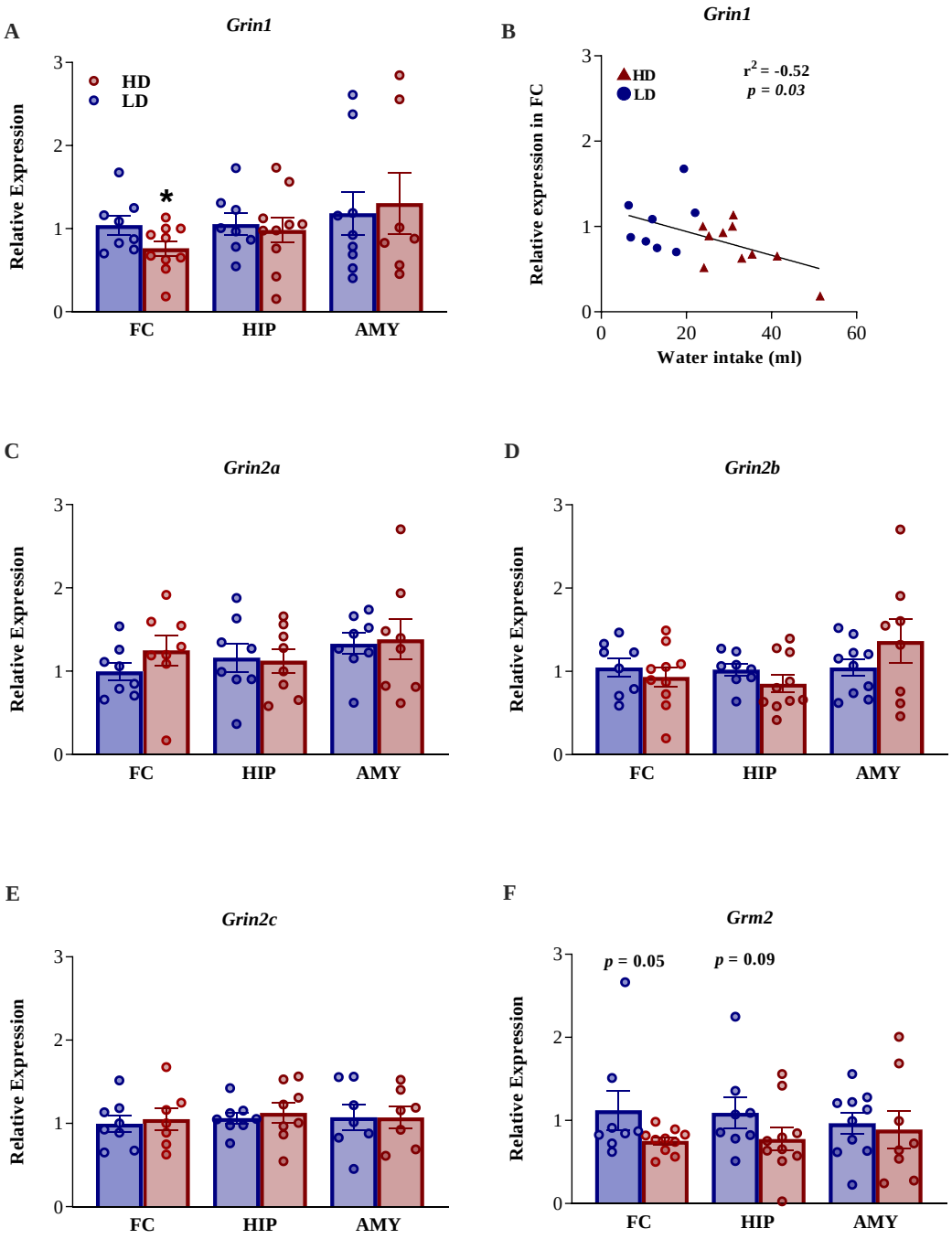

Figure 30. Glutamatergic relative expression (± SEM) of *Grin1* (A), *Grin2a* (C), *Grin2b* (D), *Grin2c* (E), and *Grm2* (F) of high drinker (HD; n = 10) and low drinker rats (LD; n = 10) in the frontal cortex, the hippocampus and the amygdala. Significant negative correlation between *Grin1* relative expression in the frontal cortex and water intake on SIP (B). Statistical analyses indicate significant differences between LD and HD (*p < 0.05) in the relative expression of *Grin1* in the frontal cortex and significant negative correlation between *Grin1* relative expression in the frontal cortex and the water intake.

5.3.4. Neuroplasticity genes relative expression in LD and HD rats

HD rats showed a significant reduction in *Bdnf* mRNA expression levels in FC (t = 1.84, df = 16, p < 0.05; d = 0.874). A trend, in the same way, was observed in HIP (t = 1.43, df = 16, p = 0.08) compared to the LD rats. No significant differences were found in *Bdnf* mRNA expression levels in AMY (t = - 0.31, df = 16, p = 0.62) between HD and LD rats (Figure 31 A). There was a significant negative correlation between the water intake on SIP and the relative expression of *Bdnf* in the FC (R2 = -0.67, p = 0.002) (Figure 31 B). No significant negative correlations were found between water intake on SIP and *Bdnf* mRNA expression levels in HIP (R2 = -0.19, p = 0.44) and in AMY (R2 =- 0.13, p = 0.60) (Table 8).

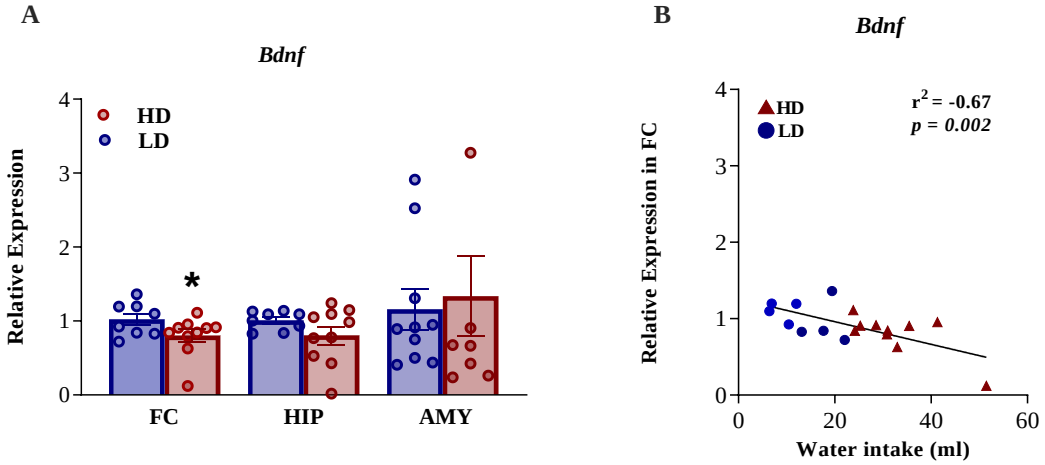

Figure 31. *Bdnf* relative expression (± SEM) of high drinkers (HD; n = 10) and low drinkers rats (LD; n = 10) in the frontal cortex, the hippocampus and the amygdala (A). Significant negative correlation between *Bdnf* relative expression in the frontal cortex and water intake on SIP (B). Statistical analyses indicate significant differences between LD and HD (*p < 0.05) in the relative expression of *Bdnf* in the frontal cortex and significant negative correlation between *Bdnf* relative expression in the frontal cortex and the water intake.

5.4. DISCUSSION

We found that HD rats have a significantly decreased mRNA expression level of *Htr2a*, *Grin1*, and *Bdnf* in the FC compared to LD rats. Also, the mRNA expression levels of *Htr2a* in the FC correlated negatively with compulsive drinking on SIP. Thus, rats that consumed more water showed less mRNA expression levels of *Htr2a*. This is in accordance with previous research in our laboratory, which revealed HD rats selected by SIP had a specific reduction of 5-HT2A receptor binding in FC compared to LD rats (Mora et al., 2018). In contrast, Roman High Avoidance (RHA) rats characterized as impulsive and by a compulsive drinking profile on SIP (Moreno et al., 2010), show higher 5-HT2A binding in the FC compared to Roman Low Avoidance (RLA) rats (Klein et al., 2014). However, a recent study did not find differences in *Htr2a* gene expression in FC between RHA and RLA rats (Fomsgaard et al., 2018). According to our findings, rats that showed a high inflexibility in a spatial discrimination reversal learning task had lower serotonin 5-HT2A receptor binding in the orbitofrontal cortex compared to low-perseverative rats (Barlow et al., 2015). Studies using single photon emission computed tomography found that dogs with compulsive behaviors have lower serotonin 5-HT2A receptor availability in the FC (Vermeire et al. 2012). On the contrary, red junglefowl (Gallus gallus) chicks characterized by a higher expression of *Htr2a*, are less flexible in a discriminative and reversal learning task (Boddington et al., 2020). Animal models of individual differences for example: on depression, have shown that the expression of *Htr2a* is reduced in the FC of Flinders Sensitive Line (FSL) compared to their control strain Flinders Resistant Line (FRL) (Du Jardin et al., 2017). Moreover, dogs with anxiety disorders have lower serotonin 5-HT2A binding in the FC (Vermeire et al., 2009). Furthermore, pharmacological studies have found that serotonin 5-TH2A/C receptor agonists reduce compulsive drinking on SIP. Thus, the systemic administration of DOI in a dose-dependent manner only reduced compulsive drinking on SIP in HD rats compared to LD rats, which supports the notion that serotonin 5-HT2A receptors have a key role in compulsive behavior (Navarro et al., 2015). In addition, the activation of prefrontal serotonin 5-HT2A/c receptors by direct micro-infusion of DOI in the medial prefrontal cortex decreased compulsive drinking in HD rats (Mora et al., 2018). Serotonin 5-HT2A receptors have a role in cognitive flexibility since the blockade of these receptors leads to impairments in reversal learning

(Boulougouris, Glennon, and Robbins, 2008). Microinfusion of the 5-HT2A receptor antagonist M100907 in the orbitofrontal cortex leads to a higher perseveration during reversal learning and potentiated self-grooming behavior in BTBR mice, a mouse model of autism (Amodeo et al., 2014). A high expression of *Htr2a* has been found in FC, HIP, and AMY of the central nervous system of adult rats, which constitute components of the brain circuits implicated in memory extinction (Cornea-Hébert et al., 1999). The activation of serotonin 5-HT2A receptors facilitates the consolidation and extinction of trace and delay-cued fear memory (Stackman et al., 2013). Clinical studies have also implicated the serotonin *Htr2a* receptor in different psychopathological disorders. In vivo studies in drug-naive OCD patients show a reduction in 5-HT2A receptors availability in the FC (Perani et al., 2008). A reduction in mRNA expression levels of *Htr2a* has also been observed in bipolar disorder patients (López-Figueroa et al., 2004), and schizophrenia patients (Hurlemann et al., 2008), a finding consistent with postmortem autoradiography studies which showed reduced 5-HT2A binding in the FC of schizophrenic patients (Matsumoto et al., 2005).

In the present study, HD rats did not differ significantly in the mRNA expression level of *Htr2c* in FC, HIP, and AMY compared with LD rats. Moreover, no significant correlations were found between water intake on SIP and mRNA expression level of *Htr2c*. Overall, there is very limited evidence to suggest the involvement of the 5-HT2C receptor in OCD (Sinopoli et al., 2017). However, studies support that 5-HT2C has a role in this disorder. Previous research shows that DOI decreased compulsive drinking in HD rats on SIP (Navarro et al., 2015) while the 5-HT2C receptor antagonist SB242084 did not affect compulsive drinking on SIP (Mora et al., 2018). Also, the administration of WAY-163909, a serotonin 5-HT2C antagonist, decreased adjunctive drinking on SIP (Rosenzweig-Lipson et al., 2007), while the 5-HT2C receptor antagonist SB242084 increased drinking on SIP (Higgins et al., 2020). The activation of 5-HT2C induced excessive self-grooming behavior in rats (Gráf, 2006), and 5-HT2C receptor knockout mice exhibited compulsive-like behaviors (Chou-Green et al., 2003). Notwithstanding, serotonin 5-HT2C receptors have been associated with cognitive flexibility and reversal learning. Preclinical studies with rats have found that the administration of 5-HT2C receptor antagonist SB242084 improved learning performance (Alsiö et al., 2015; Boulougouris, Glennon, and Robbins, 2008). In clinical studies, *Htr2c* mRNA expression levels have

been shown to be reduced in the FC on unmedicated and medicated schizophrenic patients (Castensson et al., 2003, 2005). On the contrary, a recent meta-analysis found no significant associations between *Htr2c* polymorphism and OCD (Taylor, 2013).

The assessment of the glutamatergic *Grin1* gene, which encodes NMDA receptor subunit 1, revealed a significant reduction in FC in HD rats and an inverse correlation between its expression and compulsive drinking on SIP. Previous studies pointed towards the relation between *Grin1* and compulsive behavior in rats, as for example, different correlations between the mRNA expression of *Grin1* in the dorsomedial prefrontal cortex and exposure to cocaine, in which limited access to cocaine negatively correlates with the mRNA expression levels, have been described (Ploense in 2018). Nevertheless, prolonged exposure increased the mRNA levels (Ploense et al. 2018). However, a recent study in rats after 10-day cocaine abstinence has shown no significant differences in *Grin1* expression in FC or HIP (Smaga et al. 2021). Similarly, authors did not describe any significant difference in the mRNA levels of *Grin1* in RHA high avoidance rats in the same brain areas (Elfving in 2019). Besides, other preclinical studies confirmed the implication of *Grin1* in fear memory and extinction processes, showing that the deleting of knocking out *Grin1* strongly facilitates fear memory formation and retention and attenuates extinction of a cued fear response (Gafford et al. 2014; Hirsch et al. 2015). Likewise, *Grin1* had been considered an important gene in memory acquisition (Chen et al. 2018). Thus, mutant mice expressed deficits in spatial working memory in the Morris Water Maze paradigm (Kew et al. 2000). Also, mutant mice presented abnormal anxiety-like behaviors in the light/dark transition and the Elevated Plus Maze Test, a deficient contextual and cued fear memory in the FCT, and impaired working memory in the eight-RAM (Umemori et al. 2013). *Grin1* has been considered as a susceptibility gene candidate for some neuropsychiatric disorders, including schizophrenia, bipolar disorder, and attention-deficit/hyperactivity disorder (Umemori et al. 2013; Mundo et al. 2003). Moreover, *Grin1* seems to have a relevant role in schizophrenia disorder (Liu et al. 2019; Zhao et al. 2009; Hung et al. 2002).

The assessment of the glutamatergic *Grin2b* and *Grm2* genes revealed no significant differences in gene expression between compulsive HD rats and LD rats. However, a previous study showed that high avoidance rats expressed increased levels of *Grin2b*

and decreased levels of *Grm2*, both in the FC (Elfving et al. 2019). Moreover, contradictory results have been found concerning the association between *Grin2b* mRNA expression and the experience of stressful and anxious experiences in rats. Stress induced by maternal separation did not affect the expression levels of *Grin2b* in the FC, nor in the HIP (Masrour et al. 2018). *Grin2b* knockout mice are considered as a model of autism and intellectual disability (Shin et al. 2020; O'Roak et al. 2012). Consistently, *Grin2b* variants had been associated with the susceptibility to develop OCD in humans (Bozorgmehr et al. 2017; Alonso et al. 2012; Arnorld et al. 2004). Less information is available referring to *Grm2* genes. Research exploring the effect of the lack of *Grm2* in the prelimbic cortex in alcohol consumption using knockout rats reported no significant differences in alcohol intake (Ding et al. 2017). Other studies showed an upregulation of *Grm2* proteins in the FC and the ventral tegmental area after protocols of mild stress (Liao et al. 2021). Clinical research suggested that *Grm2* may play a key role in the pathophysiology of methamphetamine-induced psychosis (Tsunoka et al. 2010). Furthermore, Gonzalez-Maeso detected downregulation of *Grm2* in post-mortem human brains from untreated schizophrenic subjects (Gonzalez-Maeso et al. 2008).

The assessment of the *Bdnf* gene revealed a significant reduction in FC in HD rats and an inverse correlation between its expression and compulsive drinking on SIP. BDNF and its receptors are involved in the regulation of synaptic plasticity processes and synaptic communication (Orhan et al. 2021; Kowianski et al., 2018; Favalli et al., 2012). In preclinical studies, upregulation of *Bdnf* expression in the Nucleus Accumbens was observed in rats showing successful extinction in morphine-conditioned place preference (Martínez-Rivera et al. 2019). In addition, increased BDNF protein in prefrontal and hippocampal regions produces extinction facilitation in fear-conditioned rats (Peters et al. 2009; Rosas-Vidal et al. 2014). Besides, decreased *Bdnf* expression level in different brain areas has been reported in animal models of depression and isolation (Elfving et al. 2010), but a review pointed out that the downregulation of *Bdnf* expression might be associated with increased anxiety-like symptoms, such as shorter time spent in the open arms of an elevated plus maze, increased immobility in the FST, or reduced sucrose preference (reviewed in Murínová et al. 2017). However, the *Bdnf* expression level has been shown to be increased in high avoidance RHA rats in FC (Elfving et al. 2019). Moreover, recent data suggest that the downregulation in the locus coeruleus of *Arc* mRNA levels,

another plasticity marker, is associated with the tendency to develop compulsive behavior on SIP (Velazquez-Sanchez, et al. 2023). Furthermore, clinical studies demonstrated decreased BDNF serum levels in OCD and schizophrenia patients when compared to control participants (Maina et al. 2010; Favalli et al. 2012).

Chapter 6. General discussion

The findings of the present Doctoral Thesis contribute to develop our knowledge about the behavioral, pharmacological and genetic alterations associated with the compulsive HD phenotype selected by SIP. Besides, it contributes to the development of new therapeutic and diagnose targets, such as possible biomarkers of vulnerability to compulsive spectrum disorders, helping to know more about the prognosis of this disorder and develop more individualized treatments. Hence, an overview of the key findings of the present Doctoral Thesis could be summarized in following points:

1. **Persistence behavior and resistance to memory extinction**. Compulsive drinking behavior during SIP might contribute to the development of perseverative responses and to the formation of resistant memories. Recent meta-analysis revealed that persistence checking behavior is associated with the memory confidence and accuracy detriment in OCD population (Jondani et al. 2023). Nevertheless, evidence regarding memory impairment in OCD is inconclusive, as we can find mixed findings. Memory deficits may be related to specific subtypes or symptom dimensions of OCD. Another possibility is that other factors such as anxiety, depression, and medication use may play a significant role in influencing memory performance in individuals with OCD. Overall, while there is some evidence to suggest that memory impairments may be present in some individuals with OCD, more research is needed to better understand the relationship between compulsivity and memory functioning.
2. **Inflexible behavior in changing environments**. Derived from this formation of resistant memories, HD rats showed inflexible responses in changing environments. According to dual-system theories, actions and choices can be supported by either a goal-oriented or a habitual system (Balleine and Dickinson, 1998). Computational studies have also made a similar distinction between model-based and model-free strategies for action selection (Daw et al., 2011). Goal-oriented choice offers flexibility but requires significant cognitive resources. In contrast, habitual or model-free be-

havior is more efficient in familiar situations where past experience provides assurance that a particular course of action is optimal, making detailed evaluation of alternatives unnecessary. However, habitual behavior lacks flexibility in response to a changing environment or changing needs. Over-reliance on habitual behavior may result in cognitive and behavioral inflexibility. It is important to note that the relationship between habitual behavior and cognitive flexibility has received little exploration, making it an important area for future research. Memory and cognitive flexibility impairments observed in OCD population, as well as in prelicnical models, point to a possible implication of glutamatercig signaling in the development and maintenance of compulsive behavior.

3. **Glutamatergic signaling affectation**. The positive impact of administering MEM and LAM on reducing compulsive drinking in HD rats on SIP implies that inhibiting glutamate, antagonizing NMDA receptors, or blocking calcium and sodium channels in pre-synaptic terminals may have therapeutic benefits. These findings support the possibility of dysregulation in glutamatergic signaling previously observed, in individuals with OCD that present a dysregulation of glutamatergic signaling in the cortico-striatal circuitry. Specifically, decreased concentrations of glutamate in the anterior cingulate cortex may be accompanied by overactivity of glutamate signaling in the striatum and orbitofrontal cortex (Pittenger et al., 2011; Ting and Feng, 2011; Milad and Rauch, 2012). Some researchers propose that the therapeutic effect of MEM in OCD patients may be due to functional disconnection between the hippocampus and critical frontal regions (Vlček et al., 2018) or by its ability to decrease glutamate levels in the hippocampus (Glodzik et al., 2009). Based on these findings, it could be hypothesized that the differences in compulsive behavior observed in HD rats selected by SIP may be attributed to altered function of glutamatergic NMDA receptors that impact firing in cortical neurons in the mPFC and affect glutamatergic, dopaminergic, and serotoninergic signaling in the striatum. Conversely, the lack of effect of NAC on compulsive intake in HD rats on SIP suggests that specific stimulation of the presynaptic terminal could have a differential effect, making evident the need for deeper exploration. That possibly altered fuction of glutamatergic NMDA receptors leads to the idea of an also altered genetic expression of NMDA-related genes in compulsive population.

4. **Gene expression alteration**. As expected by the results at neuropharmacology level, HD compulsive rats showed dysregulated expression of serotonergic, glutamatergic and neuroplasticity genes in the FC. These findings reveal the key role of that specific region alteration of the gene expression in compulsive individuals, pointing toward a possible lack of plasticity in this area as the cause of the compulsive symptoms. Clinical studies revealed the association between a single nucleotide polymorphism in serotonergic, glutamatergic and neuroplasticity genes and compulsive spectrum disorders (Beheshti et al. 2023). Moreover, medial pre-FC has been demonstrated to present functional and structural abnormalities in OCD populations (Yang et al. 2023). Additional research could help clarify the distinct roles of dysregulated serotonergic, glutamatergic and neuroplasticity gene expression in neurotransmission and finally in the resulting behavior. This would enhance our understanding of how these mechanisms contribute to susceptibility to compulsive behaviors.

All of these findings point towards new research questions to wonder about the effect of these molecular alterations in inhibitory control and the associated symptoms. In this sense, we hypothesize that FC might be controlling the top-down system and managing other brain regions. A possible lack of plasticity in the frontal cortex might be causing inflexibility and interfering with the extinction of a prominent behavior, as the compulsive drinking in stressing conditions as SIP.

Data supporting this hypothesis are as follows: (1) FC is a brain area widely associated with executive control and associated functions such as working memory, cognitive flexibility, and inhibitory control, domains altered in HD compulsive phenotype (experiments 1 and 2). (2) In the gene expression analysis (experiment 4), HD compulsive rats, in the FC, presented a downregulated expression of *Htr2a*, *Grin1* and *Bdnf* genes. Multiple studies provide reliable support for a large additive genetic contribution to liability to OCD, as well as to associated symptoms, having both shared and unique genetic risks (Mahjani et al. 2021). (3) Glutamate, a neurotransmitter implicated in the signaling of the cortico-striatal circuitry, has shown to be altered in the HD compulsive phenotype (experiment 2). OCD populations present altered concentrations of glutamatergic neurotransmitter in the anterior cingulate cortex, the striatum and the orbitofrontal cortex (Milad and Rauch 2012; Pittenger et al., 2011; Ting and Feng 2011).

Finally, it is worth to highlight that all the experiments have been carried out using male rats, as previous statistics showed a higher occurrence of OCD in males. However, recent research suggest that females are affected at a slightly higher rate than males (Mathes et al. 2019). Hence, future research lines in preclinical models of OCD could try to replicate previous data using female animals.

Future studies might determine the underlying mechanisms associated with the suggested lack of plasticity in the FC linked to the acquisition and maintenance of compulsive behavior and the associated symptoms. Genetic, proteic, histological and functional analyses could help to determine microstructural changes and intra-circuit functional connections contributing to vulnerability to compulsivity, enhancing our understanding of the compulsive phenotype and related behaviors. This study has clinical relevance as it may lead to the identification of a new target for assessment and treatment, utilizing psychopharmacology, surgery, or neurostimulation strategies, based on the affected behavioral and cognitive domains in different neuropsychiatric disorders.

Chapter 7. Conclusions

According to the exposed results, the conclusions of the present doctoral thesis are presented as follows.

1. The behavioral characterization (1st experiment set) revealed that HD compulsive rats showed compulsivity in different paradigms and a higher fear behavior, considered as comorbid behavior related to compulsive spectrum disorders according to the clinical literature. Moreover, HD rats exhibited inflexibility and damaged working and reference memory, cognitive processes altered in compulsive spectrum disorders patients.
   a) HD rats exhibited compulsivity on MBT, evidenced by a higher number of marbles partially buried (2/3) compared to LD rats (Experiment 1A).
   b) There were no differences between groups neither in depressive-like behaviors, nor in anxiety-like behaviors, measured by FST and EPM (Experiment 1A).
   c) HD rats presented an increased fear behavior profile on FCT, shown by a higher percentage of freezing time in the first block of the retrieval day as well as across the following blocks, compared to LD rats (Experiment 1A).
   d) HD rats showed cognitive inflexibility on MWM, evidenced by a higher latency to find the platform in Reversed MWM compared to LD rats (Experiment 1B).
   e) Reference and working memory were impaired on RAM in HD rats, expressed by a higher number of accumulative errors compared to LD rats (Experiment 1B).
   f) Stimulus processing and novelty reaction did not differ in HD rats compared to LD rats on NOR (Experiment 1B).
2. The pharmacological study (2nd experiment set) provides new evidence that MEM and LAM reduced, in a dose-dependent manner, compulsive intake in HD rats on SIP, and did not affect LD behavior, suggesting a potential role of antagonizing NMDA receptor or blocking calcium and sodium channels in presynaptic terminals. In contrast, the lack of effect of NAC in compulsive drinking of HD rats, pointing towards

the possible relevance of the different effect by the specific stimulation of the presynaptic terminal.

a) The blockage of the glutamate release, by the increase of the glutathione produced by the administration of NAC, did not show any therapeutic potential in the reduction of compulsive drinking on SIP (Experiment 2).
b) The blockage of the glutamate signaling, produced by the antagonism of the NMDA receptor antagonist by the administration of MEM, reduced the compulsive behavior on SIP in a dose dependent manner (Experiment 2).
c) The blockage of presynaptic glutamate release, through the blockage of the sodium channels produced by the administration of LAM, reduced in a dose dependent manner compulsive drinking on SIP, eliminating differences between groups at the highest dose (Experiment 2).

3. The gene expression analysis (3rd experiment set) revealed downregulated mRNA expression levels of *Htr2a*, *Grin1*, and *Bdnf* genes in the FC of HD compulsive rats, pointing towards a possible lack of plasticity in the frontal cortex compared to LD rats.
   a) HD rats exhibited a downregulated level of *Htr2a* in FC compared to LD rats and the mRNA expression of *Htr2a* in the FC negatively correlates with the amount of water intake on SIP (Experiment 3).
   b) Both groups did not differ in the mRNA expression of *Htr2a* in the other brain areas studied (Experiment 3).
   c) There were no differences between groups in the mRNA expression of *Htr2c* in any of the areas analyzed (Experiment 3).
   d) HD rats showed a downregulated level of *Grin1* in FC compared to LD rats and the mRNA expression of *Grin1* in the FC negatively correlates with the amount of water intake on SIP (Experiment 3).
   e) Both groups did not differ in the mRNA expression of *Grin1* in the other two brain areas analyzed (Experiment 3).
   f) No differences were found between groups in the mRNA expression of *Grin2a*, *Grin2b*, *Grin2c* or *Grm2* in any of the areas analyzed (Experiment 3).

g) HD rats showed a downregulated level of *Bdnf* in FC compared to LD rats and the mRNA expression of *Bdnf* in the FC negatively correlates with the amount of water intake on SIP (Experiment 3).

h) HD and LD rats did not differ in the expression of *Bdnf* in the other brain areas analyzed (Experiment 3).

Hypotheses	**Hypotheses support**
1st experimental set: behavioral characterization.	
Experiment 1A: comorbid behaviors in compulsive rats selected by SIP.	
HD animals might show compulsive-like behavior on Marble Burying Test.	
HD animals might express depressive-like behavior using the Forced Swimming Test.	
HD animals might present anxiety-like behavior on the Elevated Plus Maze Test.	
HD animals might show a higher fear behavior on Fear Conditioning Test.	
Experiment 1B: cognitive processing in compulsive rats selected by SIP.	
HD animals might show an impaired reference memory on Morris Water Maze.	
HD animals might present behavioral inflexibility by reversing the conditions in Morris Water Maze.	
HD rats will present an impaired reference and working memory using the Radial Arm Maze.	
HD animals might show an impaired stimuli processing and a higher novelty reaction on the Novel Object Recognition Test.	

Table 9. Degree of support obtained for each hypothesis proposed. Green, yellow and red indicate that results confirm our hypothesis completely, partially, or they do not, respectively.

Hypotheses	Hypotheses support
2nd experimental set: pharmacological study.	
Experiment 2: glutamatergic modulation on SIP.	
The acute administration of NAC will reduce compulsive behavior on SIP.	
The acute administration of MEM will reduce compulsive drinking on SIP.	
The acute administration of LAM will reduce compulsive drinking behavior on SIP.	
3rd experimental set: gene expression analysis.	
Experiment 3: genetic expression analysis of serotoninergic, glutamatergic and neuroplasticity related genes in rats selected by SIP.	
HD rats will present a downregulation in the genetic expression of serotonergic genes, *Htr2a* and *Htr2c*, in the areas analyzed.	
HD rats will present an upregulation in the genetic expression of glutamatergic genes, *Grin1*, *Grin2a*, *Grin2b*, *Grin2c* and *Grm2*, in the areas analyzed.	
HD rats will present a downregulation in the genetic expression of *Bdnf* gene, in the areas analyzed.	

Table 9 (continued). Degree of support obtained for each hypothesis proposed. Green, yellow and red indicate that results confirm our hypothesis completely, partially, or they do not, respectively.

Acronyms, initialisms and abbreviations

5-CSRT. 5-Choice Serial Reaction Time Task.

AMY. Amygdala.

ANOVA. Analysis of variance.

BDNF. Brain Derived Neurotrophic Factor.

BLA. Basolateral amygdala.

cDNA. Complementary DNA.

DDT. Delay-Discounting Task.

DNA. Desoxyribonucleic acid.

EPM. Elevated Plus Maze.

FC. Frontal cortex.

FCT. Fear Conditioning Test.

FST. Forced Swimming Test.

FT. Fixed time.

GWAS. Genome-wide association study

HD. High drinkers.

HIP. Hippocampus.

LAM. Lamotrigine.

LD. Low drinkers.

MBT. Marble Burying Test.

MEM. Memantine.

mGlu2. Metabotropic glutamate receptor type II.

mPFC. Medial prefrontal cortex.

MRI. Magnetic resonance imaging.

mRNA. Messenger RNA.

MWM. Morris Water Maze.

NAC. N- Acetylcysteine.

NMDA. N-methyl-D-aspartate receptor.

NOR. Novel Object Recognition Test.

OCD. Obsessive-compulsive disorder.

OCRDs. Obsessive-Compulsive and Related Disorders.

PA. Passive Avoidance.

PRLT. Probabilistic Reversal Learning Task.

RAM. Radial Arm Maze.

RDoC. The Research Domain Criteria.

rGT. Rodent Gambling Task (rGT).

RLT. Reversal Learning Task.

RNA. Ribonucleic acid.

RT-qPCR. Quantitative reverse transcription polymerase chain reaction.

SIP. Schedule-Induced Polydipsia.

SSRIs. Selective serotonin reuptake inhibitors.

TCAs. Tricyclic antidepressants.

VDS. Variable Delay-to-Signal.

Y-BOCS. Yale-Brown Obsessive-Compulsive Scale.

List of figures

List of tables

Bibliography

Abarca, J., Gysling, K., Roth, R. H., & Bustos, G. (1995). Changes in extracellular levels of glutamate and aspartate in rat substantia nigra induced by dopamine receptor ligands: in vivo microdialysis studies. *Neurochemical Research*, *20*(2), 159–169. http://www.ncbi.nlm.nih.gov/pubmed/7783840

Abbasi Jondani, J., Yazdkhasti, F., & Abedi, A. (2023). Memory confidence and memory accuracy deterioration following repeated checking: A systematic review and meta-analysis. *Journal of Behavior Therapy and Experimental Psychiatry*, *81*, 101855. https://doi.org/10.1016/J.JBTEP.2023.101855

Abreu, A. C., Mora, S., Tristán, A. I., Martín-González, E., Prados-Pardo, Á., Moreno, M., & Fernández, I. (2022). NMR-based Metabolomics and Fatty Acid Profiles to Unravel Biomarkers in Preclinical Animal Models of Compulsive Behavior. *Journal of Proteome Research*, *21*(3), 612–622. https://doi.org/10.1021/ACS.JPROTEOME.1C00857

Afshar, H., Roohafza, H., Mohammad-Beigi, H., Haghighi, M., Jahangard, L., Shokouh, P., Sadeghi, M., & Hafezian, H. (2012). N-acetylcysteine add-on treatment in refractory obsessive-compulsive disorder: a randomized, double-blind, placebo-controlled trial. *Journal of Clinical Psychopharmacology*, *32*(6), 797–803. https://doi.org/10.1097/JCP.0b013e318272677d

Agarwal, V., Yaduvanshi, R., Arya, A., Gupta, P. K., & Sitholey, P. (2016). A study of phenomenology, psychiatric co-morbidities, social and adaptive functioning in children and adolescents with OCD. *Asian Journal of Psychiatry*, *22*, 69–73. https://doi.org/10.1016/j.ajp.2016.04.005

Agnoli L., Mainolfi P., Invernizzi R. W., Carli M. (2013). Dopamine D1-like and D2-like receptors in the dorsal striatum control different aspects of attentional performance in the five-choice serial reaction time task under a condition of increased activity of

corticostriatal inputs. Neuropsychopharmacology 38, 701–714. 10.1038/npp.2012.236

Alaux-Cantin, S., Buttolo, R., Houchi, H., Jeanblanc, J., & Naassila, M. (2015). Memantine reduces alcohol drinking but not relapse in alcohol-dependent rats. *Addiction Biology*, *20*(5), 890–901. https://doi.org/10.1111/adb.12177

Albelda, N., Bar-On, N., & Joel, D. (2010). The role of NMDA receptors in the signal attenuation rat model of obsessive–compulsive disorder. *Psychopharmacology*, *210*(1), 13–24. https://doi.org/10.1007/s00213-010-1808-9

Alén, F., Orio, L., Gorriti, M. Á., de Heras, R. G., Ramírez-López, M. T., Pozo, M. Á., & de Fonseca, F. R. (2013). Increased alcohol consumption in rats after subchronic antidepressant treatment. *The International Journal of Neuropsychopharmacology*, *16*(08), 1809–1818. https://doi.org/10.1017/S1461145713000217

Alén, F., Serrano, A., Gorriti, M. Á., Pavón, F. J., Orio, L., de Heras, R. G., Ramírez-López, M. T., Antón, M., Pozo, M. Á., & Rodríguez de Fonseca, F. (2014). The administration of atomoxetine during alcohol deprivation induces a time-limited increase in alcohol consumption after relapse. *The International Journal of Neuropsychopharmacology*, *17*(11), 1905–1910. https://doi.org/10.1017/S146114571400087X

Alonso, P., Gratacós, M., Segalàs, C., Escaramís, G., Real, E., Bayés, M., Labad, J., López-Solà, C., Estivill, X., & Menchón, J. M. (2012). Association between the NMDA glutamate receptor GRIN2B gene and obsessive-compulsive disorder. *Journal of Psychiatry & Neuroscience : JPN*, *37*(4), 273–281. https://doi.org/10.1503/jpn.110109

Alsiö, J., Nilsson, S. R. O., Gastambide, F., Wang, R. A. H., Dam, S. A., Mar, A. C., Tricklebank, M., & Robbins, T. W. (2015). The role of 5-HT2C receptors in touchscreen visual reversal learning in the rat: a cross-site study. *Psychopharmacology*, *232*(21–22), 4017–4031. https://doi.org/10.1007/S00213-015-3963-5

American Psychiatric Association. (2013a). *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Association. https://doi.org/10.1176/appi.books.9780890425596

American Psychiatric Association. (2013b). *Guía de consulta de los criterios diagnósticos del DSM-5®*. American Psychiatric Publishing. https://doi.org/10.1176/appi.books.9780890425657

Amodeo, D. A., Yi, J., Sweeney, J. A., Ragozzino, M. E., Vijayaraghavan, S., Powell, S., & Lewis, M. H. (2014). *Oxotremorine treatment reduces repetitive behaviors in BTBR T+ tf/J mice*. https://doi.org/10.3389/fnsyn.2014.00017

Andersen, S. L., Greene-Colozzi, E. A., & Sonntag, K. C. (2010). A novel, multiple symptom model of obsessive-compulsive-like behaviors in animals. *Biological Psychiatry*, *68*(8), 741–747. https://doi.org/10.1016/J.BIOPSYCH.2010.05.011

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. *Journal of Visualized Experiments : JoVE*, *82*, 50978. https://doi.org/10.3791/50978

Arnold, P. D., Rosenberg, D. R., Mundo, E., Tharmalingam, S., Kennedy, J. L., & Richter, M. A. (2004). Association of a glutamate (NMDA) subunit receptor gene (GRIN2B) with obsessive-compulsive disorder: A preliminary study. *Psychopharmacology*, *174*(4), 530–538. https://doi.org/10.1007/s00213-004-1847-1

Baer, L., Trivedi, M. H., Huz, I., Rush, A. J., Wisniewski, S. R., & Fava, M. (2015). Prevalence and Impact of Obsessive-Compulsive Symptoms in Depression. *The Journal of Clinical Psychiatry*, *76*(12), 1668–1674. https://doi.org/10.4088/JCP.14m09670

Baker, D. A., McFarland, K., Lake, R. W., Shen, H., Tang, X.-C., Toda, S., & Kalivas, P. W. (2003). Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nature Neuroscience*, *6*(7), 743–749. https://doi.org/10.1038/nn1069

Baker, D. A., McFarland, K., Lake, R. W., Shen, H., Toda, S., & Kalivas, P. W. (2003). N-acetyl cysteine-induced blockade of cocaine-induced reinstatement. *Annals of the New York Academy of Sciences*, *1003*, 349–351. http://www.ncbi.nlm.nih.gov/pubmed/14684458

Baribeau, D. A., Dupuis, A., Paton, T. A., Hammill, C., Scherer, S. W., Schachar, R. J., Arnold, P. D., Szatmari, P., Nicolson, R., Georgiades, S., Crosbie, J., Brian, J., Iaboni, A., Kushki, A., Lerch, J. P., & Anagnostou, E. (2019). Structural neuroimaging correlates of social deficits are similar in autism spectrum disorder and attention-deficit/hyperactivity disorder: analysis from the POND Network. *Translational Psychiatry 2019 9:1*, *9*(1), 1–14. https://doi.org/10.1038/s41398-019-0382-0

Barlow, R. L., Alsiö, J., Jupp, B., Rabinovich, R., Shrestha, S., Roberts, A. C., Robbins, T. W., & Dalley, J. W. (2015). Markers of serotonergic function in the orbitofrontal cortex and dorsal raphé nucleus predict individual variation in spatial-discrimination serial reversal learning. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *40*(7), 1619–1630. https://doi.org/10.1038/NPP.2014.335

Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition*, *50*(1–3), 7–15. https://doi.org/10.1016/0010-0277(94)90018-3

Beheshti, M., Rabiei, N., Taghizadieh, M., Eskandari, P., Mollazadeh, S., Dadgostar, E., Hamblin, M. R., Salmaninejad, A., Emadi, R., Mohammadi, A. H., & Mirazei, H. (2023). Correlations between single nucleotide polymorphisms in obsessive-compulsive disorder with the clinical features or response to therapy. *Journal of Psychiatric Research*, *157*, 223–238. https://doi.org/10.1016/J.JPSYCHIRES.2022.11.025

Bejerot, S., Schlette, P., Ekselius, L., Adolfsson, R., & Von Knorring, L. (1998). Personality disorders and relationship to personality dimensions measured by the Temperament and Character Inventory in patients with obsessive-compulsive disorder. *Acta Psychiatrica Scandinavica*, *98*(3), 243–249. https://doi.org/10.1111/J.1600-0447.1998.TB10075.X

Balleine BW, Dickinson A. Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology. 1998 Apr-May;37(4-5):407-19. doi: 10.1016/s0028-3908(98)00033-1

Benatti, B., Dell'Osso, B., Arici, C., Hollander, E., & Altamura, A. C. (2013). Characterizing impulsivity profile in patients with obsessive–compulsive disorder.

Http://Dx.Doi.Org/10.3109/13651501.2013.855792, *18*(3), 156–160. https://doi.org/10.3109/13651501.2013.855792

Benzina, N., Mallet, L., Burguière, E., N'Diaye, K., & Pelissolo, A. (2016). Cognitive Dysfunction in Obsessive-Compulsive Disorder. *Current Psychiatry Reports*, *18*(9), 80. https://doi.org/10.1007/s11920-016-0720-3

Berardi, A., Trezza, V., & Campolongo, P. (2012). Modeling specific phobias and post-traumatic stress disorder in rodents: the challenge to convey both cognitive and emotional features. *Reviews in the Neurosciences*, *23*(5–6). https://doi.org/10.1515/revneuro-2012-0054

Boddington, R., Gómez Dunlop, C. A., Garnham, L. C., Ryding, S., Abbey-Lee, R. N., Kreshchenko, A., & Løvlie, H. (2020). The relationship between monoaminergic gene expression, learning, and optimism in red junglefowl chicks. *Animal Cognition*, *23*(5), 901–911. https://doi.org/10.1007/S10071-020-01394-Z

Boisseau, C. L., Thompson-Brenner, H., Caldwell-Harris, C., Pratt, E., Farchione, T., & Harrison Barlow, D. (2012). Behavioral and cognitive impulsivity in obsessive–compulsive disorder and eating disorders. *Psychiatry Research*, *200*(2–3), 1062–1066. https://doi.org/10.1016/J.PSYCHRES.2012.06.010

Boulougouris, V., Chamberlain, S. R., & Robbins, T. W. (2009). Cross-species models of OCD spectrum disorders. *Psychiatry Research*, *170*(1), 15–21. https://doi.org/10.1016/j.psychres.2008.07.016

Bozorgmehr, A., Ghadirivasfi, M., & Shahsavand Ananloo, E. (2017). Obsessive-compulsive disorder, which genes? Which functions? Which pathways? An integrated holistic view regarding OCD and its complex genetic etiology. *Journal of Neurogenetics*, *31*(3), 153–160. https://doi.org/10.1080/01677063.2017.1336236

Brett, L. P., & Levine, S. (1979). Schedule-induced polydipsia suppresses pituitary-adrenal activity in rats. *Journal of Comparative and Physiological Psychology*, *93*(5), 946–956. https://doi.org/10.1037/H0077619

Brock, H., & Hany, M. (2023). Obsessive-Compulsive Disorder. *StatPearls*. https://pubmed.ncbi.nlm.nih.gov/31985955/

Bruno, A., Micò, U., Pandolfo, G., Mallamace, D., Abenavoli, E., Di Nardo, F., D'Arrigo, C., Spina, E., Zoccali, R. A., & Muscatello, M. R. A. (2012). Lamotrigine augmentation of serotonin reuptake inhibitors in treatment-resistant obsessive–compulsive disorder: a double-blind, placebo-controlled study. *Journal of Psychopharmacology*, *26*(11), 1456–1462. https://doi.org/10.1177/0269881111431751

Canals, J., Hernández-Martínez, C., Cosi, S., & Voltas, N. (2012). The epidemiology of obsessive–compulsive disorder in Spanish school children. *Journal of Anxiety Disorders*, *26*(7), 746–752. https://doi.org/10.1016/J.JANXDIS.2012.06.003

Cardona, D., López-Crespo, G., Sánchez-Amate, M. C., Flores, P., & Sánchez-Santed, F. (2011). Impulsivity as Long-Term Sequelae After Chlorpyrifos Intoxication: Time Course and Individual Differences. *Neurotoxicity Research*, *19*(1), 128–137. https://doi.org/10.1007/s12640-009-9149-3

Cardona, D., López-Grancha, M., López-Crespo, G., Nieto-Escamez, F., Sánchez-Santed, F., & Flores, P. (2006). Vulnerability of long-term neurotoxicity of chlorpyrifos: effect on schedule-induced polydipsia and a delay discounting task. *Psychopharmacology*, *189*(1), 47–57. https://doi.org/10.1007/s00213-006-0547-4

Carli M., Invernizzi R. W. (2014). Serotoninergic and dopaminergic modulation of cortico-striatal circuit in executive and attention deficits induced by NMDA receptor hypofunction in the 5-choice serial reaction time task. Front. Neural Circuits 8:58. 10.3389/fncir.2014.00058

Castensson, A., Åberg, K., McCarthy, S., Saetre, P., Andersson, B., & Jazin, E. (2005). Serotonin receptor 2C (HTR2C) and schizophrenia: Examination of possible medication and genetic influences on expression levels. *American Journal of Medical Genetics - Neuropsychiatric Genetics*, *134 B*(1), 84–89. https://doi.org/10.1002/ajmg.b.30151

Castensson, A., Emilsson, L., Sundberg, R., & Jazin, E. (2003). Decrease of serotonin receptor 2C in schizophrenia brains identified by high-resolution mRNA expression analysis. *Biological Psychiatry*, *54*(11), 1212–1221. https://doi.org/10.1016/S0006-3223(03)00526-2

Cavedini, P., Riboldi, G., D'annucci, A., Belotti, P., Cisima, M., & Bellodi, L. (2002). Decision-making heterogeneity in obsessive-compulsive disorder: Ventromedial prefrontal cortex function predicts different treatment outcomes. *Neuropsychologia*, *40*(2), 205–211. https://doi.org/10.1016/S0028-3932(01)00077-X

Cavedini, P., Zorzi, C., Baraldi, C., Patrini, S., Salomoni, G., Bellodi, L., Freire, R. C., & Perna, G. (2012). The somatic marker affecting decisional processes in obsessive-compulsive disorder. *Cognitive Neuropsychiatry*, *17*(2), 177–190. https://doi.org/10.1080/13546805.2011.614152

Chamberlain, S. R., Fineberg, N. A., Blackwell, A. D., Robbins, T. W., & Sahakian, B. J. (2006). Motor Inhibition and Cognitive Flexibility in Obsessive-Compulsive Disorder and Trichotillomania. *American Journal of Psychiatry*, *163*(7), 1282–1284. https://doi.org/10.1176/ajp.2006.163.7.1282

Chamberlain, S. R., Solly, J. E., Hook, R. W., Vaghi, M. M., & Robbins, T. W. (2021). Cognitive Inflexibility in OCD and Related Disorders. *Current Topics in Behavioral Neurosciences*, *49*, 125–145. https://doi.org/10.1007/7854_2020_198/FIGURES/6

Charntikov, S., Pittenger, S. T., Pudiak, C. M., & Bevins, R. A. (2018). The effect of N - acetylcysteine or bupropion on methamphetamine self-administration and methamphetamine–triggered reinstatement of female rats. *Neuropharmacology*, *135*, 487–495. https://doi.org/10.1016/j.neuropharm.2018.03.021

Chen, J. Y., Campos, C. A., Jarvie, B. C., & Palmiter, R. D. (2018). Parabrachial CGRP Neurons Establish and Sustain Aversive Taste Memories. *Neuron*, *100*(4), 891-899.e5. https://doi.org/10.1016/j.neuron.2018.09.032

Cheung, H., Kamp, D., & Harris, E. (1992). An in vitro investigation of the action of lamotrigine on neuronal voltage-activated sodium channels. *Epilepsy Research*, *13*(2), 107–112. http://www.ncbi.nlm.nih.gov/pubmed/1334455

Chou-Green, J. M., Holscher, T. D., Dallman, M. F., & Akana, S. F. (2003). Compulsive behavior in the 5-HT2C receptor knockout mouse. *Physiology and Behavior*, *78*(4–5), 641–649. https://doi.org/10.1016/S0031-9384(03)00047-7

Cohen, S. J., & Stackman, R. W. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behavioural Brain Research*, *285*, 105–117. https://doi.org/10.1016/J.BBR.2014.08.002

Cornea-Hébert, V., Riad, M., Wu, C., Singh, S. K., & Descarries, L. (1999). Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. *Journal of Comparative Neurology*, *409*(2), 187–209. https://doi.org/10.1002/(SICI)1096-9861(19990628)409:2<187::AID-CNE2>3.0.CO;2-P

Cunningham, M. O., & Jones, R. S. G. (2000). The anticonvulsant, lamotrigine decreases spontaneous glutamate release but increases spontaneous GABA release in the rat entorhinal cortex in vitro. *Neuropharmacology*, *39*(11), 2139–2146. https://doi.org/10.1016/S0028-3908(00)00051-4

Cunningham-Williams, R. M., & Cottler, L. B. (2001). The epidemiology of pathological gambling. *Seminars in Clinical Neuropsychiatry*, *6*(3), 155–166. http://www.ncbi.nlm.nih.gov/pubmed/11447567

Curzon, P., Rustay, N. R., & Browman, K. E. (2009). Cued and Contextual Fear Conditioning for Rodents. In *Methods of Behavior Analysis in Neuroscience*. CRC Press/Taylor & Francis. http://www.ncbi.nlm.nih.gov/pubmed/21204331

Danysz, W., & Parsons, C. G. (1998). Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. *Pharmacological Reviews*, *50*(4), 597–664. http://www.ncbi.nlm.nih.gov/pubmed/9860805

da Rocha, F. F., Alvarenga, N. B., Malloy-Diniz, L., & Corrêa, H. (2011). Decision-making impairment in obsessive-compulsive disorder as measured by the Iowa Gambling Task. *Arquivos de Neuro-Psiquiatria*, *69*(4), 642–647. https://doi.org/10.1590/S0004-282X2011000500013

Daw ND, Gershman SJ, Seymour B, Dayan P, Dolan RJ. Model-based influences on humans' choices and striatal prediction errors. Neuron. 2011 Mar 24;69(6):1204-15. doi: 10.1016/j.neuron.2011.02.027

de Brouwer, G., & Wolmarans, D. W. (2018). Back to basics: A methodological perspective on marble-burying behavior as a screening test for psychiatric illness. *Behavioural Processes*, *157*, 590–600. https://doi.org/10.1016/J.BEPROC.2018.04.011

de Bruin, J. P. C., Sànchez-Santed, F., Heinsbroek, R. P. W., Donker, A., & Postmes, P. (1994). A behavioural analysis of rats with damage to the medial prefrontal cortex using the morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation. *Brain Research*, *652*(2), 323–333. https://doi.org/10.1016/0006-8993(94)90243-7

de Leon, J., Verghese, C., Tracy, J. I., Josiassen, R. C., & Simpson, G. M. (1994). Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. *Biological Psychiatry*, *35*(6), 408–419. http://www.ncbi.nlm.nih.gov/pubmed/8018788

Delille, H. K., Mezler, M., & Marek, G. J. (2013). The two faces of the pharmacological interaction of mGlu2 and 5-HT2A – Relevance of receptor heterocomplexes and interaction through functional brain pathways. *Neuropharmacology*, *70*, 296–305. https://doi.org/10.1016/J.NEUROPHARM.2013.02.005

Den Ouden, L., Suo, C., Albertella, L., Greenwood, L. M., Lee, R. S. C., Fontenelle, L. F., Parkes, L., Tiego, J., Chamberlain, S. R., Richardson, K., Segrave, R., & Yücel, M. (2022). Transdiagnostic phenotypes of compulsive behavior and associations with psychological, cognitive, and neurobiological affective processing. *Translational Psychiatry*, *12*(1). https://doi.org/10.1038/S41398-021-01773-1

Denys, D. (2011). Obsessionality & compulsivity: A phenomenology of obsessive-compulsive disorder. *Philosophy, Ethics, and Humanities in Medicine*, *6*(1), 1–7. https://doi.org/10.1186/1747-5341-6-3/METRICS

Didriksen, M., Olsen, G. M., & Christensen, A. V. (1993). Effect of clozapine upon schedule-induced polydipsia (SIP) resembles neither the actions of dopamine D1 nor D2 blockade. *Psychopharmacology*, *113*(2), 250–256. http://www.ncbi.nlm.nih.gov/pubmed/7855190

Ding, Z. M., Ingraham, C. M., Hauser, S. R., Lasek, A. W., Bell, R. L., & McBride, W. J. (2017). Reduced Levels of mGlu2 Receptors within the Prelimbic Cortex Are Not

Associated with Elevated Glutamate Transmission or High Alcohol Drinking. *Alcoholism, Clinical and Experimental Research*, *41*(11), 1896–1906. https://doi.org/10.1111/ACER.13488

Dong, J., Zhou, Q., Wei, Z., Yan, S., Sun, F., & Cai, X. (2018). Protein kinase A mediates scopolamine-induced mTOR activation and an antidepressant response. *Journal of Affective Disorders*, *227*, 633–642. https://doi.org/10.1016/j.jad.2017.11.041

Du Jardin, K. G., Müller, H. K., Sanchez, C., Wegener, G., & Elfving, B. (2017). Gene expression related to serotonergic and glutamatergic neurotransmission is altered in the flinders sensitive line rat model of depression: Effect of ketamine. *Synapse (New York, N.Y.)*, *71*(1), 37–45. https://doi.org/10.1002/SYN.21940

Dwyer, J. M., Platt, B. J., Sukoff Rizzo, S. J., Pulicicchio, C. M., Wantuch, C., Zhang, M.-Y., Cummons, T., Leventhal, L., Bender, C. N., Zhang, J., Kowal, D., Lu, S., Rajarao, S. J. R., Smith, D. L., Shilling, A. D., Wang, J., Butera, J., Resnick, L., Rosenzweig-Lipson, S., … Beyer, C. E. (2010). Preclinical characterization of BRL 44408: antidepressant- and analgesic-like activity through selective α2A-adrenoceptor antagonism. *The International Journal of Neuropsychopharmacology*, *13*(09), 1193–1205. https://doi.org/10.1017/S1461145709991088

Egashira, N., Okuno, R., Harada, S., Matsushita, M., Mishima, K., Iwasaki, K., Nishimura, R., Oishi, R., & Fujiwara, M. (2008). Effects of glutamate-related drugs on marble-burying behavior in mice: Implications for obsessive–compulsive disorder. *European Journal of Pharmacology*, *586*(1–3), 164–170. https://doi.org/10.1016/j.ejphar.2008.01.035

Elfving, B., Müller, H. K., Oliveras, I., Østerbøg, T. B., Rio-Alamos, C., Sanchez-Gonzalez, A., Tobeña, A., Fernandez-Teruel, A., & Aznar, S. (2019). Differential expression of synaptic markers regulated during neurodevelopment in a rat model of schizophrenia-like behavior. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *95*. https://doi.org/10.1016/J.PNPBP.2019.109669

Elfving, B., Plougmann, P. H., Müller, H. K., Mathé, A. A., Rosenberg, R., & Wegener, G. (2010). Inverse correlation of brain and blood BDNF levels in a genetic rat model

of depression. *International Journal of Neuropsychopharmacology*, *13*(5), 563–572. https://doi.org/10.1017/S1461145709990721

Escher, T., Call, S. B., Blaha, C. D., & Mittleman, G. (2006). Behavioral effects of aminoadamantane class NMDA receptor antagonists on schedule-induced alcohol and self-administration of water in mice. *Psychopharmacology*, *187*(4), 424–434. https://doi.org/10.1007/s00213-006-0465-5

Escobar, A. P., Cornejo, F. A., Olivares-Costa, M., González, M., Fuentealba, J. A., Gysling, K., España, R. A., & Andrés, M. E. (2015). Reduced dopamine and glutamate neurotransmission in the nucleus accumbens of quinpirole-sensitized rats hints at inhibitory D2 autoreceptor function. *Journal of Neurochemistry*, *134*(6), 1081–1090. https://doi.org/10.1111/jnc.13209

Exner, C., Kohl, A., Zaudig, M., Langs, G., Lincoln, T. M., & Rief, W. (2009). Metacognition and episodic memory in obsessive-compulsive disorder. *Journal of Anxiety Disorders*, *23*(5), 624–631. https://doi.org/10.1016/j.janxdis.2009.01.010

Falk, J. (1961). Production of polydipsia in normal rats by an intermittent food schedule. *Science (New York, N.Y.)*, *133*(3447), 195–196. http://www.ncbi.nlm.nih.gov/pubmed/13698026

Falk, J. L. (1971). The nature and determinants of adjunctive behavior. *Physiology & Behavior*, *6*(5), 577–588. http://www.ncbi.nlm.nih.gov/pubmed/5004684

Favalli, G., Li, J., Belmonte-de-Abreu, P., Wong, A. H. C., & Daskalakis, Z. J. (2012). The role of BDNF in the pathophysiology and treatment of schizophrenia. *Journal of Psychiatric Research*, *46*(1), 1–11. https://doi.org/10.1016/J.JPSYCHIRES.2011.09.022

Ferreira, G. M., Yücel, M., Dawson, A., Lorenzetti, V., & Fontenelle, L. F. (2017). Investigating the role of anticipatory reward and habit strength in obsessive-compulsive disorder. *CNS Spectrums*, *22*(3), 295–304. https://doi.org/10.1017/S1092852916000535

Figee, M., Vink, M., De Geus, F., Vulink, N., Veltman, D. J., Westenberg, H., & Denys, D. (2011). Dysfunctional reward circuitry in obsessive-compulsive disorder. *Biological Psychiatry*, *69*(9), 867–874. https://doi.org/10.1016/J.BIOPSYCH.2010.12.003

Fineberg, N. A., Apergis-Schoute, A. M., Vaghi, M. M., Banca, P., Gillan, C. M., Voon, V., Chamberlain, S. R., Cinosi, E., Reid, J., Shahper, S., Bullmore, E. T., Sahakian, B. J., & Robbins, T. W. (2018). Mapping Compulsivity in the DSM-5 Obsessive Compulsive and Related Disorders: Cognitive Domains, Neural Circuitry, and Treatment. *The International Journal of Neuropsychopharmacology*, *21*(1), 42–58. https://doi.org/10.1093/ijnp/pyx088

Fineberg, N. A., Brown, A., Reghunandanan, S., & Pampaloni, I. (n.d.). *Evidence-based pharmacotherapy of obsessive-compulsive disorder*. https://doi.org/10.1017/S1461145711001829

Fineberg, N. A., Chamberlain, S. R., Goudriaan, A. E., Stein, D. J., Vanderschuren, L. J. M. J., Gillan, C. M., Shekar, S., Gorwood, P. A. P. M., Voon, V., Morein-Zamir, S., Denys, D., Sahakian, B. J., Moeller, F. G., Robbins, T. W., & Potenza, M. N. (2014). New developments in human neurocognition: clinical, genetic, and brain imaging correlates of impulsivity and compulsivity. *CNS Spectrums*, *19*(01), 69–89. https://doi.org/10.1017/S1092852913000801

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

Fineberg, N. A., Hollander, E., Pallanti, S., Walitza, S., Grünblatt, E., Dell'Osso, B. M., Albert, U., Geller, D. A., Brakoulias, V., Janardhan Reddy, Y. C., Arumugham, S. S., Shavitt, R. G., Drummond, L., Grancini, B., De Carlo, V., Cinosi, E., Chamberlain, S. R., Ioannidis, K., Rodriguez, C. I., … Menchon, J. M. (2020). Clinical advances in obsessive-compulsive disorder: a position statement by the International College of Obsessive-Compulsive Spectrum Disorders. *International Clinical Psychopharmacology*, *35*(4), 173–193. https://doi.org/10.1097/YIC.0000000000000314

Fineberg, N. A., Potenza, M. N., Chamberlain, S. R., Berlin, H. A., Menzies, L., Bechara, A., Sahakian, B. J., Robbins, T. W., Bullmore, E. T., & Hollander, E. (2010). Probing compulsive and impulsive behaviors, from animal models to endophenotypes: A narrative review. *Neuropsychopharmacology*, *35*(3), 591–604. https://doi.org/10.1038/npp.2009.185

Fink, L. H., Anastasio, N. C., Fox, R. G., Rice, K. C., Moeller, F. G., & Cunningham, K. A. (2015). Individual Differences in Impulsive Action Reflect Variation in the Cortical Serotonin 5-HT2A Receptor System. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *40*(8), 1957–1968. https://doi.org/10.1038/NPP.2015.46

Flores, Á., Fullana, M. À., Soriano-Mas, C., & Andero, R. (2018). Lost in translation: how to upgrade fear memory research. *Molecular Psychiatry*, *23*(11), 2122–2132. https://doi.org/10.1038/s41380-017-0006-0

Flores, P., Sánchez-Kuhn, A., Merchán, A., Vilches, O., García-Martín, S., Moreno, M., Flores, P., Sánchez-Kuhn, A., Merchán, A., Vilches, O., García-Martín, S., & Moreno, M. (2014). Schedule-Induced Polydipsia: Searching for the Endophenotype of Compulsive Behavior. *World Journal of Neuroscience*, *4*(3), 253–260. https://doi.org/10.4236/WJNS.2014.43029

Fole, A., Miguéns, M., Morales, L., González-Martín, C., Ambrosio, E., & Del Olmo, N. (2017). Lewis and Fischer 344 rats as a model for genetic differences in spatial learning and memory: Cocaine effects. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *76*, 49–57. https://doi.org/10.1016/J.PNPBP.2017.02.024

Fomsgaard, L., Moreno, J. L., de la Fuente Revenga, M., Brudek, T., Adamsen, D., Rio-Alamos, C., Saunders, J., Klein, A. B., Oliveras, I., Cañete, T., Blazquez, G., Tobeña, A., Fernandez-Teruel, A., Gonzalez-Maeso, J., & Aznar, S. (2018). Differences in 5-HT2A and mGlu2 Receptor Expression Levels and Repressive Epigenetic Modifications at the 5-HT2A Promoter Region in the Roman Low- (RLA-I) and High- (RHA-I) Avoidance Rat Strains. *Molecular Neurobiology*, *55*(3), 1998–2012. https://doi.org/10.1007/s12035-017-0457-y

Fribourg, M., Moreno, J. L., Holloway, T., Provasi, D., Baki, L., Mahajan, R., Park, G., Adney, S. K., Hatcher, C., Eltit, J. M., Ruta, J. D., Albizu, L., Li, Z., Umali, A., Shim, J., Fabiato, A., MacKerell, A. D., Brezina, V., Sealfon, S. C., … Logothetis, D. E. (2011). Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. *Cell*, *147*(5), 1011–1023. https://doi.org/10.1016/j.cell.2011.09.055

Fyer, A. J., Schneier, F. R., Simpson, H. B., Choo, T. H., Tacopina, S., Kimeldorf, M. B., Steinglass, J. E., Wall, M., & Walsh, B. T. (2020). Heterogeneity in Fear Processing across and within Anxiety, Eating, and Compulsive Disorders. *Journal of Affective Disorders*, *275*, 329–338. https://doi.org/10.1016/J.JAD.2020.03.091

Gafford, G., Jasnow, A. M., & Ressler, K. J. (2014). Grin1 receptor deletion within CRF neurons enhances fear memory. *PLoS ONE*, *9*(10). https://doi.org/10.1371/journal.pone.0111009

Gardner, R., & Wilson, D. R. (2004). Sociophysiology and Evolutionary Aspects of Psychiatry. *Textbook of Biological Psychiatry*, 597–625. https://doi.org/10.1002/0471468975.CH20

Geller, D. A., McGuire, J. F., Orr, S. P., Pine, D. S., Britton, J. C., Small, B. J., Murphy, T. K., Wilhelm, S., & Storch, E. A. (2017). Fear conditioning and extinction in pediatric obsessive-compulsive disorder. *Annals of Clinical Psychiatry : Official Journal of the American Academy of Clinical Psychiatrists*, *29*(1), 17–26. http://www.ncbi.nlm.nih.gov/pubmed/28207912

Ghaleiha, A., Entezari, N., Modabbernia, A., Najand, B., Askari, N., Tabrizi, M., Ashrafi, M., Hajiaghaee, R., & Akhondzadeh, S. (2013). Memantine add-on in moderate to severe obsessive-compulsive disorder: randomized double-blind placebo-controlled study. *Journal of Psychiatric Research*, *47*(2), 175–180. https://doi.org/10.1016/j.jpsychires.2012.09.015

Gillan, C. M., Kalanthroff, E., Evans, M., Weingarden, H. M., Jacoby, R. J., Gershkovich, M., Snorrason, I., Campeas, R., Cervoni, C., Crimarco, N. C., Sokol, Y., Garnaat, S. L., McLaughlin, N. C. R., Phelps, E. A., Pinto, A., Boisseau, C. L., Wilhelm, S., Daw, N. D., & Simpson, H. B. (2020). Comparison of the Association Between

Goal-Directed Planning and Self-reported Compulsivity vs Obsessive-Compulsive Disorder Diagnosis. *JAMA Psychiatry*, *77*(1), 77–85. https://doi.org/10.1001/JAMAPSYCHIATRY.2019.2998

Gillan, C. M., & Robbins, T. W. (2014). Goal-directed learning and obsessive-compulsive disorder. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1655). https://doi.org/10.1098/rstb.2013.0475

Glodzik, L., De Santi, S., Rich, K. E., Brys, M., Pirraglia, E., Mistur, R., Switalski, R., Mosconi, L., Sadowski, M., Zetterberg, H., Blennow, K., & de Leon, M. J. (2009). Effects of Memantine on Cerebrospinal Fluid Biomarkers of Neurofibrillary Pathology. *Journal of Alzheimer's Disease*, *18*(3), 509–513. https://doi.org/10.3233/JAD-2009-1183

González-Maeso, J., Ang, R. L., Yuen, T., Chan, P., Weisstaub, N. V., López-Giménez, J. F., Zhou, M., Okawa, Y., Callado, L. F., Milligan, G., Gingrich, J. A., Filizola, M., Meana, J. J., & Sealfon, S. C. (2008). Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature*, *452*(7183), 93–97. https://doi.org/10.1038/nature06612

Gráf M. 5-HT2c receptor activation induces grooming behaviour in rats: possible correlations with obsessive-compulsive disorder. Neuropsychopharmacol Hung. 2006 Mar;8(1):23-8. PMID: 16841562.

Grant, J. E., Redden, S. A., Leppink, E. W., Odlaug, B. L., & Chamberlain, S. R. (2016). Psychosocial dysfunction associated with skin picking disorder and trichotillomania. *Psychiatry Research*, *239*, 68–71. https://doi.org/10.1016/j.psychres.2016.03.004

Grassi, G., Figee, M., Ooms, P., Righi, L., Nakamae, T., Pallanti, S., Schuurman, R., & Denys, D. (2018). Impulsivity and decision-making in obsessive-compulsive disorder after effective deep brain stimulation or treatment as usual. *CNS Spectrums*, *23*(5), 333–339. https://doi.org/10.1017/S1092852918000846

Grassi, G., Makris, N., & Pallanti, S. (2020). Addicted to compulsion: assessing three core dimensions of addiction across obsessive-compulsive disorder and gambling disorder. *CNS Spectrums*, *25*(3), 392–401. https://doi.org/10.1017/S1092852919000993

Grassi, G., Pallanti, S., Righi, L., Figee, M., Mantione, M., Denys, D., Piccagliani, D., Rossi, A., & Stratta, P. (2015). Think twice: Impulsivity and decision making in obsessive–compulsive disorder. *Journal of Behavioral Addictions*, *4*(4), 263–272. https://doi.org/10.1556/2006.4.2015.039

Haghighi, M., Jahangard, L., Mohammad-Beigi, H., Bajoghli, H., Hafezian, H., Rahimi, A., Afshar, H., Holsboer-Trachsler, E., & Brand, S. (2013). In a double-blind, randomized and placebo-controlled trial, adjuvant memantine improved symptoms in inpatients suffering from refractory obsessive-compulsive disorders (OCD). *Psychopharmacology*, *228*(4), 633–640. https://doi.org/10.1007/s00213-013-3067-z

Hajheidari, S., Miladi-Gorji, H., & Bigdeli, I. (2017). Environmental Enrichment Prevents Methamphetamine-Induced Spatial Memory Deficits and Obsessive-Compulsive Behavior in Rats. *Iranian Journal of Psychiatry*, *12*(1), 8–14. https://pubmed.ncbi.nlm.nih.gov/28496496/

Higgins, G. A., Brown, M., St John, J., MacMillan, C., Silenieks, L. B., & Thevarkunnel, S. (2020). Effects of 5-HT2C receptor modulation and the NA reuptake inhibitor atomoxetine in tests of compulsive and impulsive behaviour. *Neuropharmacology*, *170*. https://doi.org/10.1016/J.NEUROPHARM.2020.108064

Higley, J., Hasert, M., Suomi, S., & Linnoila, M. (1998). The Serotonin Reuptake Inhibitor Sertraline Reduces Excessive Alcohol Consumption in Nonhuman Primates: Effect of Stress. *Neuropsychopharmacology*, *18*(6), 431–443. https://doi.org/10.1016/S0893-133X(97)00180-2

Hirsch, S. J., Regmi, N. L., Birnbaum, S. G., & Greene, R. W. (2015). CA1-specific deletion of NMDA receptors induces abnormal renewal of a learned fear response. *Hippocampus*, *25*(11), 1374–1379. https://doi.org/10.1002/HIPO.22457

Hirschtritt, M. E., Bloch, M. H., & Mathews, C. A. (2017). Obsessive-Compulsive Disorder: Advances in Diagnosis and Treatment. *JAMA*, *317*(13), 1358–1367. https://doi.org/10.1001/JAMA.2017.2200

Hogg, S., & Dalvi, A. (2004). Acceleration of onset of action in schedule-induced polydipsia: combinations of SSRI and 5-HT1A and 5-HT1B receptor antagonists. *Pharmacology, Biochemistry, and Behavior*, *77*(1), 69–75. http://www.ncbi.nlm.nih.gov/pubmed/14724043

Hung, C. C., Chen, H. Y., & Chen, C. H. (2002). Systematic mutation analysis of the human glutamate receptor, ionotropic, N-methyl-D-aspartate 1 gene(GRIN1) in schizophrenic patients. *Psychiatric Genetics*, *12*(4), 225–230. https://doi.org/10.1097/00041444-200212000-00005

Hurlemann, R., Matusch, A., Kuhn, K. U., Berning, J., Elmenhorst, D., Winz, O., Kolsch, H., Zilles, K., Wagner, M., Maier, W., & Bauer, A. (2008). 5-HT2A receptor density is decreased in the at-risk mental state. *Psychopharmacology*, *195*(4), 579–590. https://doi.org/10.1007/s00213-007-0921-x

Hurley, M. M., Resch, J. M., Maunze, B., Frenkel, M. M., Baker, D. A., & Choi, S. (2016). N-acetylcysteine decreases binge eating in a rodent model. *International Journal of Obesity*, *40*(7), 1183–1186. https://doi.org/10.1038/ijo.2016.31

Hussain, A., Dar, M. A., Wani, R. A., Shah, M. S., Jan, M. M., Malik, Y. A., Chandel, R. K., & Margoob, M. A. (2015). Role of lamotrigine augmentation in treatment-resistant obsessive compulsive disorder: a retrospective case review from South Asia. *Indian Journal of Psychological Medicine*, *37*(2), 154–158. https://doi.org/10.4103/0253-7176.155613

Iftene, F., Bowie, C., Milev, R., Hawken, E., Talikowska-Szymczak, E., Potopsingh, D., Hanna, S., Mulroy, J., Groll, D., & Millson, R. (2013). Identification of primary polydipsia in a severe and persistent mental illness outpatient population: a prospective observational study. *Psychiatry Research*, *210*(3), 679–683. https://doi.org/10.1016/j.psychres.2013.04.011

Javitt, D. C., Schoepp, D., Kalivas, P. W., Volkow, N. D., Zarate, C., Merchant, K., Bear, M. F., Umbricht, D., Hajos, M., Potter, W. Z., & Lee, C.-M. (2011). Translating Glutamate: From Pathophysiology to Treatment. *Sci Transl Med*, *3*(102). https://doi.org/10.1126/scitranslmed.3002804

Joel, D., & Avisar, A. (2001). Excessive lever pressing following post-training signal attenuation in rats: a possible animal model of obsessive compulsive disorder? *Behavioural Brain Research*, *123*(1), 77–87. http://www.ncbi.nlm.nih.gov/pubmed/11377731

Jung WH, Kang DH, Han JY, Jang JH, Gu BM, Choi JS, Jung MH, Choi CH, Kwon JS. (2011). Aberrant ventral striatal responses during incentive processing in unmedicated patients with obsessive-compulsive disorder. Acta Psychiatr Scand. 2011 May;123(5):376-86. doi: 10.1111/j.1600-0447.2010.01659.x.

Katerberg, H., Cath, D. C., Denys, D. A. J. P., Heutink, P., Polman, A., Van Nieuwerburgh, F. C. W., Deforce, D. L. D., Bochdanovits, Z., Van Balkom, A. J. L. M., & Den Boer, J. A. (2010). The role of the COMT Val(158)Met polymorphism in the phenotypic expression of obsessive-compulsive disorder. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *153B*(1), 167–176. https://doi.org/10.1002/AJMG.B.30971

Katerberg, H., Lochner, C., Cath, D. C., De Jonge, P., Bochdanovits, Z., Moolman-Smook, J. C., Hemmings, S. M. J., Carey, P. D., Stein, D. J., Sondervan, D., Den Boer, J. A., Van Balkom, A. J. L. M., Polman, A., & Heutink, P. (2009). The role of the brain-derived neurotrophic factor (BDNF) val66met variant in the phenotypic expression of obsessive-compulsive disorder (OCD). *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics : The Official Publication of the International Society of Psychiatric Genetics*, *150B*(8), 1050–1062. https://doi.org/10.1002/AJMG.B.30930

Kew, J. N. C., Koester, A., Moreau, J. L., Jenck, F., Ouagazzal, A. M., Mutel, V., Richards, J. G., Trube, G., Fischer, G., Montkowski, A., Hundt, W., Reinscheid, R. K., Pauly-Evers, M., Kemp, J. A., & Bluethmann, H. (2000). Functional consequences of reduction in NMDA receptor glycine affinity in mice carrying targeted point mutations in the glycine binding site. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(11), 4037–4049. https://doi.org/10.1523/JNEUROSCI.20-11-04037.2000

Khalkhali, M., Aram, S., Zarrabi, H., Kafie, M., & Heidarzadeh, A. (2016). Lamotrigine Augmentation Versus Placebo in Serotonin Reuptake Inhibitors-Resistant Obsessive-Compulsive Disorder: A Randomized Controlled Trial. *Iranian Journal of Psychiatry*, *11*(2), 104–114. http://www.ncbi.nlm.nih.gov/pubmed/27437007

Klein, A. B., Ultved, L., Adamsen, D., Santini, M. A., Tobeña, A., Fernandez-Teruel, A., Flores, P., Moreno, M., Cardona, D., Knudsen, G. M., Aznar, S., & Mikkelsen, J. D. (2014). 5-HT2A and mGlu2 receptor binding levels are related to differences in impulsive behavior in the Roman Low- (RLA) and High- (RHA) avoidance rat strains. *Neuroscience*, *263*, 36–45. https://doi.org/10.1016/j.neuroscience.2013.12.063

Kodaira, M., Iwadare, Y., Ushijima, H., Oiji, A., Kato, M., Sugiyama, N., Sasayama, D., Usami, M., Watanabe, K., & Saito, K. (2012). Poor performance on the Iowa gambling task in children with obsessive-compulsive disorder. *Annals of General Psychiatry*, *11*, 25. https://doi.org/10.1186/1744-859X-11-25

Kowiański, P., Lietzau, G., Czuba, E., Waśkow, M., Steliga, A., & Moryś, J. (2018). BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. *Cellular and Molecular Neurobiology*, *38*(3), 579–593. https://doi.org/10.1007/s10571-017-0510-4

Krügel, U., Schraft, T., Regenthal, R., Illes, P., & Kittner, H. (2004). Purinergic modulation of extracellular glutamate levels in the nucleus accumbens in vivo. *International Journal of Developmental Neuroscience*, *22*(7), 565–570. https://doi.org/10.1016/j.ijdevneu.2004.07.009

Lafleur, D. L., Pittenger, C., Kelmendi, B., Gardner, T., Wasylink, S., Malison, R. T., Sanacora, G., Krystal, J. H., & Coric, V. (2006). N-acetylcysteine augmentation in serotonin reuptake inhibitor refractory obsessive-compulsive disorder. *Psychopharmacology*, *184*(2), 254–256. https://doi.org/10.1007/s00213-005-0246-6

Lebourgeois, S., González-Marín, M. C., Jeanblanc, J., Naassila, M., & Vilpoux, C. (2017). Effect of N-acetylcysteine on motivation, seeking and relapse to ethanol self-administration. *Addiction Biology*, *23*(2), 643–652. https://doi.org/10.1111/adb.12521

Liao, W., Liu, Y., Wang, L., Cai, X., Xie, H., Yi, F., Huang, R., Fang, C., Xie, P., & Zhou, J. (2021). Chronic mild stress-induced protein dysregulations correlated with susceptibility and resiliency to depression or anxiety revealed by quantitative proteomics of the rat prefrontal cortex. *Translational Psychiatry*, *11*(1). https://doi.org/10.1038/S41398-021-01267-0

Li, N., He, X., Qi, X., Zhang, Y., & He, S. (2010). The mood stabilizer lamotrigine produces antidepressant behavioral effects in rats: role of brain-derived neurotrophic factor. *Journal of Psychopharmacology*, *24*(12), 1772–1778. https://doi.org/10.1177/0269881109359102

Liu, Y. P., Ding, M., Zhang, X. C., Liu, Y., Xuan, J. F., Xing, J. X., Xia, X., Yao, J., & Wang, B. J. (2019). Association between polymorphisms in the GRIN1 gene 5′ regulatory region and schizophrenia in a northern Han Chinese population and haplotype effects on protein expression in vitro. *BMC Medical Genetics*, *20*(1). https://doi.org/10.1186/s12881-019-0757-3

López-Figueroa, A. L., Norton, C. S., López-Figueroa, M. O., Armellini-Dodel, D., Burke, S., Akil, H., López, J. F., & Watson, S. J. (2004). Serotonin 5-HT1A, 5-HT1B, and 5-HT2A receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. *Biological Psychiatry*, *55*(3), 225–233. https://doi.org/10.1016/j.biopsych.2003.09.017

López-Grancha, M., Lopez-Crespo, G., Sanchez-Amate, M. C., & Flores, P. (2008). Individual differences in schedule-induced polydipsia and the role of gabaergic and dopaminergic systems. *Psychopharmacology*, *197*(3), 487–498. https://doi.org/10.1007/s00213-007-1059-6

Lu, C.-W., Lin, T.-Y., & Wang, S.-J. (2010). Memantine depresses glutamate release through inhibition of voltage-dependent Ca2+ entry and protein kinase C in rat cerebral cortex nerve terminals: An NMDA receptor-independent mechanism. *Neurochemistry International*, *57*(2), 168–176. https://doi.org/10.1016/j.neuint.2010.05.010

Mahjani B, Bey K, Boberg J, Burton C. (2021) Genetics of obsessive-compulsive disorder. *Psychol Med*., 51(13):2247-2259. doi: 10.1017/S0033291721001744

Maina, G., Rosso, G., Zanardini, R., Bogetto, F., Gennarelli, M., & Bocchio-Chiavetto, L. (2010). Serum levels of brain-derived neurotrophic factor in drug-naïve obsessive-compulsive patients: A case-control study. *Journal of Affective Disorders*, *122*(1–2), 174–178. https://doi.org/10.1016/j.jad.2009.07.009

Malkova, N. V., Gallagher, J. J., Yu, C. Z., Jacobs, R. E., & Patterson, P. H. (2014). Manganese-enhanced magnetic resonance imaging reveals increased DOI-induced brain activity in a mouse model of schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(24). https://doi.org/10.1073/PNAS.1323287111

Marinova, Z., Chuang, D.-M., & Fineberg, N. (2017a). Glutamate-Modulating Drugs as a Potential Therapeutic Strategy in Obsessive-Compulsive Disorder. *Current Neuropharmacology*, *15*(7), 977–995. https://doi.org/10.2174/1570159X15666170320104237

Martijena, I. D., Bustos, S. G., Bertotto, M. E., & Molina, V. A. (2005). Antidepressants attenuate both the enhanced ethanol intake and ethanol-induced anxiolytic effects in diazepam withdrawn rats. *European Neuropsychopharmacology*, *15*(1), 119–130. https://doi.org/10.1016/J.EURONEURO.2004.05.009

Martínez-Rivera, F. J., Martínez, N. A., Martínez, M., Ayala-Pagán, R. N., Silva, W. I., & Barreto-Estrada, J. L. (2019). Neuroplasticity transcript profile of the ventral striatum in the extinction of opioid-induced conditioned place preference. *Neurobiology of Learning and Memory*, *163*. https://doi.org/10.1016/j.nlm.2019.107031

Martín-González, E., Olmedo-Córdoba, M., Flores, P., & Moreno- Montoya, M. (2022). Differential neurobiological markers in phenotype-stratified rats modeling high or low vulnerability to compulsive behavior: A narrative review. *Current Neuropharmacology*, *21*. https://doi.org/10.2174/1570159X21666221121091454

Martín-González, E., Olmedo-Córdoba, M., Prados-Pardo, Á., Cruz- Garzón, D. J., Flores, P., Mora, S., & Moreno, M. (n.d.). Behavioral Domains in Compulsive Rats: Implications for Understanding Compulsive Spectrum Disorders. *Frontiers in Behavioral Neuroscience*, *17*, 105. https://doi.org/10.3389/FNBEH.2023.1175137

Martín-González, E., Olmedo-Córdoba, M., Prados-Pardo, Á., Cruz-Garzón, D. J., Flores, P., Mora, S., & Moreno, M. (2022). Socioemotional deficit and HPA axis time response in high compulsive rats selected by schedule-induced polydipsia. *Hormones and Behavior*, *142*. https://doi.org/10.1016/J.YHBEH.2022.105170

Martín-González, E., Prados-Pardo, Á., Mora, S., Flores, P., & Moreno, M. (2018). Do psychoactive drugs have a therapeutic role in compulsivity? Studies on schedule-induced polydipsia. *Psychopharmacology*, *235*(2), 419–432. https://doi.org/10.1007/s00213-017-4819-y

Martin, V., Huber, M., Rief, W., & Exner, C. (2008). Comparative cognitive profiles of obsessive-compulsive disorder and schizophrenia. *Archives of Clinical Neuropsychology*, *23*(5), 487–500. https://doi.org/10.1016/j.acn.2008.03.006

Masrour, F. F., Peeri, M., Azarbayjani, M. A., & Hosseini, M. J. (2018). Voluntary Exercise During Adolescence Mitigated Negative the Effects of Maternal Separation Stress on the Depressive-Like Behaviors of Adult Male Rats: Role of NMDA Receptors. *Neurochemical Research*, *43*(5), 1067–1074. https://doi.org/10.1007/s11064-018-2519-6

Mathes, B.M., Morabito, D.M. & Schmidt, N.B. Epidemiological and Clinical Gender Differences in OCD. Curr Psychiatry Rep 21, 36 (2019). https://doi.org/10.1007/s11920-019-1015-2

Matsumoto, I., Inoue, Y., Iwazaki, T., Pavey, G., & Dean, B. (2005). 5-HT2A and muscarinic receptors in schizophrenia: A postmortem study. *Neuroscience Letters*, *379*(3), 164–168. https://doi.org/10.1016/j.neulet.2004.12.059

Menzies, L., Chamberlain, S. R., Laird, A. R., Thelen, S. M., Sahakian, B. J., & Bullmore, E. T. (2008). Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: The orbitofronto-striatal model revisited. *Neurosci Biobehav Rev*, *32*(3), 525–549. https://doi.org/10.1016/j.neubiorev.2007.09.005

Merchán, A., Sánchez-Kuhn, A., Prados-Pardo, A., Gago, B., Sánchez-Santed, F., Moreno, M., & Flores, P. (2019). Behavioral and biological markers for predicting

compulsive-like drinking in schedule-induced polydipsia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *93*, 149–160. https://doi.org/10.1016/J.PNPBP.2019.03.016

Milad, M. R., & Rauch, S. L. (2012). Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways. *Trends in Cognitive Sciences*, *16*(1), 43–51. https://doi.org/10.1016/j.tics.2011.11.003

Mitra, S., Bastos, C. P., Bates, K., Pereira, G. S., & Bult-Ito, A. (2016). Ovarian Sex Hormones Modulate Compulsive, Affective and Cognitive Functions in A Non-Induced Mouse Model of Obsessive-Compulsive Disorder. *Frontiers in Behavioral Neuroscience*, *10*, 215. https://doi.org/10.3389/fnbeh.2016.00215

Mittleman, G., Blaha, C. D., & Phillips, A. G. (1992). Pituitary-Adrenal and Dopaminergic Modulation of Schedule-Induced Polydipsia: Behavioral and Neurochemical Evidence. *Behavioral Neuroscience*, *106*(2), 408–420. https://doi.org/10.1037/0735-7044.106.2.408

Mittleman, G., Rosner, A. L., & Schaub, C. L. (1994). Polydipsia and dopamine: behavioral effects of dopamine D1 and D2 receptor agonists and antagonists. *The Journal of Pharmacology and Experimental Therapeutics*, *271*(2), 638–650. http://www.ncbi.nlm.nih.gov/pubmed/7965779

Mittleman, G., Whishaw, I. Q., Jones, G. H., Koch, M., & Robbins, T. W. (1990). Cortical, Hippocampal, and Striatal Mediation of Schedule-Induced Behaviors. *Behavioral Neuroscience*, *104*(3), 399–409. https://doi.org/10.1037/0735-7044.104.3.399

Modarresi, A., Chaibakhsh, S., Koulaeinejad, N., & Koupaei, S. R. (2019). A systematic review and meta-analysis: Memantine augmentation in moderate to severe obsessive-compulsive disorder. *Psychiatry Research*, *282*. https://doi.org/10.1016/J.PSYCHRES.2019.112602

Monaghan, D. T., Yao, D., & Cotman, C. W. (1985). l -[3 H]Glutamate binds to kainate-, NMDA- and AMPA-sensitive binding sites: an autoradiographic analysis. *Brain Research*, *340*(2), 378–383. https://doi.org/10.1016/0006-8993(85)90936-9

Mora, S., Merchán, A., Aznar, S., Flores, P., & Moreno, M. (2020). Increased amygdala and decreased hippocampus volume after schedule-induced polydipsia in high drinker compulsive rats. *Behavioural Brain Research*, *390*. https://doi.org/10.1016/J.BBR.2020.112592

Mora, S., Merchán, A., Vilchez, O., Aznar, S., Klein, A. B., Ultved, L., Campa, L., Suñol, C., Flores, P., & Moreno, M. (2018). Reduced cortical serotonin 5-HT2A receptor binding and glutamate activity in high compulsive drinker rats. *Neuropharmacology*, *143*, 10–19. https://doi.org/10.1016/J.NEUROPHARM.2018.09.004

Moran M. M., McFarland K., Melendez R. I., Kalivas P. W., Seamans J. K. (2005). Cystine/glutamate exchange regulates metabotropic glutamate receptor presynaptic inhibition of excitatory transmission and vulnerability to cocaine seeking. J. Neurosci. 25, 6389–6393. 10.1523/JNEUROSCI.1007-05.2005

Morein-Zamir, S., Fineberg, N. A., Robbins, T. W., & Sahakian, B. J. (2010). Inhibition of thoughts and actions in obsessive-compulsive disorder: extending the endophenotype? *Psychological Medicine*, *40*(2), 263–272. https://doi.org/10.1017/S003329170999033X

Moreno, M., Cardona, D., Gómez, M. J., Sánchez-Santed, F., Tobeña, A., Fernández-Teruel, A., Campa, L., Suñol, C., Escarabajal, M. D., Torres, C., & Flores, P. (2010). Impulsivity characterization in the Roman high- and low-avoidance rat strains: behavioral and neurochemical differences. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *35*(5), 1198–1208. https://doi.org/10.1038/npp.2009.224

Moreno, M., & Flores, P. (2012). Schedule-induced polydipsia as a model of compulsive behavior: neuropharmacological and neuroendocrine bases. *Psychopharmacology*, *219*(2), 647–659. https://doi.org/10.1007/s00213-011-2570-3

Moreno, M., Gutiérrez-Ferre, V. E., Ruedas, L., Campa, L., Suñol, C., & Flores, P. (2012). Poor inhibitory control and neurochemical differences in high compulsive drinker rats selected by schedule-induced polydipsia. *Psychopharmacology*, *219*(2), 661–672. https://doi.org/10.1007/s00213-011-2575-y

Moreno-Montoya, M., Olmedo-Córdoba, M., & Martín-González, E. (2022). Negative valence system as a relevant domain in compulsivity: review in a preclinical model of compulsivity. *Emerging Topics in Life Sciences*, *6*(5), 491–500. https://doi.org/10.1042/ETLS20220005

Mundo, E., Tharmalingham, S., Neves-Pereira, M., Dalton, E. J., Macciardi, F., Parikh, S. V., Bolonna, A., Kerwin, R. W., Arranz, M. J., Makoff, A. J., & Kennedy, J. L. (2003). Evidence that the N-methyl-D-aspartate subunit 1 receptor gene (GRIN1) confers susceptibility to bipolar disorder. *Molecular Psychiatry*, *8*(2), 241–245. https://doi.org/10.1038/SJ.MP.4001218

Murínová, J., Hlaváčová, N., Chmelová, M., & Riečanský, I. (2017). The Evidence for Altered BDNF Expression in the Brain of Rats Reared or Housed in Social Isolation: A Systematic Review. *Frontiers in Behavioral Neuroscience*, *11*, 101. https://doi.org/10.3389/fnbeh.2017.00101

Murphy, E. R., Fernando, A. B. P., Urcelay, G. P., Robinson, E. S. J., Mar, A. C., Theobald, D. E. H., Dalley, J. W., & Robbins, T. W. (2011). *Impulsive behaviour induced by both NMDA receptor antagonism and GABA A receptor activation in rat ventromedial prefrontal cortex*. https://doi.org/10.1007/s00213-011-2572-1

Naranjo, C. A., Sellers, E. M., & Lawrin, M. O. (1986). Modulation of ethanol intake by serotonin uptake inhibitors. *The Journal of Clinical Psychiatry*, *47 Suppl*, 16–22. http://www.ncbi.nlm.nih.gov/pubmed/3007443

Navarro, S. V., Gutiérrez-ferre, V., Flores, P., & Moreno, M. (2015). *Activation of serotonin 5-HT2 A receptors inhibits high compulsive drinking on schedule-induced polydipsia*. 683–697. https://doi.org/10.1007/s00213-014-3699-7

Navarro, S. V, Alvarez, R., Colomina, M. T., Sanchez-Santed, F., Flores, P., & Moreno, M. (2017). Behavioral Biomarkers of Schizophrenia in High Drinker Rats: A Potential Endophenotype of Compulsive Neuropsychiatric Disorders. *Schizophrenia Bulletin*, *43*(4), 778–787. https://doi.org/10.1093/schbul/sbw141

Nezgovorova, V., Reid, J., Fineberg, N. A., & Hollander, E. (2022). Optimizing first line treatments for adults with OCD. *Comprehensive Psychiatry*, *115*. https://doi.org/10.1016/J.COMPPSYCH.2022.152305

Niciu, M. J., Henter, I. D., Luckenbaugh, D. A., Zarate, C. A., & Charney, D. S. (2014). Glutamate receptor antagonists as fast-acting therapeutic alternatives for the treatment of depression: ketamine and other compounds. *Annual Review of Pharmacology and Toxicology*, *54*(1), 119–139. https://doi.org/10.1146/annurev-pharmtox-011613-135950

NIMH » Research Domain Criteria (RDoC). (n.d.). Retrieved May 16, 2023, from https://www.nimh.nih.gov/research/research-funded-by-nimh/rdoc

O'Kearney, R. (2007). Motivation and emotions in the cognitive theory of obsessive-compulsive disorder. *Https://Doi.Org/10.1080/00049530108255114*, *53*(1), 7–9. https://doi.org/10.1080/00049530108255114

Orhan, C., Erten, F., Er, B., Tuzcu, M., Şahin, N., Durmaz Kurşun, Ö. E., Juturu, V., & Şahin, K. (2021). Lutein/zeaxanthin isomers regulate neurotrophic factors and synaptic plasticity in trained rats. *Turkish Journal of Medical Sciences*, *51*(4), 2167–2176. https://doi.org/10.3906/sag-2101-264

O'Roak, B. J., Vives, L., Fu, W., Egertson, J. D., Stanaway, I. B., Phelps, I. G., Carvill, G., Kumar, A., Lee, C., Ankenman, K., Munson, J., Hiatt, J. B., Turner, E. H., Levy, R., O'Day, D. R., Krumm, N., Coe, B. P., Martin, B. K., Borenstein, E., … Shendure, J. (2012). Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science*, *338*(6114), 1619–1622. https://doi.org/10.1126/science.1227764

Palit, A., Roy, P. K., & Saha, P. K. (2022). Role of Prospective Memory in Obsessive Compulsive Disorder. *Indian Journal of Psychological Medicine*, *44*(6), 586–591. https://doi.org/10.1177/02537176221100846

Pallanti, S., Hollander, E., Bienstock, C., Koran, L., Leckman, J., Marazziti, D., Pato, M., Stein, D., & Zohar, J. (2002). Treatment non-response in OCD: methodological issues and operational definitions. *The International Journal of Neuropsychopharmacology*, *5*(2), 181–191. https://doi.org/10.1017/S1461145702002900

Patel, D. D., Laws, K. R., Padhi, A., Farrow, J. M., Mukhopadhaya, K., Krishnaiah, R., & Fineberg, N. A. (2010). The neuropsychology of the schizo-obsessive subtype of

schizophrenia: a new analysis. *Psychological Medicine*, *40*(6), 921–933. https://doi.org/10.1017/S0033291709991255

Pauls, D. L., Abramovitch, A., Rauch, S. L., & Geller, D. A. (2014). Obsessive-compulsive disorder: an integrative genetic and neurobiological perspective. *Nature Reviews. Neuroscience*, *15*(6), 410–424. https://doi.org/10.1038/NRN3746

Paydary, K., Akamaloo, A., Ahmadipour, A., Pishgar, F., Emamzadehfard, S., & Akhondzadeh, S. (2016). N-acetylcysteine augmentation therapy for moderate-to-severe obsessive-compulsive disorder: randomized, double-blind, placebo-controlled trial. *Journal of Clinical Pharmacy and Therapeutics*, *41*(2), 214–219. https://doi.org/10.1111/jcpt.12370

Pellón, R., Ruíz, A., Moreno, M., Claro, F., Ambrosio, E., & Flores, P. (2011). Individual differences in schedule-induced polydipsia: Neuroanatomical dopamine divergences. *Behavioural Brain Research*, *217*(1), 195–201. https://doi.org/10.1016/J.BBR.2010.10.010

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, *14*(3), 149–167. https://doi.org/10.1016/0165-0270(85)90031-7

Perani, D., Garibotto, V., Gorini, A., Moresco, R. M., Henin, M., Panzacchi, A., Matarrese, M., Carpinelli, A., Bellodi, L., & Fazio, F. (2008). In vivo PET study of 5HT(2A) serotonin and D(2) dopamine dysfunction in drug-naive obsessive-compulsive disorder. *NeuroImage*, *42*(1), 306–314. https://doi.org/10.1016/J.NEUROIMAGE.2008.04.233

Pereira, V. S., Casarotto, P. C., Hiroaki-Sato, V. A., Sartim, A. G., Guimarães, F. S., & Joca, S. R. L. (2013). Antidepressant- and anticompulsive-like effects of purinergic receptor blockade: involvement of nitric oxide. *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology*, *23*(12), 1769–1778. https://doi.org/10.1016/j.euroneuro.2013.01.008

Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learning and Memory*, *16*(5), 279–288. https://doi.org/10.1101/lm.1041309

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience*, *106*(2), 274–285. https://doi.org/10.1037/0735-7044.106.2.274

Pinto, A., Steinglass, J. E., Greene, A. L., Weber, E. U., & Simpson, H. B. (2014). Capacity to Delay Reward Differentiates Obsessive-Compulsive Disorder and Obsessive-Compulsive Personality Disorder. *Biological Psychiatry*, *75*(8), 653–659. https://doi.org/10.1016/J.BIOPSYCH.2013.09.007

Pittenger, C., Bloch, M. H., & Williams, K. (2011). Glutamate abnormalities in obsessive compulsive disorder: neurobiology, pathophysiology, and treatment. *Pharmacology & Therapeutics*, *132*(3), 314–332. https://doi.org/10.1016/j.pharmthera.2011.09.006

Pittenger, C., Krystal, J. H., & Coric, V. (2005). Initial evidence of the beneficial effects of glutamate-modulating agents in the treatment of self-injurious behavior associated with borderline personality disorder. *The Journal of Clinical Psychiatry*, *66*(11), 1492–1493. http://www.ncbi.nlm.nih.gov/pubmed/16420092

Platt, B., Beyer, C. E., Schechter, L. E., & Rosenzweig-Lipson, S. (2008). Schedule-Induced Polydipsia: A Rat Model of Obsessive-Compulsive Disorder. In *Current Protocols in Neuroscience*. John Wiley & Sons, Inc. https://doi.org/10.1002/0471142301.ns0927s43

Ploense, K. L., Vieira, P., Bubalo, L., Olivarria, G., Carr, A. E., Szumlinski, K. K., & Kippin, T. E. (2018). Contributions of prolonged contingent and non-contingent cocaine exposure to escalation of cocaine intake and glutamatergic gene expression. *Psychopharmacology*, *235*(5), 1347–1359. https://doi.org/10.1007/s00213-017-4798-z

Porsolt, R. D., Le Pichon, M., & Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature*, *266*(5604), 730–732. https://doi.org/10.1038/266730a0

Pozzi L., Baviera M., Sacchetti G., Calcagno E., Balducci C., Invernizzi R. W., et al.. (2011). Attention deficit induced by blockade of N-methyl d-aspartate receptors in

the prefrontal cortex is associated with enhanced glutamate release and cAMP response element binding protein phosphorylation: role of metabotropic glutamate receptors 2/3. Neuroscience 176, 336–348. 10.1016/j.neuroscience.2010.11.060

Prabhavalkar, K. S., Poovanpallil, N. B., & Bhatt, L. K. (2015). Management of bipolar depression with lamotrigine: an antiepileptic mood stabilizer. *Frontiers in Pharmacology*, *6*, 242. https://doi.org/10.3389/fphar.2015.00242

Prica, C., Hascoet, M., & Bourin, M. (2008). Antidepressant-like effect of lamotrigine is reversed by veratrine: A possible role of sodium channels in bipolar depression. *Behavioural Brain Research*, *191*(1), 49–54. https://doi.org/10.1016/J.BBR.2008.03.007

Price, R. B., Nock, M. K., Charney, D. S., & Mathew, S. J. (2009). Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biological Psychiatry*, *66*(5), 522–526. https://doi.org/10.1016/j.biopsych.2009.04.029

Rajendram, R., Kronenberg, S., Burton, C. L., & Arnold, P. D. (2017). Glutamate Genetics in Obsessive-Compulsive Disorder: A Review. *Journal of the Canadian Academy of Child and Adolescent Psychiatry = Journal de l'Academie Canadienne de Psychiatrie de l'enfant et de l'adolescent*, *26*(3), 205–213. https://pubmed.ncbi.nlm.nih.gov/29056983/

Redden, S. A., Leppink, E. W., & Grant, J. E. (2015). Clinical and cognitive correlates of young adult at-risk gamblers with and without depression. *Annals of Clinical Psychiatry : Official Journal of the American Academy of Clinical Psychiatrists*, *27*(4), 261–266. http://www.ncbi.nlm.nih.gov/pubmed/26554367

Reimer, A. E., Ribeiro De Oliveira, A., Belo Diniz, J., Hoexter, M. Q., Chiavegatto, S., & Lira Brandão, M. (2015). Rats with differential self-grooming expression in the elevated plus-maze do not differ in anxiety-related behaviors. *Behavioural Brain Research*, *292*, 370–380. https://doi.org/10.1016/j.bbr.2015.06.036

Reisberg, B., Doody, R., Stöffler, A., Schmitt, F., Ferris, S., Möbius, H. J., & Memantine Study Group. (2003). Memantine in Moderate-to-Severe Alzheimer's Disease. *New*

England Journal of Medicine, *348*(14), 1333–1341. https://doi.org/10.1056/NEJMoa013128

Réus, G. Z., Stringari, R. B., Kirsch, T. R., Fries, G. R., Kapczinski, F., Roesler, R., & Quevedo, J. (2010). Neurochemical and behavioural effects of acute and chronic memantine administration in rats: Further support for NMDA as a new pharmacological target for the treatment of depression? *Brain Research Bulletin*, *81*(6), 585–589. https://doi.org/10.1016/J.BRAINRESBULL.2009.11.013

Rickelt, J., Viechtbauer, W., Lieverse, R., Overbeek, T., van Balkom, A. J., van Oppen, P., van den Heuvel, O. A., Marcelis, M., Eikelenboom, M., Tibi, L., & Schruers, K. R. (2016). The relation between depressive and obsessive-compulsive symptoms in obsessive-compulsive disorder: Results from a large, naturalistic follow-up study. *Journal of Affective Disorders*, *203*, 241–247. https://doi.org/10.1016/j.jad.2016.06.009

Robbins, T. W., & Koob, G. F. (1980). Selective disruption of displacement behaviour by lesions of the mesolimbic dopamine system. *Nature 1980 285:5764*, *285*(5764), 409–412. https://doi.org/10.1038/285409a0

Robbins, T. W., Vaghi, M. M., & Banca, P. (2019). Obsessive-Compulsive Disorder: Puzzles and Prospects. In *Neuron* (Vol. 102, Issue 1). https://doi.org/10.1016/j.neuron.2019.01.046

Rodriguez, M. M., Overshiner, C., Leander, J. D., Li, X., Morrow, D., Conway, R. G., Nelson, D. L., Briner, K., & Witkin, J. M. (2017). Behavioral Effects of a Novel Benzofuranyl-Piperazine Serotonin-2C Receptor Agonist Suggest a Potential Therapeutic Application in the Treatment of Obsessive-Compulsive Disorder. *Frontiers in Psychiatry*, *8*. https://doi.org/10.3389/FPSYT.2017.00089

Rosas-Vidal, L. E., Do-Monte, F. H., Sotres-Bayon, F., & Quirk, G. J. (2014). Hippocampal-prefrontal BDNF and memory for fear extinction. *Neuropsychopharmacology*, *39*(9), 2161–2169. https://doi.org/10.1038/npp.2014.64

Rosenzweig-Lipson, S., Sabb, A., Stack, G., Mitchell, P., Lucki, I., Malberg, J. E., Grauer, S., Brennan, J., Cryan, J. F., Sukoff Rizzo, S. J., Dunlop, J., Barrett, J. E., & Marquis, K. L. (2007). Antidepressant-like effects of the novel, selective, 5-HT2C receptor

agonist WAY-163909 in rodents. *Psychopharmacology*, *192*(2), 159–170. https://doi.org/10.1007/s00213-007-0710-6

Ruscio, A. M., Stein, D. J., Chiu, W. T., & Kessler, R. C. (2010). The epidemiology of obsessive-compulsive disorder in the National Comorbidity Survey Replication. *Molecular Psychiatry*, *15*(1), 53–63. https://doi.org/10.1038/MP.2008.94

Seif, T., Simms, J. A., Lei, K., Wegner, S., Bonci, A., Messing, R. O., & Hopf, F. W. (2015). D-Serine and D-Cycloserine Reduce Compulsive Alcohol Intake in Rats. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *40*(10), 2357–2367. https://doi.org/10.1038/npp.2015.84

Sela, V. R., Hattanda, I., Albrecht, C. M., De Almeida, C. B., Obici, S., Cortez, D. A., & Audi, E. A. (2010). Effect of xanthone from Kielmeyera coriacea stems on serotonergic neurons of the median raphe nucleus. *Phytomedicine*, *17*(3–4), 274–278. https://doi.org/10.1016/j.phymed.2009.07.002

Shin, W., Kim, K., Serraz, B., Cho, Y. S., Kim, D., Kang, M., Lee, E. J., Lee, H., Bae, Y. C., Paoletti, P., & Kim, E. (2020). Early correction of synaptic long-term depression improves abnormal anxiety-like behavior in adult GluN2B-C456Y-mutant mice. *PLoS Biology*, *18*(4). https://doi.org/10.1371/journal.pbio.3000717

Şimşek, Ş., Gençoğlan, S., Yüksel, T., Kaplan, I., & Alaca, R. (2016). Cortisol and Brain-Derived Neurotrophic Factor Levels Prior to Treatment in Children With Obsessive-Compulsive Disorder. *The Journal of Clinical Psychiatry*, *77*(7), e855–e859. https://doi.org/10.4088/JCP.15M10146

Sinopoli, V. M., Burton, C. L., Kronenberg, S., & Arnold, P. D. (2017). A review of the role of serotonin system genes in obsessive-compulsive disorder. *Neuroscience and Biobehavioral Reviews*, *80*, 372–381. https://doi.org/10.1016/J.NEUBIOREV.2017.05.029

Simone J. J., McCormick C. M. (2017). Intracellular signaling and plasma hormone profiles associated with the expression of unconditioned and conditioned fear and anxiety in female rats. Physiol. Behav 169, 234–244. 10.1016/j.physbeh.2016.12.002

Smaga, I., Wydra, K., Suder, A., Frankowska, M., Sanak, M., Caffino, L., Fumagalli, F., & Filip, M. (2021). The NMDA Receptor Subunit (GluN1 and GluN2A) Modulation Following Different Conditions of Cocaine Abstinence in Rat Brain Structures. *Neurotoxicity Research*, *39*(3), 556–565. https://doi.org/10.1007/s12640-021-00350-0

Snodgrass, S. H., & Allen, J. D. (1989). Time-response effects of pimozide on operant behavior and schedule-induced polydipsia. *Pharmacology, Biochemistry, and Behavior*, *32*(4), 949–955. http://www.ncbi.nlm.nih.gov/pubmed/2798543

Sohn, S. Y., Kang, J. I., Namkoong, K., & Kim, S. J. (2014). Multidimensional Measures of Impulsivity in Obsessive-Compulsive Disorder: Cannot Wait and Stop. *PLOS ONE*, *9*(11), e111739. https://doi.org/10.1371/JOURNAL.PONE.0111739

Stackman, R. W., Zhang, G., Sgeirsdóttir, H. N., Cohen, S. J., Munchow, A. H., & Barrera, M. P. (2013). Stimulation of serotonin 2A receptors facilitates consolidation and extinction of fear memory in C57BL/6J mice. *Neuropharmacology*, *64*(1), 403–413. https://doi.org/10.1016/J.NEUROPHARM.2012.06.007

Stewart, S., Eric Jenike, Þ. A., Dianne Hezel, Þ. M., Denise Egan Stack, Þ., Dodman, N. H., Shuster, L., & Jenike, M. A. (2010). A Single-Blinded Case-Control Study of Memantine in Severe Obsessive-Compulsive Disorder. *Journal of Clinical Psychopharmacology*, *30*(1), 34–39. https://doi.org/10.1097/JCP.0b013e3181c856de

Ströhle, A., Gensichen, J., & Domschke, K. (2018). The Diagnosis and Treatment of Anxiety Disorders. *Deutsches Arzteblatt International*, *155*(37), 611–620. https://doi.org/10.3238/arztebl.2018.0611

Strom, N. I., Soda, T., Mathews, C. A., & Davis, L. K. (2021). A dimensional perspective on the genetics of obsessive-compulsive disorder. *Translational Psychiatry 2021 11:1*, *11*(1), 1–11. https://doi.org/10.1038/s41398-021-01519-z

Szechtman, H., Sulis, W., & Eilam, D. (1998). Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD). *Behavioral Neuroscience*, *112*(6), 1475–1485. http://www.ncbi.nlm.nih.gov/pubmed/9926830

Taj M J, R. J., Ganesh, S., Shukla, T., Deolankar, S., Nadella, R. K., Sen, S., Purushottam, M., Reddy, Y. C. J., Jain, S., & Viswanath, B. (2018). BDNF gene and obsessive compulsive disorder risk, symptom dimensions and treatment response. *Asian Journal of Psychiatry*, *38*, 65–69. https://doi.org/10.1016/J.AJP.2017.10.014

Taylor, G. T., Lerch, S., & Chourbaji, S. (2017). Marble burying as compulsive behaviors in male and female mice. *Acta Neurobiologiae Experimentalis*, *77*(3), 254–260. http://www.ncbi.nlm.nih.gov/pubmed/29182616

Taylor, S. (2013). Molecular genetics of obsessive–compulsive disorder: a comprehensive meta-analysis of genetic association studies. *Molecular Psychiatry*, *18*(7), 799–805. https://doi.org/10.1038/mp.2012.76

Thamby, A., & Jaisoorya, T. S. (2019). Antipsychotic augmentation in the treatment of obsessive-compulsive disorder. *Indian Journal of Psychiatry*, *61*(Suppl 1), S51–S57. https://doi.org/10.4103/PSYCHIATRY.INDIANJPSYCHIATRY_519_18

Ting, J. T., & Feng, G. (2011). Neurobiology of obsessive-compulsive disorder: insights into neural circuitry dysfunction through mouse genetics. *Current Opinion in Neurobiology*, *21*(6), 842–848. https://doi.org/10.1016/j.conb.2011.04.010

Torres, A. R., Ferrão, Y. A., Shavitt, R. G., Diniz, J. B., Costa, D. L. C., Rosário, M. C. do, Miguel, E. C., & Fontenelle, L. F. (2014). Panic Disorder and Agoraphobia in OCD patients: Clinical profile and possible treatment implications. *Comprehensive Psychiatry*, *55*(3), 588–597. https://doi.org/10.1016/j.comppsych.2013.11.017

Torres, A. R., Fontenelle, L. F., Shavitt, R. G., Ferrão, Y. A., do Rosário, M. C., Storch, E. A., & Miguel, E. C. (2016). Comorbidity variation in patients with obsessive–compulsive disorder according to symptom dimensions: Results from a large multicentre clinical sample. *Journal of Affective Disorders*, *190*, 508–516. https://doi.org/10.1016/j.jad.2015.10.051

Tsunoka, T., Kishi, T., Kitajima, T., Okochi, T., Okumura, T., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., Naitoh, H., Inada, T., Ujike, H., Yamada, M., Uchimura, N., Sora, I., Iyo, M., Ozaki, N., & Iwata, N. (2010). Association analysis of GRM2 and HTR2A with methamphetamine-induced psychosis and schizophrenia in

the Japanese population. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(4), 639–644. https://doi.org/10.1016/j.pnpbp.2010.03.002

Tükel, R., Gürvit, H., Ertekin, B. A., Oflaz, S., Ertekin, E., Baran, B., Kalem, Ş. A., Kandemir, P. E., Özdemiroğlu, F. A., & Atalay, F. (2012). Neuropsychological function in obsessive-compulsive disorder. *Comprehensive Psychiatry*, *53*(2), 167–175. https://doi.org/10.1016/j.comppsych.2011.03.007

Umemori, J., Takao, K., Koshimizu, H., Hattori, S., Furuse, T., Wakana, S., & Miyakawa, T. (2013). ENU-mutagenesis mice with a non-synonymous mutation in Grin1 exhibit abnormal anxiety-like behaviors, impaired fear memory, and decreased acoustic startle response. *BMC Research Notes*, *6*(1). https://doi.org/10.1186/1756-0500-6-203

Vaghi, M. M., Vértes, P. E., Kitzbichler, M. G., Apergis-Schoute, A. M., van der Flier, F. E., Fineberg, N. A., Sule, A., Zaman, R., Voon, V., Kundu, P., Bullmore, E. T., & Robbins, T. W. (2017). Specific Frontostriatal Circuits for Impaired Cognitive Flexibility and Goal-Directed Planning in Obsessive-Compulsive Disorder: Evidence From Resting-State Functional Connectivity. *Biological Psychiatry*, *81*(8), 708–717. https://doi.org/10.1016/j.biopsych.2016.08.009

Vazdarjanova A., McGaugh J. L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. Proc. Natl. Acad. Sci. U S A 95, 15003–15007. 10.1073/pnas.95.25.15003

Velazquez-Sanchez, C., Muresan, L., Marti-Prats, L., & Belin, D. (2023). The development of compulsive coping behaviour is associated with a downregulation of Arc in a Locus Coeruleus neuronal ensemble. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *48*(4). https://doi.org/10.1038/S41386-022-01522-Y

Vermeire, S. T., Audenaert, K. R., Dobbeleir, A. A., De Meester, R. H., De Vos, F. J., & Peremans, K. Y. (2009). Evaluation of the brain 5-HT2A receptor binding index in dogs with anxiety disorders, measured with 123I-5I-R91150 and SPECT. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *50*(2), 284–289. https://doi.org/10.2967/JNUMED.108.055731

Vlček, P., Polák, J., Brunovský, M., & Horáček, J. (2018). Role of Glutamatergic System in Obsessive-Compulsive Disorder with Possible Therapeutic Implications. *Pharmacopsychiatry*, *51*(06), 229–242. https://doi.org/10.1055/s-0043-118665

Wald, R., Dodman, N., & Shuster, L. (2009). The combined effects of memantine and fluoxetine on an animal model of obsessive compulsive disorder. *Experimental and Clinical Psychopharmacology*, *17*(3), 191–197. https://doi.org/10.1037/a0016402

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, *2*(2), 322–328. https://doi.org/10.1038/nprot.2007.44

Wang Y, Mathews CA, Li Y, Lin Z, Xiao Z. Brain-derived neurotrophic factor (BDNF) plasma levels in drug-naïve OCD patients are lower than those in healthy people, but are not lower than those in drug-treated OCD patients. J Affect Disord [Internet]. 2011 Sep [cited 2023 Mar 10];133(1–2):305–10. Available from: https://pubmed.ncbi.nlm.nih.gov/21616543/

WHO | World Health Organization. (2018). *WHO*. https://www.who.int/gho/mortality_burden_disease/en/

Woods-Kettelberger, A., Kongsamut, S., Smith, C. P., Winslow, J. T., & Corbett, R. (1997). Animal models with potential applications for screening compounds for the treatment of obsessive-compulsive disorder. *Expert Opinion on Investigational Drugs*, *6*(10), 1369–1381. https://doi.org/10.1517/13543784.6.10.1369

Xie, X., Lancaster, B., Peakman, T., & Garthwaite, J. (1995). Interaction of the antiepileptic drug lamotrigine with recombinant rat brain type IIA Na+ channels and with native Na+ channels in rat hippocampal neurones. *Pflugers Archiv : European Journal of Physiology*, *430*(3), 437–446. http://www.ncbi.nlm.nih.gov/pubmed/7491269

Yadin, E., Friedman, E., & Bridger, W. H. (1991). Spontaneous alternation behavior: an animal model for obsessive-compulsive disorder? *Pharmacology, Biochemistry, and Behavior*, *40*(2), 311–315. http://www.ncbi.nlm.nih.gov/pubmed/1839567

Yang, Z., Xiao, S., Su, T., Gong, J., Qi, Z., Chen, G., Chen, P., Tang, G., Fu, S., Yan, H., Huang, L., & Wang, Y. (2023). A multimodal meta-analysis of regional functional

and structural brain abnormalities in obsessive-compulsive disorder. *European Archives of Psychiatry and Clinical Neuroscience*. https://doi.org/10.1007/S00406-023-01594-X

Zhao, X., Li, H., Shi, Y., Tang, R., Chen, W., Liu, J., Feng, G., Shi, J., Yan, L., Liu, H., & He, L. (2006). Significant association between the genetic variations in the 5′ end of the N-methyl-D-aspartate receptor subunit gene GRIN1 and schizophrenia. *Biological Psychiatry*, *59*(8), 747–753. https://doi.org/10.1016/j.biopsych.2005.10.023

Funding, dissemination, and academic activities

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DISSEMINATION

Publications directly related to this thesis

The research developed in the present doctoral thesis has been published and disseminated through various forms, as categorized below by type of publication.

Articles

Prados-Pardo Á., Martín-González E., Mora S., Merchán A., Flores P., Moreno M. (2019). Increased Fear Memory and Glutamatergic Modulation in Compulsive Drinker Rats Selected by Schedule-Induced Polydipsia. Front Behav Neurosci. May 7; 13:100. doi: 10.3389/fnbeh.2019.00100. PMID: 31133835; PMCID: PMC6514533.

Prados-Pardo Á., Martín-González E., Mora S., Martín C., Olmedo-Córdoba M., Pérez-Fernández C., Sánchez-Santed F., Moreno-Montoya M. Reduced expression of the *Htr2a*, *Grin1*, and *Bdnf* genes and cognitive inflexibility in a model of high compulsive rats. Molecular Neurobiology. (Submitted).

Oral communications

Prados-Pardo Á., & Martín-González E. Investigación preclínica en trastornos neuropsicopatológicos. I Jornadas del Papel de la Mujer en la Neurociencia: de la Investigación a la Innovación. Almería (Spain). 12 Febr 2018

Prados-Pardo A., Martín-González E., Mora S., Merchán A., Flores P., Moreno M. Increased fear memory and glutamatergic modulation in compulsive drinker rats selected by schedule-induced polydipsia. III International Psychobiology meeting. Granada (Spain). 29-31 May 2019.

Prados-Pardo A., Martín-González E., Mora S., Martín C., Merchán A., Flores P., Moreno M. Behavioural inflexibility under negative outcomes and glutamatergic modulation in high compulsive rats selected by schedule-induced polydipsia. XXXI international SEPC conference. Málaga (Spain). 16-18 Sept 2019.

Prados-Pardo Á., Martín-González E., Mora S., Martín C., Pérez-Fernández C., Sánchez-Santed F. Moreno M. Alteración de genes precursores de receptores NMDA en ratas compulsivas. II Jornadas de la Mujer en Neurociencia Clínica y Experimental. Almería (Spain). 11 Febr 2021.

Prados-Pardo Á., Martín-González E., Mora S., Martín C., Sánchez-Salvador L., Olmedo- Córdoba M., Pérez-Fernández C., Sánchez-Santed F. Moreno M. Cognitive inflexibility mediated by memory impairment and decreased frontal gene expression of *Htr2a*, *Grin1*, and *Bdnf* in a preclinical model of compulsivity. International Forum for Comparative Psychology. Held online by the University of Almería (Spain). 23-24 Sept 2021.

Posters

Prados-Pardo, Á., Martín-González, E., Mora, S., Flores, P., Moreno, M. Compulsivity, a common trait in different neuropsychiatric disorders: preclinical studies on schedule-induced polydipsia. II Jornadas de Investigación en Salud, Psicología y Psiquiatría. Almería (Spain). 15-16 March 2018.

Prados-Pardo, Á., Martín-González, E., Mora, S., Flores, P. and Moreno, M. Compulsivity, a common trait in different neuropsychiatric disorders: preclinical studies in schedule-induced polydipsia. II Congreso Iberoamericano de Neuropsicología. Almería (Spain). 3-5 May 2018.

Prados-Pardo, Á., Martín-González, E., Mora, S., Flores, P. and Moreno, M. New psychoactive drugs in a preclinical model of compulsivity. II Congreso Iberoamericano de Neuropsicología. Almería (Spain). 3-5 May 2018.

Prados-Pardo, Á., Martín-González, E., Mora, S., Merchán, A., Flores, P. and Moreno, M. Glutamatergic drugs in compulsivity: preclinical studies in schedule-induced polydipsia. XI FENS Forum. Berlín (Germany). 07-11 July 2018.

Prados-Pardo, Á., Martín-González, E., Mora, S., Merchán A., Flores, P. and Moreno, M. Compulsivity, a common trait in different neuropsychiatric disorders: preclinical studies on schedule-induced polydipsia. EBPS Workshop: Using Computational Approaches to Build a Two-way Bridge. Cambridge (UK). 29-31 July 2018.

Martín-González, E., **Prados-Pardo, Á.**, Mora, S., Merchán, A., Flores, P. and Moreno, M. Glutamatergic modulators as a new tool to treat compulsivity: preclinical studies on schedule-induced polydipsia. *EBPS Workshop: Using Computational Approaches to Build a Two-way Bridge.* Cambridge (UK). 29-31 July 2018.

Prados-Pardo, Á., Martín-González, E., Mora, S., Merchán, A., Flores, P. and Moreno, M. Glutamatergic modulators as a new tool to treat compulsivity: preclinical studies on schedule-induced polydipsia. XXX international SEPC conference. Ávila (Spain). 12-14 Sept 2018.

Prados-Pardo Á., Martín-González E., Mora S., Martín C., Merchán A., Flores P., Moreno M. Altered memory retrieval and reduced 5-HT2a receptors in basolateral amygdala in high compulsive rats selected by schedule-induced polydipsia. EBPS Biennial Meeting 2019. Braga (Portugal). 28-31 Aug 2019.

Prados-Pardo Á., Martín-González E., Mora S., Martín C., Merchán A, Flores P., Moreno M. Behavioural inflexibility under negative outcomes and glutamatergic modulation in high compulsive rats selected by schedule-induced polydipsia. XXXI international SEPC conference. Málaga (Spain). 16-18 Sept 2019.

Prados-Pardo Á., Martín C., Martín-González E., Mora S., Perez-Fernandez C., Sánchez-Santed F., Flores P., Moreno M. Altered memory retrieval and NMDA and 5-HT2A gene receptors in high compulsive rats. XII FENS 2020 Virtual Forum. 11-15 July 2020.

Prados-Pardo Á., Martín-González E., Mora S., Martín C., Sánchez-Salvador L., Olmedo-Córdoba M., Pérez-Fernández C., Sánchez-Santed F., Moreno M. Altered memory retrieval and frontal gene expression of *Htr2a*, *Grin1*, and *Bdnf* in a preclinical model of compulsivity. EBPS Biennial Meeting 2021. Online. 13-16 July 2021.

Prados-Pardo Á., Martín-González E., Mora S., Pérez-Fernández C., Sánchez-Salvador L., Martín C., Sánchez-Santed F., Moreno M. Reduced cortical *Htr2a* gene expression in high compulsive drinker rats. 49th Meeting of the European Brain and Behaviour. Lausanne (Switzerland). 4-7 Sept 2021.

Prados-Pardo Á., Martín-González E., Mora S., Olmedo-Córdoba M., Moreno M. Chemogenetic orbito frontal cortex inhibition and chemogenetic amygdala activation in high compulsive rats. *XIII FENS Forum 2022.* París, France. 9-13 July 2022.

Prados-Pardo Á., Martín-González E., Mora S., Olmedo-Córdoba M., Moreno M. A chemogenetic approach to disentangle the brain circuit function in high compulsive rats. *IV International Psychobiology Meeting.* Valencia, Spain. 20-22 July 2022.

Prados-Pardo Á., Martín-González E., Mora S., Olmedo-Córdoba M., Moreno M. Chemogenetic orbito frontal cortex and amygdala circuit activation and inhibition in high compulsive rats. *XXXII International SEPC conference.* Almería, Spain. 21-23 Sept 2022.

Collaborations and other research topics

During the development of this doctoral thesis, the collaboration with other researchers have led to the following publications.

Articles

Martín-González E., **Prados-Pardo Á.**, Mora S., Flores P., Moreno M. (2018) Do psychoactive drugs have a therapeutic role in compulsivity? Studies on schedule-induced polydipsia. Psychopharmacology (Berl). 2018 Feb; 235(2):419-432. doi: 10.1007/s00213-017-4819-y. Epub 2018 Jan 8. PMID: 29313138.

Merchán A., Sánchez-Kuhn A., **Prados-Pardo A.**, Gago B., Sánchez-Santed F., Moreno M., Flores P. (2019). Behavioral and biological markers for predicting compulsive-like drinking in schedule-induced polydipsia. Prog Neuropsychopharmacol Biol Psychiatry. 2019 Jul 13; 93:149-160. doi: 10.1016/j.pnpbp.2019.03.016. Epub 2019 Mar 30. PMID: 30940483.

Mora S., Martín-González E., **Prados-Pardo Á.**, Moreno J., López M.J., Pilar-Cuellar F., Castro E., Díaz Á., Flores P., Moreno M. (2020). Increased vulnerability to impulsive behavior after streptococcal antigen exposure and antibiotic treatment in rats. Brain Behav Immun. Oct; 89:675-688. doi: 10.1016/j.bbi.2020.08.010. Epub 2020 Aug 13. PMID: 32798664.

Mora S., Martín-González E., **Prados-Pardo Á.**, Flores P., Moreno M. (2021). Increased Compulsivity in Adulthood after Early Adolescence Immune Activation: Preclinical Evidence. Int J Environ Res Public Health. Apr 28;18(9):4684. doi: 10.3390/ijerph18094684. PMID: 33924858; PMCID: PMC8125663.

Sánchez-Salvador L., **Prados-Pardo Á.**, Martín-González E., Olmedo-Córdoba M., Mora S., Moreno M. (2021). The Role of Social Stress in the Development of Inhibitory Control Deficit: A Systematic Review in Preclinical Models. Int J Environ Res Public Health. May 6;18(9):4953. doi: 10.3390/ijerph18094953. PMID: 34066570; PMCID: PMC8124175.

Abreu A.C., Mora S., Tristán A.I., Martín-González E., **Prados-Pardo Á.**, Moreno M., Fernández I. (2022). NMR-based Metabolomics and Fatty Acid Profiles to Unravel Biomarkers in Preclinical Animal Models of Compulsive Behavior. J Proteome Res. Mar 4;21(3):612-622. doi: 10.1021/acs.jproteome.1c00857. Epub 2022 Feb 10. PMID: 35142515; PMCID: PMC8902800.

Martín-González E., Olmedo-Córdoba M., **Prados-Pardo Á.**, Cruz-Garzón D. J., Flores P., Mora S., Moreno M. (2022). Socioemotional deficit and HPA axis time response in high compulsive rats selected by schedule-induced polydipsia. Horm Behav. 2022 Jun; 142:105170. doi: 10.1016/j.yhbeh.2022.105170. Epub 2022 Mar 31. PMID: 35367739.

Martín-González E., Olmedo-Córdoba M., **Prados-Pardo Á.**, Cruz-Garzón D. J., Flores P., Mora S., Moreno M. (2023). Behavioral Domains in Compulsive Rats: Implications for Understanding Compulsive Spectrum Disorders. Front. Behav. Neurosci. 2023 May; 17. doi: 10.3389/fnbeh.2023.1175137.

Oral communications

Mora S., Martín-González E., **Prados-Pardo Á.**, Flores P., Moreno M. Group-A streptococcus exposure and inhibitory control deficit: preclinical studies. III International Psychobiology meeting. Granada (Spain). 29-31 May 2019.

Olmedo-Córdoba M., Martín-González E., **Prados-Pardo Á.**, Mora S, Moreno M. Propuesta de estudio de memoria emocional, corticosterona y metabolómica en una población de ratas compulsivas. *II Jornadas de la Mujer en Neurociencia Clínica y Experimental.* Almería (Spain). 11 Feb 2021.

Martín-González E., **Prados-Pardo A.**, Cruz-Garzón D.J., Olmedo-Córdoba M., Cruz-Garzón D.J., Sawiak S.J., Dalley J.W., Ramos-Cabrer P., Flores P., Mora S., Moreno M. Mapeando el control de impulsos: una caracterización conductual y neuroestructural de un fenotipo compulsivo de ratas. II Jornadas de la Mujer en Neurociencia Clínica y Experimental. Almería (Spain). 11 Feb 2021.

Mora S., Ruiz-Sobremazas D., Martín-González E., **Prados-Pardo Á.**, Moreno M. High-fat diet induces long-term vulnerability to impulsive behavior: preclinical studies.

International Forum for Comparative Psychology. Held online by the University of Almería (Spain). 23-24 Sept 2021.

Martín-González E., Olmedo-Córdoba M., **Prados-Pardo Á.**, Sawiak S.J., Daley J.W., Ramos-Cabrer P., Padro D., Mora S., Moreno M. Altered neuroanatomical mechanisms of a compulsive phenotype selected by Schedule-Induced Polydipsia. IV International Psychobiology Meeting. Valencia, Spain. 20-22 July 2022.

Posters

Martín-González, E., **Prados-Pardo, Á.**, Mora, S., Flores, P. and Moreno, M. Potential therapeutic effect of psychoactive drugs in compulsivity. *II International Psychobiology Meeting.* Ávila (Spain). 19-21 July 2017. Poster.

Martín-González, E., **Prados-Pardo, Á.**, Mora, S., Flores, P. and Moreno, M. Do psychoactive drugs play a therapeutic role in compulsivity? *Biennial EBPS meeting.* Heraklion, (Greece). 31 Aug - 03 Sept 2017.

Mora, S., Martín-González, E. **Prados-Pardo, Á.**, Flores, P. and Moreno, M. Stress and immune activation: effects on inhibitory control deficit. Biennial EBPS meeting. Heraklion, (Greece). 31 Aug - 03 Sept 2017.

Martín-González, E., **Prados-Pardo, Á.**, Mora, S., Flores, P., Moreno, M. Potencial use of psychoactive drugs as compulsive disorders treatment. A preclinic study with a model of control inhibitory deficit. *II Jornadas de Investigación en Salud, Psicología y Psiquiatría.* Almería (Spain). 15-16 March 2018. Poster.

Mora, S., Martín-González, E., **Prados-Pardo, Á.**, Flores, P. and Moreno, M. Stress and early immune activation: an animal model of inhibitory control deficit. II Congreso Iberoamericano de Neuropsicología. Almería (Spain). 3-5 May 2018.

Martín-González, E., **Prados-Pardo, Á.**, Mora, S., Moreno, J., López, M.J., Flores, P. and Moreno, M. Long-term increased impulsive response after group A streptococcal antigen and antibiotic exposition in adolescent rats. *XI FENS Forum.* Berlín (Germany). 07-11 July 2018.

Martín-González, E., **Prados-Pardo, Á.**, Mora, S., Merchán, A., Flores, P. and Moreno, M. Preclinical study on a common trait of different neuropsychiatric disorders: Compulsivity. *XXX international SEPC conference.* Ávila (Spain). 12-14 Sept 2018.

Martín-González, E., **Prados-Pardo, Á.**, Mora, S., Flores, P. and Moreno, M. Individual differences in the modulation of compulsive drinking behavior using psychedelic drugs. *XXXI international SEPC conference.* Málaga (Spain). 16-18 Sept 2019.

Martín-González, E., **Prados-Pardo, Á.**, Mora, S., Merchán, A., Flores, P. and Moreno, M. New psychopharmacological target for compulsive behaviours: direct and indirect modulation of GLU on schedule-induced polydipsia. *EBPS Biennial Meeting 2019.* Braga (Portugal). 28-31 Aug 2019.

Mora S., Martín-González E., **Prados-Pardo Á.**, Flores P, Moreno M. Increased impulsivity and brain cytokine alterations after streptococcal and antibiotic exposure in rats. EBPS Biennial Meeting 2019. Braga (Portugal). 28-31 Aug 2019.

Olmedo-Córdoba M., Martín-González E., **Prados-Pardo Á.**, Cruz-Garzón D.J., Flores P., Mora S., Moreno M. Resistant emotional memory and blunted neuroendocrine response of HPA axis in a compulsive phenotype of rats. XII FENS 2020 Virtual Forum. 11-15 July 2020.

Mora S., Abreu A.C., Martín-González E., **Prados-Pardo Á.**, Tristán A.I., Fernández I., Moreno M. Metabolomic alterations underlying compulsive behavior in a preclinical model. EBPS Biennial Meeting 2021. Online. 13-16 July 2021.

Olmedo-Córdoba M., Martín-González E., Prados-Pardo Á., Cruz-Garzón D.J., Flores P., Mora S., Moreno M. Resistant emotional memory and blunted HPA axis response stress in a compulsive phenotype of rats. EBPS Biennial Meeting 2021. Online. 13-16 July 2021.

Ruiz-Sobremazas D., Martín-González E., **Prados-Pardo Á.**, Moreno M., S. Mora S. The effect of high-fat diet on impulsivity: lessons from a preclinical model. EBPS Biennial Meeting 2021. Online. 13-16 July 2021.

Martín-González E,. Olmedo-Córdoba M., **Prados-Pardo Á.**, Cruz-Garzón D.J., Flores P., Mora S., Moreno M. Impaired decision-making and social regulation in a compulsive phenotype of rats selected by Schedule-Induced Polydipsia. *EBPS Biennial Meeting 2021.* Online. 13-16 July 2021.

Martín-González E,. Olmedo-Córdoba M., **Prados-Pardo Á.**, Cruz-Garzón D.J., Flores P., Mora S., Moreno M. How uncertainty affects decision-making in a compulsive phenotype of rats selected by Schedule-Induced Polydipsia. 49th Meeting of the European Brain and Behaviour. Lausanne (Switzerland). 4-7 Sept 2021.

Mora S., Ruiz-Sobremazas D., Martín-González E., **Prados-Pardo A.**, Moreno M. Increased impulsivity by chronic high-fat diet: evidences in a preclinical study. 49th Meeting of the European Brain and Behaviour. Lausanne (Switzerland). 4-7 Sept 2021.

Martín-González E., Olmedo-Córdoba M., **Prados-Pardo A.**, Cruz-Garzón D.J., Sawiak S.J., Dalley J.W., Ramos-Cabrer P., Flores P., Mora S., Moreno M. Risky decision-making and morphological abnormalities in acompulsive phenotype: a study on Schedule-Induced polydipsia. *3rd SEJYD Meeting*. Madrid (Spain). 16 Nov 2021.

Olmedo-Córdoba M., Martín-González E., **Prados-Pardo Á.**, Cruz-Garzón D.J., Mora S, Moreno M. Resistant emotional memory and blunted neuroendocrine response of HPA axis in a compulsive phenotype of rats. *International Forum for Comparative Psychology.* Held online by the University of Almería (Spain). 23-24 Sept 2021.

Martín-González E., Olmedo-Córdoba M., **Prados-Pardo Á.**, Sawiak S.J., Daley J.W., Ramos-Cabrer P., Padro D., Mora S., Moreno M. Neurostructural abnormalities in High Drinkers compulsive rats selected by Schedule-Induced Polydipsia. *XIII FENS Forum 2022.* París, France. 9-13 July 2022.

Olmedo-Córdoba M., Martín-González E., **Prados-Pardo Á.**, Moreno M. Effects of Transcranial direct-current stimulation on medial prefrontal cortex in a preclinical model of compulsivity. *XIII FENS Forum 2022.* París, France. 9-13 July 2022.

De las Heras-Martínez N., Rodríguez A., López-Hernández T., Olmedo-Córdoba M., Martín-González E., **Prados-Pardo Á.**, Cruz-Garzón D.J.; Martinez-Sánchez P., Moreno M. Impulsive behavior assessment in a preclinical model of stroke. *XIII FENS Forum 2022.* París, France. 9-13 July 2022.

López-Hernández, T., De las Heras-Martínez, N., Olmedo-Córdoba M., **Prados-Pardo Á.**, Cruz-Garzón D.J., Moreno M., Martín-González E. Individual differences in risk decision-making strategies: an study using a rat gambling task. *IV International Psychobiology Meeting.* Valencia, Spain. 20-22 July 2022.

Olmedo-Córdoba M., López-Hernández T., **Prados-Pardo Á.**, De Las Heras-Martínez N., Martín-González E., Moreno M. Effects Transcranial direct-current stimulation on frontal cortex in a preclinical model of compulsivity. *IV International Psychobiology Meeting.* Valencia, Spain. 20-22 July 2022.

De las Heras-Martínez N., Rodríguez A., López-Hernández T., Olmedo-Córdoba M., Martín-González E., **Prados-Pardo Á.**, J Cruz-Garzón D.; Martinez-Sánchez P., Moreno M. The effects in risky decision making in a preclinical model of ischemic stroke. *IV International Psychobiology Meeting.* Valencia, Spain. 20-22 July 2022.

Olmedo-Córdoba M., López-Hernández T., **Prados-Pardo Á.**, De Las Heras-Martínez N., Martín-González E., Moreno M. Efficacy of two types of Transcranial direct-current stimulation on frontal cortex in a preclinical model of compulsivity. *XXXII International SEPC conference.* Almería, Spain. 21-23 Sept 2022.

Martín-González E., González-Rodríguez A., Olmedo-Córdoba M., **Prados-Pardo Á.**, Mora S., Moreno M. Is there any influence of compulsivity on decision-making behavior? Preclinical evidence. *XXXII International SEPC conference.* Almería, Spain. 21-23 Sept 2022.

De las Heras-Martínez N., Rodríguez A., López-Hernández T., Olmedo-Córdoba M., Martín-González E., **Prados-Pardo Á.**, Cruz-Garzón D.J.; Martinez-Sánchez P., Moreno M. Is there any effects in risky decision making behavior after a ischemic stroke? Preclinical evidences. *XXXII International SEPC conference.* Almería, Spain. 21-23 Sept 2022.

López-Hernández, T., De las Heras-Martínez, N., Olmedo-Córdoba M., **Prados-Pardo Á.**, Cruz-Garzón D.J., Moreno M., Martín-González E. Rodent version of the Iowa Gambling Task: Individual differences in risk decision-making behavior. Preclinical evidences. *XXXII International SEPC conference.* Almería, Spain. 21-23 Sept 2022.

Martín-González E., Olmedo-Córdoba M., **Prados-Pardo Á.**, Cruz-Garzón D.J., Sawiak S.J., Dalley J.W., Ramos-Cabrer P., Padro D., Mora S., Moreno M. Neurobehavioral and emotional alterations in a preclinical model of compulsivity. *SFN meeting 2022.* 12-16 Nov 2022. San Diego, US.

ACADEMIC ACTIVITIES

Supervision of bachelor/master's theses

Martín C. (2019). Preclinical study in compulsivity as a common feature in different neuropsychological disorders. Bachelor's thesis. BSc in Psychology (University of Almería, Spain). Grade: "Sobresaliente". Supervisors: **Prados-Pardo A.**, Moreno M.

Lozano N. (2020). Role of serotonin in cognitive flexibility: a review of results in translationals tasks, reversal learning and probabilistic reversal learning. Bachelor's thesis. BSc in Psychology (University of Almería, Spain). Grade: "Sobresaliente". Supervisors: **Prados-Pardo A.**, Mora S.

Martínez M. (2020). Role of Intestinal Dysbiosis and Neuroinflammation in the Etiopathogenesis of Compulsive behavior: A Systematic Review. Bachelor's thesis. BSc in Psychology (University of Almería, Spain). Grade: "Sobresaliente". Supervisors: **Prados-Pardo A.**, Moreno M.

Salvador L. (2021). "Análisis de la Expresión Genética en ratas compulsivas altas bebedoras seleccionadas mediante Polidipsia Inducida por Programa (PIP)". Master's thesis. MSc in the Nervous System (University of Almería, Spain). Grade: "Sobresaliente". Supervisors: **Prados-Pardo A.**, Moreno M.

Other activities

Participation in the European Researcher's night, in the framework Open Research, approved by the European Commission within the Marie Sklodowska-Curie Actions. Organizer: Research Results Transfer Office (OTRI) from the University of Almería. (2017-present).

Participation in the Week of Sciences, aimed at high-school students, at the University of Almería. (2017-present).

Participation in scientific divulgation talks in the secondary schools of Almería on the occasion of the celebration of February 11: World Day of Women and Girls in Science. Organizer: University of Almería. (2017-present).

Participation and co-creator of “Women in Neuroscience Talks” on the occasion of the celebration of February 11: World Day of Women and Girls in Science. Organizer: University of Almería. 2022. (2017-present).

Appendix

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
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Increased Fear Memory and Glutamatergic Modulation in Compulsive Drinker Rats Selected by Schedule-Induced Polydipsia

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Compulsive behavior is observed in several neuropsychiatric disorders such as obsessive-compulsive disorder (OCD), anxiety, depression, phobia, and schizophrenia. Thus, compulsivity has been proposed as a transdiagnostic symptom with a highly variable pharmacological treatment. Recent evidence shows that glutamate pharmacotherapy may be of benefit in impaired inhibitory control. The purpose of the present study was: first, to test the comorbidity between compulsivity and other neuropsychiatric symptoms on different preclinical behavioral models; second, to assess the therapeutic potential of different glutamate modulators in a preclinical model of compulsivity. Long Evans rats were selected as either high (HD) or low (LD) drinkers corresponding with their water intake in schedule-induced polydipsia (SIP). We assessed compulsivity in LD and HD rats by marble burying test (MBT), depression by forced swimming test (FST), anxiety by elevated plus maze (EPM) and fear behavior by fear conditioning (FC) test. After that, we measured the effects of acute administration (i.p.) of glutamatergic drugs: N-Acetylcysteine (NAC; 25, 50, 100 and 200 mg/kg), memantine (3.1 and 6.2 mg/kg) and lamotrigine (15 and 30 mg/kg) on compulsive drinking on SIP. The results obtained showed a relation between high compulsive drinking on SIP and a higher number of marbles partially buried in MBT, as well as a higher percentage of freezing on the retrieval day of FC test. We did not detect any significant differences between LD and HD rats in FST, nor in EPM. The psychopharmacological study of glutamatergic drugs revealed that memantine and lamotrigine, at all doses tested, decreased compulsive water consumption in HD rats compared to LD rats on SIP. NAC did not produce any significant effect on SIP. These results indicate that the symptom clusters of different forms of compulsivity and phobia might be found in the compulsive phenotype of HD rats selected by SIP. The effects of memantine and lamotrigine in HD rats point towards a dysregulation in the glutamatergic signaling as a possible underlying mechanism in the vulnerability

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to compulsive behavior on SIP. Further studies on SIP, could help to elucidate the therapeutic role of glutamatergic drugs as a pharmacological strategy on compulsive spectrum disorders.

Keywords: compulsivity, schedule-induced polydipsia, marble burying test, forced swimming test, elevated plus maze test, fear conditioning, glutamatergic modulators

INTRODUCTION

Compulsivity has been defined as "the performance of repetitive, unwanted and functionally impairing overt or covert behavior without adaptive function according to either rigid rules or as a means of avoiding perceived negative consequences" (Fineberg et al., 2014). It is one of the principal symptoms in obsessive-compulsive disorder (OCD), that affects 2%–3% of the population and is considered as one of the ten leading neuropsychiatric disorders of disability (WHO, 2018). In the Diagnostic and Statistical Manual of Mental Disorders (5th edn), the *obsessive-compulsive and related disorders family* state that the course of OCD is often complicated by the co-occurrence of other disorders, including anxiety, specific phobia, depression, bipolar disorder, schizophrenia, and eating disorders as common comorbid pathologies (DSM-5; American Psychiatric Association, 2013). Indeed, compulsive behavior has been proposed as a trans-diagnostic symptom being comorbid especially with general anxiety disorders and depression (Gillan et al., 2017). For example, Torres et al. (2014, 2016) found that OCD patients, evaluated using the Dimensional Yale-Brown Obsessive–Compulsive Scale and Structured Clinical Interview for DSM-IV-TR Axis I Disorders, presented a lifetime prevalence of: 15.3% panic disorder (Torres et al., 2014), 56.4% major depression, 34.6% social phobia, 34.3% generalized anxiety disorder, and 31.4% specific phobia (Torres et al., 2016). Despite these studies, there are few experimental approaches in animals that have characterized the comorbidity with other altered pathological behaviors in preclinical models of compulsivity.

The clinical treatment of compulsivity in OCD patients has been focused on Serotonin reuptake inhibitors (SRIs), such as fluvoxamine, fluoxetine, sertraline, paroxetine and citalopram (reviewed in Fineberg and Gale, 2005). However, recent studies point out that up to 40% of patients do not respond successfully to SRIs treatment (Marinova et al., 2017). Recent studies suggest that glutamate-modulating drugs seem to have a beneficial effect in reducing compulsive symptoms in humans (Marinova et al., 2017) maybe because of its fundamental role in neuronal plasticity, learning, and memory (Javitt et al., 2011). Glutamate, the major excitatory neurotransmitter in the brain, is highly implicated in the cortico-striatal-thalamic circuit (Ting and Feng, 2011), the proposed neuroanatomical basis in compulsive deficit (reviewed in Menzies et al., 2008; Fineberg et al., 2010); which present a rich glutamatergic receptor density (Monaghan et al., 1985). A dysregulation of glutamatergic signaling in the cortico-striatal circuitry has been suggested in OCD, with reduced glutamatergic concentrations in the anterior cingulate cortex, as well as overactivity of glutamatergic signaling in the striatum and orbitofrontal cortex (Pittenger et al., 2011; Ting and Feng, 2011; Milad and Rauch, 2012).

Preclinical and clinical data have shown evidence that glutamatergic drugs could be a promising potential benefit in compulsive disorders. The N-Acetylcysteine (NAC), glutathione (GSH) precursor and a cell-permeable antioxidant, decrease the synaptic glutamate release (Moran et al., 2005). In clinical studies, NAC treatment has been shown to be effective in SRI-resistant OCD patients (Lafleur et al., 2006). Chronic treatment of NAC in OCD patients, 10–12 weeks, reduced the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS; Afshar et al., 2012; Paydary et al., 2016). Moreover, it has also shown to improve symptomatology in other psychiatric syndromes, including depression, bipolar disorder, suicidality, and self-injurious behavior (Pittenger et al., 2005; Price et al., 2009; Niciu et al., 2014). In a preclinical study using an acute administration of 100 mg/kg of NAC reduced ethanol self-administration and ethanol-seeking behavior (Lebourgeois et al., 2018). Furthermore, memantine (MEM), an uncompetitive N-Methyl-D-aspartate (NMDA) receptor antagonist, that is currently employed in the treatment of Alzheimer disease (Reisberg et al., 2003) has also shown a beneficial effect in compulsivity. MEM reduce glutamate release through inhibition of voltage-dependent calcium channel and protein kinase C (Lu et al., 2010). In OCD patients, MEM reduced the Y-BOCS scores after chronic treatment with MEM (Ghaleiha et al., 2013; Haghighi et al., 2013). Preclinical studies showed that acute administration of 25 mg/kg MEM suppressed ethanol self-administration in non-dependent rats and decreased by half the one of post-dependent rats during acute withdrawal (Alaux-Cantin et al., 2015). Besides, the administration of MEM (10 mg/kg) and amantadine, another uncompetitive NMDA receptor antagonists (30 mg/kg), significantly inhibited compulsive marble burying in mice (Egashira et al., 2008). Moreover, the combination of MEM and fluoxetine reduced scratching behavior, considered as an effective model for studying compulsive behavior (Wald et al., 2009). Lamotrigine (LAM) is an established anticonvulsant drug, with antiepileptic activity due to the inhibition of the voltage-sensitive neuronal membrane sodium channels, inhibition of the excitatory amino acids release such as glutamate and aspartate, and blockade of the calcium-channel (Cheung et al., 1992; Xie et al., 1995; Cunningham and Jones, 2000; Prabhavalkar et al., 2015). A clinical study with chronic treatment with LAM evidenced a decrease in Y-BOCS scores in OCD patients, in addition to the Hamilton Rating Scale for Depression scores, the Clinical Global Impression-Improvement scores and the obsession and compulsion subscales (Bruno et al., 2012; Khalkhali et al., 2016). Besides, preclinical research showed

that 15 and 30 mg/kg acute treatment of LAM significantly reduced immobility in the forced swimming test (FST; Li et al., 2010). However, there is insufficient preclinical research on the therapeutic role of these glutamate release modulators on reducing compulsive behaviors.

Schedule-induced polydipsia (SIP), a model of compulsive behavior (Moreno and Flores, 2012), is characterized by the development of an adjunctive behavior of repetitive drinking in food-deprived animals which are exposed to intermittent food-reinforcement schedules (Falk, 1961, 1966). An analogous phenomenon, called psychogenic polydipsia, which involves compulsive non-regulatory fluid consumption, is observed in 6%–20% of psychiatric patients (Evenson et al., 1987; de Leon et al., 1994, 2002; Dundas et al., 2007; Iftene et al., 2013). SIP is considered an animal model of compulsive drinking effective for studying the compulsive phenotype and modeling different psychopathologies related to compulsive spectrum disorders (Moreno and Flores, 2012; Hawken and Beninger, 2014; Belin-Rauscent et al., 2016). The individual differences observed on SIP acquisition support the selection of high compulsive drinking rats (HD) vs. low drinker rats (LD). In our laboratory, we have found consistent differences between these two populations in the inhibitory response deficit. Thus, HD rats selected by SIP have shown increased perseverative–compulsive responses under extinction conditions on the 5-Choice Serial Reaction Time task (5-CSRT; Moreno et al., 2012); impulsive decision making on the delay-discounting task (Cardona et al., 2011); less latent inhibition effect, considered as a behavioral model of schizophrenia, and augmented behavioral inflexibility in a spatial reversal learning task, characteristic in OCD patients (Navarro et al., 2017). Thus, HD and LD rats selected by SIP has shown consistent behavioral differences among different behavioral paradigms. Otherwise, SIP is considered a good model for researching the psychopharmacology of the compulsive phenotype (Platt et al., 2008; Moreno and Flores, 2012; Rodriguez et al., 2017). Indeed, studies on SIP revealed the efficacy of antipsychotic (haloperidol, clozapine, and pimozide) and antidepressant (fluoxetine) drugs in reducing SIP water intake (Snodgrass and Allen, 1989; Didriksen et al., 1993; Mittleman et al., 1994; Hogg and Dalvi, 2004; Dwyer et al., 2010). In HD rats selected by SIP, citalopram and the serotonin 5-$HT_{2A/C}$ receptor agonist DOI reduced compulsive drinking (Navarro et al., 2017). Moreover, a recent study has revealed that HD rats showed cortical reduced serotonin 5-HT_{2A} receptor binding and increased serotonin and reduced glutamate efflux compared to LD rats (Mora et al., 2018). Therefore, the study of comorbid altered behaviors and the effect of glutamatergic drugs in compulsive HD rats selected by SIP could help for a better characterization of the compulsive endophenotype and explore new possible pharmacological targets for its treatment.

According to the previous clinical data, in the present study, first, we have explored the presence of other altered behaviors, including other forms of compulsivity and typical comorbid symptoms, such as depression, general anxiety and pathological fear disorder in the high compulsive drinker rats HD selected by SIP. The animal models selected to achieve this goal has been: the marble burying test (MBT) as a assay of compulsive-like behavior (Egashira et al., 2008; de Brouwer and Wolmarans, 2018); the FST developed in Porsolt et al. (1977) as an animal model of depression that assess learned helplessness; the elevated plus maze test (EPM) as a behavioral measure of anxiety for rodents (Pellow et al., 1985); and finally, the fear conditioning (FC) to test aversive learning considered as a behavioral paradigm that model specific phobias (Berardi et al., 2012). Furthermore, as a second goal, we assessed the efficacy of different glutamatergic drugs in reducing compulsive drinking on SIP. We explored the dose-response effects of acute administration of NAC, MEM, and LAM in reducing compulsive drinking on SIP. The results are discussed regarding the contributions of the characterization of comorbid altered behaviors in the compulsive phenotype rat population HD selected by SIP and the implication of the glutamatergic modulators as a new pharmacological strategy for compulsive neuropsychiatric disorders.

MATERIALS AND METHODS

Subjects

A total of 16 male Long Evans rats (Janvier Labs, Le Genest-Saint-Isle, France) weighing between 250–350 g at the start of the experiments were used in the present study. The animals were housed four rats per cage (50 × 15 × 25 cm) at 22°C, with a 12:12-h light-dark cycle (lights off at 08:00 h) and food and water provided *ad libitum*. Before SIP training and after 10 days of habituation, rats were gradually reduced to 85% of their free-feeding body weight through controlled feeding, and their body weights were maintained at this level of deprivation throughout the experiments. Food was provided daily 30 min after each experimental session. All testing was performed between 9:00 and 15:00 h. All the procedures were conducted following the Spanish Royal Decree 53/2013 on the protection of experimental animals, the European Community Directive (2010/63/EU) for animal experiments and approved by the University of Almería Animal Research Committee.

SIP Procedure

A complete description of the SIP procedure has been previously described (Moreno and Flores, 2012). First, over two successive days, we assessed the amount of water consumed by each rat in 60 min (baseline). Unlimited access to a bottle of water was provided (100 ml), and 60 food reward pellets were placed together (45 mg of dustless pellets; catalog number 259901-PE-45/50T TSE Systems, Germany). After one session of habituation to the SIP chambers (35 × 25 × 34 cm), the animals were exposed to a fixed time 60-s (FT-60s) schedule of food reward pellet presentation for 60-min sessions. During each SIP session, a bottle of water (100 ml) was positioned opposite the food-magazine in the SIP chamber, the amount of water intake was recorded at the end of the test session. The licking behavior to the bottle of water was detected when the animal touches the metal drinking tube (spout) of the bottle. The spout is connected to the metal grid of the SIP chamber,

where the animal stands, by an electronic circuit with a low current, less than ten microAmp, inappreciable to the animal. When the rat touches the water spout of the bottle, this closes the circuit, producing a 50 ms pulse, which registers a lick. The scheduling and recording of the experimental events are controlled using a computer and the commercial software Med PC (Cibertec SA, Madrid, Spain). For each rat, we recorded the following measures: the total amount of water (milliliters) removed from the bottle, the total number of licks to the bottle, and the total entries to the food magazine. After 20 daily sessions, the animals were separated into two specific populations, HD and LD, according to whether their rates of drinking (average for each animal over the last five sessions) were above or below the group median, respectively (the number of animals in each group of LD and HD rats was $n = 8$).

Experimental Design

The order of the behavioral assessment and drug testing are summarized in **Figure 1**.

Experiment 1

Behavioral Assessment

We examined the presence of other altered behaviors considered as comorbid symptoms for compulsivity in high compulsive animals selected by SIP. We assessed compulsive-like behavior on MBT (Taylor et al., 2017), depressive-like behavior on FST (Yan et al., 2010), anxiety-like behavior on EPM (Pellow et al., 1985) and specific phobia behavior on FC (Berardi et al., 2012) in LD and HD rats selected by SIP (n per group = 8). The screening in each test commenced at least 1 week after the previous one.

Experiment 2

Glutamatergic Drugs

The behavioral effects of acute systemic administration of different glutamatergic drugs were tested in both groups of LD and HD rats in SIP (n per group = 8). We explored the effects of acute intraperitoneal injections (i.p.) of NAC (25, 50, 100 and 200 mg/kg), MEM (3.1 and 6.2 mg/kg) and LAM (15 and 30 mg/kg) in LD and HD rats in SIP. The drug doses, the injection time of 60 min before behavioral testing, were selected based on previous experiments (Li et al., 2010; Réus et al., 2010; Lebourgeois et al., 2018). All animals received drugs according to a fully randomized Latin-square design, separated by a minimum of 72 h between drug test sessions. There was a wash-out period of 1 week between each drug tested (animals continued performing SIP sessions during this week). The experimental sessions were led on Tuesdays and Fridays, and baseline testing was accomplished on Mondays and Thursdays. On Wednesdays, animals performed SIP procedure, but the results were not analyzed.

Behavioral Assessment

MBT began placing the rat into a corner of the cage containing nine marbles, being careful to place the rat on bedding as far from marbles as possible. Animals were allowed to remain in the cage undisturbed for 30 min. Rats were returned to its home cage after test completion, taking extreme care not to move or dislodge the marbles in the process of removing the subject from the cage. The number of marbles partially and completely buried was counted by two observers blinded to the experimental groups. We found a great concordance between observers. A marble was scored as partially buried if two-thirds of its surface area is covered by bedding and completely buried if all the surface area is covered by bedding (Angoa-Pérez et al., 2013).

FST was performed in a plastic cylinder containing 20 cm in diameter and 40 cm in height water temperature was 23–25°C, and the depth of water was set to prevent animals from touching the bottom. Rats swam in the cylinder for 2 min. The time

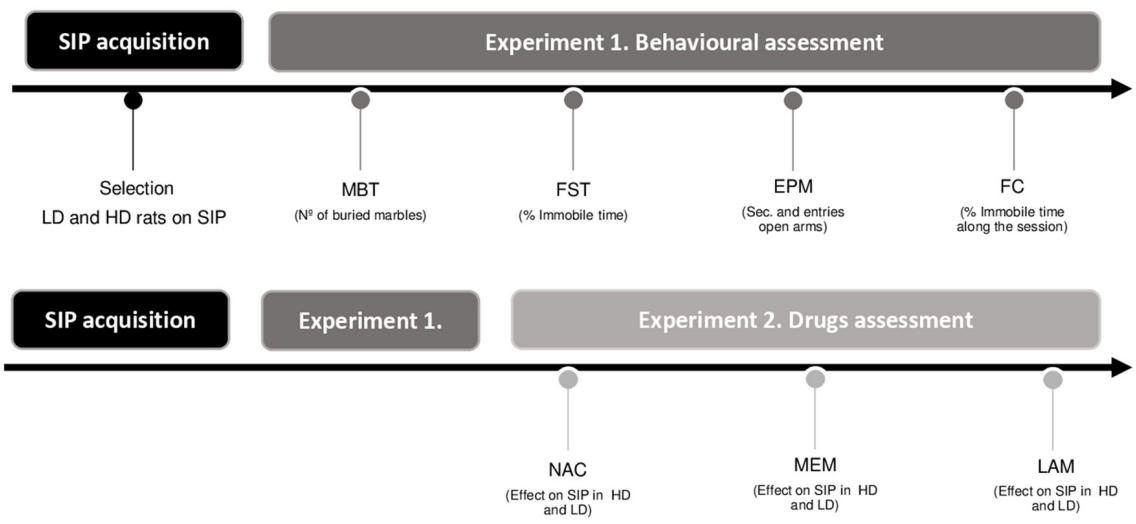

FIGURE 1 | Experimental procedure illustrated in a timetable (SIP, schedule-induced polydipsia; LD, low drinkers; HD, high drinkers; MBT, marble burying test; FST, forced swimming test; EPM, elevated plus maze test; FC, fear conditioning test; NAC, N-Acetylcysteine; MEM, memantine; and LAM, lamotrigine).

each animal spent immobile during the last min of the test was counted by two observers blinded to the experimental groups. We found a great concordance between observers. Immobility was defined as floating or absolute lack of motion (i.e., the absence of all movements except those required to maintain balance; Dong et al., 2018).

For EPM rats were placed at the junction of the four arms of the maze, facing an open arm, and entries/duration in each arm was recorded by a video-tracking system and observer simultaneously for 10 min. We found a good concordance in data collected with both methods. An increase in open arm activity (duration and/or entries) reflects anti-anxiety behavior (Walf and Frye, 2007).

FC started placing the rat into a novel set of cages with a shock grid floor capable of delivering foot-shock where, after 3 min exploration period, they received three pairings of a 10 s light (82 lx) with a shock (0.5 mA during 1 s). The light-shock trials were delivered after a 3-min acclimation time, the inter-lights intervals were 1 min, and the rats remained in the chambers for an additional minute after the last shock. Next day rats were allowed a 3 min exploration period after which they were presented with 22 lights (10 s, 82 lx, 1 min inter-lights interval) in the absence of a foot shock (Simone and McCormick, 2017). The freezing time was counted by the Video Freeze Software (Med PC) which detected changes at the pixel level from one video frame to the next. Hence, data can reflect the total time animals spent in motionless during the session, the percentage of time motionless and the number of freezing episodes.

Drugs

We explored the effects of acute intraperitoneal injections (i.p.) of NAC (25, 50, 100 and 200 mg/kg; Lebourgeois et al., 2018), MEM (3.1 and 6.2 mg/kg; Li et al., 2010) and LAM (15 and 30 mg/kg; Réus et al., 2010) in LD and HD rats in SIP. NAC [(2R)-2-(Acetylamino)-3-mercapto propanamide] and MEM [3, 5-Dimethyl-tricyclo (3.3.1.13, 7) decan-1-amine hydrochloride] were dissolved in 0.9% saline. LAM [6-(2, 3-Dichlorophenyl)-1, 2, 4-triazine-3, 5-diamine] was suspended in 1% Tween-80 in 0.9% saline. All drugs were purchased from Sigma-Aldrich (Madrid, Spain). The injection volumes were 1 ml/kg for all drugs. For all drug solutions, the final pH was adjusted to approximately 6.4 using 0.1 M NaOH, and they were aliquoted after preparation and frozen at −80°C before use.

Data Analyses

Behavioral data on SIP acquisition were analyzed using two-way repeated-measure analysis of variance (ANOVA), with "group" (LD and HD) as the between-subject factor and "sessions" (20 sessions) as the within-subject factor. The differences on the MBT, FST, EPM, and FC of the behavioral assessment in LD and HD were studied using Student's *t*-test (*T*-test). When appropriate, the effect size of the group differences was calculated using Cohen's d (*d*; mean difference divided by pooled standard deviation). The differences on FC blocks and the effects of the different drugs in LD and HD on SIP were analyzed using two-way repeated-measure ANOVA, with group (LD and HD) as the between-subject factor and "percentage of freezing" (percentage of time spent on freezing during the different blocks of the retrieval day) or "drug" (different doses of drug and vehicle) as the repeated within-subject factor. When appropriate, the effect size of the group differences was calculated using eta-squared (η^2). *Post hoc* comparisons were performed using the Newman-Keuls test. Statistical significance was set at $p < 0.05$. All analyses were computed using Statistica software (version 6.0).

RESULTS

LD and HD Selected by SIP

The mean water intake and licks in LD and HD during the acquisition and maintenance of SIP is shown in **Figures 2A,B**. In the experimental phase, the mean water intake over the last 5 days of SIP was 4.3 ± 0.6 and 11.2 ± 1.9 ml for LD and HD, respectively (**Figure 2C**). The number of licks also showed SIP acquisition. The mean total licks averaged across the last 5 days of SIP were 885.1 ± 202.9 and 2742.9 ± 536.9 for LD and HD, respectively (**Figure 2D**).

ANOVA revealed significant differences in water intake according to the interaction between SIP acquisition sessions and LD vs. HD (SIP session effect: $F_{(19,266)} = 11.759$, $p < 0.001$; group effect: $F_{(1,14)} = 10.332$, $p < 0.01$; interaction SIP session × group effect: $F_{(19,266)} = 2.58$, $p < 0.001$). This difference was also confirmed by the significant interaction observed in the total number of licks (SIP session effect: $F_{(19,266)} = 11.890$, $p < 0.001$; group effect: $F_{(1,14)} = 13.647$, $p < 0.01$; interaction SIP session × group effect: $F_{(19,266)} = 3.38$, $p < 0.001$). *Post hoc* analysis indicated significant differences between the LD and HD animals in the water intake at session 6 ($p < 0.01$) onwards. Furthermore, animals in the HD group significantly increased their consumption of water from session 4 ($p < 0.05$) compared to session 1. Differences between the LD and HD groups in the number of total licks at session 6 ($p < 0.05$) were also observed, and HD rats increased their number of licks from session 5 ($p < 0.001$) compared to session 1. We also found significant differences in the number of magazine entries according to the interaction between SIP acquisition sessions and LD vs. HD (session × group effect: $F_{(19,266)} = 2.124$; $p < 0.01$; session effect: $F_{(19,266)} = 4.515$, $p < 0.001$; group effect: $F_{(1,14)} = 5.577$, $p < 0.05$). Differences between the LD and HD groups in the number magazine entries at session 11 ($p < 0.001$ were also observed, and HD rats increased their number of magazine entries from session 6 ($p < 0.05$) compared to session 1.

Experiment 1

Behavioral Assessment

Marble Burying Test

The number of marbles partially (2/3) and completely buried by LD and HD rats on MBT are shown in **Figure 3A**. *T*-test and the effect sizes by Cohen's d showed that HD rats had a significantly increased number of marbles partially (2/3) buried compared to LD rats ($df = 14$; T-test = −2.22; $p < 0.05$; $d = 1.186$). There was no significant effect on the number of marbles completely buried between LD and HD rats ($df = 14$; T-test = 1.14; $p = 0.27$).

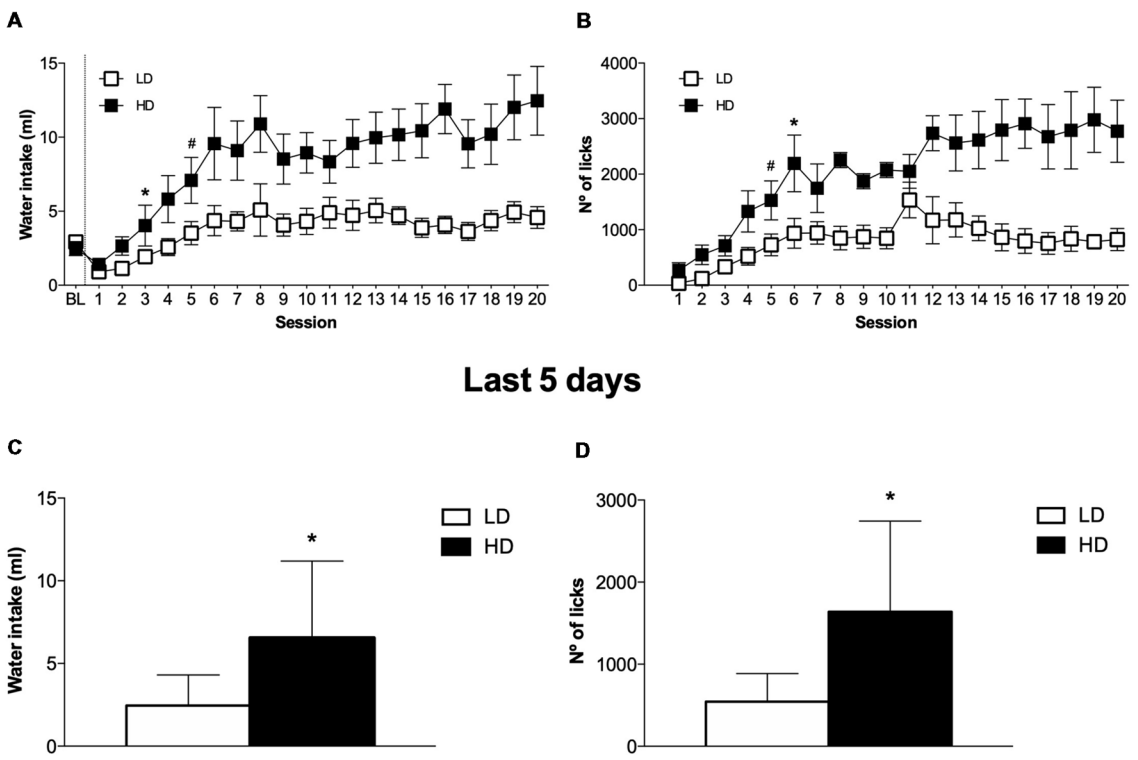

FIGURE 2 | The mean (± SEM) water intake **(A)** and number of licks **(B)** in FT-60s across 20 sessions of SIP. The mean (± SEM) water intake **(C)** and number of licks **(D)** in FT-60s across the last five sessions of SIP acquisition. Statistical analyses indicate significant differences between low drinkers (LD, $n = 8$) and high drinkers (HD, $n = 8$; $^{*}p < 0.05$). Significant differences between sessions were found from session 1 ($^{\#}p < 0.05$).

Forced Swimming Test

The percentage of immobile time of LD and HD rats on FST are shown in **Figure 3B**. *T*-test showed no significant difference in the percentage of immobile time between LD and HD rats ($df = 14$; T-test = 0.35; $p = 0.72$).

Elevated Plus Maze Test

The time LD and HD rats spent on the open arm before changing to the other, and the number of entries in the open arm on EPM are shown in **Figures 3C,D**. *T*-test showed that there was no significant difference in the mean time and the number of entries in the open arms between LD and HD rats ($df = 14$; T-test = −0.09; $p = 0.92$; $df = 14$; T-test = 0.86; $p = 0.40$). The mean time LD and HD rats spent on the closed arm before changing to the other was 1.53 ± 0.35 and 1.83 ± 0.39, respectively. The mean number of entries in the closed arm on EPM was 9.38 ± 0.67 for LD rats and 8.88 ± 1.24 for HD rats. *T*-test showed that there was no significant difference in the mean time and the number of entries in the closed arms between LD and HD rats ($df = 14$; T-test = −0.60; $p = 0.56$; $df = 14$; T-test = 0.38; $p = 0.71$). The mean time LD and HD rats spent on one arm before changing to another one was 1.53 ± 0.06 and 1.70 ± 0.08, respectively. The mean number of entries in open and closed arms was 18.50 ± 1.14 for LD rats and 16.38 ± 1.23 for HD rats. *T*-test showed that there was no significant difference in the mean time and the number of entries in open and closed arms between LD and HD rats ($df = 14$; T-test = −1.85; $p = 0.08$; $df = 14$; T-test = 1.35; $p = 0.20$).

Fear Conditioning

The percentage of freezing time of LD and HD rats on FC during the acquisition day, the percentage of freezing time during the contextual fear test and the cued fear test at the retrieval day, as well as the percentage of freezing during the different blocks of trials on the retrieval day, is shown in **Figures 3E–H**. No significant differences were found in the percentage of freezing time spent by LD and HD rats during the acquisition day ($df = 14$; T-test = −0.45; $p = 0.65$), nor in the contextual fear test on the retrieval day ($df = 14$; T-test = −1.51; $p = 0.15$). However, *T*-test and effect sizes by Cohen's d revealed a significant increase in the percentage of freezing time spent by HD compared to LD rats during the cue presentation on retrieval day ($df = 14$; T-test = −3.12; $p < 0.01$; $d = 1.67$). The analyses of the 4 blocks of trials on the retrieval day by ANOVA and η^2 revealed that both, LD and HD rats, significantly reduced the percentage of freezing time in the different blocks of the retrieval day (Trial effect: $F_{(3,42)} = 36.64$; $p < 0.001$;

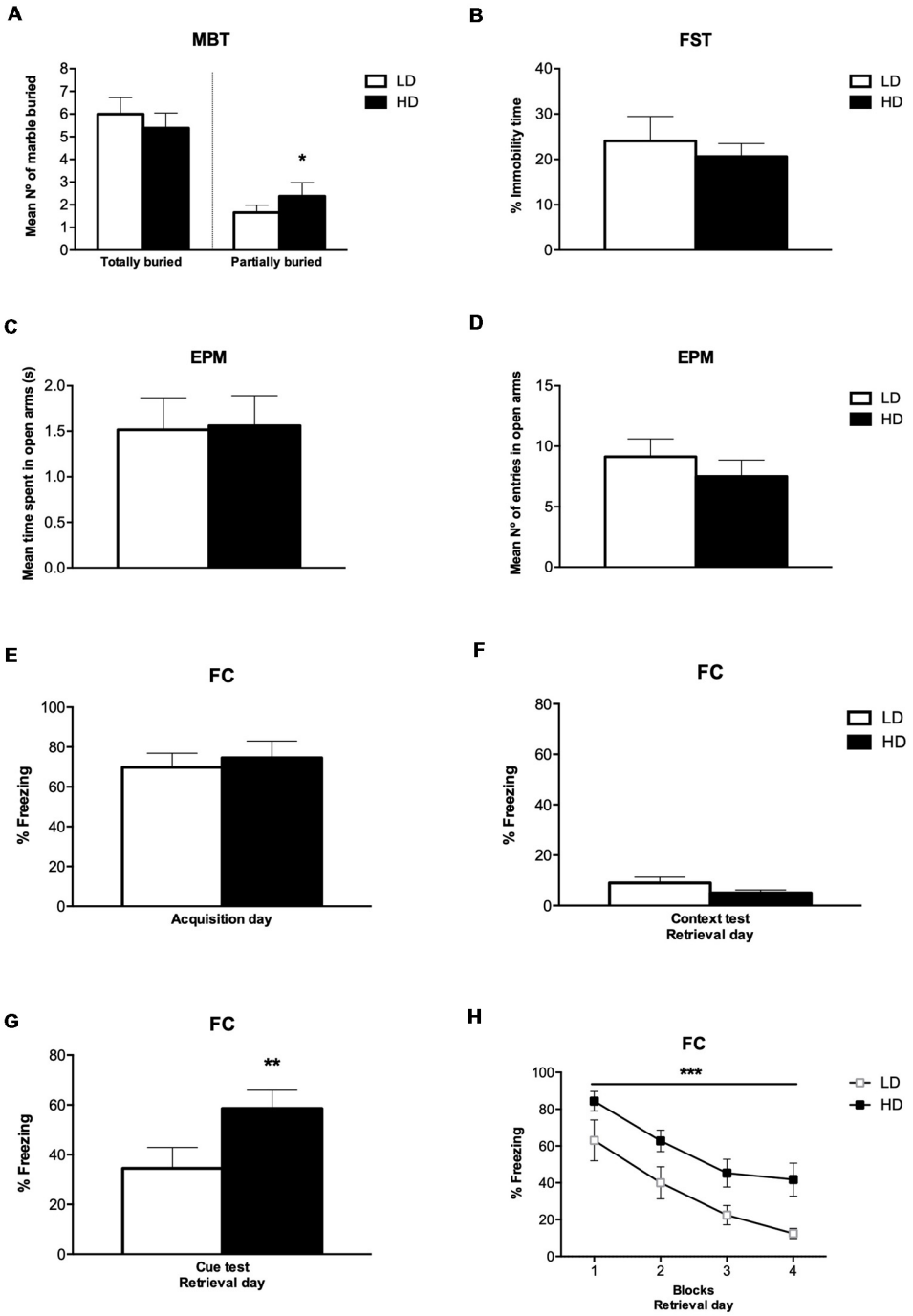

FIGURE 3 | Behavioral assessment after SIP procedure. **(A)** MBT scores of low drinkers (LD, $n = 8$) and high drinkers (HD, $n = 8$) rats, **(B)** percentage of immobile time LD and HD rats spent on forced swimming test (FST), **(C)** mean number of entries by LD and HD rats on the open arms in elevated plus maze test (EPM), **(D)** seconds spent by LD and HD rats on the open arms in EPM, **(E)** percentage of freezing LD and HD rats exhibited during fear acquisition day, **(F)** percentage of freezing LD and HD rats exhibited during contextual fear test on retrieval day, **(G)** percentage of freezing LD and HD rats exhibited during cued fear test on retrieval day, and **(H)** percentage of freezing LD and HD rats exhibited during the four blocks of time (6 min per block) at cued fear test on retrieval day of fear conditioning procedure (FC). Data are expressed as the means ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ indicate significant differences between groups.

$\eta^2 = 0.931$); whether the significant increased percentage of freezing time spent by HD compared to LD rats was maintained through the four blocks of trials on the retrieval day (group effect: $F_{(1,14)} = 9.73$; $p < 0.01$; $\eta^2 = 0.933$). No significant differences were observed by group × trial interaction ($F_{(3,42)} = 0.27$; $p = 0.84$).

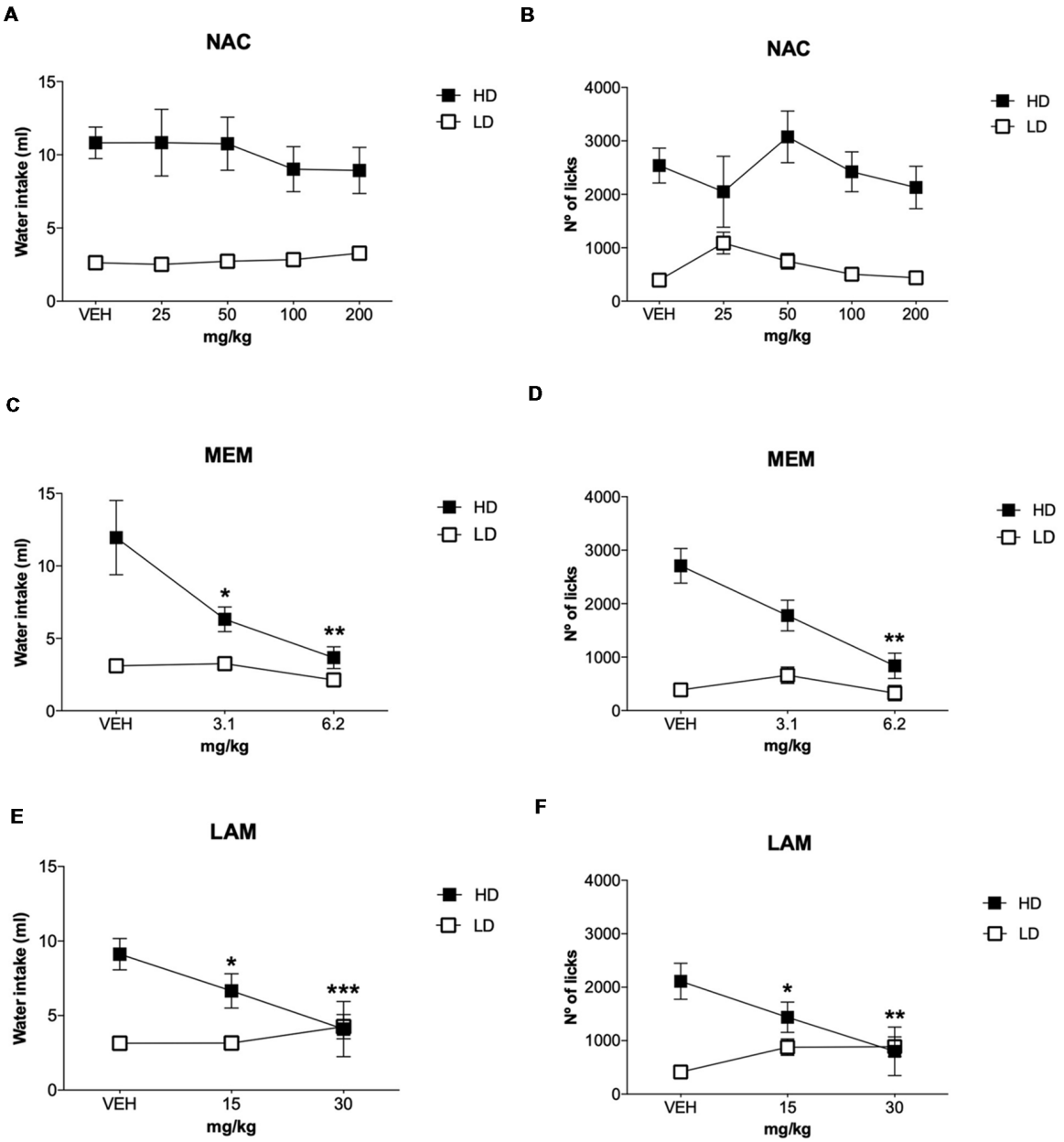

FIGURE 4 | Glutamatergic drugs on SIP. Effects of **(A,B)** NAC, **(C,D)** memantine (MEM), and **(E,F)** lamotrigine (LAM) administration on water intake and number of licks in low drinkers (LD, $n = 8$) and high drinkers (HD, $n = 8$) rats on SIP. Data are expressed as the means ± SEM. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ indicate significant differences vs. vehicle administration in the same group of rats.

Experiment 2

Glutamatergic Drugs

N-Acetylcysteine

The effects of NAC on water intake and licks in SIP are shown in **Figures 4A,B**, and the number of magazine entries after NAC administration are shown in **Table 1**. ANOVA showed that NAC did not induce significant differences in water intake (group × drug interaction, $F_{(4,56)} = 0.63$, $p = 0.64$; group effect, $F_{(1,14)} = 109.15$, $p < 0.001$; drug effect, $F_{(4,56)} = 0.38$, $p = 0.82$), total licks (group × drug interaction, $F_{(4,56)} = 0.57$, $p = 0.68$; group effect, $F_{(1,14)} = 111.89$, $p < 0.001$ drug effect, $F_{(4,56)} = 0.90$, $p = 0.47$), and magazine entries (group × drug interaction, $F_{(4,56)} = 0.28$, $p = 0.89$; group effect, $F_{(1,14)} = 8.41$, $p < 0.05$; drug effect, $F_{(4,56)} = 0.51$, $p = 0.73$).

Memantine

The effects of MEM on water intake and total licks in SIP are shown in **Figures 4C,D**. Effects of MEM on magazine entries are depicted in **Table 1**. MEM significantly reduced compulsive

TABLE 1 | Effects of N-Acetylcysteine (NAC), memantine (MEM) and lamotrigine (LAM) on total magazine entries in low drinkers (LD, $n = 8$) and high drinkers (HD, $n = 8$) rats on schedule-induced polydipsia (SIP).

	Total magazine entries	
	LD	HD
N-Acetylcysteine		
Vehicle	996.59 ± 126.34	2,052.68 ± 314.41
25 mg/kg	1,087.19 ± 205.43	2,047.67 ± 664.21
50 mg/kg	1,011.57 ± 162.58	2,041.77 ± 488.66
100 mg/kg	987.75 ± 199.97	2,095.57 ± 676.96
200 mg/kg	1,006.29 ± 183.00	1,428.29 ± 247.77
Memantine		
Vehicle	961.13 ± 144.41	1,584.11 ± 206.60
3.1 mg/kg	992.71 ± 197.17	1,173.43 ± 116.98
6.2 mg/kg	930.00 ± 222.24	811.67 ± 182.63
Lamotrigine		
Vehicle	1,058.71 ± 134.80	1,192.29 ± 111.46
15 mg/kg	1,093.57 ± 152.87	1,286.14 ± 145.31
30 mg/kg	1,135.69 ± 233.17	554.27 ± 162.55*

*Data are expressed as the means ± SEM. *$p < 0.05$ indicate significant differences vs. vehicle administration in the same group of rats.*

water intake in HD rats compared to LD rats (group × drug interaction, $F_{(2,28)} = 4.51$, $p < 0.05$; group effect, $F_{(1,14)} = 24.05$, $p < 0.001$; drug effect, $F_{(2,28)} = 8.42$, $p < 0.01$; $\eta^2 = 0.930$). *Post hoc* analyses revealed that MEM reduced dose-dependent water intake in HD rats at both doses: 3.1 ($p < 0.05$) and 6.2 mg/kg ($p < 0.001$) compared with vehicle in the same group. MEM did not affect water intake in LD rats. The comparison between LD and HD revealed a dose dependent reduction of the significant differences in water intake disappearing at the highest dose (vehicle, $p = 0.0001$; 3.1 mg/kg, $p = 0.041$; 6.2, $p = 0.572$). Moreover, MEM also significantly reduced the total licks in HD rats compared with the LD group (group × drug interaction, $F_{(2,28)} = 6.04$, $p < 0.01$; group effect, $F_{(1,14)} = 16.96$, $p < 0.05$; drug effect, $F_{(2,28)} = 5.50$, $p < 0.01$; $\eta^2 = 0.730$). *Post hoc* comparison confirmed a decrease in the total licks in the HD group at the highest dose used 6.2 mg/kg ($p < 0.001$) compared with vehicle in the same group. Differences between LD and HD remained significant at all doses tested. MEM administration did not affect the number of magazine entries in both groups of rats (group × drug interaction: $F_{(2,28)} = 2.663$; $p = 0.087$; drug effect: $F_{(2,28)} = 2.507$; $p = 0.099$; group effect: $F_{(1,14)} = 1.569$; $p = 0.23$).

Lamotrigine

The effects of LAM on water intake and total licks in SIP are shown in **Figures 4E,F**. The effects of LAM on magazine entries in SIP are shown in **Table 1**. LAM significantly reduced compulsive water intake in HD rats compared to LD rats (group × drug interaction: $F_{(2,28)} = 11.396$, $p < 0.0002$; group effect: $F_{(1,14)} = 5.187$, $p < 0.05$; drug effect: $F_{(2,28)} = 3.532$, $p < 0.05$; $\eta^2 = 0.882$). *Post hoc* analyses revealed that LAM reduced dose-dependent water intake in HD rats at both doses: 15 ($p < 0.05$) and 30 mg/kg ($p < 0.01$) compared with vehicle in the same group. LAM reversed the significant differences on water intake between LD and HD rats on SIP (vehicle, $p = 0.008$; 15 mg/kg $p = 0.16$; 30 mg/kg, $p = 0.914$). LAM did not affect water intake in LD rats. Moreover, LAM also significantly reduced the total licks in HD rats compared with the LD group (group × drug interaction, $F_{(2,28)} = 11.40$, $p < 0.001$; group effect, $F_{(1,14)} = 5.18$, $p < 0.05$; drug effect, $F_{(2,28)} = 3.53$, $p < 0.05$; $\eta^2 = 0.870$). *Post hoc* comparison showed a dose dependent decrease in the total licks in the HD group at both doses used 15 mg/kg ($p < 0.05$) and 30 mg/kg ($p < 0.001$) compared with vehicle in the same group. The comparison between LD and HD revealed a dose dependent reduction of the significant differences in the number of licks disappearing at the highest dose (vehicle, $p = 0.0001$; 15 mg/kg $p = 0.005$; 30 mg/kg, $p = 0.86$). LAM administration reduced magazine entries in both groups of rats (group × drug interaction: $F_{(2,28)} = 3.61$, $p < 0.05$; group effect: $F_{(1,14)} = 0.19$, $p = 0.67$; drug effect: $F_{(2,28)} = 4.65$, $p < 0.05$; 0.931). *Post hoc* analyses revealed a decrease in magazine entries in HD rats only at the highest dose tested 30 mg/kg ($p < 0.05$) compared with vehicle and with the LD group.

DISCUSSION

The present study investigated the presence of possible comorbid symptoms (compulsive, depressive, anxious and fear behavior) in animals selected by high compulsive drinking behavior on SIP, HD rats. Moreover, we investigated the therapeutic potential of glutamatergic drugs for reducing compulsive drinking behavior in HD rats on SIP. The findings showed that HD rats, characterized by excessive and persistent compulsive drinking on SIP, also exhibited a compulsive behavior on MBT by a higher number of marbles partially buried (2/3) compared to LD rats. Besides, compulsive HD rats selected by SIP had an increased fear behavior profile on FC, showed by a higher percentage of freezing time in the first block of the retrieval day as well as across the following blocks, compared to LD rats. These differences between HD and LD rats might not be attributed to individual differences in reactivity to novelty. HD rats selected by SIP did not differ in spontaneous locomotor reactivity to novelty compared with LD rats (Moreno et al., 2012). Moreover, in the present study, no significant differences were found in the number of magazine entries, considered as a control measure of motor activity or motivational behavior (Navarro et al., 2015), between HD and LD on SIP.

The acute administration of glutamatergic drugs revealed that MEM and LAM reduced, in a dose-dependent manner, compulsive intake in HD rats on SIP, and did not affect LD behavior. Hence, the observed effect cannot be considered as a compensatory behavior by the use of these treatments. Moreover, we discard other possible side effects, as in previous studies the selected doses of NAC, MEM, and LAM did not affect locomotor activity in rats (Li et al., 2010; Réus et al., 2010; Lebourgeois et al., 2018). However, NAC administration did not selectively affect compulsive intake in SIP, as LD and HD kept significant differences at all doses administrated.

Assessment of Comorbid Behaviors on Compulsive HD Rats

HD rats selected by SIP showed comorbidity with compulsive behavior on MBT, by a significantly increased number of marbles partially buried compared to LD rats. Previous studies have found that HD rats selected by SIP showed other behavioral

compulsivity forms such as compulsive lever pressing, during the pre-training phase to assess latent inhibition (Navarro et al., 2017), proposed as an OCD model (Joel and Avisar, 2001); and behavioral inflexibility in a spatial reversal task (Navarro et al., 2017). In contrast, other studies on rats with high levels of grooming, considered as a compulsive-like behavior, have shown a reduced number of marbles buried in MBT, showing a negative correlation between these factors (Reimer et al., 2015). The reason for these contradictory results could be due to the fact that compulsivity is not a unitary phenomenon and can be expressed by different forms (Fineberg et al., 2018).

The assessment of depressive behavior revealed that LD and HD rats selected by SIP did not exhibit any differences in depressive-like behavior measured on FST. The compulsive HD rats might not have depression signs as a comorbid behavior. Nevertheless, other preclinical studies have shown associations between depressive and compulsive behavior in the same individuals. For example, the administration of 8-OH-DPAT, a 5-HT_{1A} agonist, proposed as an OCD model (Yadin et al., 1991), increased the immobility time on FST (Sela et al., 2010). Moreover, the administration of the purinergic receptor P2R antagonist [pyridoxalphosphate-6-azophenyl-2', 4'-disulfonic acid tetrasodium salt (PPADS)] in Swiss mice, reduced depressive-like behavior in the FST, as well as compulsive-like behavior in MBT (Pereira et al., 2013). The effect of antidepressants on addictions, considered as compulsive disorders, has created some controversy. On the one hand, some preclinical studies have demonstrated reductions in alcohol addiction subsequently to the administration of different 5-HT receptors agonists (Naranjo et al., 1986; Higley et al., 1998; Martijena et al., 2005). On the other hand, the possibility that antidepressant treatment might increase susceptibility to alcoholism has been overlooked (Alén et al., 2013, 2014). Moreover, several clinical studies have shown that pathological gambling, associated with elevated compulsivity, frequently co-occurs with major depression (Cunningham-Williams and Cottler, 2001; Baer et al., 2015; Redden et al., 2015; Agarwal et al., 2016; Grant et al., 2016; Rickelt et al., 2016). More research is needed to clarify the relation between depressive and compulsive behavior.

Anxiety behavior measured by EPM did not show any significant differences between HD and LD rats selected by SIP. Nevertheless, we have replicated the results published in 2008 by López-Grancha, in which there were no differences in the EPM between LD and HD rats selected by SIP (López-Grancha et al., 2008). Moreover, animals with distinct levels of self-grooming emission, considered as a compulsive-like behavior, did not differ in the exploration of the EPM (Reimer et al., 2015). In contrast, a previous study has shown that an increased compulsive behavior in the MBT has also been accompanied by increased anxiety response in the EPM and open-field test in the same animals (Mitra et al., 2016). These contradictory results posit the relevance of the study on individual differences, using populations more prone to a behavioral deficit. Self-grooming and MBT might be evaluating different kinds of compulsivity, as well as anxiety is also a neuropsychological domain that could be expressed by different symptoms (reviewed in Ströhle et al., 2018). For instance, compulsive drinkers HD rats selected by SIP did not differ in anxiety-like behavior assessed using EPM to LD rats, while they differed in anxiety-like behavior measured by freezing time on the retrieval day in FC.

The assessment of fear behavior by FC revealed that HD rats selected by SIP showed a significantly augmented percentage of freezing time compared to LD rats during cued-fear memory on the retrieval day. Thus, HD and LD rats had no differences in the percentage of freezing time on the acquisition day, nor in the exploration period when exposed to the fear context on the retrieval day. Previous findings in our laboratory, have shown that under extinction conditions, HD rats had a greater increase in perseverative responses, considered as compulsive behavior, compared to LD rats on 5-CSRT (Moreno et al., 2012). Moreover, HD rats have shown increased c-Fos activity in the basolateral amygdala compared with LD rats (Merchán et al., 2019). The basolateral amygdala, as an essential structure in the neural system for FC (Phillips and LeDoux, 1992; Vazdarjanova and McGaugh, 1998), is highly implicated in cued-related fear memories and not essential for contextual FC (reviewed in Curzon et al., 2009). HD animals selected by SIP might be a convenient phenotype to study the neuronal basis of individual differences in habit formation under extinction conditions. Thus, in HD rats, a possible alteration in the basolateral amygdala might underlie the observed increased cued-fear memory on FC that possibly also affect the vulnerability to develop compulsive behaviors. In this sense, clinical studies demonstrated that OCD patients continued to exhibit a differential skin conductance response to the conditioned stimuli in the extinction phase of fear conditioned computer task, while control participants extinguished fear (Geller et al., 2017). Translational neuroscience studying fear could help us to better understand brain circuitry underlying fear behavior, although the translation of animal model results into the clinic is limited and more research is needed (Flores et al., 2018).

Effects of Glutamatergic Drugs on Compulsive Rats on SIP

The administration of NAC (25, 50, 100 and 200 mg/kg) revealed no significant differences in the water intake nor LD, nor in HD rats on SIP. Conversely, previous research has demonstrated that NAC (90 mg/kg), chronically and systemically administered, resulted in significant reductions of compulsive binge eating in a rodent model (Hurley et al., 2016). NAC systemically administrated has been demonstrated to abolish the recovery of compulsive cocaine-seeking behavior in a rodent model through augmenting the glutamate/cystine antiporter activity and reestablishing the concentration of extracellular glutamate in the nucleus accumbens (Baker et al., 2003a,b). Moreover, the acute administration of NAC at 100 mg/kg reduced motivation, seeking and relapse to self-administration of ethanol in rats (Lebourgeois et al., 2018). However, acute injections of NAC (0, 30, 60, or 120 mg/kg) did not have any result on self-administration of methamphetamine in rats (Charntikov et al., 2018). Some clinical studies have suggested the possible therapeutic role of NAC in OCD patients, showing a

reduction in the scores of the Y-BOCS after treatment with NAC during 10 and 12 weeks respectively (Afshar et al., 2012; Paydary et al., 2016).

The acute systemic administration of MEM, 3.1 and 6.2 mg/kg, decreased compulsive drinking in HD rats on SIP, compared to LD rats that remain unaffected. Hence, these results could not be considered as a general effect on rats exposed to SIP, pointing towards the neuropsychopharmacological effects of MEM might be involved in the vulnerability to compulsive non-regulatory drinking on SIP. In contrast, previous studies, have found that acute administration of MEM at 5 and 25 mg/kg in mice, did not affect water intake on SIP, but revealed a reduction in regulatory drinking (Escher et al., 2006). Although in this study, mice were not selected according to the rate of compulsive drinking. However, in the same study MEM have been found as a useful treatment for reducing compulsive alcohol intake, the administration of MEM 10 and 25 mg/kg significantly reduced alcohol drinking in mice on SIP (Escher et al., 2006). Moreover, findings revealed that acute administration of 10 mg/kg MEM significantly inhibited compulsive behavior in MBT without affecting locomotor activity in mice (Egashira et al., 2008). Furthermore, acute administration of 25 mg/kg MEM blocked ethanol self-administration in non-dependent rats, as well as it decreased by half the one of post-dependent rats during acute withdrawal (Alaux-Cantin et al., 2015). Otherwise, compulsive lever pressing, proposed as an OCD model (Joel and Avisar, 2001), was not affected by an NMDA antagonist (MK 801), while an NMDA partial agonist (D-cycloserine) decreased this behavior (Albelda et al., 2010). In this sense, the present results also contrast with the no effect found after ketamine administration in HD and LD rats on SIP (Martín-González et al., 2018). Though both ketamine and MEM typify the same kind of drugs, they diverge in voltage dependence and blocking kinetics (Danysz and Parsons, 1998). In human studies, MEM showed a therapeutic role in obsessive-compulsive patients, by reducing the Y-BOCS scores after chronic treatment with MEM during 8 weeks (Ghaleiha et al., 2013) and 12 weeks (Stewart et al., 2010; Haghighi et al., 2013). Other study investigating MEM augmentation of risperidone treatment in children with autism spectrum disorders revealed that the group receiving MEM showed significant improvements in the subscales: irritability, stereotypic behavior, and hyperactivity of the Aberrant Behavior Checklist-Community (Ghaleiha et al., 2013).

Our data showed that the administration of LAM, 15 and 30 mg/kg, significantly decreased compulsive water drinking in HD rats, compared to LD rats, on SIP. There are few preclinical studies on the behavioral effects of LAM, most of them related to as an anti-depressant like effect. The acute administration of LAM at 16 and 32 mg/kg of LAM induced a reduction in immobility time in the FST (Prica et al., 2008). Similarly, LAM at 15 and 30 mg/kg significantly reduced immobility in the FST (Li et al., 2010). In human studies, have evidenced that 16 weeks of treatment with LAM in obsessive-compulsive patients significantly reduced the Y-BOCS scores, as well as the Hamilton Rating Scale for Depression scores and the Clinical Global Impression-Severity scores (Bruno et al., 2012). More recently, two other studies using adjunctive treatment of LAM in addition to SRIs treatment led in treatment-resistant OCD patients during 8 and 12 weeks respectively, revealed a greater reduction in total YBOCS scores in LAM group (Hussain et al., 2015; Khalkhali et al., 2016).

Collectively, the beneficial effects of MEM and LAM administration in reducing compulsive drinking in HD rats on SIP suggest a therapeutic role for glutamate inhibition, antagonizing NMDA receptor or blocking calcium and sodium channels in pre-synaptic terminals. In contrast, the lack of effect of NAC in compulsive intake in HD rats on SIP posits the idea of the possible relevance of the differential effect by the specific stimulation of the presynaptic terminal. These results support the possible dysregulation in glutamatergic signal previously observed, in which HD rats selected by SIP showed a decreased basal level of glutamate in the medial prefrontal cortex (mPFC), restored by serotonin 5-$HT_{2A/C}$ agonist DOI (Mora et al., 2018). Moreover, the effects of glutamatergic drugs MEM and LAM suggest a possible modulatory role in the neuroanatomic and neurochemical alterations observed in dopamine D_2 receptors and 5-HT_{2A} receptors in HD rats selected by SIP (Pellón et al., 2011; Moreno et al., 2012; Mora et al., 2018).

Preclinical studies on compulsivity, using the dopamine D_2 and D_3 receptor agonist quinpirole (QNP) in rats (Szechtman et al., 1998), have also evidenced a dysregulation by an increased glutamate release in the subtantia nigra and a lower extracellular concentration in the nucleus accumbens (Abarca et al., 1995; Krügel et al., 2004; Escobar et al., 2015). Therefore, the proposed underlying mechanism in compulsivity of the QNP-OCD model was associated with decreased dopaminergic and glutamatergic neurotransmission in the mPFC to the nucleus accumbens, pointing toward a loss of executive control (Escobar et al., 2015). Furthermore, NMDA dependent glutamate neurotransmission in the cortico-striatal circuitry seems to play a central role by the functional interaction with serotonin and dopamine receptors in executive response control and compulsivity measured by the 5-CSRT (reviewed in Carli and Invernizzi, 2014). In example, the local infusions of NMDA receptor antagonist 3-((R)-2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid ((R)-CPP) in the mPFC and also in the infralimbic cortex impaired accuracy and increased premature and perseverative responding, raising glutamate, dopamine, and GABA release in the dorsomedial striatum (Pozzi et al., 2011; Murphy et al., 2011; Agnoli et al., 2013). Similarly, in OCD patients, a dysregulation of glutamatergic signaling in the cortico-striatal circuitry has been suggested, with decreased concentrations of glutamate in the anterior cingulate cortex, accompanied by overactivity of the glutamate signaling in the striatum and orbitofrontal cortex (Pittenger et al., 2011; Ting and Feng, 2011; Milad and Rauch, 2012). Other authors proposed that the beneficial effect of MEM in OCD patients could be mediated by functional disconnection of the hippocampus with critical frontal regions (Vlček et al., 2018), by its effect on decreasing glutamate level in the hippocampus (Glodzik et al., 2009). Finally, we could hypothesize that according to these results, a possible explanation under the differences in compulsive HD rats selected by SIP might be an altered function of glutamatergic NMDA receptors that affect firing in cortical neurons in mPFC and affect

glutamatergic, as well as dopaminergic and serotoninergic signal in the striatum.

CONCLUSION

The exploration of other possible comorbid behaviors in compulsive HD rats selected by SIP indicated a relation with another form of compulsivity, measured by marble burying, and an increased vulnerability to cued fear behavior showed by an increased percentage of freezing time on FC compared to LD rats. No differences were found in the assessment of the depressive behavior on FST, nor in anxious behavior on EPM, replicating previous results from our laboratory (López-Grancha et al., 2008). The acute administration of glutamatergic drugs on SIP revealed that MEM and LAM dose-dependently and selectively decreased compulsive intake in HD rats, and did not affect LD on SIP. However, NAC did not affect compulsive drinking on SIP. These differences might be due to the specific action of the drugs on the presynaptic terminal. Further studies might disentangle the specific implication of the fear learning component and the dysregulation in glutamatergic neurotransmission, and its relation with the dopamine $D_{2/3}$ and serotonergic 5-HT_{2A} receptors, in the mechanisms of vulnerability to compulsive behavior in HD rats on SIP.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of "the Spanish Royal Decree 53/2013 on the protection of experimental animals, the European Community Directive (2010/63/EU) for animal experiments." The protocol was approved by the University of Almería Animal Research Committee.

AUTHOR CONTRIBUTIONS

MM and PF designed research. ÁP-P, EM-G, SM and AM performed research. ÁP-P and EM-G analyzed data. ÁP-P and MM wrote the manuscript with the help of the other authors.

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REFERENCES

Abarca, J., Gysling, K., Roth, R. H., and Bustos, G. (1995). Changes in extracellular levels of glutamate and aspartate in rat substantia nigra induced by dopamine receptor ligands: *in vivo* microdialysis studies. *Neurochem. Res.* 20, 159–169. doi: 10.1007/bf00970540

Afshar, H., Roohafza, H., Mohammad-Beigi, H., Haghighi, M., Jahangard, L., Shokouh, P., et al. (2012). N-acetylcysteine add-on treatment in refractory obsessive-compulsive disorder: a randomized, double-blind, placebo-controlled trial. *J. Clin. Psychopharmacol.* 32, 797–803. doi: 10.1097/jcp.0b013e318272677d

Agarwal, V., Yaduvanshi, R., Arya, A., Gupta, P. K., and Sitholey, P. (2016). A study of phenomenology, psychiatric co-morbidities, social and adaptive functioning in children and adolescents with OCD. *Asian. J. Psychiatr.* 22, 69–73. doi: 10.1016/j.ajp.2016.04.005

Agnoli, L., Mainolfi, P., Invernizzi, R. W., and Carli, M. (2013). Dopamine D1-like and D2-like receptors in the dorsal striatum control different aspects of attentional performance in the five-choice serial reaction time task under a condition of increased activity of corticostriatal inputs. *Neuropsychopharmacology* 38, 701–714. doi: 10.1038/npp.2012.236

Alaux-Cantin, S., Buttolo, R., Houchi, H., Jeanblanc, J., and Naassila, M. (2015). Memantine reduces alcohol drinking but not relapse in alcohol-dependent rats. *Addict. Biol.* 20, 890–901. doi: 10.1111/adb.12177

Albelda, N., Bar-On, N., and Joel, D. (2010). The role of NMDA receptors in the signal attenuation rat model of obsessive-compulsive disorder. *Psychopharmacology* 210, 13–24. doi: 10.1007/s00213-010-1808-9

Alén, F., Orio, L., Gorriti, M. Á., de Heras, R. G., Ramírez-López, M. T., Pozo, M. Á., et al. (2013). Increased alcohol consumption in rats after subchronic antidepressant treatment. *Int. J. Neuropsychopharmacol.* 16, 1809–1818. doi: 10.1017/s1461145713000217

Alén, F., Serrano, A., Gorriti, M. Á., Pavón, F. J., Orio, L., de Heras, R. G., et al. (2014). The administration of atomoxetine during alcohol deprivation induces a time-limited increase in alcohol consumption after relapse. *Int. J. Neuropsychopharmacol.* 17, 1905–1910. doi: 10.1017/s1461145714000087x

American Psychiatric Association. (2013). *Guía de Consulta de los Criterios Diagnósticos del DSM-5®*. Washington, DC: American Psychiatric Publishing.

Angoa-Pérez, M., Kane, M. J., Briggs, D. I., Francescutti, D. M., and Kuhn, D. M. (2013). Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviors in mice. *J. Vis. Exp.* 82:50978. doi: 10.3791/50978

Baer, L., Trivedi, M. H., Huz, I., Rush, A. J., Wisniewski, S. R., and Fava, M. (2015). Prevalence and impact of obsessive-compulsive symptoms in depression. *J. Clin. Psychiatry* 76, 1668–1674. doi: 10.4088/JCP.14m09670

Baker, D. A., McFarland, K., Lake, R. W., Shen, H., Tang, X.-C., Toda, S., et al. (2003a). Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nat. Neurosci.* 6, 743–749. doi: 10.1038/nn1069

Baker, D. A., McFarland, K., Lake, R. W., Shen, H., Toda, S., and Kalivas, P. W. (2003b). N-acetyl cysteine-induced blockade of cocaine-induced reinstatement. *Ann. N Y Acad. Sci.* 1003, 349–351. doi: 10.1196/annals.1300.023

Belin-Rauscent, A., Daniel, M. L., Puaud, M., Jupp, B., Sawiak, S., Howett, D., et al. (2016). From impulses to maladaptive actions: the insula is a neurobiological gate for the development of compulsive behavior. *Mol. Psychiatry* 21, 491–499. doi: 10.1038/mp.2015.140

Berardi, A., Trezza, V., and Campolongo, P. (2012). Modeling specific phobias and posttraumatic stress disorder in rodents: the challenge to convey both cognitive and emotional features. *Rev. Neurosci.* 23, 645–657. doi: 10.1515/revneuro-2012-0054

Bruno, A., Micò, U., Pandolfo, G., Mallamace, D., Abenavoli, E., Di Nardo, F., et al. (2012). Lamotrigine augmentation of serotonin reuptake inhibitors in treatment-resistant obsessive-compulsive disorder: a double-blind, placebo-controlled study. *J. Psychopharmacol.* 26, 1456–1462. doi: 10.1177/0269881111431751

Cardona, D., López-Crespo, G., Sánchez-Amate, M. C., Flores, P., and Sánchez-Santed, F. (2011). Impulsivity as long-term sequelae after chlorpyrifos intoxication: time course and individual differences. *Neurotox. Res.* 19, 128–137. doi: 10.1007/s12640-009-9149-3

Carli, M., and Invernizzi, R. W. (2014). Serotoninergic and dopaminergic modulation of cortico-striatal circuit in executive and attention deficits induced by NMDA receptor hypofunction in the 5-choice serial reaction time task. *Front. Neural Circuits* 8:58. doi: 10.3389/fncir.2014.00058

Charntikov, S., Pittenger, S. T., Pudiak, C. M., and Bevins, R. A. (2018). The effect of N -acetylcysteine or bupropion on methamphetamine self-administration and methamphetamine-triggered reinstatement of female rats. *Neuropharmacology* 135, 487–495. doi: 10.1016/j.neuropharm.2018.03.021

Cheung, H., Kamp, D., and Harris, E. (1992). An *in vitro* investigation of the action of lamotrigine on neuronal voltage-activated sodium channels. *Epilepsy Res.* 13, 107–112. doi: 10.1016/0920-1211(92)90065-2

Cunningham, M. O., and Jones, R. S. G. (2000). The anticonvulsant, lamotrigine decreases spontaneous glutamate release but increases spontaneous GABA release in the rat entorhinal cortex *in vitro*. *Neuropharmacology* 39, 2139–2146. doi: 10.1016/s0028-3908(00)00051-4

Cunningham-Williams, R. M., and Cottler, L. B. (2001). The epidemiology of pathological gambling. *Semin. Clin. Neuropsychiatry* 6, 155–166. doi: 10.1053/scnp.2001.22919

Curzon, P., Rustay, N. R., and Browman, K. E. (2009). "Cued and contextual fear conditioning for rodents," in *Methods of Behavior Analysis in Neuroscience*, ed. J. J. Buccafusco (Boca Raton, FL: CRC Press/Taylor and Francis), 19–37.

Danysz, W., and Parsons, C. G. (1998). Glycine and N-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. *Pharmacol. Rev.* 50, 597–664.

de Brouwer, G., and Wolmarans, D. W. (2018). Back to basics: a methodological perspective on marble-burying behavior as a screening test for psychiatric illness. *Behav. Processes* 157, 590–600. doi: 10.1016/j.beproc.2018.04.011

de Leon, J., Tracy, J., McCann, E., and McGrory, A. (2002). Polydipsia and schizophrenia in a psychiatric hospital: a replication study. *Schizophr. Res.* 57, 293–301. doi: 10.1016/s0920-9964(01)00292-4

de Leon, J., Verghese, C., Tracy, J. I., Josiassen, R. C., and Simpson, G. M. (1994). Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. *Biol. Psychiatry* 35, 408–419. doi: 10.1016/0006-3223(94)90008-6

Didriksen, M., Olsen, G. M., and Christensen, A. V. (1993). Effect of clozapine upon schedule-induced polydipsia (SIP) resembles neither the actions of dopamine D1 nor D2 blockade. *Psychopharmacology* 113, 250–256. doi: 10.1007/bf02245706

Dong, J., Zhou, Q., Wei, Z., Yan, S., Sun, F., and Cai, X. (2018). Protein kinase A mediates scopolamine-induced mTOR activation and an antidepressant response. *J. Affect. Disord.* 227, 633–642. doi: 10.1016/j.jad.2017.11.041

Dundas, B., Harris, M., and Narasimhan, M. (2007). Psychogenic polydipsia review: etiology, differential and treatment. *Curr. Psychiatry Rep.* 9, 236–241. doi: 10.1007/s11920-007-0025-7

Dwyer, J. M., Platt, B. J., Sukoff Rizzo, S. J., Pulicicchio, C. M., Wantuch, C., Zhang, M. Y., et al. (2010). Preclinical characterization of BRL 44408: antidepressant- and analgesic-like activity through selective α2A-adrenoceptor antagonism. *Int. J. Neuropsychopharmacol.* 13, 1193–1205. doi: 10.1017/s1461145709991088

Egashira, N., Okuno, R., Harada, S., Matsushita, M., Mishima, K., Iwasaki, K., et al. (2008). Effects of glutamate-related drugs on marble-burying behavior in mice: implications for obsessive-compulsive disorder. *Eur. J. Pharmacol.* 586, 164–170. doi: 10.1016/j.ejphar.2008.01.035

Escher, T., Call, S. B., Blaha, C. D., and Mittleman, G. (2006). Behavioral effects of aminoadamantane class NMDA receptor antagonists on schedule-induced alcohol and self-administration of water in mice. *Psychopharmacology* 187, 424–434. doi: 10.1007/s00213-006-0465-5

Escobar, A. P., Cornejo, F. A., Olivares-Costa, M., González, M., Fuentealba, J. A., Gysling, K., et al. (2015). Reduced dopamine and glutamate neurotransmission in the nucleus accumbens of quinpirole-sensitized rats hints at inhibitory D2 autoreceptor function. *J. Neurochem.* 134, 1081–1090. doi: 10.1111/jnc.13209

Evenson, R. C., Jos, C. J., and Mallya, A. R. (1987). Prevalence of polydipsia among public psychiatric patients. *Psychol. Rep.* 60, 803–807. doi: 10.2466/pr0.1987.60.3.803

Falk, J. (1961). Production of polydipsia in normal rats by an intermittent food schedule. *Science* 133, 195–196. doi: 10.1126/science.133.3447.195

Falk, J. (1966). Schedule-induced polydipsia as a function of fixed interval length. *J. Exp. Anal. Behav.* 9, 37–39. doi: 10.1901/jeab.1966.9-37

Fineberg, N. A., Apergis-Schoute, A. M., Vaghi, M. M., Banca, P., Gillan, C. M., Voon, V., et al. (2018). Mapping compulsivity in the DSM-5 obsessive compulsive and related disorders: cognitive domains, neural circuitry and treatment. *Int. J. Neuropsychopharmacol.* 21, 42–58. doi: 10.1093/ijnp/pyx088

Fineberg, N. A., Chamberlain, S. R., Goudriaan, A. E., Stein, D. J., Vanderschuren, L. J. M. J., Gillan, C. M., et al. (2014). New developments in human neurocognition: clinical, genetic and brain imaging correlates of impulsivity and compulsivity. *CNS Spectr.* 19, 69–89. doi: 10.1017/s1092852913000801

Fineberg, N. A., and Gale, T. M. (2005). Evidence-based pharmacotherapy of obsessive-compulsive disorder. *Int. J. Neuropsychopharmacol.* 8, 107–129. doi: 10.1017/S1461145704004675

Fineberg, N. A., Potenza, M. N., Chamberlain, S. R., Berlin, H. A., Menzies, L., Bechara, A., et al. (2010). Probing compulsive and impulsive behaviors, from animal models to endophenotypes: a narrative review. *Neuropsychopharmacology* 35, 591–604. doi: 10.1038/npp.2009.185

Flores, Á., Fullana, M. A., Soriano-Mas, C., and Andero, R. (2018). Lost in translation: how to upgrade fear memory research. *Mol. Psychiatry* 23, 2122–2132. doi: 10.1038/s41380-017-0006-0

Geller, D. A., McGuire, J. F., Orr, S. P., Pine, D. S., Britton, J. C., Small, B. J., et al. (2017). Fear conditioning and extinction in pediatric obsessive-compulsive disorder. *Ann. Clin. Psychiatry* 29, 17–26.

Ghaleiha, A., Entezari, N., Modabbernia, A., Najand, B., Askari, N., Tabrizi, M., et al. (2013). Memantine add-on in moderate to severe obsessive-compulsive disorder: randomized double-blind placebo-controlled study. *J. Psychiatr. Res.* 47, 175–180. doi: 10.1016/j.jpsychires.2012.09.015

Gillan, C. M., Fineberg, N. A., and Robbins, T. W. (2017). A trans-diagnostic perspective on obsessive-compulsive disorder. *Psychol. Med.* 47, 1528–1548. doi: 10.1017/S0033291716002786

Glodzik, L., De Santi, S., Rich, K. E., Brys, M., Pirraglia, E., Mistur, R., et al. (2009). Effects of memantine on cerebrospinal fluid biomarkers of neurofibrillary pathology. *J. Alzheimers Dis.* 18, 509–513. doi: 10.3233/JAD-2009-1183

Grant, J. E., Redden, S. A., Leppink, E. W., Odlaug, B. L., and Chamberlain, S. R. (2016). Psychosocial dysfunction associated with skin picking disorder and trichotillomania. *Psychiatry Res.* 239, 68–671. doi: 10.1016/j.psychres.2016.03.004

Haghighi, M., Jahangard, L., Mohammad-Beigi, H., Bajoghli, H., Hafezian, H., Rahimi, A., et al. (2013). In a double-blind, randomized and placebo-controlled trial, adjuvant memantine improved symptoms in inpatients suffering from refractory obsessive-compulsive disorders (OCD). *Psychopharmacology* 228, 633–640. doi: 10.1007/s00213-013-3067-z

Hawken, E. R., and Beninger, R. J. (2014). The amphetamine sensitization model of schizophrenia symptoms and its effect on schedule-induced polydipsia in the rat. *Psychopharmacology* 231, 2001–2008. doi: 10.1007/s00213-013-3345-9

Higley, J., Hasert, M., Suomi, S., and Linnoila, M. (1998). The serotonin reuptake inhibitor sertraline reduces excessive alcohol consumption in nonhuman primates: effect of stress. *Neuropsychopharmacology* 18, 431–443. doi: 10.1016/s0893-133x(97)00180-2

Hogg, S., and Dalvi, A. (2004). Acceleration of onset of action in schedule-induced polydipsia: combinations of SSRI and 5-HT1A and 5-HT1B receptor antagonists. *Pharmacol. Biochem. Behav.* 77, 69–75. doi: 10.1016/j.pbb.2003.09.020

Hurley, M. M., Resch, J. M., Maunze, B., Frenkel, M. M., Baker, D. A., and Choi, S. (2016). N-acetylcysteine decreases binge eating in a rodent model. *Int. J. Obes.* 40, 1183–1186. doi: 10.1038/ijo.2016.31

Hussain, A., Dar, M. A., Wani, R. A., Shah, M. S., Jan, M. M., Malik, Y. A., et al. (2015). Role of lamotrigine augmentation in treatment-resistant obsessive compulsive disorder: a retrospective case review from South Asia. *Indian J. Psychol. Med.* 37, 154–158. doi: 10.4103/0253-7176.155613

Iftene, F., Bowie, C., Milev, R., Hawken, E., Talikowska-Szymczak, E., Potopsingh, D., et al. (2013). Identification of primary polydipsia in a severe and persistent mental illness outpatient population: a prospective observational study. *Psychiatry Res.* 210, 679–683. doi: 10.1016/j.psychres.2013.04.011

Javitt, D. C., Schoepp, D., Kalivas, P. W., Volkow, N. D., Zarate, C., Merchant, K., et al. (2011). Translating glutamate: from pathophysiology to treatment. *Sci. Transl. Med.* 3:102mr2. doi: 10.1126/scitranslmed.3002804

Joel, D., and Avisar, A. (2001). Excessive lever pressing following post-training signal attenuation in rats: a possible animal model of obsessive compulsive disorder? *Behav. Brain Res.* 123, 77–87. doi: 10.1016/s0166-4328(01)00201-7

Khalkhali, M., Aram, S., Zarrabi, H., Kafie, M., and Heidarzadeh, A. (2016). Lamotrigine augmentation versus placebo in serotonin reuptake inhibitors-resistant obsessive-compulsive disorder: a randomized controlled trial. *Iran. J. Psychiatry* 11, 104–114.

Krügel, U., Schraft, T., Regenthal, R., Illes, P., and Kittner, H. (2004). Purinergic modulation of extracellular glutamate levels in the nucleus accumbens *in vivo*. *Int. J. Dev. Neurosci.* 22, 565–570. doi: 10.1016/j.ijdevneu.2004.07.009

Lafleur, D. L., Pittenger, C., Kelmendi, B., Gardner, T., Wasylink, S., Malison, R. T., et al. (2006). N-acetylcysteine augmentation in serotonin reuptake inhibitor refractory obsessive-compulsive disorder. *Psychopharmacology* 184, 254–256. doi: 10.1007/s00213-005-0246-6

Lebourgeois, S., González-Marín, M. C., Jeanblanc, J., Naassila, M., and Vilpoux, C. (2018). Effect of N-acetylcysteine on motivation, seeking and relapse to ethanol self-administration. *Addict. Biol.* 23, 643–652. doi: 10.1111/adb.12521

Li, N., He, X., Qi, X., Zhang, Y., and He, S. (2010). The mood stabilizer lamotrigine produces antidepressant behavioral effects in rats: role of brain-derived neurotrophic factor. *J. Psychopharmacol.* 24, 1772–1778. doi: 10.1177/0269881109359102

López-Grancha, M., Lopez-Crespo, G., Sanchez-Amate, M. C., and Flores, P. (2008). Individual differences in schedule-induced polydipsia and the role of gabaergic and dopaminergic systems. *Psychopharmacology* 197, 487–498. doi: 10.1007/s00213-007-1059-6

Lu, C. W., Lin, T. Y., and Wang, S. J. (2010). Memantine depresses glutamate release through inhibition of voltage-dependent Ca^{2+} entry and protein kinase C in rat cerebral cortex nerve terminals: an NMDA receptor-independent mechanism. *Neurochem. Int.* 57, 168–176. doi: 10.1016/j.neuint.2010.05.010

Marinova, Z., Chuang, D.-M., and Fineberg, N. (2017). Glutamate-modulating drugs as a potential therapeutic strategy in obsessive-compulsive disorder. *Curr. Neuropharmacol.* 15, 977–995. doi: 10.2174/1570159X15666170320104237

Martijena, I. D., Bustos, S. G., Bertotto, M. E., and Molina, V. A. (2005). Antidepressants attenuate both the enhanced ethanol intake and ethanol-induced anxiolytic effects in diazepam withdrawn rats. *Eur. Neuropsychopharmacol.* 15, 119–130. doi: 10.1016/j.euroneuro.2004.05.009

Martín-González, E., Prados-Pardo, Á., Mora, S., Flores, P., and Moreno, M. (2018). Do psychoactive drugs have a therapeutic role in compulsivity? Studies on schedule-induced polydipsia. *Psychopharmacology* 235, 419–432. doi: 10.1007/s00213-017-4819-y

Menzies, L., Chamberlain, S. R., Laird, A. R., Thelen, S. M., Sahakian, B. J., and Bullmore, E. T. (2008). Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited. *Neurosci. Biobehav. Rev.* 32, 525–549. doi: 10.1016/j.neubiorev.2007.09.005

Merchán, A., Mora, S., Gago, B., Rodriguez-Ortega, E., Fernández-Teruel, A., Puga, J. L., et al. (2019). Excessive habit formation in schedule-induced polydipsia: microstructural analysis of licking among rat strains and involvement of the orbitofrontal cortex. *Genes Brain Behav.* 18:e12489. doi: 10.1111/gbb.12489

Milad, M. R., and Rauch, S. L. (2012). Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways. *Trends Cogn. Sci.* 16, 43–51. doi: 10.1016/j.tics.2011.11.003

Mitra, S., Bastos, C. P., Bates, K., Pereira, G. S., and Bult-Ito, A. (2016). Ovarian sex hormones modulate compulsive, affective and cognitive functions in a non-induced mouse model of obsessive-compulsive disorder. *Front. Behav. Neurosci.* 10:215. doi: 10.3389/fnbeh.2016.00215

Mittleman, G., Rosner, A. L., and Schaub, C. L. (1994). Polydipsia and dopamine: behavioral effects of dopamine D1 and D2 receptor agonists and antagonists. *J. Pharmacol. Exp. Ther.* 271, 638–650.

Monaghan, D. T., Yao, D., and Cotman, C. W. (1985). L-[^{3H}]Glutamate binds to kainate-, NMDA- and AMPA-sensitive binding sites: an autoradiographic analysis. *Brain Res.* 340, 378–383. doi: 10.1016/0006-8993(85)90936-9

Mora, S., Merchán, A., Vilchez, O., Aznar, S., Klein, A. B., Ultved, L., et al. (2018). Reduced cortical serotonin 5-HT2A receptor binding and glutamate activity in high compulsive drinker rats. *Neuropharmacology* 143, 10–19. doi: 10.1016/j.neuropharm.2018.09.004

Moran, M. M., McFarland, K., Melendez, R. I., Kalivas, P. W., and Seamans, J. K. (2005). Cystine/glutamate exchange regulates metabotropic glutamate receptor presynaptic inhibition of excitatory transmission and vulnerability to cocaine seeking. *J. Neurosci.* 25, 6389–6393. doi: 10.1523/JNEUROSCI.1007-05.2005

Moreno, M., and Flores, P. (2012). Schedule-induced polydipsia as a model of compulsive behavior: neuropharmacological and neuroendocrine bases. *Psychopharmacology* 219, 647–659. doi: 10.1007/s00213-011-2570-3

Moreno, M., Gutiérrez-Ferre, V. E., Ruedas, L., Campa, L., Suñol, C., and Flores, P. (2012). Poor inhibitory control and neurochemical differences in high compulsive drinker rats selected by schedule-induced polydipsia. *Psychopharmacology* 219, 661–672. doi: 10.1007/s00213-011-2575-y

Murphy, E. R., Fernando, A. B., Urcelay, G. P., Robinson, E. S., Mar, A. C., Theobald, D. E., et al. (2011). Impulsive behavior induced by both NMDA receptor antagonism and GABAA receptor activation in rat ventromedial prefrontal cortex. *Psychopharmacology* 219, 401–410. doi: 10.1007/s00213-011-2572-1

Naranjo, C. A., Sellers, E. M., and Lawrin, M. O. (1986). Modulation of ethanol intake by serotonin uptake inhibitors. *J. Clin. Psychiatry* 47, 16–22.

Navarro, S. V., Alvarez, R., Colomina, M. T., Sanchez-Santed, F., Flores, P., and Moreno, M. (2017). Behavioral biomarkers of schizophrenia in high drinker rats: a potential endophenotype of compulsive neuropsychiatric disorders. *Schizophr. Bull.* 43, 778–787. doi: 10.1093/schbul/sbw141

Niciu, M. J., Henter, I. D., Luckenbaugh, D. A., Zarate, C. A. Jr., and Charney, D. S. (2014). Glutamate receptor antagonists as fast-acting therapeutic alternatives for the treatment of depression: ketamine and other compounds. *Annu. Rev. Pharmacol. Toxicol.* 54, 119–139. doi: 10.1146/annurev-pharmtox-011613-135950

Paydary, K., Akamaloo, A., Ahmadipour, A., Pishgar, F., Emamzadehfard, S., and Akhondzadeh, S. (2016). N-acetylcysteine augmentation therapy for moderate-to-severe obsessive-compulsive disorder: randomized, double-blind, placebo-controlled trial. *J. Clin. Pharm. Ther.* 41, 214–219. doi: 10.1111/jcpt.12370

Pellón, R., Ruíz, A., Moreno, M., Claro, F., Ambrosio, E., and Flores, P. (2011). Individual differences in schedule-induced polydipsia: neuroanatomical dopamine divergences. *Behav. Brain Res.* 217, 195–201. doi: 10.1016/j.bbr.2010.10.010

Pellow, S., Chopin, P., File, S. E., and Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* 14, 149–167. doi: 10.1016/0165-0270(85)90031-7

Pereira, V. S., Casarotto, P. C., Hiroaki-Sato, V. A., Sartim, A. G., Guimarães, F. S., and Joca, S. R. L. (2013). Antidepressant- and anticompulsive-like effects of purinergic receptor blockade: involvement of nitric oxide. *Eur. Neuropsychopharmacol.* 23, 1769–1778. doi: 10.1016/j.euroneuro.2013.01.008

Phillips, R. G., and LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* 106, 274–285. doi: 10.1037/0735-7044.106.2.274

Pittenger, C., Bloch, M. H., and Williams, K. (2011). Glutamate abnormalities in obsessive compulsive disorder: neurobiology, pathophysiology, and treatment. *Pharmacol. Ther.* 132, 314–332. doi: 10.1016/j.pharmthera.2011.09.006

Pittenger, C., Krystal, J. H., and Coric, V. (2005). Initial evidence of the beneficial effects of glutamate-modulating agents in the treatment of self-injurious behavior associated with borderline personality disorder. *J. Clin. Psychiatry* 66, 1492–1493. doi: 10.4088/jcp.v66n1121d

Platt, B., Beyer, C. E., Schechter, L. E., and Rosenzweig-Lipson, S. (2008). Schedule-induced polydipsia: a rat model of obsessive-compulsive disorder. *Curr. Protoc. Neurosci.* 9:9.27. doi: 10.1002/0471142301.ns0927s43

Porsolt, R. D., Le Pichon, M., and Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266, 730–732. doi: 10.1038/266730a0

Pozzi, L., Baviera, M., Sacchetti, G., Calcagno, E., Balducci, C., Invernizzi, R. W., et al. (2011). Attention deficit induced by blockade of N-methyl d-aspartate receptors in the prefrontal cortex is associated with enhanced glutamate release and cAMP response element binding protein phosphorylation: role of metabotropic glutamate receptors 2/3. *Neuroscience* 176, 336–348. doi: 10.1016/j.neuroscience.2010.11.060

Prabhavalkar, K. S., Poovanpallil, N. B., and Bhatt, L. K. (2015). Management of bipolar depression with lamotrigine: an antiepileptic mood stabilizer. *Front. Pharmacol.* 6:242. doi: 10.3389/fphar.2015.00242

Prica, C., Hascoet, M., and Bourin, M. (2008). Antidepressant-like effect of lamotrigine is reversed by veratrine: a possible role of sodium channels in bipolar depression. *Behav. Brain Res.* 191, 49–54. doi: 10.1016/j.bbr.2008.03.007

Price, R. B., Nock, M. K., Charney, D. S., and Mathew, S. J. (2009). Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol. Psychiatry* 66, 522–526. doi: 10.1016/j.biopsych.2009.04.029

Redden, S. A., Leppink, E. W., and Grant, J. E. (2015). Clinical and cognitive correlates of young adult at-risk gamblers with and without depression. *Ann. Clin. Psychiatry* 27, 261–266.

Reimer, A. E., de Oliveira, A. R., Diniz, J. B., Hoexter, M. Q., Chiavegatto, S., and Brandão, M. L. (2015). Rats with differential self-grooming expression in the elevated plus-maze do not differ in anxiety-related behaviors. *Behav. Brain Res.* 292, 370–380. doi: 10.1016/j.bbr.2015.06.036

Reisberg, B., Doody, R., Stöffler, A., Schmitt, F., Ferris, S., Möbius, H. J., et al. (2003). Memantine in moderate-to-severe Alzheimer's disease. *N. Engl. J. Med.* 348, 1333–1341. doi: 10.1056/NEJMoa013128

Réus, G. Z., Stringari, R. B., Kirsch, T. R., Fries, G. R., Kapczinski, F., Roesler, R., et al. (2010). Neurochemical and behavioral effects of acute and chronic memantine administration in rats: further support for NMDA as a new pharmacological target for the treatment of depression? *Brain Res. Bull.* 81, 585–589. doi: 10.1016/j.brainresbull.2009.11.013

Rickelt, J., Viechtbauer, W., Lieverse, R., Overbeek, T., van Balkom, A. J., van Oppen, P., et al. (2016). The relation between depressive and obsessive-compulsive symptoms in obsessive-compulsive disorder: results from a large, naturalistic follow-up study. *J. Affect. Disord.* 203, 241–247. doi: 10.1016/j.jad.2016.06.009

Rodriguez, M. M., Overshiner, C., Leander, J. D., Li, X., Morrow, D., Conway, R. G., et al. (2017). Behavioral effects of a novel benzofuranyl-piperazine serotonin-2C receptor agonist suggest a potential therapeutic application in the treatment of obsessive-compulsive disorder. *Front. Psychiatry* 8:89. doi: 10.3389/fpsyt.2017.00089

Sela, V. R., Hattanda, I., Albrecht, C. M., De Almeida, C. B., Obici, S., Cortez, D. A., et al. (2010). Effect of xanthone from Kielmeyera coriacea stems on serotonergic neurons of the median raphe nucleus. *Phytomedicine* 17, 274–278. doi: 10.1016/j.phymed.2009.07.002

Simone, J. J., and McCormick, C. M. (2017). Intracellular signaling and plasma hormone profiles associated with the expression of unconditioned and conditioned fear and anxiety in female rats. *Physiol. Behav* 169, 234–244. doi: 10.1016/j.physbeh.2016.12.002

Snodgrass, S. H., and Allen, J. D. (1989). Time-response effects of pimozide on operant behavior and schedule-induced polydipsia. *Pharmacol. Biochem. Behav.* 32, 949–955. doi: 10.1016/0091-3057(89)90064-6

Stewart, S. E., Jenike, E. A., Hezel, D. M., Stack, D. E., Dodman, N. H., Shuster, L., et al. (2010). A single-blinded case-control study of memantine in severe obsessive-compulsive disorder. *J. Clin. Psychopharmacol.* 30, 34–39. doi: 10.1097/jcp.0b013e3181c856de

Ströhle, A., Gensichen, J., and Domschke, K. (2018). The diagnosis and treatment of anxiety disorders. *Dtsch. Arztebl. Int.* 155, 611–620. doi: 10.3238/arztebl.2018.0611

Szechtman, H., Sulis, W., and Eilam, D. (1998). Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD). *Behav. Neurosci.* 112, 1475–1485. doi: 10.1037//0735-7044.112.6.1475

Taylor, G. T., Lerch, S., and Chourbaji, S. (2017). Marble burying as compulsive behaviors in male and female mice. *Acta Neurobiol. Exp.* 77, 254–260. doi: 10.21307/ane-2017-059

Ting, J. T., and Feng, G. (2011). Neurobiology of obsessive-compulsive disorder: insights into neural circuitry dysfunction through mouse genetics. *Curr. Opin. Neurobiol.* 21, 842–848. doi: 10.1016/j.conb.2011.04.010

Torres, A. R., Ferrão, Y. A., Shavitt, R. G., Diniz, J. B., Costa, D. L. C., Rosário, M. C., et al. (2014). Panic disorder and agoraphobia in OCD patients: clinical profile and possible treatment implications. *Compr. Psychiatry* 55, 588–597. doi: 10.1016/j.comppsych.2013.11.017

Torres, A. R., Fontenelle, L. F., Shavitt, R. G., Ferrão, Y. A., do Rosário, M. C., Storch, E. A., et al. (2016). Comorbidity variation in patients with obsessive-compulsive disorder according to symptom dimensions: results from a large multicentre clinical sample. *J. Affect. Disord.* 190, 508–516. doi: 10.1016/j.jad.2015.10.051

Vazdarjanova, A., and McGaugh, J. L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. *Proc. Natl. Acad. Sci. U S A* 95, 15003–15007. doi: 10.1073/pnas.95.25.15003

Vlček, P., Polák, J., Brunovský, M., and Horáček, J. (2018). Role of glutamatergic system in obsessive-compulsive disorder with possible therapeutic implications. *Pharmacopsychiatry* 51, 229–242. doi: 10.1055/s-0043-118665

Wald, R., Dodman, N., and Shuster, L. (2009). The combined effects of memantine and fluoxetine on an animal model of obsessive compulsive disorder. *Exp. Clin. Psychopharmacol.* 17, 191–197. doi: 10.1037/a0016402

Walf, A. A., and Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat. Protoc.* 2, 322–328. doi: 10.1038/nprot.2007.44

WHO. (2018). *Global Health Observatory (GHO) Data.* World Health Organization. Available online at: https://www.who.int/gho/mortality_burden_disease/en/. [Accessed on January 20, 2019].

Xie, X., Lancaster, B., Peakman, T., and Garthwaite, J. (1995). Interaction of the antiepileptic drug lamotrigine with recombinant rat brain type IIA Na^+ channels and with native Na^+ channels in rat hippocampal neurons. *Pflugers Arch.* 430, 437–446. doi: 10.1007/bf00373920

Yadin, E., Friedman, E., and Bridger, W. H. (1991). Spontaneous alternation behavior: an animal model for obsessive-compulsive disorder? *Pharmacol. Biochem. Behav.* 40, 311–315. doi: 10.1016/0091-3057(91)90559-k

Yan, H.-C., Cao, X., Das, M., Zhu, X.-H., and Gao, T.-M. (2010). Behavioral animal models of depression. *Neurosci. Bull.* 26, 327–337. doi: 10.1007/s12264-010-0323-7

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Reduced expression of the *Htr2a*, *Grin1*, and *Bdnf* genes and cognitive inflexibility in a model of high compulsive rats

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Abstract

Compulsivity is a core symptom in different psychopathological disorders, characterized by excessive behaviors and behavioral inflexibility. In preclinical studies, the selection of high drinker (HD) versus low drinker (LD) rats by schedule-induced polydipsia (SIP) is a valid model of compulsivity. HD rats shows a compulsive phenotype with a reduction in serotonin 2A (5-HT2A) receptor binding levels in the frontal cortex (FC). However, is necessary further explore the cognitive components regarding inflexibility in the compulsive HD rats, as well as, the related brain gene expression. Here, first, we assessed spatial memory and cognitive inflexibility by Morris Water Maze (MWM), working and reference memory by Radial Arm Maze, and behavioral deficits in stimulus processing by novel object recognition test. Second, we analyed the genetic expression of 5HT2A, 5HT2C, glutamate NMDA receptors, and brain-derived neurotrophic factor (BDNF) in FC, hippocampus, and amygdala. HD rats confirmed a cognitive inflexibility profile in the reversal condition in MWM compared to LD rats, while no differences were observed in stimulus processing, spatial and working memory. Moreover, HD rats showed a reduced expression of the *Htr2a, Grin1*, and *Bdnf* genes in FC. Furthermore, there was a negative correlation between the relative expression of the *Htr2a*, *Grin1* and *Bdnf* genes in FC and the level of compulsive water intake in HD rats on SIP. These data reveal that cognitive inflexibility may not be associated with memory or stimulus processing alterations, but may result from a region-specific alteration on FC of the gene expression *Htr2a*, *Grin1*, and *Bdnf* in compulsive individuals.

Keywords

Compulsivity; Schedule-induced polydipsia; Cognitive inflexibility; *Htr2a, Grin1* and *Bdnf;* Frontal cortex.

Introduction

Compulsivity is defined as actions inappropriate to the situation that persist, have no obvious relationship to the overall goal, and often result in undesirable consequences (1). It is one of the principal symptoms of obsessive-compulsive disorder (OCD), which affects 2%–3% of the population and is ranked among the ten leading neuropsychiatric causes of disability (2). It is associated with other disorders such as anorexia nervosa, trichotillomania, and excoriation disorder, but is also present in depression, bipolar disorder, schizophrenia, eating disorders, and addiction (3). Thus, meta-analyses identify at least 5 different endophenotypes of OCD due to the symptomatic heterogeneity of these groups of patients, suggesting that OCD could be a consequence of dysfunctional circuits that regulate response inhibition, cognitive flexibility, planning (and goal-directed behavior), working memory, and error monitoring (1). According to the clinical data, OCD patients present behavioral inflexibility in the Wisconsin Card Sorting Test and persistent skin conductance response in the extinction phase of the fear conditioning test (4,5). However, memory deficits in OCD has not been disentangled (6).

Schedule-induced polydipsia (SIP), a model of compulsive behavior (7) recently reviewed (8) has been demonstrated to be effective in the study of different related disorders such as schizophrenia (9), alcohol intake (10,11) and OCD (12). SIP is characterized by the development of an adjunctive behavior of excessive drinking in food-deprived animals exposed to intermittent food-reinforcement schedules (13). After the SIP procedure, rats can be divided into two different populations according to their levels of water intake: high drinkers (HD) considered as high compulsive, and low drinkers (LD) considered as noncompulsive rats. Compulsive HD rats have a well differentiated behavioral profile compared to non-compulsive LD rats characterized by deficits in cognitive inflexibility and maintenance of maladaptive memories shown by resistance to extinction, for example, an increased number of trials and perseverative errors in different protocols of the spatial Reversal Learning Task (14,15), increased number in perseverative responses under extinction conditions in the attentional 5-choice serial reaction time task (5-CSRTT) (16), long-lasting in extinction in fear conditioning (17) and passive avoidance (18). Here, we wanted to further delve into these observations and investigate if compulsive HD rats' behavioral inflexibility is mediated by memory impairments, so we decided to explore stimulus processing by the Novel Object Recognition test (NOR), reference and working memory by Radial Arm Maze (RAM), and cognitive flexibility by reversed Morris Water Maze (MWM).

The pharmacological treatment in patients with OCD is focused on selective serotonin reuptake inhibitors (SSRIs) (19). Nevertheless, up to 40% of patients with OCD do not respond successfully to this treatment and glutamate modulating drugs have been proposed as a potential treatment for OCD (20). Genome-wide association study has shown that vulnerability to OCD is associated with genetic polymorphisms in the serotonin transporter (*Sert*) and *Htr2a* (21). Moreover, recent studies have evidence that variations in glutamatergic genes such as the SAPAP (DLGAP) family, SLC1A1, and GRIN / GRIK proteins can lead to dysfunctional glutamate signaling in OCD [reviewed in (22)]. Many studies support that there is an association between brain derived neurotrophic factor (BDNF) gene polymorphism and OCD (23,24), reporting a lower BDNF serum level in OCD patients (25,26). The compulsive HD rats selected by SIP have shown reduced cortical 5-HT2A binding levels compared to LD rats (27). Indeed, the systemic and prefrontal administration of the serotonin 5-TH2A/C receptor agonist DOI reduced compulsive water intake in HD rats on SIP (27,28). Moreover, the systemic administration of glutamatergic drugs such as memantine, an uncompetitive NMDA receptor antagonist, and lamotrigine, which inhibits excitatory amino acid release such as glutamate; also reduced compulsive water intake in HD rats on SIP (17).

Based on these neuropharmacological studies and the behavioral profile of HD rats, the present study aimed to investigate the possible memory deficit associated with cognitive inflexibility in compulsive HD rats selected by SIP, as well as the alterations in gene expression that could contribute as underlying mechanisms of this specific trait. Therefore, after the selection of compulsive HD and noncompulsive LD rats on SIP: First, we evaluated possible behavioral deficits in memory-related tasks, such as spatial memory and cognitive inflexibility by MWM, working and reference memory by RAM, and stimulus processing by NOR. Second, we analyzed the genetic expression, related to memory and cognitive inflexibility, of the serotonergic *Htr2a* and *Htr2c*, glutamatergic *Grin1*, *Grin2a*, *Grin2b*, *Grin2c* and *Grm2* and *Bdnf* genes in the following neuroanatomical areas: frontal cortex (FC), hippocampus (HIP) and amygdala (AMY), related to the cortico-limbic circuit and compulsive drinking on SIP (29). The results are discussed regarding the relevance of memory deficit and cognitive inflexibility, and the alteration in the relative expression of the genes analyzed as possible biomarkers of vulnerability to compulsive spectrum disorders.

Materials and Methods

Animals

In this study 40 male Wistar rats (Envigo, RMS Spain) weighing approximately 230 to 250 g were used in this study; 20 for experiment 1 and 20 for experiment 2. The animals were housed in four rats/cages (50 × 35 × 20 cm) kept in a temperature controlled environment at 22 ± 2 °C, humidity (50 ± 10%), with a 12:12 h light-dark cycle (light off at 08:00 h) and food and water provided ad libitum. After ten days of habituation and before behavioral tasks, rats were gradually reduced to 85% of their free-feeding body weight through controlled feeding and their body weights were maintained at this level of deprivation throughout the experiment. The food was provided by daily feedings of lab food approximately 30 minutes (min) after each experimental session. All tests were performed between 9:00 am and 2:00 pm. The animals were around 3 months old when the experiment started and finished it with 5 months of age. All procedures were carried out following the Spanish Royal Decree 53/ 2013 on the protection of experimental animals, the European Community Directive 2010/63/EU for animal experiments and complies with the ARRIVE guidelines for animal research. The Animal Research Committee of the University of Almeria approved the experiments described here, and the authors declare that the research shows commitment to the 3Rs principle (replacement, reduction, refinement).

Behavioral selection: SIP procedure

The complete SIP procedure has been previously described (30). Rats were tested in eight operant SIP chambers (35 x 25 x 34 cm). Before the SIP procedure and over two consecutive days, the amount of water consumed by each rat in 60 min (baseline) was measured. There was unlimited access to a bottle of water and sixty food reward pellets were placed together (Noyes 45 mg dustless reward pellets; TSE Systems, Germany). The animals were then exposed to a fixed 60 seconds (FT-60 s) schedule of food pellet presentation in 60 min sessions. During each session, 100 mL of freshwater bottles were provided. After 20 daily sessions, the animals were separated into two specific populations, HD and LD, according to drinking rates (average of each animal over the last five sessions) above or below the group median, respectively. The following measures were recorded for each rat: a) the total amount of water (milliliters) removed from the bottle, b) the total number of licks to the bottle, and c) the total number of entries into the food magazine.

Experimental design

The order of behavioral assessment and gene expression analyses are summarized in Figure 1.

Experiment 1. Behavioral assessment. We examined cognitive flexibility, different types of memory, and stimulus processing, considered as possibly altered processes in compulsive populations (31), in HD and LD rats selected by SIP. We explored spatial memory and cognitive flexibility by carrying out MWM, assessed working and reference memory by RAM, and analyzed stimuli processing and novelty reactivity by NOR. The screening for each test commenced at least one week after the previous one.

Experiment 2. Gene expression. After the last session of SIP, all rats were sacrificed by rapid decapitation after induction of anesthesia by inhalation of 4% isoflurane, to extract the brain and obtain structure samples, by fresh dissection, which would later be subject to analyses: FC, HIP and AMY. The collected samples were immediately frozen on dry ice to prevent degradation of the RNA. The samples were then stored at -80 ° C until use. All the material used in this procedure was autoclaved (Class B P Selecta) and treated with ZAP RNA (Sigma Aldrich) to avoid contamination and degradation of the genetic material. The samples were then isolated, quantified and diluted to 100 ng/uL. This concentration was used for cDNA synthesis (20uL). Twenty microliters of that cDNA was then diluted (1:4 factor) and this dilution was finally used for the qPCR reaction.

Behavioral assessment

Morris water maze MWM

The MWM protocol used follows the guidelines defined by De Bruin (32), with minor changes. The water maze test was carried out in a black circular pool with an inner diameter of 150 cm and walls 34 cm high. It was filled with tap water to a depth of 30 cm. The water was at room temperature (22 ± 2 °C). The pool was divided into four quadrants of equal size: A, B, C, and D; with A opposite D and B opposite C. A removable circular escape platform (diameter: 10 cm) could be positioned at only one location in each of the four quadrants (in the middle of a quadrant, with the center 30 cm away from the wall). Two types of platform were used: an invisible one painted black, always 1.5 cm below the water surface, and a visible gray one, always 1.5 cm above the surface. Both platforms had a rough surface that provided enough grip for the animal to climb on top of it. Release sites were marked outside of the pool, each directly opposite to either of the four possible platform positions. The walls of the room were equipped with a variety of spatial cues that remained unchanged throughout the experiment. A video camera was used to record behavioral activities during transfer tests using Ethovision 3.1. (Noldus). Behavioral procedures: During a total of 9 days, the animals were trained and tested using the following:
Spatial training (days 1-4).The invisible white platform was placed for half of the animals in each group in quadrant B and for the other half in the opposite quadrant (C). Training was carried out in 4-trial sessions with each animal released into the pool from one of the four release sites. The sequence of the four release sites varied from session to session, but was identical for all animals within one session. The animal was released into the pool with its head facing the wall, and the time to reach the hidden platform was recorded with a stopwatch (escape latency). If the platform was not located within the maximum trial duration of 90 s, the animal was removed from the water and placed on the platform. In either case, the animal was left on top of the platform for 30 s. In between the successive trials of one session, the animal was placed in a black plastic bucket for a 30-s intertrial interval period. During

this period, fecal boluses (if present) were removed from the pool, and the transparent wall was wiped clean. Following the last trial of a session, the animal was dried with a cloth towel and placed in a clean cage. There were two sessions a day with an interval of approximately 3 h.

Reinstating memory (day 5)

On the afternoon of the fifth day, the animals were again subjected to a spatial training session with the platform in its original position.

Reversal test (days 6-7)

Following 2 days without behavioral training or testing, the reversal test began. The platform was now placed in the quadrant opposite to the one used during spatial training for two test sessions (each consisting of two reversal sessions, four trials per session); otherwise, all training procedures were identical to the ones described for spatial training.

Visually Cued task (days 8-9)

One day after the completion of reversal training, animals were subjected to the visually cued task. Instead of a white invisible platform, a grey visible platform was used, extending 1.5 cm above the surface. Although the release site of the animal remained the same (always opposite to the quadrant where the platform was during spatial training), the position of the platform varied from trial to trial. The sequence of these positions was the same for all animals. Otherwise, procedures were as described for the spatial training phase.

Radial Arm Maze RAM

The RAM protocol consists of three consecutive phases: habituation (2 days), learning (4 days maximal), and test (4 days), as described Fole in 2017 (33). Rats were trained every day, twice per day. Each rat was placed on the central platform and the maze could be visited for 10 min. Each rat was placed in the radial maze in a random order that changed every day. The radial maze was cleaned between each animal with diluted ethanol (70%) and absorb paper to minimize olfactory intra-maze cues. The performance of the animals was recorded on a computer. Data considered were arm entries; total trial time; first entry latency. With these data, the number of errors in working memory (WM) and reference memory (RM) were counted. Every entry in an already visited arm was considered a WM error. Entries in a non-rewarded arm were considered as RM errors. The behavioral procedure was as follows:

Habituation. This habituation allowed rats to adapt to the maze and to collect pellets at the end of their arms. All arms were kept baited during the trial period. On the first day, some pellets were placed along the arms to invite the animals to go to the end of the arms.

Acquisition. The animals performed 4 trials per session (2 sessions per day) until they reached the minimum values during three consecutive sessions. A trial was finished when: 10 min past or the animal visited the 8 arms at least one time. The criterion consisted in doing either no error for 8 entries or at maximum 1 error for 9 entries.

Test. This protocol aimed to test the memorization of the task. Rat performance was evaluated by the number of errors and the rank of the first error. An error was defined as the rat returning to a previously visited arm, i.e., crossing the first beam of the arm.

Novel object recognition test NOR

The NOR protocol (34) consists of three consecutive phases: habituation (day 1), acquisition (day 2), and test (day 2). Each rat was placed in the center of the arena and allowed to explore for 5 min. The arena was cleaned between each animal with water and absorbent paper to minimize intra-cue olfactory noise. The animal performance was recorded on a computer using Ethovision 3.1. (Noldus). The data considered were speed, mobility, percentage of time in contact with objects (time in contact with the

new or old object / total time), percentage of time near the objects (time near the new or old object / total time), and percentage of time in the neutral zone (time in the neutral zone / total time). Behavioral procedure was as follows:
Habituation. This habituation allowed accustoming the rats to the maze by free exploration of the arena for 5 min. No objects were placed in the arena in this phase.
Acquisition. 24 h after habituation, two identical objects were placed in opposite quadrants of the arena. The rats were placed in the center of the arena, equidistant from the two identical objects, and allowed to explore freely for 5 min. All sessions were recorded and analyzed.
Test. Two hours after the acquisition phase, one object used during acquisition (the familiar object) and one novel object were placed in the opposite quadrants of the arena. The animals were allowed to explore for 10 min. Sessions were recorded, and we analyzed the same variables as in the acquisition phase.

Gene Expression

Real-time Quantitative Polymerase Chain Reaction

Using this technique, a multitude of copies of a particular nucleotide sequence can be generated in vitro from a small amount of genetic material from structures that have been carefully extracted beforehand. Thus, before exponential replication of the sequences of interest, a series of processes had to be developed to enable the polymerase chain reaction itself, as well as its reliability. First, RNA was extracted from samples of the three structures (frontal cortex, hippocampus, and amygdala) and purified using trizol reagent (Invitrogen) according to the manufacturer's instructions. The trizol reagent reliably extracts and purifies RNA from the samples by maintaining RNA integration through inhibition of RNAase activity and destroying cellular components in the homogenization of samples. Thus, a separation of different layers was obtained by adding chloroform, which is in the aqueous layer where the RNA is located; therefore, the RNA pellet was collected and, by employing isopropyl alcohol and suitable centrifugation, the RNA pellet was precipitated. The supernatant was then removed and the RNA pellet was washed with 75% ethanol. Finally, the remaining ethanol was removed and allowed to dry. Once the process was completed, the final number of samples to be analyzed was obtained: 18 frontal cortex, 18 amygdala, and 18 hippocampus. Secondly, RNA quality assessment was carried out by electrophoresis. Electrophoresis is a technique that uses the polar character of the genetic material (negative charge given by the phosphate groups) to consequently move it from the negative pole to the positive pole on a solid matrix (agarose gel) when a certain voltage is applied. First, the agarose gel was prepared by dissolving agarose in Milli-Q water. The resulting mixture was heated until the agarose was perfectly dissolved. The solution was then poured into a gel holder. A comb installed in the gel holder shaped the wells where the samples were placed once the loading buffer was added to the samples and the gel was perfectly solidified and placed in the electrophoresis cuvette. The next step was the application of voltage that would cause the desired electrophoretic shift. To visualize the fragments, the gel had to be stained with an intercalating agent that binds to the genetic material. The marker used was ethidium bromide, which was handled with special caution because of its mutagenic nature. The last step was fluorescence spectrometry, whereby ultraviolet light is applied to cause the ethidium bromide to emit fluorescence, thus separating the fragments according to molecular weight. This process was repeated for the samples of each structure. Furthermore, RNA was quantified by fluorescence signaling with a Qubit® fluorometer (Life Technologies). This specific and sensitive process allowed us to know the concentration and quality, and to discard degradation and/or contamination of the samples. This process was repeated for the samples of each structure. The samples were then subjected to a Turbo DNA-free treatment in which the DNAases remove contaminating

genomic DNA from the preparations that will then be retrotranscribed. An inactivating reagent was used to stop the effect of DNAases. The removal of these contaminants allows for a PCR with less interference. Finally, complementary DNA (cDNA) was obtained from messenger RNA. Retrotransscription allows RNA to be used to obtain complementary DNA (cDNA) from a reverse transcriptase enzyme. In our case, cDNA was synthesized from DNA-free total RNA using the Maxima First Strand® cDNA synthesis kit (Thermo Scientific), using a mixture of random hexamers and 18-mer oligo (dT) as primers. This process was repeated for samples of each structure. The cDNA samples were stored at -80 ° C until the real-time quantitative polymerase chain reaction (qPCR) analysis.

Real-time qPCR

Gene expression analyses was performed by RT-qPCR using the SYBR Green PCR Master Mix kit on a Step-One real-time PCR system (Applied Biosystems) and a pair of specific primers for each gene analyzed (Table 1). The appropriate efficiency of the primers was controlled by serial dilutions (dilution factor 1:10). The Gapdh housekeeping gene was used as an internal reference for gene expression analyses. The absence of gDNA contamination in the RNA sample analyzed by RT-qPCR was demonstrated using a specific amplicon of an intron section of the Gapdh gene as a control. The melting curves were analyzed to ensure the specificity of the amplification. This process was repeated for samples of each structure.

Statistical Analyses

The SIP acquisition data were analyzed using a two-way repeated measures analysis of variance (ANOVA), with a between-subject factor (group: HD and LD) and a within-subject factor (session: 20 sessions). Experiment 1: The mean latency and speed in MWM, the speed and number of errors in RAM, and the percentage of time spent in each zone in NOR by LD and HD rats were compared using two-way repeated measures ANOVA, with a between-subject factor (group: HD and LD) and a within-subject factor (sessions). *Post hoc* comparisons were made using the Bonferroni test. Experiment 2: The study of the differences in gene expression between HD and LD was analyzed using Student's t-test for each of the genes in the three different structures. To assess the relationship between water intake levels in SIP and relative gene expression, correlations were calculated using Pearson correlation analysis. The effect size of the group differences was calculated using Cohen's d or η^2. Partial eta-squared values of 0.01, 0.06, and 0.14 and Cohen's d values of 0.2, 0.5, and 0.8 are considered to reflect small, medium and large effects, respectively (35). Statistical significance was set at $p < 0.05$. All analyses were computed using the Statistica software package (version 8.0). GraphPad Prism (version 9.0) was used for the graphs presented in the results section.

Results

LD and HD selected by SIP

Figure 2 (experiment 1) and Supplementary figure S1 (experiment 2) show the mean water intake in LD and HD during the acquisition and maintenance of SIP during 20 sessions. In experiment 1 on SIP, the mean water intake during the last 5 days of SIP was 25.9 ± 5.1 mL for HD and 6.1 ± 0.8 for LD, respectively. The number of licks also showed SIP acquisition (data not shown). The mean total licks averaged over the last 5 days of SIP were 3987.4 ± 820.7 and 1441.1 ± 243 for HD and LD, respectively. ANOVA revealed significant differences in water intake according to the interaction between the SIP acquisition sessions and the group ($F(1,18) = 34.26$, $p < 0.001$, $\eta^2p = 0.16$).

Furthermore, differences in water consumption were observed for the session effect ($F(19, 342) = 10.38$, $p < 0.001$) and the group effect ($F(19, 342) = 5.47$, $p < 0.001$). ANOVA also showed a significant interaction in the total number of licks (interaction SIP session × group effect: $F(1, 18) = 12.18$, $p < 0.001$, $d = 0.92$; session effect: $F(19,342) = 8.66$, $p < 0.001$; group effect: $F(19, 342) = 3.27$, $p < 0.001$). Post hoc comparisons indicated that SIP induced differences in drinking rates between the 20 sessions in both groups. The differences between LD and HD were evident in water intake from session 6 ($p < 0.01$) onwards. Furthermore, compared to session 1, the animals in the HD group significantly increased their water consumption from session 6 ($p < 0.01$). Differences between the HD and LD groups were also observed in the number of total licks in session 8 ($p < 0.01$) were also observed, and HD rats increased their number of licks in session 8 ($p < 0.01$) compared to session 1. There were significant differences between LD and HD animals in total magazine entries according to session effect ($F(19, 342) = 5.431$, $p < 0.001$). However, there were no significant differences according to the interaction between the SIP acquisition sessions and LD vs. HD (interaction SIP session × group effect: $F(19, 342) = 0.933$, $p = 0.54$) and group effect ($F(1,18) = 0.039$, $p = 0.84$).

In experiment 2, the mean water intake for the last 5 days of SIP was 32.51 ± 2.42 ml for HD and 13.36 ± 1.61 for LD, respectively. The number of licks also showed SIP acquisition (data not shown). The mean total licks averaged during the last 5 days of SIP were 1014.8 ± 320.92 and 765.89 ± 242.2 for HD and LD, respectively (data not shown). ANOVA revealed significant differences in water intake according to the interaction between SIP acquisition sessions and the group (interaction effect of SIP session x group: $F(19,342) = 4.48$, $p < 0.001$, $\eta^2p = 0.17$). Furthermore, differences in water consumption were observed for the session effect ($F(19, 342) = 18.46$, $p < 0.001$) and the group effect ($F(1,18) = 29.31$, $p < 0.001$). ANOVA also showed a significant interaction in the total number of licks (interaction SIP session × group effect: $F(19,342) = 23.95$, $p < 0.001$; session effect: $F(19,342) = 38.56$, $p < 0.001$; group effect: $F(1,18) = 85.42$, $p < 0.001$).

Post hoc comparisons indicated that SIP induced differences in drinking rates across the 20 sessions in both groups. Differences between LD and HD were evident in water intake from session 7 ($p < 0.05$) onwards. Furthermore, compared to session 1, animals in the HD group significantly increased their water consumption from session 7 ($p < 0.01$). Differences between the HD and LD groups were also observed in the number of total licks at session 7 ($p < 0.01$) were also observed, and HD rats increased their number of licks from session 7 ($p < 0.05$) compared to session 1. There were significant differences between LD and HD animals in total magazine entries according to session effect ($F(19,342) = 4.76$, $p < 0.001$). However, there were no significant differences according to the interaction between SIP acquisition sessions and LD vs. HD (interaction SIP session × group effect: $F(19,342) = 0.33$, $p = 0.42$) and group effect ($F(1,18) = 0.40$, $p = 0.61$).

Experiment 1. Behavioral assessment

Morris water maze. Figure 3A shows the latency to the platform during the spatial training and reinstating session spent by LD and HD rats. No significant differences were observed between the groups ($F(8,624) = 1,09$, $p = 0,37$). Figure 3B shows the latency to the platform during the reversal test by LD and HD rats. Significant differences between LD and HD rats were found in MWM test 1. ANOVA revealed that HD rats spent more time finding the platform compared to LD rats ($F(18,1) = 5.90$, $p < 0.05$, $\eta^2p = 0.25$). No significant differences were found between the LD and HD rats in latency in MWM test 2 ($F(1, 18) = 0.13$, $p = 0.7$). LD and HD rats do not show significant differences in swimming speed during reversal (neither in Test 1 nor in Test 2) (Figure 3C).

Radial Arm Maze. Significant differences in the number of memory errors accumulated in RAM by LD and HD rats were observed (Figure 4). ANOVA revealed that HD rats committed a higher number of accumulated working memory errors (Figure 4A) compared to LD rats ($F(126, 7) = 3.64$, $p < 0.01$, $\eta^2p = 0.17$). Additionally, HD rats committed a higher number of accumulated reference memory errors (Figure 4B) compared to LD rats ($F(126, 7) = 4.41$, $p < 0.001$, $\eta^2p = 0.20$). Furthermore, HD rats committed a higher number of accumulated total errors (Figure 4C) compared to LD rats ($F(126, 7) = 4.08$, $p < 0.001$, $\eta^2p = 0.19$).

Novel object recognition test. Supplementary figure S2 shows the performance of LD and HD rats in the NOR test. No significant differences were found between rats with LD and HD in the percentage of time in contact with the object, near the object, or in the neutral zone ($F(4,15) = 0.223$, $p = 0.92$).

Experiment 2. Gene expression

Relative expression of serotonergic genes in LD and HD rats. Figure 5 shows the mRNA expression levels of the *Htr2a* and *Htr2c* genes in different brain structures in HD and LD rats selected by SIP. HD rats showed significantly lower Htr2a mRNA expression levels in FC ($t = 2.23$, $df = 16$, $p < 0.05$; $d = 1.06$) compared to LD rats. No differences were found in the expression level of *Htr2a* mRNA gene in HIP ($t = - 0.61$, $df = 16$, $p = 0.72$), nor in AMY ($t = - 0.91$, $df = 16$, $p = 0.81$) between LD and HD rats (Figure 5A). A significant negative correlation was found between water intake on SIP and *Htr2a* mRNA expression levels in FC ($R2 = -0.57$, $p = 0.01$) (Figure 5B). However, no significant correlations were found between water intake and *Htr2a* mRNA expression levels in HIP ($R2 = -0.01$, $p = 0.96$) and AMY ($R2 = 0.25$, $p = 0.30$). No differences were found in *Htr2c* mRNA expression levels in FC ($t = 0.71$, $df = 16$, $p = 0.25$), nor in HIP ($t = 1.08$, $df = 16$, $p = 0.15$) nor in AMY ($t = - 0.49$, $df = 16$, $p = 0.68$) between HD and LD rats (Figure 5C). No significant correlations were found between water intake and *Htr2a* mRNA expression levels in HIP ($R2 = -0.01$, $p = 0.96$) and AMY ($R2 = 0.25$, $p = 0.30$). Furthermore, there were no significant correlations between water intake in SIP and *Htr2c* mRNA expression levels in FC ($R2 = -0.21$, $p = 0.41$) (Figure 5D) nor in HIP ($R2 = 0.36$, $p = 0.14$) nor in AMY ($R2 = -0.09$, $p = 0.72$) (Table 2).

Relative expression of glutamatergic genes in LD and HD rats. Figure 6 shows the expression levels of different glutamatergic genes in different brain structures in HD and LD rats selected by SIP. HD rats showed a significant reduction in *Grin1* mRNA expression levels in FC ($t = 1.95$, $df = 16$, $p < 0.05$; $d = 0.93$) compared to LD rats. A significant negative correlation was also observed between water intake in SIP and relative expression of *Grin1* in FC ($R2 = -0.52$, $p = 0.03$) (Figure 6B). No significant differences were found in *Grin1* mRNA expression levels in HIP ($t = 0.36$, $df = 16$, $p = 0.36$) nor in AMY ($t = - 0.28$, $df = 14$, $p = 0.61$) between HD and LD rats (Figure 6A). No differences were found in *Grin2a* expression levels in FC ($t = - 1.20$, $df = 14$, $p = 0.88$), in HIP ($t = 0.17$, $df = 14$, $p = 0.43$) and in AMY ($t = - 0.19$, $df = 14$, $p = 0.57$) between HD and LD rats (Figure 6C). Furthermore, no significant differences were found in *Grin2b* expression levels in FC ($t = 0.70$, $df = 16$, $p = 0.25$), in HIP ($t = 1,24$, $df = 16$, $p = 0.12$) and in AMY ($t = - 1.22$, $df = 16$, $p = 0.88$) between HD and LD rats (Figure 6D). Additionally, we did not find any significant differences in *Grin2c* expression levels in FC ($t = - 0.32$, $df = 13$, $p = 0.63$), in HIP ($t = - 0.44$, $df = 14$, $p = 0.67$) and in AMY ($t = 0.01$, $df = 12$, $p = 0.50$) between HD and LD rats (Figure 6E). There was a nonsignificant trend to decrease *Grm2* levels mRNA expression in FC ($t = 1.68$, $df = 16$, $p = 0.05$) and in HIP ($t = 1.38$, $df = 16$, $p = 0.09$) between HD and LD rats. No significant differences were found in AMY ($t = 0.31$, $df = 16$, $p = 0.38$) between HD and LD rats (Figure 6F). Comparison between drinking levels in SIP and mRNA expression levels of the rest of the glutamatergic genes analyzed revealed no significant correlations. The correlation between water intake in SIP and *Grin1* mRNA expression levels: in HIP ($R2 = -0.13$,

p = 0.61) and AMY (R2 = -0.09, p = 0.70) is shown in Table 2. No significant differences between water intake in SIP and *Grin2a* mRNA expression levels: in FC (R2 = -0.34, p = 0.20), in HIP (R2 = -0.33, p = 0.97) and in AMY (R2 = 0.92, p = 0.40); *Grin2b*: in FC (R2 = -0.48, p = 0.17), in HIP (R2 = -0.45, p = 0.17) and in AMY (R2 = 0.32, p = 0.90); and *Grin2a*: in FC (R2 = -0.74, p = 0.47), in HIP (R2 = -0.36, p = 0.17) and AMY (R2 = 0.22, p = 0.40) (Table 2). There were no significant correlations between water intake in SIP and *Grin1* mRNA expression levels of *Grm2* in FC (R2 = -0.13, p = 0.62), in HIP (R2 = 0.02, p = 0.94) and in AMY (R2 = -0.06, p = 0.82) (Table 2).

Relative expression of BDNF genes in LD and HD rats. HD rats showed a significant reduction in *Bdnf* mRNA expression levels in FC (t = 1.84, df = 16, p < 0.05; d = 0.874). A trend was observed in the same way in HIP (t = 1.43, df = 16, p = 0.08) compared to LD rats. No significant differences were found in the expression levels of *Bdnf* mRNA in AMY (t = - 0.31, df = 16, p = 0.62) between HD and LD rats (Figure 7A). There was a significant negative correlation between water intake in SIP and the relative expression of *Bdnf* in FC (R2 = -0.67, p = 0.002) (Figure 7B). No significant negative correlations were found between water intake in SIP and *Bdnf* mRNA expression levels in HIP (R2 = -0.19, p = 0.44) and in AMY (R2 =- 0.13, p = 0.60) (Table 2).

Discussion

The present results confirm the differences in behavioral inflexibility and the 5-HT2A receptor previously described by us in compulsive HD rats selected by SIP (8, 36). We report that the behavioral inflexibility in compulsive HD rats, expressed by an increase in latency to find the platform in the reversal phase of MWM, might also be associated with other components of memory deficits reflected by an increased number of memory errors in RAM compared to LD rats. Furthermore, we expanded our knowledge about the reduced binding of the 5-HT2A receptor in HD rats selected by SIP (27), previously described, by revealing a decreased expression of the serotonin *Htr2a*, *Grin1* and *Bdnf* genes in FC compared to LD rats. Thus, the present data point to a memory deficit in the compulsive phenotype linked to cognitive inflexibility in which the expression of the *Htr2a*, *Grin1*, and *Bdnf* genes in FC could play a key role in its functionality.

Assessment of inflexible behaviors and memory impairments in compulsive HD rats

HD rats and LD rats did not show differences in learning in the acquisition phase of MWM. However, we observed an inflexible behavior of HD rats, as they spent more time finding the platform in the reversal phase of MWM. Previous findings have strongly demonstrated that HD rats have an inflexible and perseverant profile. In the reverse learning task HD rats needed more trials to reach the criterion compared to LD rats (14), as well as they performed more incorrect perseverative responses (14,15). Furthermore, Moreno reported an increase in perseverative responses on the 5-choice serial reaction time task (5-CSRTT) under extinction conditions compared to LD rats (16). Also, HD rats expressed an increased habit behavior in the reinforcer devaluation paradigm by a greater number of lever presses during the devaluation test day (15). Moreover, HD rats exhibited a higher resistance to extinct fear behavior, although they did not differ in acquisition compared to LD rats (17,18). In the fear conditioning test, we observed an increased percentage of freezing behavior on the retrieval day (17), as well as we find a sustained higher latency to enter the dark compartment during the last extinction session of the passive avoidance test (18). According to our preclinical compulsive model, OCD patients have decreased behavioral flexibility as they committed more perseverative errors with a pronounced trend towards poorer performance in the Wisconsin Card Sorting Test [reviewed in (4)].

In addition, patients with OCD have shown a deficit in fear renewal and extinction recall in fear conditioning paradigms (37), with a different skin conductance response in the extinction phase (5).

The persevering behavior profile shown by HD rats might be related to memory deficits, as revealed by the increased number of working memory errors and reference memory errors in RAM compared to LD rats. Few preclinical studies have investigated the role of memory in compulsive behavior. However, in 2010 Andersen observed that rats exposed to clomipramine in early life, considered as an OCD model, had an impaired working memory in a win-shift task, shown by an increased number of errors and longer time to enter each arm than control rats (38). Interestingly, the use of enriched environments reduced spatial memory impairments in MWM and compulsive grooming behavior induced by methamphetamine (39). The systemic administration of d-cycloserine and d-serine, NMDA modulators that enhance memory, reduced compulsive aversion-resistant alcohol drinking (40). Clinical studies evidenced that OCD patients present a deficit in verbal episodic memory, by an impaired performance in Wechsler Memory Scale-Revised relative to controls and non-verbal memory (41). Similarly, OCD patients appear to have poorer performance than control subjects when evaluating verbal and visual memory using the Wechsler Adult Intelligence Scale-Revised (42). Other researchers assessing neuropsychological skills in OCD patients also found that verbal memory was impaired in these patients measured by California Verbal Learning Test (43).

Genetic expression in compulsive HD rats

We found that HD rats have a significantly lower level of mRNA expression of *Htr2a*, *Grin1* and *Bdnf* in FC compared to LD rats. Furthermore, *Htr2a* mRNA expression levels in FC were negatively correlated with compulsive drinking on SIP. This is in accordance with previous research in our laboratory, which revealed that HD rats selected by SIP had a specific reduction in 5-HT2A receptor binding in FC compared to LD rats (27). In contrast, Roman High Avoidance (RHA) rats characterized as impulsive and by a compulsive drinking profile on SIP (44), show higher 5-HT2A binding in FC compared to Roman Low Avoidance (RLA) rats (45). However, a recent study did not find differences in *Htr2a* gene expression in FC between RHA and RLA rats (46). According to our findings, rats that showed high inflexibility in a spatial discrimination Reversal Learning Task had lower serotonin 5-HT2A receptor binding in the orbitofrontal cortex compared to low-perseverative rats (47). Studies using single photon emission computed tomography found that dogs with compulsive behaviors have lower serotonin 5-HT2A receptor availability in the FC (48). On the contrary, red junglefowl (Gallus gallus) chicks characterized by higher expression of *Htr2a*, are less flexible in a discriminative and Reversal Learning Task (49). Animal models of individual differences for example: on depression, have shown that the expression of *Htr2a* is reduced in the FC of Flinders Sensitive Line (FSL) compared to their control strain Flinders Resistant Line (FRL) (50). Moreover, dogs with anxiety disorders have lower serotonin 5-HT2A binding in the FC (48). Furthermore, pharmacological studies have found that serotonin 5-TH2A/C receptor agonists reduce compulsive drinking on SIP (27,28). Thus, the systemic administration of DOI in a dose-dependent manner only reduced compulsive drinking on SIP in HD rats compared to LD rats, which supports the notion that serotonin 5-HT2A receptors have a key role in compulsive behavior (28). Also, the activation of prefrontal serotonin 5-HT2A/C receptors by direct micro-infusion of DOI in the medial prefrontal cortex decreased compulsive drinking in HD rats (27). Serotonin 5-HT2A receptors have a role in cognitive flexibility since the blockade of these receptors leads to impairments in reversal learning (51). Microinfusion of the 5-HT2A receptor antagonist M100907 in the orbitofrontal cortex leads to a higher perseveration during reversal learning and potentiated self-grooming behavior in BTBR mice, a mouse model of autism (52). Also, a high expression of *Htr2a* has been found in FC, HIP, and AMY of adult rats, which constitute components of the brain circuits implicated in memory extinction (53). The activation of

serotonin 5-HT2A receptors facilitates the consolidation and extinction of trace and delay-cued fear memory (54). Clinical studies have also implicated the serotonin *Htr2a* receptor in different psychopathological disorders. In vivo PET studies in drug-naive OCD patients show a reduction in the availability of 5-HT2A receptors in FC (55). A reduction in mRNA expression levels of *Htr2a* has also been observed in patients with bipolar disorder (56), and schizophrenia (57); a finding consistent with postmortem autoradiography studies that showed reduced binding of 5-HT2A in the FC (58).

In the present study, HD rats did not differ significantly in the level of *Htr2c mRNA expression* in FC, HIP, and AMY compared to LD rats. Furthermore, no significant correlations were found between water intake on SIP and the level of *Htr2c mRNA expression.* Overall, there is very little evidence to suggest the involvement of the 5-HT2C receptor in OCD (59). However, studies support that 5-HT2C plays a role in this disorder. Previous research shows that DOI decreased compulsive drinking in HD rats on SIP (28), while the SB242084 did not affect compulsive drinking on SIP (27). Also, the administration of WAY-163909, a serotonin 5-HT2C antagonist, decreased adjunctive drinking on SIP (60), while the 5-HT2C receptor antagonist SB242084 increased drinking behavior on SIP (61). 5-HT2C receptor knockout mice exhibited compulsive-like behaviors (62). Notwithstanding, serotonin 5-HT2C receptors have been associated with cognitive flexibility and reversal learning. Preclinical studies with rats have found that the administration of the 5-HT2C receptor antagonist SB242084 improved learning performance (51,63). In clinical studies, *Htr2c* mRNA expression levels have been shown to be reduced in the FC in unmedicated and medicated schizophrenic patients (64,65). On the contrary, a recent meta-analysis found no significant associations between *Htr2c* polymorphism and OCD (21).

The assessment of the glutamatergic *Grin1* gene, which encodes NMDA receptor subunit 1, revealed a significant reduction in FC in HD rats and an inverse correlation between its expression and compulsive drinking in SIP. Previous studies pointed toward the relationship between *Grin1* and compulsive behavior in rats, as, for example, Ploense in 2018 described different correlations between Grin1 mRNA expression in the dorsomedial PFC and cocaine exposure, in which limited access to cocaine negatively correlates with mRNA expression levels. Nevertheless, prolonged exposure increased the mRNA levels (66). However, a recent study in rats after 10 days of cocaine abstinence has not shown significant differences in *Grin1* expression in FC or HIP (67). Similarly, other study did not describe any significant differences in Grin1 mRNA levels in RHA high avoidance rats in the same brain areas (68). Besides, other preclinical studies confirmed the implication of *Grin1* in fear memory and extinction processes, showing that deletion of *Grin1* strongly facilitates the formation and retention of fear memory and attenuates the extinction of a cued fear response (69,70). Likewise, *Grin1* is considered an important gene in memory acquisition (71). Thus, mutant mice expressed deficits in spatial working memory in the MWM (72). Also, mutant mice presented abnormal anxiety-like behaviors in the light/dark transition and the elevated plus maze tests, a deficient contextual and cued fear memory in the fear conditioning test, and impaired working memory in the RAM test (73). *Grin1* has been considered as a susceptibility gene candidate for some neuropsychiatric disorders, including schizophrenia, bipolar disorder, and attention-deficit/hyperactivity disorder (73,74). Moreover, *Grin1* seems to have a relevant role in schizophrenia disorder (75–77).

The assessment of the glutamatergic *Grin2b* and *Grm2* genes revealed no significant differences in gene expression between compulsive HD rats and LD rats. However, a previous study showed that rats with high avoidance behavior expressed increased levels of *Grin2b* and decreased levels of *Grm2*, both in the FC (68). Moreover, contradictory results have been found regarding the association between *Grin2b* mRNA expression and the experience of stressful and anxious experiences in rats. Stress induced by maternal separation did not affect *Grin2b* expression levels in the FC, nor in the HIP (78).

Grin2b knockout mice are considered as a model of autism and intellectual disability (79,80). Consistently, *Grin2b* variants had been associated with the susceptibility to develop OCD in humans (81–83). Less information is available on *Grm2* genes. Research exploring the effect of the lack of *Grm2* in the prelimbic cortex in alcohol consumption using knockout rats did not report significant differences in alcohol intake (84). Other study showed an upregulation of *Grm2* proteins in the FC and ventral tegmental area after protocols of mild stress (85). Clinical research suggested that *Grm2* may play a key role in the pathophysiology of methamphetamine-induced psychosis (86).

The assessment of the *Bdnf* gene revealed a significant reduction in FC in HD rats and an inverse correlation between its expression and compulsive drinking on SIP. BDNF and its receptors are involved in the regulation of synaptic plasticity processes and synaptic communication (87–89). In preclinical studies, upregulation of *Bdnf* expression was observed in the nucleus accumbens of rats showing successful extinction in morphine-conditioned place preference (90). In addition, increased BDNF protein in the prefrontal and hippocampal regions produces extinction facilitation in fear-conditioned rats (91,92). Besides, decreased *Bdnf* expression level in different areas of the brain has been reported in animal models of depression and isolation (93), but a review pointed out that downregulation of *Bdnf* expression could be associated with increased anxiety-like symptoms, such as shorter time spent in the open arms of an elevated plus maze, increased immobility in the forced swimming test, or reduced sucrose preference [reviewed in (94)]. However, the level of *Bdnf* expression has been shown to be increased in high avoidance RHA rats in FC (68). Moreover, recent data suggest that down-regulation of *Arc* mRNA levels in the locus coeruleus, another plasticity marker, is associated with the tendency to develop compulsive behavior on SIP (95). Furthermore, clinical studies demonstrated decreased serum levels of BDNF in patients with OCD and schizophrenia compared to control participants (89,96).

Conclusions

The present study suggests that the compulsivity expressed by HD rats in SIP could be under memory deficits that cause compulsive and inflexible behavior, as these rats also presented inflexibility in the reversal phase of MWM and impaired memory in RAM. Low mRNA expression levels of *Htr2a*, *Grin1*, and *Bdnf* in the FC of HD rats are related to increased compulsive drinking on SIP. Furthermore, the results of the present study show an inverse relationship between the levels of mRNA expression of the *Htr2a*, *Grin1* and *Bdnf* genes in the FC and the level of water intake in the SIP, that is, increased compulsive drinking on SIP, less relative gene expression. No differences were found in the levels of mRNA expression of the rest of the genes analyzed between high-compulsive HD rats and LD rats selected by SIP. Collectively and according to the reviewed literature, the down-regulation of the expression levels of *Htr2a*, *Grin1*, and *Bdnf* mRNA related to compulsive drinking in SIP points to a possible lack of plasticity in the FC, causing inflexibility and interfering with the extinction of a prominent behavior, thus compulsive drinking under stressing conditions such as SIP. Future studies should explore the link between high and low mRNA expression of the serotonergic, glutamatergic, and *Bdnf* genes and the development of behavioral alterations related to impulsivity and compulsivity. This approach could help us to understand vulnerability biomarkers that could guide new neuropsychopharmacological treatments for compulsive spectrum disorders.

Ethics approval

All procedures were carried out following the Spanish Royal Decree 53/ 2013 on the protection of experimental animals, the European Community Directive 2010/63/EU for animal experiments and complies with the ARRIVE guidelines for animal research. The Animal Research Committee of the University of Almeria approved the experiments described here, and the authors declare that the research shows commitment to the 3Rs principle (replacement, reduction, refinement).

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Authors' contributions

Ángeles Prados-Pardo and Margarita Moreno contributed to the conception and design of the study. Ángeles Prados-Pardo, Elena Martín-González, Santiago Mora, Carlos Martín, and Manuela Olmedo-Córdoba collected and analyzed the data. Cristian Perez-Fernandez contributed with methodological assistance. Fernando Sánchez-Santed contributed to the data interpretation. Ángeles Prados-Pardo wrote the first draft of the manuscript. Margarita Moreno supervised all the experimental processes. Margarita Moreno also had contributions with the resources, the project administration, and the funding acquisition. All authors contributed to the manuscript revision, and read, and approved the submitted version.

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References

1. Robbins TW, Vaghi MM, Banca P. Obsessive-Compulsive Disorder: Puzzles and Prospects. Vol. 102, Neuron. 2019.
2. WHO | World Health Organization. WHO [Internet]. 2018 [cited 2019 Jan 22]; Available from: https://www.who.int/gho/mortality_burden_disease/en/
3. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders [Internet]. American Psychiatric Association; 2013 [cited 2018 Oct 15]. Available from: https://psychiatryonline.org/doi/book/10.1176/appi.books.9780890425596
4. Benzina N, Mallet L, Burguière E, N'Diaye K, Pelissolo A. Cognitive Dysfunction in Obsessive-Compulsive Disorder. Curr Psychiatry Rep [Internet]. 2016 Sep 16 [cited 2018 Oct 15];18(9):80. Available from: http://link.springer.com/10.1007/s11920-016-0720-3
5. Geller DA, McGuire JF, Orr SP, Pine DS, Britton JC, Small BJ, et al. Fear conditioning and extinction in pediatric obsessive-compulsive disorder. Ann Clin Psychiatry [Internet]. 2017 [cited 2019 Jan 15];29(1):17–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28207912
6. Palit A, Roy PK, Saha PK. Role of Prospective Memory in Obsessive Compulsive Disorder. Indian J Psychol Med. 2022 Nov 1;44(6):586–91.
7. Moreno M, Flores P. Schedule-induced polydipsia as a model of compulsive behavior: neuropharmacological and neuroendocrine bases. Psychopharmacology (Berl) [Internet]. 2012 Jan 24 [cited 2018 Oct 28];219(2):647–59. Available from: http://link.springer.com/10.1007/s00213-011-2570-3
8. Martín-González E, Olmedo-Córdoba M, Flores P, Moreno- Montoya M. Differential neurobiological markers in phenotype-stratified rats modeling high or low vulnerability to compulsive behavior: A narrative review. Curr Neuropharmacol [Internet]. 2022 Nov 22 [cited 2023 Mar 10];21. Available from: https://pubmed.ncbi.nlm.nih.gov/36411566/
9. Hawken ER, Beninger RJ. The amphetamine sensitization model of schizophrenia symptoms and its effect on schedule-induced polydipsia in the rat. Psychopharmacology (Berl) [Internet]. 2014 May 16 [cited 2017 May 1];231(9):2001–8. Available from: http://link.springer.com/10.1007/s00213-013-3345-9
10. Ford MM. Applications of schedule-induced polydipsia in rodents for the study of an excessive ethanol intake phenotype. Alcohol [Internet]. 2014 May [cited 2017 May 1];48(3):265–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24680665
11. Fouyssac M, Puaud M, Ducret E, Marti-Prats L, Vanhille N, Ansquer S, et al. Environment-dependent behavioral traits and experiential factors shape addiction vulnerability. Eur J Neurosci [Internet]. 2021 Mar 1 [cited 2023 Mar 10];53(6):1794–808. Available from: https://pubmed.ncbi.nlm.nih.gov/33332672/
12. Woods A, Smith C, Szewczak M, Dunn RW, Cornfeldt M, Corbett R. Selective serotonin re-uptake inhibitors decrease schedule-induced polydipsia in rats: a potential model for obsessive compulsive disorder. Psychopharmacology (Berl) [Internet]. 1993 [cited 2017 May 1];112(2–3):195–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7871019
13. Falk J. Production of polydipsia in normal rats by an intermittent food schedule. Science [Internet]. 1961 Jan 20 [cited 2017 Apr 17];133(3447):195–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13698026
14. Navarro S V, Alvarez R, Colomina MT, Sanchez-Santed F, Flores P, Moreno M. Behavioral Biomarkers of Schizophrenia in High Drinker Rats: A Potential Endophenotype of Compulsive Neuropsychiatric Disorders. Schizophr Bull [Internet]. 2017 [cited 2019 Feb 24];43(4):778–87. Available from: http://creativecommons.

15. Merchán A, Sánchez-Kuhn A, Prados-Pardo A, Gago B, Sánchez-Santed F, Moreno M, et al. Behavioral and biological markers for predicting compulsive-like drinking in schedule-induced polydipsia. Prog Neuropsychopharmacol Biol Psychiatry [Internet]. 2019 Jul 13 [cited 2023 Mar 10];93:149–60. Available from: https://pubmed.ncbi.nlm.nih.gov/30940483/
16. Moreno M, Gutiérrez-Ferre VE, Ruedas L, Campa L, Suñol C, Flores P. Poor inhibitory control and neurochemical differences in high compulsive drinker rats selected by schedule-induced polydipsia. Psychopharmacology (Berl) [Internet]. 2012 Jan 24 [cited 2018 Nov 26];219(2):661–72. Available from: http://link.springer.com/10.1007/s00213-011-2575-y
17. Prados-Pardo Á, Martín-González E, Mora S, Merchán A, Flores P, Moreno M. Increased Fear Memory and Glutamatergic Modulation in Compulsive Drinker Rats Selected by Schedule-Induced Polydipsia. Front Behav Neurosci [Internet]. 2019 Apr 30 [cited 2023 Mar 10];13. Available from: https://pubmed.ncbi.nlm.nih.gov/31133835/
18. Martín-González E, Olmedo-Córdoba M, Prados-Pardo Á, Cruz-Garzón DJ, Flores P, Mora S, et al. Socioemotional deficit and HPA axis time response in high compulsive rats selected by schedule-induced polydipsia. Horm Behav [Internet]. 2022 Jun 1 [cited 2023 Mar 10];142. Available from: https://pubmed.ncbi.nlm.nih.gov/35367739/
19. Fineberg NA, Chamberlain SR, Goudriaan AE, Stein DJ, Vanderschuren LJMJ, Gillan CM, et al. New developments in human neurocognition: clinical, genetic, and brain imaging correlates of impulsivity and compulsivity. CNS Spectr [Internet]. 2014 Feb 11 [cited 2018 Oct 15];19(01):69–89. Available from: https://www.cambridge.org/core/product/identifier/S1092852913000801/type/journal_article
20. Marinova Z, Chuang DM, Fineberg N. Glutamate-Modulating Drugs as a Potential Therapeutic Strategy in Obsessive-Compulsive Disorder. Curr Neuropharmacol [Internet]. 2017 [cited 2018 Oct 16];15(7):977–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28322166
21. Taylor S. Molecular genetics of obsessive–compulsive disorder: a comprehensive meta-analysis of genetic association studies. Mol Psychiatry [Internet]. 2013 Jul 5 [cited 2018 Oct 28];18(7):799–805. Available from: http://www.nature.com/articles/mp201276
22. Rajendram R, Kronenberg S, Burton CL, Arnold PD. Glutamate Genetics in Obsessive-Compulsive Disorder: A Review. J Can Acad Child Adolesc Psychiatry [Internet]. 2017 Oct 1 [cited 2023 Mar 10];26(3):205–13. Available from: https://pubmed.ncbi.nlm.nih.gov/29056983/
23. Katerberg H, Lochner C, Cath DC, De Jonge P, Bochdanovits Z, Moolman-Smook JC, et al. The role of the brain-derived neurotrophic factor (BDNF) val66met variant in the phenotypic expression of obsessive-compulsive disorder (OCD). Am J Med Genet B Neuropsychiatr Genet [Internet]. 2009 Dec 5 [cited 2023 Mar 10];150B(8):1050–62. Available from: https://pubmed.ncbi.nlm.nih.gov/19219856/
24. Taj M J RJ, Ganesh S, Shukla T, Deolankar S, Nadella RK, Sen S, et al. BDNF gene and obsessive compulsive disorder risk, symptom dimensions and treatment response. Asian J Psychiatr [Internet]. 2018 Dec 1 [cited 2023 Mar 10];38:65–9. Available from: https://pubmed.ncbi.nlm.nih.gov/29079096/
25. Şimşek Ş, Gençoğlan S, Yüksel T, Kaplan I, Alaca R. Cortisol and Brain-Derived Neurotrophic Factor Levels Prior to Treatment in Children With Obsessive-Compulsive Disorder. J Clin Psychiatry [Internet]. 2016 Jul 1 [cited 2023 Mar 10];77(7):e855–9. Available from: https://pubmed.ncbi.nlm.nih.gov/27314567/
26. Wang Y, Mathews CA, Li Y, Lin Z, Xiao Z. Brain-derived neurotrophic factor (BDNF) plasma levels in drug-naïve OCD patients are lower than those in healthy people, but are not lower than those in drug-treated OCD patients. J Affect Disord [Internet]. 2011 Sep [cited 2023 Mar 10];133(1–2):305–10. Available from: https://pubmed.ncbi.nlm.nih.gov/21616543/

27. Mora S, Merchán A, Vilchez O, Aznar S, Klein AB, Ultved L, et al. Reduced cortical serotonin 5-HT2A receptor binding and glutamate activity in high compulsive drinker rats. Neuropharmacology [Internet]. 2018 Dec 1 [cited 2019 Jan 15];143:10–9. Available from: https://www.sciencedirect.com/science/article/pii/S0028390818306270?via%3Dihub
28. Navarro SV, Gutiérrez-ferre V, Flores P, Moreno M. Activation of serotonin 5-HT2 A receptors inhibits high compulsive drinking on schedule-induced polydipsia. 2015;683–97.
29. Mora S, Merchán A, Aznar S, Flores P, Moreno M. Increased amygdala and decreased hippocampus volume after schedule-induced polydipsia in high drinker compulsive rats. Behavioural brain research [Internet]. 2020 Jul 15 [cited 2023 Mar 10];390. Available from: https://pubmed.ncbi.nlm.nih.gov/32417273/
30. Moreno M, Flores P. Schedule-induced polydipsia as a model of compulsive behavior: Neuropharmacological and neuroendocrine bases. Psychopharmacology (Berl). 2012;219(2):647–59.
31. Pittenger C. Disorders of memory and plasticity in psychiatric disease. Dialogues Clin Neurosci [Internet]. 2013 Dec [cited 2023 Mar 10];15(4):455–63. Available from: https://pubmed.ncbi.nlm.nih.gov/24459412/
32. Fole A, Miguéns M, Morales L, González-Martín C, Ambrosio E, Del Olmo N. Lewis and Fischer 344 rats as a model for genetic differences in spatial learning and memory: Cocaine effects. Prog Neuropsychopharmacol Biol Psychiatry [Internet]. 2017 Jun 2 [cited 2023 Mar 10];76:49–57. Available from: https://pubmed.ncbi.nlm.nih.gov/28263897/
33. Cohen SJ, Stackman RW. Assessing rodent hippocampal involvement in the novel object recognition task. A review. Behavioural brain research [Internet]. 2015 May 5 [cited 2023 Mar 10];285:105–17. Available from: https://pubmed.ncbi.nlm.nih.gov/25169255/
34. Statistical Power Analysis for the Behavioral Sciences - Jacob Cohen - Google Libros [Internet]. [cited 2023 Mar 16]. Available from: https://books.google.es/books?hl=es&lr=&id=rEe0BQAAQBAJ&oi=fnd&pg=PP1&ots=sw_ZHtUPs9&sig=FB7Ht7UeZXro1MzjNUgzZ6Qu0K8&redir_esc=y#v=onepage&q&f=false
35. Moreno-Montoya M, Olmedo-Córdoba M, Martín-González E. Negative valence system as a relevant domain in compulsivity: review in a preclinical model of compulsivity. Emerg Top Life Sci [Internet]. 2022 Dec 9 [cited 2023 Mar 10];6(5):491–500. Available from: https://pubmed.ncbi.nlm.nih.gov/36377776/
36. Fyer AJ, Schneier FR, Simpson HB, Choo TH, Tacopina S, Kimeldorf MB, et al. Heterogeneity in Fear Processing across and within Anxiety, Eating, and Compulsive Disorders. J Affect Disord [Internet]. 2020 Oct 1 [cited 2023 Mar 10];275:329–38. Available from: https://pubmed.ncbi.nlm.nih.gov/32734926/
37. Andersen SL, Greene-Colozzi EA, Sonntag KC. A novel, multiple symptom model of obsessive-compulsive-like behaviors in animals. Biol Psychiatry [Internet]. 2010 Oct 15 [cited 2023 Mar 10];68(8):741–7. Available from: https://pubmed.ncbi.nlm.nih.gov/20619828/
38. Hajheidari S, Miladi-Gorji H, Bigdeli I. Environmental Enrichment Prevents Methamphetamine-Induced Spatial Memory Deficits and Obsessive-Compulsive Behavior in Rats. Iran J Psychiatry [Internet]. 2017 Jan [cited 2023 Mar 10];12(1):8–14. Available from: https://pubmed.ncbi.nlm.nih.gov/28496496/
39. Seif T, Simms JA, Lei K, Wegner S, Bonci A, Messing RO, et al. D-Serine and D-Cycloserine Reduce Compulsive Alcohol Intake in Rats. Neuropsychopharmacology [Internet]. 2015 Sep [cited 2018 Aug 19];40(10):2357–67. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25801502
40. Exner C, Kohl A, Zaudig M, Langs G, Lincoln TM, Rief W. Metacognition and episodic memory in obsessive-compulsive disorder. J Anxiety Disord. 2009 Jun;23(5):624–31.

41. Martin V, Huber M, Rief W, Exner C. Comparative cognitive profiles of obsessive-compulsive disorder and schizophrenia. Archives of Clinical Neuropsychology. 2008;23(5):487–500.
42. Tükel R, Gürvit H, Ertekin BA, Oflaz S, Ertekin E, Baran B, et al. Neuropsychological function in obsessive-compulsive disorder. Compr Psychiatry. 2012 Feb;53(2):167–75.
43. Moreno M, Cardona D, Gómez MJ, Sánchez-Santed F, Tobeña A, Fernández-Teruel A, et al. Impulsivity characterization in the Roman high- and low-avoidance rat strains: behavioral and neurochemical differences. Neuropsychopharmacology [Internet]. 2010;35(5):1198–208. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3055403&tool=pmcentrez&rendertype=abstract
44. Klein AB, Ultved L, Adamsen D, Santini MA, Tobeña A, Fernandez-Teruel A, et al. 5-HT2A and mGlu2 receptor binding levels are related to differences in impulsive behavior in the Roman Low- (RLA) and High- (RHA) avoidance rat strains. Neuroscience. 2014 Mar 28;263:36–45.
45. Fomsgaard L, Moreno JL, de la Fuente Revenga M, Brudek T, Adamsen D, Rio-Alamos C, et al. Differences in 5-HT2A and mGlu2 Receptor Expression Levels and Repressive Epigenetic Modifications at the 5-HT2A Promoter Region in the Roman Low- (RLA-I) and High- (RHA-I) Avoidance Rat Strains. Mol Neurobiol. 2018 Mar 1;55(3):1998–2012.
46. Barlow RL, Alsiö J, Jupp B, Rabinovich R, Shrestha S, Roberts AC, et al. Markers of serotonergic function in the orbitofrontal cortex and dorsal raphé nucleus predict individual variation in spatial-discrimination serial reversal learning. Neuropsychopharmacology [Internet]. 2015 Feb 4 [cited 2023 Mar 10];40(7):1619–30. Available from: https://pubmed.ncbi.nlm.nih.gov/25567428/
47. Vermeire ST, Audenaert KR, Dobbeleir AA, De Meester RH, De Vos FJ, Peremans KY. Evaluation of the brain 5-HT2A receptor binding index in dogs with anxiety disorders, measured with 123I-5I-R91150 and SPECT. J Nucl Med [Internet]. 2009 Feb 1 [cited 2023 Mar 10];50(2):284–9. Available from: https://pubmed.ncbi.nlm.nih.gov/19164223/
48. Boddington R, Gómez Dunlop CA, Garnham LC, Ryding S, Abbey-Lee RN, Kreshchenko A, et al. The relationship between monoaminergic gene expression, learning, and optimism in red junglefowl chicks. Anim Cogn [Internet]. 2020 Sep 1 [cited 2023 Mar 10];23(5):901–11. Available from: https://pubmed.ncbi.nlm.nih.gov/32440792/
49. Du Jardin KG, Müller HK, Sanchez C, Wegener G, Elfving B. Gene expression related to serotonergic and glutamatergic neurotransmission is altered in the flinders sensitive line rat model of depression: Effect of ketamine. Synapse [Internet]. 2017 Jan 1 [cited 2023 Mar 10];71(1):37–45. Available from: https://pubmed.ncbi.nlm.nih.gov/27589698/
50. Boulougouris V, Chamberlain SR, Robbins TW. Cross-species models of OCD spectrum disorders. Psychiatry Res [Internet]. 2009 Nov [cited 2019 Jan 19];170(1):15–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165178108002370
51. Amodeo DA, Yi J, Sweeney JA, Ragozzino ME, Vijayaraghavan S, Powell S, et al. Oxotremorine treatment reduces repetitive behaviors in BTBR T+ tf/J mice. 2014 [cited 2017 May 3]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4131251/pdf/fnsyn-06-00017.pdf
52. Cornea-Hébert V, Riad M, Wu C, Singh SK, Descarries L. Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. Journal of Comparative Neurology. 1999 Jun 28;409(2):187–209.
53. Stackman RW, Zhang G, Sgeirsdóttir HN, Cohen SJ, Munchow AH, Barrera MP. Stimulation of serotonin 2A receptors facilitates consolidation and extinction of fear memory in C57BL/6J

mice. Neuropharmacology [Internet]. 2013 Jan [cited 2023 Mar 10];64(1):403–13. Available from: https://pubmed.ncbi.nlm.nih.gov/22722027/
54. Perani D, Garibotto V, Gorini A, Moresco RM, Henin M, Panzacchi A, et al. In vivo PET study of 5HT(2A) serotonin and D(2) dopamine dysfunction in drug-naive obsessive-compulsive disorder. Neuroimage [Internet]. 2008 Aug 1 [cited 2023 Mar 10];42(1):306–14. Available from: https://pubmed.ncbi.nlm.nih.gov/18511303/
55. López-Figueroa AL, Norton CS, López-Figueroa MO, Armellini-Dodel D, Burke S, Akil H, et al. Serotonin 5-HT1A, 5-HT1B, and 5-HT2A receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. Biol Psychiatry. 2004 Feb 1;55(3):225–33.
56. Hurlemann R, Matusch A, Kuhn KU, Berning J, Elmenhorst D, Winz O, et al. 5-HT2A receptor density is decreased in the at-risk mental state. Psychopharmacology (Berl). 2008 Jan;195(4):579–90.
57. Matsumoto I, Inoue Y, Iwazaki T, Pavey G, Dean B. 5-HT2A and muscarinic receptors in schizophrenia: A postmortem study. Neurosci Lett. 2005 May 13;379(3):164–8.
58. Sinopoli VM, Burton CL, Kronenberg S, Arnold PD. A review of the role of serotonin system genes in obsessive-compulsive disorder. Neurosci Biobehav Rev [Internet]. 2017 Sep 1 [cited 2023 Mar 10];80:372–81. Available from: https://pubmed.ncbi.nlm.nih.gov/28576508/
59. Rosenzweig-Lipson S, Sabb A, Stack G, Mitchell P, Lucki I, Malberg JE, et al. Antidepressant-like effects of the novel, selective, 5-HT2C receptor agonist WAY-163909 in rodents. Psychopharmacology (Berl). 2007 Jun;192(2):159–70.
60. Higgins GA, Brown M, St John J, MacMillan C, Silenieks LB, Thevarkunnel S. Effects of 5-HT2C receptor modulation and the NA reuptake inhibitor atomoxetine in tests of compulsive and impulsive behaviour. Neuropharmacology [Internet]. 2020 Jun 15 [cited 2023 Mar 10];170. Available from: https://pubmed.ncbi.nlm.nih.gov/32222404/
61. Chou-Green JM, Holscher TD, Dallman MF, Akana SF. Compulsive behavior in the 5-HT2C receptor knockout mouse. Physiol Behav. 2003;78(4–5):641–9.
62. Alsiö J, Nilsson SRO, Gastambide F, Wang RAH, Dam SA, Mar AC, et al. The role of 5-HT2C receptors in touchscreen visual reversal learning in the rat: a cross-site study. Psychopharmacology (Berl) [Internet]. 2015 Nov 1 [cited 2023 Mar 10];232(21–22):4017–31. Available from: https://pubmed.ncbi.nlm.nih.gov/26007324/
63. Castensson A, Emilsson L, Sundberg R, Jazin E. Decrease of serotonin receptor 2C in schizophrenia brains identified by high-resolution mRNA expression analysis. Biol Psychiatry. 2003 Dec 1;54(11):1212–21.
64. Castensson A, Åberg K, McCarthy S, Saetre P, Andersson B, Jazin E. Serotonin receptor 2C (HTR2C) and schizophrenia: Examination of possible medication and genetic influences on expression levels. American Journal of Medical Genetics - Neuropsychiatric Genetics. 2005 Apr 5;134 B(1):84–9.
65. Ploense KL, Vieira P, Bubalo L, Olivarria G, Carr AE, Szumlinski KK, et al. Contributions of prolonged contingent and non-contingent cocaine exposure to escalation of cocaine intake and glutamatergic gene expression. Psychopharmacology (Berl). 2018 May 1;235(5):1347–59.
66. Smaga I, Wydra K, Suder A, Frankowska M, Sanak M, Caffino L, et al. The NMDA Receptor Subunit (GluN1 and GluN2A) Modulation Following Different Conditions of Cocaine Abstinence in Rat Brain Structures. Neurotox Res. 2021 Jun 1;39(3):556–65.
67. Elfving B, Müller HK, Oliveras I, Østerbøg TB, Rio-Alamos C, Sanchez-Gonzalez A, et al. Differential expression of synaptic markers regulated during neurodevelopment in a rat model of schizophrenia-like behavior. Prog Neuropsychopharmacol Biol Psychiatry [Internet]. 2019 Dec 20 [cited 2023 Mar 10];95. Available from: https://pubmed.ncbi.nlm.nih.gov/31228641/

68. Gafford G, Jasnow AM, Ressler KJ. Grin1 receptor deletion within CRF neurons enhances fear memory. PLoS One. 2014 Oct 23;9(10).
69. Hirsch SJ, Regmi NL, Birnbaum SG, Greene RW. CA1-specific deletion of NMDA receptors induces abnormal renewal of a learned fear response. Hippocampus [Internet]. 2015 Nov 1 [cited 2023 Mar 10];25(11):1374–9. Available from: https://pubmed.ncbi.nlm.nih.gov/25786918/
70. Chen JY, Campos CA, Jarvie BC, Palmiter RD. Parabrachial CGRP Neurons Establish and Sustain Aversive Taste Memories. Neuron. 2018 Nov 21;100(4):891-899.e5.
71. Kew JNC, Koester A, Moreau JL, Jenck F, Ouagazzal AM, Mutel V, et al. Functional consequences of reduction in NMDA receptor glycine affinity in mice carrying targeted point mutations in the glycine binding site. J Neurosci [Internet]. 2000 Jun 1 [cited 2023 Mar 10];20(11):4037–49. Available from: https://pubmed.ncbi.nlm.nih.gov/10818139/
72. Umemori J, Takao K, Koshimizu H, Hattori S, Furuse T, Wakana S, et al. ENU-mutagenesis mice with a non-synonymous mutation in Grin1 exhibit abnormal anxiety-like behaviors, impaired fear memory, and decreased acoustic startle response. BMC Res Notes [Internet]. 2013 [cited 2023 Mar 10];6(1). Available from: https://pubmed.ncbi.nlm.nih.gov/23688147/
73. Mundo E, Tharmalingham S, Neves-Pereira M, Dalton EJ, Macciardi F, Parikh S V., et al. Evidence that the N-methyl-D-aspartate subunit 1 receptor gene (GRIN1) confers susceptibility to bipolar disorder. Mol Psychiatry [Internet]. 2003 [cited 2023 Mar 10];8(2):241–5. Available from: https://pubmed.ncbi.nlm.nih.gov/12610658/
74. Liu YP, Ding M, Zhang XC, Liu Y, Xuan JF, Xing JX, et al. Association between polymorphisms in the GRIN1 gene 5′ regulatory region and schizophrenia in a northern Han Chinese population and haplotype effects on protein expression in vitro. BMC Med Genet. 2019 Jan 31;20(1).
75. Zhao X, Li H, Shi Y, Tang R, Chen W, Liu J, et al. Significant association between the genetic variations in the 5′ end of the N-methyl-D-aspartate receptor subunit gene GRIN1 and schizophrenia. Biol Psychiatry. 2006 Apr 15;59(8):747–53.
76. Hung CC, Chen HY, Chen CH. Systematic mutation analysis of the human glutamate receptor, ionotropic, N-methyl-D-aspartate 1 gene(GRIN1) in schizophrenic patients. Psychiatr Genet [Internet]. 2002 Dec [cited 2023 Mar 10];12(4):225–30. Available from: https://pubmed.ncbi.nlm.nih.gov/12454527/
77. Masrour FF, Peeri M, Azarbayjani MA, Hosseini MJ. Voluntary Exercise During Adolescence Mitigated Negative the Effects of Maternal Separation Stress on the Depressive-Like Behaviors of Adult Male Rats: Role of NMDA Receptors. Neurochem Res. 2018 May 1;43(5):1067–74.
78. Shin W, Kim K, Serraz B, Cho YS, Kim D, Kang M, et al. Early correction of synaptic long-term depression improves abnormal anxiety-like behavior in adult GluN2B-C456Y-mutant mice. PLoS Biol. 2020 Apr 1;18(4).
79. O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science (1979). 2012 Dec 21;338(6114):1619–22.
80. Bozorgmehr A, Ghadirivasfi M, Shahsavand Ananloo E. Obsessive-compulsive disorder, which genes? Which functions? Which pathways? An integrated holistic view regarding OCD and its complex genetic etiology. J Neurogenet [Internet]. 2017 Jul 3 [cited 2023 Mar 10];31(3):153–60. Available from: https://pubmed.ncbi.nlm.nih.gov/28608743/
81. Alonso P, Gratacós M, Segalàs C, Escaramís G, Real E, Bayés M, et al. Association between the NMDA glutamate receptor GRIN2B gene and obsessive-compulsive disorder. J Psychiatry Neurosci [Internet]. 2012 Jul [cited 2023 Mar 10];37(4):273–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22433450

82. Arnold PD, Rosenberg DR, Mundo E, Tharmalingam S, Kennedy JL, Richter MA. Association of a glutamate (NMDA) subunit receptor gene (GRIN2B) with obsessive-compulsive disorder: A preliminary study. Psychopharmacology (Berl). 2004;174(4):530–8.
83. Ding ZM, Ingraham CM, Hauser SR, Lasek AW, Bell RL, McBride WJ. Reduced Levels of mGlu2 Receptors within the Prelimbic Cortex Are Not Associated with Elevated Glutamate Transmission or High Alcohol Drinking. Alcohol Clin Exp Res [Internet]. 2017 Nov 1 [cited 2023 Mar 10];41(11):1896–906. Available from: https://pubmed.ncbi.nlm.nih.gov/28858384/
84. Liao W, Liu Y, Wang L, Cai X, Xie H, Yi F, et al. Chronic mild stress-induced protein dysregulations correlated with susceptibility and resiliency to depression or anxiety revealed by quantitative proteomics of the rat prefrontal cortex. Transl Psychiatry [Internet]. 2021 Jun 1 [cited 2023 Mar 10];11(1). Available from: https://pubmed.ncbi.nlm.nih.gov/33627638/
85. Tsunoka T, Kishi T, Kitajima T, Okochi T, Okumura T, Yamanouchi Y, et al. Association analysis of GRM2 and HTR2A with methamphetamine-induced psychosis and schizophrenia in the Japanese population. Prog Neuropsychopharmacol Biol Psychiatry. 2010 May;34(4):639–44.
86. Orhan C, Erten F, Er B, Tuzcu M, Şahin N, Durmaz Kurşun ÖE, et al. Lutein/zeaxanthin isomers regulate neurotrophic factors and synaptic plasticity in trained rats. Turk J Med Sci. 2021;51(4):2167–76.
87. Kowiański P, Lietzau G, Czuba E, Waśkow M, Steliga A, Moryś J. BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. Cell Mol Neurobiol. 2018 Apr 1;38(3):579–93.
88. Favalli G, Li J, Belmonte-de-Abreu P, Wong AHC, Daskalakis ZJ. The role of BDNF in the pathophysiology and treatment of schizophrenia. J Psychiatr Res [Internet]. 2012 [cited 2023 Mar 10];46(1):1–11. Available from: https://pubmed.ncbi.nlm.nih.gov/22030467/
89. Martínez-Rivera FJ, Martínez NA, Martínez M, Ayala-Pagán RN, Silva WI, Barreto-Estrada JL. Neuroplasticity transcript profile of the ventral striatum in the extinction of opioid-induced conditioned place preference. Neurobiol Learn Mem. 2019 Sep 1;163.
90. Peters J, Kalivas PW, Quirk GJ. Extinction circuits for fear and addiction overlap in prefrontal cortex. Learning and Memory. 2009 May;16(5):279–88.
91. Rosas-Vidal LE, Do-Monte FH, Sotres-Bayon F, Quirk GJ. Hippocampal-prefrontal BDNF and memory for fear extinction. Neuropsychopharmacology. 2014;39(9):2161–9.
92. Elfving B, Plougmann PH, Müller HK, Mathé AA, Rosenberg R, Wegener G. Inverse correlation of brain and blood BDNF levels in a genetic rat model of depression. International Journal of Neuropsychopharmacology. 2010 Jun;13(5):563–72.
93. Murínová J, Hlaváčová N, Chmelová M, Riečanský I. The Evidence for Altered BDNF Expression in the Brain of Rats Reared or Housed in Social Isolation: A Systematic Review. Front Behav Neurosci [Internet]. 2017 May 31 [cited 2023 Mar 10];11:101. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28620285
94. Velazquez-Sanchez C, Muresan L, Marti-Prats L, Belin D. The development of compulsive coping behaviour is associated with a downregulation of Arc in a Locus Coeruleus neuronal ensemble. Neuropsychopharmacology [Internet]. 2023 Mar 1 [cited 2023 Mar 10];48(4). Available from: https://pubmed.ncbi.nlm.nih.gov/36635597/
95. Maina G, Rosso G, Zanardini R, Bogetto F, Gennarelli M, Bocchio-Chiavetto L. Serum levels of brain-derived neurotrophic factor in drug-naïve obsessive-compulsive patients: A case-control study. J Affect Disord. 2010 Apr;122(1–2):174–8.

Figure 1. The experimental procedures are illustrated in a timetable. Two independent experiments were carried out in the present study. In both experiments, high drinkers (HD) and low drinkers (LD) populations were separated by schedule-induced polydipsia (SIP). In experiment 1 HD and LD rats were assessed by: Morris water maze test (MWM), radial arm maze test (RAM), and novel object recognition test (NOR). In experiment 2 serotonergic, glutamatergic and *Bdnf* genes were analyzed.

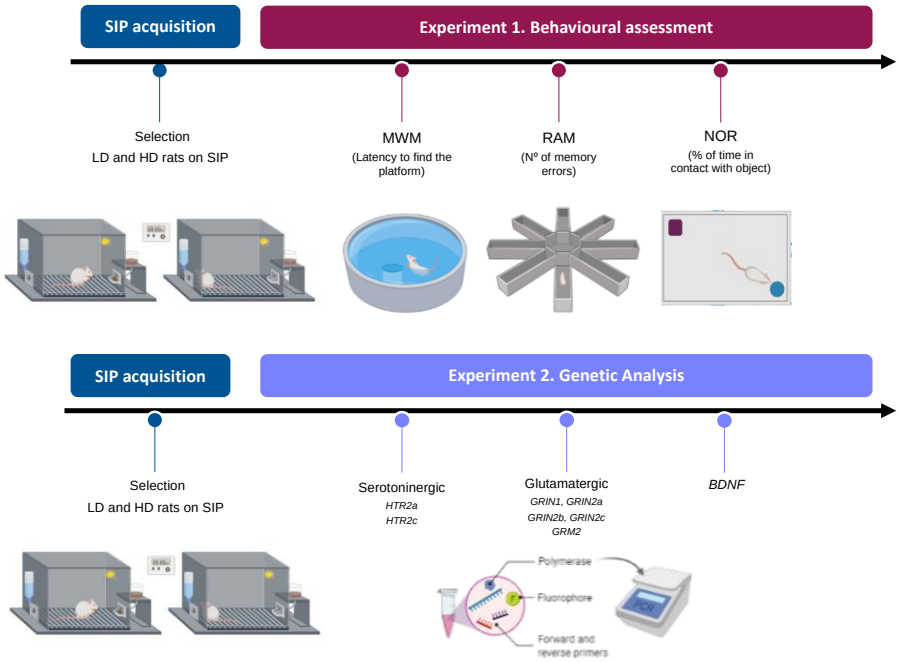

Figure 2. The mean (± SEM) water intake in mL in fixed time FT-60s across 20 sessions of schedule-induced polydipsia (SIP). Statistical analyses indicate significant differences between low drinkers (LD, n = 10) and high drinkers (HD, n = 10; *p < 0.05) from session 3. Significant differences between sessions were found from session 5 (#p < 0.05).

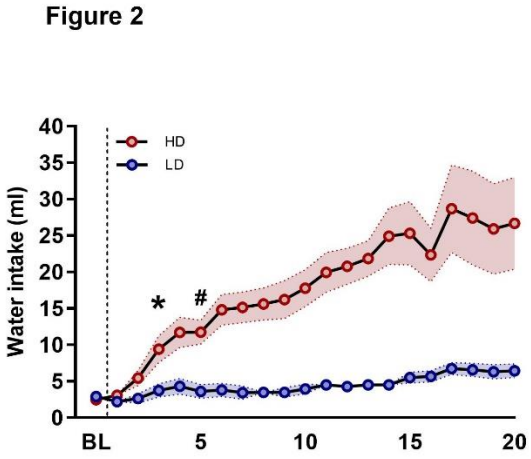

Figure 3. Latency to the platform in seconds (± SEM) in morris water maze (MWM) spent by high drinkers (HD; n = 10) and low drinkers rats (LD; n = 10) in the acquisition (A), the latency in the

reversal phase (B) and the speed in the reversal phase. Statistical analyses indicate significant differences between LD and HD (*p < 0.05) in the latency to platform in test 1.

Figure 3

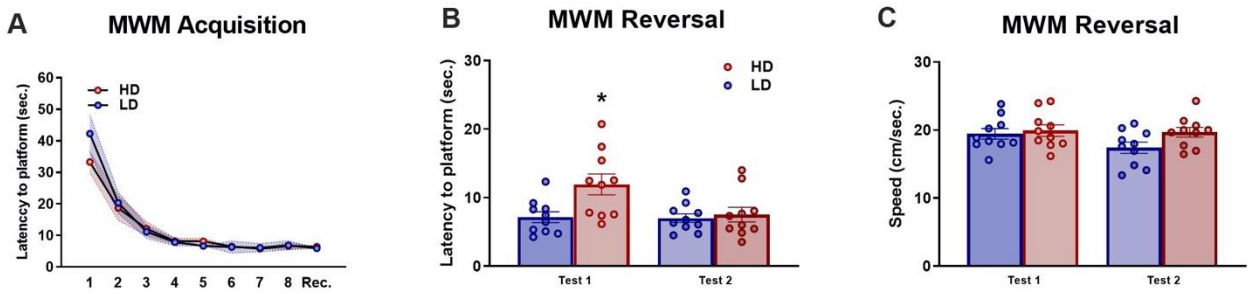

Figure 4. The cumulative number of memory errors (± SEM) committed by high drinkers (HD; n = 10) and low drinkers rats (LD; n = 10) in radial arm maze (RAM). (A) Working memory errors, (B) reference memory errors and (C) the total number of memory errors committed across the 8 sessions of RAM. Statistical analyses indicate significant differences between LD and HD (**p < 0.01; *** p < 0.001) in the number of working memory errors, reference memory errors and the total number of errors form session 4.

Figure 4

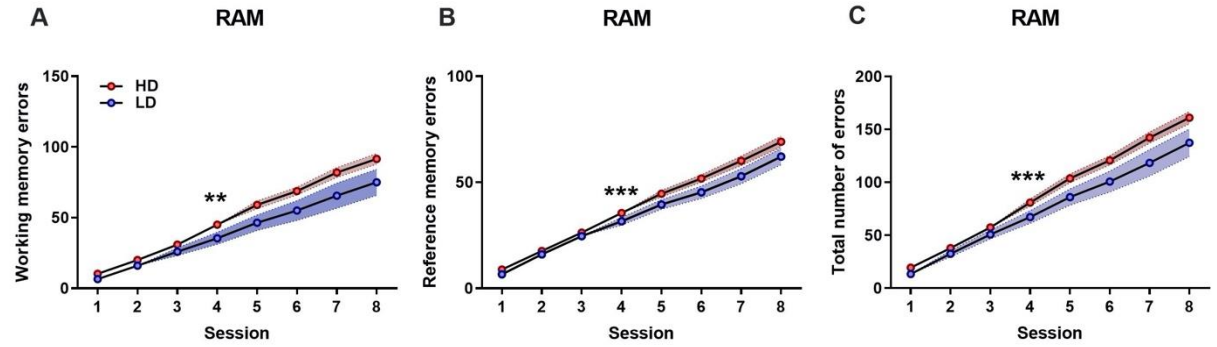

Figure 5. Serotonergic relative expression (± SEM) of *Htr2a* (A) and *Htr2c* (C) of high drinker (HD; n = 10) and low drinker rats (LD; n = 10) in the frontal cortex, the hippocampus and the amygdala. Negative correlation between *Htr2a* (B) and *Htr2c* (D) relative expression in the frontal cortex and

water intake on SIP. Statistical analyses indicate significant differences between LD and HD (*p < 0.05) in the relative expression of *Htr2a* in the frontal cortex and significant negative correlation between *Htr2a* relative expression in the frontal cortex and the water intake.

Figure 5

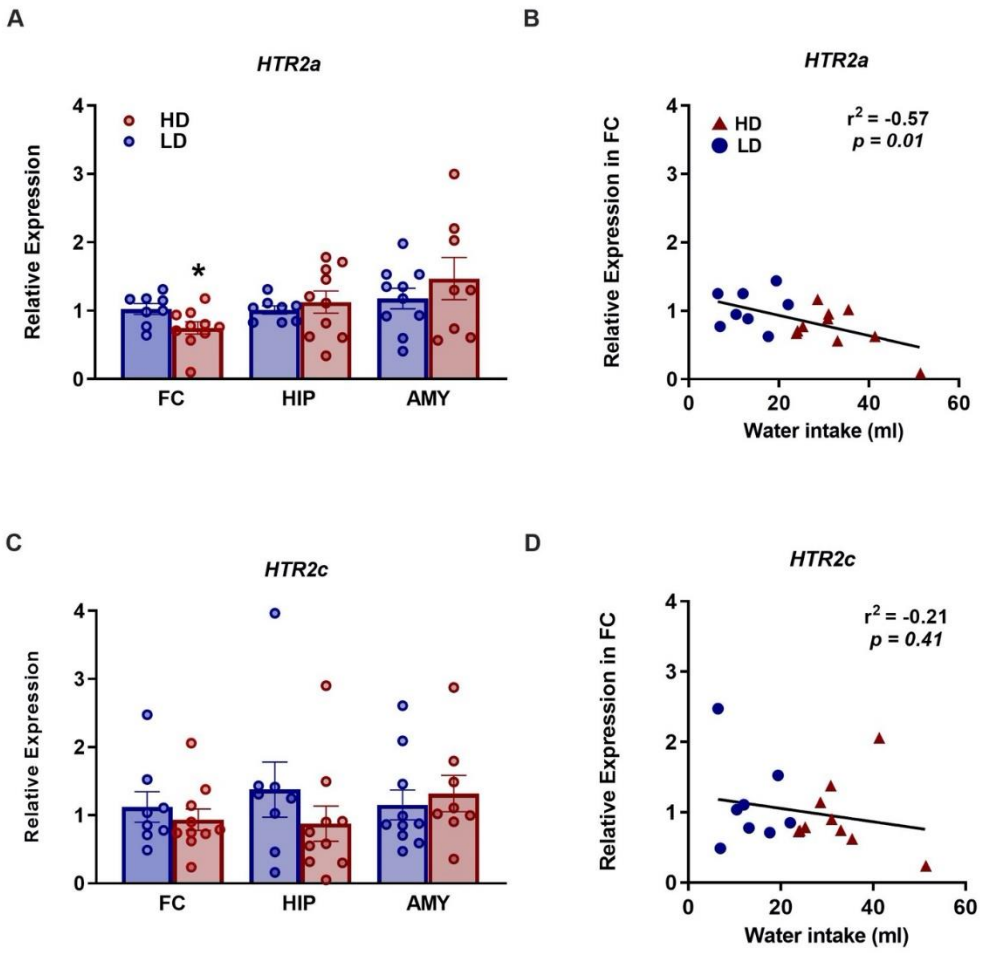

Figure 6. Glutamatergic relative expression (± SEM) of *Grin1* (A), *Grin2a* (C), *Grin2b* (D), *Grin2c* (E), and *Grm2* (F) of high drinker (HD; n = 10) and low drinker rats (LD; n = 10) in the frontal cortex, the hippocampus and the amygdala. Significant negative correlation between *Grin1* relative

expression in the frontal cortex and water intake on SIP (B). Statistical analyses indicate significant differences between LD and HD (*p < 0.05) in the relative expression of *Grin1* in the frontal cortex and significant negative correlation between *Grin1* relative expression in the frontal cortex and the water intake.

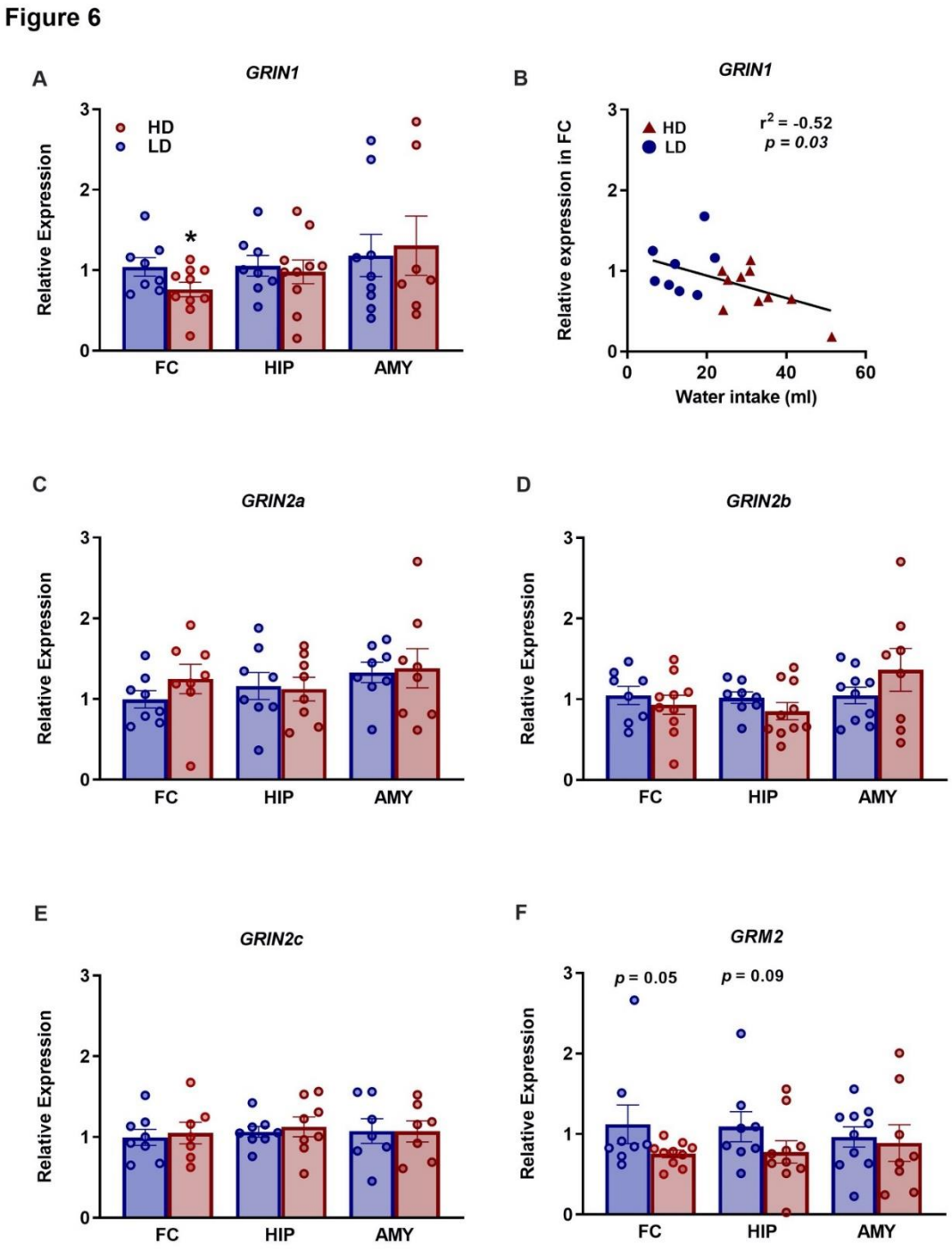

Figure 7. *Bdnf* relative expression (± SEM) of high drinkers (HD; n = 10) and low drinkers rats (LD; n = 10) in the frontal cortex, the hippocampus and the amygdala (A). Significant negative correlation between *Bdnf* relative expression in the frontal cortex and water intake on SIP (B). Statistical analyses

indicate significant differences between LD and HD (*p < 0.05) in the relative expression of *Bdnf* in the frontal cortex and significant negative correlation between *Bdnf* relative expression in the frontal cortex and the water intake.

Figure 7

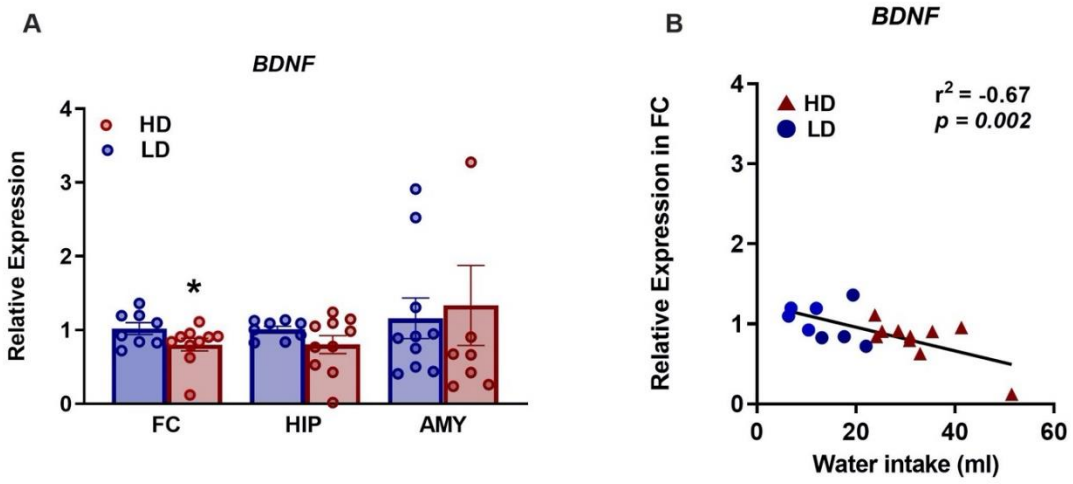

Table 1. Primers selected for the RT-qPCR study. From left to right, the name of the gene, forward primer, reverse primer, and source. Gapdh: Glyceraldehyde 3-phosphate dehydrogenase. Htr2a and c: Serotonergic receptor 2 a & b. Grin1, 2a, 2b, and 2c: Glutamatergic NMDA subunit ionotropic receptor

1, 2a, 2b, and 2c, respectively. Grm2: Glutamatergic metabotropic receptor 2. Bdnf: Brain-derived neurotrophic factor.

Gene	Forward	Reverse	Reference
Gadph	ACAACTTTGGCATTGTGGAA	GATGCAGGGATGATGTTCTG	Own design
Htr2a	AACGGTCCATCCACAGAG	AACAGGAAGAACACGATGC	Kindlundh-Högbergetal et al. 2006
Htr2c	TTGGACTGAGGGACGAAAGC	GGATGAAGAATGCCACGAAGG	Kindlundh-Högbergetalet al. 2006
Grin1	ATGGCTTCTGCATAGACC	GTTGTTTACCCGCTCCTG	Lau et al. 2013
Grin2a	AGTTCACCTATGACCTCTACC	GTTGATAGACCACTTCACCT	Lau et al. 2013
Grin2b	AAGTTCACCTATGACCTTTACC	CATGACCACCTCACCGAT	Lau et al. 2013
Grin2c	GGCCCAGCTTTTGACCTTAGT	CCTGTGACCACCGCAAGAG	Lau et al. 2013
Grm2	CTATGCCACCCACAGTGATG	GCACAGTGCGAGCAAAGTAATC	Pershina et al. 2018
Bdnf	GGTCACAGCGGCAGATAA	CCGAACATACGATTGGGTAG	Own design

Table 2. Correlations between water intake on SIP and relative gene expression. From left to right, the name of the gene, the brain structure, the R2 value, and P value. Statistical analyses indicate

significant negative correlation between Htr2a, Grin1 and Bdnf relative expression in the frontal cortex and the water intake. (*p < 0.05; ** p < 0.01).

Gene	Brain structure	R^2	P Value
Htr2a	FC	-0.57	0.01*
	HIP	-0.01	0.96
	AMY	0.25	0.30
Htr2c	FC	-0.21	0.41
	HIP	0.36	0.14
	AMY	-0.09	0.72
Grin1	FC	-0.52	0.03*
	HIP	-0.13	0.61
	AMY	-0.09	0.70
Grin2a	FC	-0.06	0.69
	HIP	0.25	0.37
	AMY	0.03	0.93
Grin2b	FC	-0.34	0.17
	HIP	-0.34	0.17
	AMY	0.22	0.40
Grin2c	FC	-0.13	0.70
	HIP	-0.05	0.81
	AMY	-0.36	0.15
Grm2	FC	-0.13	0.62
	HIP	0.02	0.94
	AMY	0.06	0.82
Bdnf	FC	-0,67	0.002**
	HIP	-0.19	0.44
	AMY	-0.13	0.60