LA BEBIDA COMPULSIVA EN POLIDIPSIA INDUCIDA POR PROGRAMA COMO RASGO DE VULNERABILIDAD PSICOPATÓLOGICA EN RATAS: IMPLICACIONES DE LA SEROTONINA EN SU DESARROLLO Y MANTENIMIENTO

COMPULSIVE DRINKING IN SCHEDULE-INDUCED POLYDIPSIA AS A FEATURE OF VULNERABILITY TO PSYCHOPATHOLOGY IN RATS: IMPLICATIONS OF SEROTONIN IN ITS DEVELOPMENT AND MAINTENANCE

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The Thesis is realised under the supervision of the doctors Margarita Moreno Montoya, Pilar Flores Cubos and Roberto Álvarez Gómez, who guarantee its quality as well as the preparation of the PhD student to achieve the doctor degree.

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A mis padres que siempre están conmigo,
mis hermanos por su complicidad y refugio.
Mi sobrino Unai, por sacar mi mejor versión.
A mi tío Guillermo, en todo lo que soy estás tú.

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Abstract

Compulsive drinking in Schedule-induced Polydipsia as a feature of vulnerability to psychopathology: implications of serotonin in its development and maintenance

Inhibitory control of behaviour plays a key role in adaptation of humans to the environment. The ability to stop an action in response to a situational change, such as negative consequences or just the achievement of the first aim of that action, it is essential to a healthy psychological and social functioning of the individual. When behaviour is repeated persistently and is not focused on a goal we call it compulsivity. Psychopathology is the most dramatic an also the most costly in social and economic terms manifestation of compulsivity. In the case of obsessive compulsive disorder (OCD), which affects approximately to 2% of general population (Ruscio et al., 2008), the cognitive impairment caused by anxiety, the recurrent intrusive thoughts and the behavioural inflexibility suffered by these patients are quite serious(van Westen et al., 2015). Furthermore, a recent multigenerational family study with humans revealed that individuals with OCD had a 12-fold increased risk of having a comorbid diagnosis of schizophrenia (Cederlöf et al., 2015), while vulnerability to schizophrenia in TOC patients has also been suggested (Peng et al., 2012). Concerning pharmacology, clinical observations showed the participation of the neurotransmitter serotonin (5-HT) in OCD (Angoa-Pérez et al., 2012) and different studies with animal and humans have showed the involvement of the serotonin receptor subtypes 5-HT2A/C in compulsive behaviour modulation (for review, see Aznar and Klein, 2013; Fineberg et al., 2010; 2011).

The present Doctoral Thesis is aimed to evaluate the participation of compulsivity as a factor of vulnerability in different symptoms of schizophrenia and the role of serotonin in the manifestation and maintenance of such compulsive behaviour. For that purpose, we start out from a well established animal model of compulsivity, Schedule-induced polydipsia (SIP; Falk, 1961, 1971), in order to obtain two different populations of high drinker compulsive rats (HD) and low drinkers rats (HD) and subsequently assess their performance in other animal models of psychopathology as Latent Inhibition (LI) and Reversal Learning (RL). Moreover, taking in account that white substance abnormalities are a schizophrenia biomarker, we analysed the corpus callosum, striatum and basolateral amygdala of non-preexposed rats of LI experiment by Myelin Basic Protein (MBP) staining technique. This group of rats was chosen to prevent possible effects of learning derived from preexposure. In the second series of the present Doctoral Thesis we evaluated the effects of chronic tryptophan (TRP) depletion, a serotonin precursor, in compulsive response on SIP and locomotor activity in the Open Field maze of Lister Hooded and Wistar rats to observe whether serotonergic manipulations results on behaviour were strain-dependent. Furthermore, we measured the levels of dopamine, noradrenalin, serotonin and its metabolite, as well as the 5-HT2A and 5-HT1A receptor binding in prefrontal cortex (PFC), striatum, nucleus accumbens, hippocampus and amygdala.

Once we tested the participation of serotonin in compulsive behaviour in SIP, a third study with Wistar rats was carried out to establish the functionality of specific serotonin receptors in compulsive behaviour. For this purpose, three cohorts of rats

were selected by SIP in HD and LD to test the effects of the systemic administration of different serotonergic compounds. In the first experiment of this series we evaluated the effects of the selective serotonin reuptake inhibitor (SSRI) citalopram (0.3, 1 and 3 mg/kg, intraperitoneal injection (i.p.)), the noradrenalin reuptake inhibitor atomoxetin (1, 2, 3 and 5 mg/kg, i.p.) and the serotonin 5-HT2A/C receptor antagonist DOI ((±)-2,5-dimethoxy-4-iodoamphetamine; 0.1, 0.3 y 0.5 mg/kg, subcutaneous administration (s.c.) on SIP. In the second experiment of this series we evaluated the effects of theserotonin 5-HT2A receptor antagonist ketanserin (0.3, 0.6, and 1 mg/kg, ip.), the high specific serotonin 5-HT2C receptor antagonist SB242048 (0.1, 0.5, 1 and 2 mg/kg, ip.), and the high specific serotonin 5-HT2A receptors antagonist M100907 (0.1, 0.5, 1 and 2 mg/kg, ip.) on SIP. In the third experiment of this series we tested the effects on SIP by the combinations of the serotonin 5-HT2Areceptor antagonists ketanserin (0.5 and 1 mg/kg, ip.), M100907 (1 mg/kg, ip.), and the 5-HT2C receptor antagonist SB2420 (1 mg/kg, ip.) with a dose of the 5-HT2A/C receptor agonist DOI (0.5 mg/kg, s.c.).

The results of the present Doctoral Thesis have provided relevant information about the presence of selective attention deficit in rats with a compulsive phenotype on SIP (HD) through LI task, besides the capacity of variable interval 60 (VI 60) to induce compulsive lever pressing in this population. We must add that when myelin differences were measured by MBP protocol, we observed less myelination in corpus callosum, striatum and basolateral amygdala in HD compulsive rats compared with LD. This phenotype of rats also showed behavioural inflexibility measured by RL

task, where they showed poorer performance in all variables measured, including perseveration errors. Nevertheless, HD compulsive rats did not show any learning deficit in this task. Concerning serotonin implication in compulsive behaviour in this compulsive phenotype of rats, theHD Wistar rats showed more sensitivity to TRP chronic depletion by diet on SIP, while Lister Hooded strain did not show such a difference. TRP chronic depletion had effect only in locomotor activity of Lister Hooded rats. Furthermore, serotonin 5-HT2A receptor binding was reduced in stritatum of compulsive HD Wistar TRP depleted rats, while serotonin 5-HT1A receptor binding was reduced in TRP depleted Lister Hooded rats. To finish, when the serotonergic drugs citalopram, atomoxetine, DOI, ketanserin, SB242084 and M100907 were administered in order to test their effect on SIP, we observed that citalopram and DOI effectively reduced compulsive drinking in HD compulsive rats compared to LD rats. Conversely, SB242084 increased compulsive drinking on SIP in HD compulsive rats compared to LD rats. However, atomoxetin, ketanserin and M100907 had no effect on SIP when administered alone. Subsequently, we observed that DOI-induced reduction in compulsive drinking on SIP was blocked by administration of M100907 and ketanserin, both of them serotonin 5-HT2A receptor antagonists in HD rats.

In conclusion, the present Doctoral Thesis provides relevant information about the presence of schizophrenia symptoms in compulsive HD rats, such as alterations in selective attention and behavioural inflexibility, and a vulnerability of this population of rats to develop compulsive behaviour under determinate reinforcing

programs. Furthermore, we add that chronic TRP depletion increases compulsive response, changes in serotonin receptor binding in specific brain areas and that the activation of serotonin 5-HT2A receptors inhibits compulsive drinking on SIP. All together, our results suggest the existence of a vulnerability to psychotic symptoms in the compulsive phenotype and the implication of the serotonergic system in such vulnerability, specifically of the serotonin 5-HT2A receptors, which may have a role in the development of these symptoms.

Resumen general

La bebida compulsiva en Polidipsia inducida por programa como rasgo de vulnerabilidad psicopatológica: la implicación de la serotonina en su desarrollo y mantenimiento

El control inhibitorio de la conducta juega un papel clave en la adaptación del ser humano al mundo que le rodea. Saber detener una determinada acción a tiempo en respuesta a un cambio situacional, ya sea la aparición de consecuencias negativas de la misma o sencillamente la consecución del objetivo por el que dicha acción se inició, es esencial para el funcionamiento psicológico y social de la persona. Cuando una conducta es repetida de forma persistente a pesar de resultar en consecuencias indeseables y además de no perseguir un objetivo concreto, hablamos de compulsividad. La manifestación más extrema de la compulsividad, a la vez de tener un alto coste a nivel económico y social, es la psicopatología. En el caso del trastorno obsesivo-compulsivo (TOC), el cual afecta aproximadamente a un 2% de la población general (Ruscio et al., 2008),el deterioro funcional causado por la ansiedad, los pensamientos intrusivos recurrentes y la inflexibilidad conductual que sufren estos pacientes es bastante severo (van Westen et al., 2015).Además, un reciente estudio multigeneracional realizado con humanos revela que los pacientes con un primer diagnóstico de TOC tienen doce veces más riesgo de tener un diagnóstico comórbido de esquizofrenia (Cederlöf et al., 2015), mientras que también se ha sugerido vulnerabilidad a la esquizofrenia en pacientes de TOC (Peng et al.,

2012). A nivel neuroquímico, existen observaciones clínicas que prueban la implicación de la serotonina en TOC (Angoa-Pérez et al., 2012) y estudios con animales y humanos han mostrado la implicación de los subtipos de receptores serotonérgicos5-HT2A/C en la conducta compulsiva (para revisión, ver Aznar y Klein, 2013; Fineberg et al., 2010; 2011).

La presente Tesis Doctoral se ha dirigido a evaluar la participación de la compulsividad como un factor de vulnerabilidad en diferentes síntomas de esquizofrenia y el papel de la serotonina en el establecimiento de la conducta compulsiva y su mantenimiento. Para ello, se ha partido de un modelo animal de compulsividad, la polidipsia inducida por programa (PIP; Falk, 1961, 1971), para obtener dos poblaciones de ratas diferentes, altas bebedoras (AB) compulsivas y bajas bebedoras (BB), y posteriormente evaluar su ejecución en otros modelos animales de psicopatología como son la Inhibición latente (IL) y el Reversal learning (RL). Además, teniendo en cuenta que las anormalidades en la sustancia blanca son un biomarcador de esquizofrenia, se realizó la técnica de tinción inmunohistoquímica Myelin Basic Protein (MBP) en el cuerpo calloso, estriado y amígdala basolateral de los cerebros del subgrupo de ratas no preexpuestas del experimento de IL. Escogimos este último grupo de animales para descartar cualquier posible efecto del aprendizaje derivado de la preexposición sobre los resultados de la tinción en dichas áreas cerebrales. En la segunda serie experimental de la presente Tesis Doctoral se evaluaron los efectos de la depleción crónica de triptófano (TRP), un precursor de la serotonina, sobre la bebida compulsiva en PIP y

la actividad locomotora en el test de campo abierto en ratas Lister Hooded y Wistar con el fin de comprobar si los efectos de las manipulaciones serotonérgicas sobre la conducta son dependientes de la raza de los animales. Además, se midieron los niveles de dopamina, adrenalina, serotonina y su metabolito, así como la unión de los receptores serotonérgicos 5-HT2A y 5-HT1A en el córtex prefrontal, estriado, núcleo accumbens, hipocampo y amígdala. Una vez probada la participación de la serotonina en la bebida compulsiva en SIP, se realizó un tercer estudio con ratas Wistar para averiguar la funcionalidad de los receptores serotonérgicos sobre la conducta compulsiva. Para este fin, se seleccionaron mediante PIP tres grupos experimentales de ratas AB y BB en los que se probaron los efectos de la administración sistémica aguda de diferentes compuestos serotonérgicos sobre la PIP. En el primer experimento se evaluaron los efectos del inhibidor selectivo de la recaptación de serotonina (ISRS) citalopram(0.3, 1 y 3 mg/kg, inyección intraperitoneal (ip.)), el inhibidor de recaptación de noradrenalina atomoxetina (1, 2, 3 y 5 mg/kg, ip.) y el agonista de los receptores serotonérgicos 5-HT2A/C DOI ((±)-2,5-dimethoxy-4-iodoamphetamine; 0.1, 0.3 y 0.5 mg/kg, administración subcutánea (s.c.) en PIP. En el segundo experimento se evaluaron los efectos del antagonista de los receptores serotonérgicos5-HT2A ketanserin (0.3, 0.6, y 1 mg/kg, ip.), del antagonista altamente especifico de los receptores serotonérgicos5-HT2A M100907 (0.1, 0.5, 1 y 2 mg/kg, ip.) y del antagonista altamente específico de los receptores serotonérgicos 5-HT2C SB242048 (0.1, 0.5, 1 y 2 mg/kg), en PIP. En el tercer experimento se evaluaron los efectos en PIP de las combinaciones de los

antagonistas de los receptores serotonérgicos 5-HT2A ketanserin (0.5 y 1 mg/kg, ip.), M100907 (1 mg/kg, ip.) y el antagonista de los receptores 5-HT2C SB242048 (1 mg/kg, ip.) con la administración del agonista de los receptores 5-HT2A/C DOI (0.5 mg/kg, s.c.).

Los resultados de la presente tesis doctoral han proporcionado información relevante sobre la presencia de un déficit de atención selectiva en ratas con fenotipo compulsivo (AB) en la tarea de IL, además de la capacidad del intervalo variable 60 (IV 60) de inducir respuesta compulsiva en ratas también en presión de palanca. A estos resultados hay que añadir que cuando se midieron las diferencias en mielina en estas ratas mediante el protocolo MBP, se observó menor mielinización en el cuerpo calloso, estriado y amígdala basolateral de las ratas AB compulsivas. Este mismo fenotipo de ratas compulsivas AB mostró inflexibilidad conductual cuando se evaluó su rendimiento en la tarea de RL, donde mostraron peor ejecución de la tarea que las ratas BB en todas las variables, así como mayor número de errores de perseveración. Sin embargo, las ratas AB no mostraron ningún déficit de aprendizaje en este modelo. Además, las ratas compulsivas AB de la raza Wistar mostraron más sensibilidad a la depleción crónica de triptófano mediante dieta en PIP, mientras que entre las ratas BB Wistar y las Lister Hooded no se obtuvieron dichas diferencias. En actividad locomotora, sin embargo, sí se encontraron diferencias entre ratas privadas y no privadas de triptófano en la raza Lister Hooded, mientras que en Wistar dicha privación no tuvo ningún efecto. Además, la unión del receptor 5-HT2A estaba reducida en el estriado de las ratas AB Wistar privadas de triptófano,

mientras que la unión del receptor 5-HT1A estaba reducida en córtex frontal de las ratas Lister Hooded privadas de triptófano. Para terminar, cuando se administraron los compuestos serotonérgico escitalopram, atomoxetina, DOI, ketanserin, SB242084y M100907 para evaluar su efecto sobre la bebida compulsiva en PIP, se observó que el citalopram y DOI redujeron la bebida compulsiva en PIP en ratas AB compulsivas comparadas con las BB. En cambio, SB242084 aumentó la bebida compulsiva en PIP en ratas AB comparadas con BB. Sin embargo, la atomoxetina, el ketanserin y el M100907 no tuvieron ningún efecto sobre la bebida en PIP cuando se administraron solos. Posteriormente, se observó que cuando la reducción de la bebida compulsiva en PIP provocada por DOI era bloqueada por la administración de M100907 y ketanserin, ambos antagonistas de del receptor serotonérgico 5-HT2A.

En conclusión, la presente Tesis Doctoral proporciona datos relevantes sobre la presencia de síntomas de esquizofrenia en ratas AB compulsivas, como son la alteración en la atención selectiva y la inflexibilidad cognitiva, y la vulnerabilidad de dichas ratas a desarrollar conducta compulsiva bajo determinados programas de reforzamiento. Además se aporta que la depleción crónica de triptófano en ratas Wistar aumenta la respuesta compulsiva, produce cambios en la unión de receptores serotonérgicos en determinadas áreas cerebrales y que la activación de los receptores serotonérgicos 5-HT2A inhiben a su vez este comportamiento compulsivo. En conjunto, nuestros resultados sugieren la existencia de vulnerabilidad a los trastornos psicóticos dentro del fenotipo compulsivo y la implicación del sistema serotonérgico en dicha vulnerabilidad, concretamente de los receptores 5-HT2A, los

cuales podrían tener papel modulador en la manifestación de los síntomas estudiados.

1. Introduction

1.1. Compulsiveness and its study: Schedule-induced polydipsia

Compulsiveness has been defined as an inappropriate response perseveration that the individual is unable to hold despite the adverse consequences it entails (Robbins and Crockett, 2010). Thus, the compulsiveness can be characterized as an insensitivity or lack of adaptation of behaviour to situational changes. That is, when a particular course of action or behaviour does not lead to the expected result or even brings unpleasant consequences and yet the person, but is aware of it, is unable to stop, we could talk about compulsiveness. For example, in the case of obsessive-compulsive disorder (OCD), this persistent repetition of the same action or sequence of actions meets most of the time the function of preventing negative consequences as perceived by the individual or states of deep unease. As happens in impulsivity, alterations in the regulation of compulsive behavior lead in the worst cases the development of mental disorders (Fineberg et al., 2010). Although OCD is probably the most characteristic clinical example, compulsiveness plays a role in the symptoms of attention deficit hyperactivity disorder (ADHD), schizophrenia, depression, pathological gambling, intake related disorders, and substance abuse (Skodol and Oldham, 1996). Compulsive behaviour also manifests itself in other neuropsychiatric disorders like autism, Kluver-Bucy syndrome or Tourette's syndrome (American Psychiatric Association, 2010). The World Health Organization estimated the prevalence of Obsessive Compulsive Disorders (TOCs) about 1 to 3% of the population in Western countries, assuming an economic cost of around $ 5

billion a year to the government of a country like the United States (World Health Organization, 2009). Thus, a distinct change in the latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V; American Psychiatric Association, 2013) has been the removal of obsessive -compulsive (OCD) anxiety disorders and the creation of a new group of disorders grouped under the heading of "OCD and related disorders" and the inclusion in this group of disorders and binge excoriation, characterized by lack inhibitory control in scratching or pinching the skin and recurrent compulsive food intake, respectively.

Schedule-induced polydipsia (SIP) is defined as the development of a non-thirsty-motivated adjunctive drinking behaviour under an intermittent reinforcement program in food-deprived animals (Falk 1961, 1966). SIP has been proposed as an animal model for the study of compulsivity (for a review, see Moreno and Flores 2012; Platt et al. 2008). Due to its characteristics of "excessiveness" and "persistence," SIP acquisition manifests as a lack of inhibitory control on fluid intake in animals. A similar phenomenon known as psychogenic polydipsia, a compulsive non-regulatory fluid consumption, is present in 6-20% of psychiatric patients with different disorders related to compulsivity symptoms, such as OCD, schizophrenia and ADHD (de Leon et al. 1994, 2002; Dundas et al. 2007; Evenson et al. 1987; Oades et al. 1998; Verghese et al. 1996). Growing evidence has shown that SIP is a useful model for the neurobiological study of compulsive response in different disorders, such as OCD (Rosenzweig-Lipson et al. 2007; Schechter et al. 2008; Toscano et al. 2008; Woods-Kettelberger et al. 1997), schizophrenia (Hawken et al. 2011; Hawken and Beninger

2013) and alcohol abuse (Gilpin et al. 2008; Mittleman et al. 2003, 2011; Wayner 2002). Indeed, clinical pharmacotherapies that are used in compulsive disorders, such as antipsychotic drugs or selective serotonin re-uptake inhibitors (SSRIs), can efficiently reduce drinking behaviour on SIP without affecting water or food regulatory intake (Didriksen et al. 1993; Mittleman et al. 1994; for a review, see Platt et al. 2008).Moreover, because the SIP procedure is sensitive to individual differences, rats can be divided according to their SIP acquisition (Dantzer et al. 1888; López-Grancha et al. 2008; Mittleman et al. 1988a,b; Moreno et al. 2012), which is highly relevant to explorations of the neurobehavioural mechanisms in subjects with a compulsivity phenotype, such as high drinker rats (HD) that are selected according to SIP (for a review, see Moreno and Flores 2012). Previous studies performed in our laboratory have shown relevant differences between HD rats and low drinker rats (LD) selected according to SIP. For example, the behavioural response to a challenge with dopaminergic drugs, such as D-amphetamine and cocaine (López-Grancha et al. 2008), divergences in the availability of dopamine D_1 and D_2receptors in mesolimbic brain areas (Pellón et al. 2011), a lack of inhibitory control in the 5-choice serial reaction time task 5-CSRTT and increased serotonin and noradrenalin activity in the amygdala in post-mortem brain analyses (Moreno et al. 2012).

Behavioral inflexibility, which is present in compulsive spectrum disorders, can be defined as the inability to change the behavior spontaneously in response to situational demands and it is a symptom shared bysome neuropsychiatric disorders

such as schizophrenia, depression and TOC (Boulougouris and Robbins, 2010). A sensitive task of strengthening both the value and the change in the response strategy is the reversal learning (RL) (Boulougouris et al 2007; Remijnse et al 2009; Waltz et al 2013), ability which recent studies have shown that both schizophrenic patients with the first psychotic episode (Leeson et al 2009; Murray et al 2008) and in chronic cases (Waltz 2013 et al.) and unmedicated (Schlagenhauf 2014 et al.) have marked deficits. In animals, spatial reversal dircrimination learning (Boulougouris et al 2007, 2008, 2009; Boulougouris and Robbins, 2010) is a similar version of RL, where subjects are trained in spatial discrimination of two levers, of which only one produces reinforcement. Once instituted response of the levers, an inversion of reinforcement occurs, so that the pressure of a lever where reinforcement produced was not provided before. Most compulsive subjects take more sessions to correctly implement the second phase. For studying animal models of OCD symptoms they are camouflaged by injury orbitofrontal cortex (OF) as well as serotonergic depletion prefrontal cortex, which has resulted in increased perseverative response RL (Boulougouris and Robbins, 2009; revised in Boulougouris et al, 2009;. Fineberg et al, 2011).

1.2 Schizophrenia

Schizophrenia is a neuropsychiatric disorder that in many cases can be highly disabling and affects approximately 1% of the world population (Insel, 2010). In addition, symptoms affect nearly all aspects of mental activity of the individual,

causing social and workplace result of these severely affected patients, resulting in isolation and psychological suffering that it entails. Although in recent years drug treatments have evolved a lot, and not forgetting that two decades ago about 25% of patients with persistent positive symptoms were resistant to treatment with clozapine (Kane et al, 1988; Rosenheck et al, 1997), yet in recent years we have seen worse in those with chronic schizophrenia patients worse cognitive functioning (Chang et al, 2013;. Harvey et al, 2009;. Hofer et al, 2011). During the course of the disorder, acute episodes are usually characterized by positive symptoms which are followed by a "state deficit" on the chronic phase (Crow, 1980). Alternating positive and negative syndromes in one patient and the heterogeneity of cognitive functions affected by them suggest the existence of complex interactions between brain systems and the influence of situational variables in the manifestation of symptoms. Psychotic symptoms affect nearly all aspects of mental activity, including perception, attention, memory and emotion. Among so-called positive symptoms, hallucinations can be defined as sensory perception in the absence of external stimuli that generate (Lindenmayer and Khan, 2012) and are one of the most characteristic symptoms of schizophrenia, auditory hallucinations being experienced by 50-70 % of patients (Hoffman et al., 2003). A strange behavior observed in schizophrenia has also been listed in positive symptomatology is are repetitive behaviors, stereotypes and ritualistic movements aimless and unstimulated motor activity, which should be differentiated from tardive dyskinesia. Inappropriate affect and sudden changes of the object of this affection may also occur. Regarding the negative symptoms of

schizophrenia, it is known to be more persistent, more stable and less likely to improve the positive (Hull et al., 1997) and that may appear long before the first psychotic episode occurs (Hafner et al., 1995). Negative symptoms include anhedonia, flat or limited affect, poverty of speech, abolition and reduced social activity (Carpenter and Kirkpatrick, 1988). Lindenmayer and Khan (2012) associated the latter group of symptoms with deterioration in general intellectual abilities and executive functions. According to the authors, the stereotypical thinking is also a negative symptom is stereotyped and unproductive thoughts or that infringe or interfere with normal thinking individual, who maintains unreasonable or excessive rigid beliefs. The formal thought disorder is the main feature of cognitive deficits in schizophrenia and recent research to understand its origin has focused on abnormalities of care, problems in using context clues and impaired executive functions.

Selective attention is the ability that allows us to differentiate and filter relevant information from the environment and ignore what is not, an essential skill in decision-making, goal-oriented behavior and good cognitive functioning in general. Since Bleuler in 1911 linked the loss of selective attention in schizophrenia, this disorder has become one of the hallmarks of the disorder and a major focus of study for treatment (Kapur, 2003; Lubow, 2005; Nuechterlein et al. 2006). Selective attention can be evaluated by the phenomenon of latent inhibition. Latent inhibition is defined as the decline in associative strength of a stimulus that has been previously presented not be followed by no consequence and is a robust

phenomenon that has been observed in a wide range of animal species, including humans. The fact that attentional processes are involved in the IL (Lubow, 1989, 2005; Lubow and Weiner, 2010; Pearce & Hall, 1980) is what has led to use this phenomenon to study neuropsychiatric disorders characterized by attentional dysfunction, especially schizophrenia, where abundant empirical evidence linking IL alterations to the symptoms thereof (Lubow, 2005; Lubow and Weiner, 2010; Weiner, 2003) exists. The data from animal models where association between IL and symptoms of schizophrenia is shown come mainly from studies with rats (Killcross et al 1994a, 1994b; Weiner, 2003). To model the changes in the IL in animals have been used both parametric and pharmacological manipulations that reproduce both poles of the phenomenon: interruption-persistence (Barak and Weiner, 2010; Knapman et al 2010; Weiner and Arad, 2009; Young et al., 2005).

1.3 Is compulsive behavior a key element in some psychotic symptoms?

There is evidence of the relationship between compulsive behavior and psychotic symptoms and the importance of contextual factors. For example, a recent study has indicated cannabis, childhood trauma and life in urban environments as modulators of the onset of psychosis in adolescents with obsessive compulsive disorder (Guloskuz et al., 2015), anxiety and emotional stress also they have been listed as modulators of psychotic experiences in OCD patients (Bortolon and Raffard, 2015). In addition, studies of several generations of families have linked OCD schizophrenia and bipolar disorder (Cederlöf et al., 2014) and some of them suggest

the existence of vulnerability to schizophrenia in OCD patients (Peng et al., 2012). In fact, in the latest edition of the DSM (2013) specifications awareness of disease in people diagnosed with OCD are established and are precisely those with lack of insight and / or delusional beliefs that manifest symptoms similar to psychosis, reflecting in some cases the proximity between both diagnostic groups.

1.4 A biomarker of schizophrenia: myelin

Several subtypes of schizophrenia have been associated with lateralization of executive functions (Levin et al., 1989), these alterations related to abnormalities in the white matter (being Federspiel et al, 2006; Walterfang et al, 2006, 2011; Bernstein et al, 2009;. Takahashi et al, 2011).. In fact, it has been found reduced oligodendrocytes in the basolateral amygdala density of patients with schizophrenia (Williams et al., 2013) .One of the proposed structural basis to explain these changes in areas of the brain is demyelination or loss of myelinatedfibers (Bernstein et al, 2015;. Xiu et al, 2015.). Indeed, alterations in myelin have been proposed as a crucial element in the cognitive impairment observed in schizophrenia (Dwork et al., 2007; Fields, 2008; Bernstein et al., 2010a), as well as other neuropsychiatric disorders associated with compulsiveness as OCD syndrome Gilles de la Tourette (Zai et al., 2004; Stewart et al., 2007; Atmaca et al, 2010;. Fan et al, 2012;.Worbe et al, 2015.).

Since the first neuroanatomical and electrophysiological studies indicated the corpus callosum of chronic and visibly thinner and smaller size of limbic structures like the amygdala schizophrenic patients (Rosenthal and Bigelow, 1972. Bigelow et al, 1983;

Bogerts et al., 1985), it was found that changes in inter- andintrahemispheric brain connectivity due to abnormal neural communication is a significant event in schizophrenia (Begré yKoenig, 2008; Schmitt et al, 2011; Bartzokis, 2012;Mulert et al, 2012; Wagner et al, 2013;Holleran et al, 2014) .In fact, the corpus callosum reduced myelination involving alterations in interhemispheric connection in the brain has been associated with reduced left dominance for language and greater duration and severity of symptoms in patients with schizophrenia, as well as the first episode in bipolar patients with psychosis (Whitford et al, 2010;. Lu et al, 2011;Holleran et al, 2014.; Leroux et al., 2015) .In addition, early psychosis has been associated with alterations in the integrity of the myelin in the corpus callosum due to low levels of polyunsaturated fatty acids in the erythrocyte membrane (Peters et al., 2013). Another structure that schizophrenia is important in the striatum, where human studies have associated with reward anticipation salience processing stimulus have found a significant correlation between the activation of this area and positive symptoms of schizophrenia (Nielsen et al. 2012) as well as abnormal powers to irrelevant stimuli (Jensen et al, 2008;. Palaniyappan and Liddle, 2012). Neuroimaging and processing of stimuli with schizophrenic patients have also shown increased amygdala activity when shown both neutral and fearful faces (Gur et al., 2007), as well as correlations between the amygdala and underactive and negative symptoms deficits in empathy (Shepherd et al., 2012).

For compulsiveness, diffusion studies tensor images showed changes in white matter with relatives of OCD patients in various cortex areas (Menzies et al., 2008b) that

match the results of a previous study with patients TOC (Szeszko et al, 2005), which led to the first authors to propose these abnormalities in the white matter as a phenotype of TOC (Menzies et al., 2008b).

1.5 Serotonin role on compulsivity

Neuroanatomical models posit the existence of different top-down circuitries that exert inhibitory control by a prefrontal cortex component over a striatal component, which may drive the compulsive and impulsive behaviour (Robbins, 2007; Brewer and Potenza, 2008). Thus, in these models, it is hypothesized that there are two different circuitries for compulsive and impulsive behaviour. In the compulsive circuit, hyperactivity within the caudate nucleus (dorso-medial striatum in the rat)and hypoactivity within the orbitofrontal cortex may result in an increase of compulsive behaviour. Similarly, in the impulsive circuit, hyperactivity within the ventral striatum or nucleus accumbens and hypoactivity within the anterior cingulate or ventromedial prefrontal cortex may lead to increase of impulsive behaviour (for review, see Fineberg et al., 2010). However, overlap between impulsive and compulsive circuitries may occur (Hollander and Wong, 1995), i.e. dysregulation of the impulsive circuit may affect the compulsive circuit and vice versa (Fineberg et al., 2010).

The serotonergic dysfunction has been linked to impulsive-compulsive spectrum disorders. Selective serotonin reuptake inhibitors (SSRIs) are effective in treating compulsory behaviors across disorders on the obsessive-compulsive spectrum.

However, some disorders with compulsive symptoms such as trichotillomania, Tourette syndrome or ADHD appears SRRI-unresponsive (Fineberg et al. 2014). Evidence from animal and human studies implicate 5-HT (5-hydroxytryptamine) system in impulsivity and compulsivity (Eagle and Baunez, 2010; Fineberg et al, 2010). Concretely, different 5-HT_2 sub-receptors have been suggested as mediating compulsivity (Aznar and Klein, 2013; Fineberg et al., 2010, 2011). Positron emission tomography (PET) studies in drug-naive OCD patients revealed reductions in serotonin 5-HT_{2A} receptor availability in the frontal cortex (Perani et al., 2008), whereas increases were found in the caudate nucleus (Adams et al., 2005). Moreover, specific correlations were found between serotonin 5-HT_{2A} receptor availability in the orbitofrontal cortex, clinical severity and age of onset of the disorder (Perani et al., 2008; Simpson et al., 2011), which suggests the important role of serotonin 5-HT_{2A} receptors in compulsive symptoms.

Under normal physiological conditions the biosynthesis of 5-HT is limited by the availability of precursor Tryptophan (TRP), an essential amino acid (Fernstrom, 1983; Gessa et al., 1974). A non-invasive and naturalistic method to reduce central 5-HT is nutritional manipulation of TRP. Rats receiving a free-TRP diet reduce 5-HT content, synthesis (Gessaet al., 1974) and release (Stancampianoet al., 1997a, 1997b). Acute administration of TRP-free diet (ATD) produces a moderate depletion in adult rats (Brown et al., 1998; Lieben et al., 2004). However, a 14-day chronic tryptophan depletion by diet (CTD) reduces brain 5-HT levels about 35-40% (Fadda et al., 2000) and 75% for 5-weeks (Vergnes and Kempf, 1981). Moreover, long-term TRP depleting

diets lead to changes in serotonergic receptors in animals increasing 5-HT_{2A} receptors density but with no effect on 5-HT_{1A} subtypes (Cahir et al., 2007; Franklin et al., 1999).

Animal studies utilizing 5-HT depletion have reported changes on inhibitory control. Serotonindepleted rats induced by administration of the selective neurotoxin 5,7 dihydroxytryptamine (5,7-DHT) showed significant increases in premature responding in 5-CSRTT (Carli and Samanin, 2000; Harrison et al., 1997a, 1997b; Winstanley et al., 2004), poorer performance of the no-go trials (Harrison et al., 1999; Masaki et al., 2006) and reversal phases of the Go/No Go task (Masaki et al., 2006), and choice of the smaller reward over larger reward in Delay Discounting Task (Mobini et al., 2000; Wogar et al., 1993; Bizot et al., 1999). Mice lacking the gene encoding brain tryptophan hydroxylase 2 (Tph2-/-), the initial and rate-limiting enzyme in the synthesis of serotonin show intense compulsive and impulsive behaviors to include extreme aggression (Angoa-Pérez et al., 2012).

Moreover, studies in rodents using TRP-free diet have demonstrated changes by increasing behaviours such as sensitivity to pain (Kantak et al., 1980), stress-reactivity (Tanke et al., 2008; Uchida et al., 2005), aggressiveness (Vergnes and Kempf, 1981), locomotor activity (Vergnes and Kempf, 1981) and sexual behaviour (Fratta et al., 1977). The increase of these behaviours may suggest a lack of inhibition due to the 5-HT brain manipulation leading to compulsive behaviour. In fact, reductions of forebrain serotonin induced compulsive cocaine seeking in rats (Pelloux et al., 2012). Although some studies have reported impaired reversal

learning by invasive 5-HT depletion (Bari et al., 2010; Wallace et al., 2014; Rygula et al., 2014; Lapiz-Bluhm et al., 2009), only one study have evaluated the effect of acute TRP depletion on compulsivity, showing no effects on reversal learning and extinction (Van derPlasse and Feenstra, 2008). No previous studies have evaluated the effect of a chronic administration of TRP-free diet in compulsive behaviour. We hypothesized that a reduction of 5-HT by chronic TRP-free diet administration will produce an increase of compulsive drinking on the SIP model that will be manifested in changes of the 5-HT$_{2A}$ receptor subtype.

1.6 Serotonin 5-HT2$_A$ receptors modulation in compulsive behaviour

Convergent evidence from animal and human studies has suggested a key role of serotonin 5-HT$_{2A/C}$ receptors subtypes in compulsive behaviours (for a review, see Aznar and Klein 2013; Fineberg et al. 2010, 2011). Positron emission tomography (PET) studies in drug-naive OCD patients revealed reductions in serotonin 5-HT$_{2A}$ receptor availability in the frontal cortex (Perani et al. 2008), whereas increases were found in the caudate nucleus (Adams et al. 2005). Moreover, specific correlations were found between serotonin 5-HT$_{2A}$ receptor availability in the orbitofrontal cortex, clinical severity and age of onset of the disorder (Perani et al. 2008; Simpson et al. 2011), which suggests a key role of serotonin 5-HT$_{2A}$ receptors in determining compulsive symptoms.

The SSRI fluoxetine, used as clinical pharmacotherapy in compulsive disorders, which exhibits appreciable affinity for serotonin $5\text{-HT}_{2A/C}$ receptors (Knight et al. 2004), has been shown to reduce compulsive water intake on SIP in a dose-dependent manner via an acute administration (Martin et al. 1998, 2002; for a review, see Platt et al. 2008), as well as by chronic low doses after the second or third week, which is consistent with the efficacy of SSRIs in the clinical treatment of OCD patients (Woods et al. 1993). Previous studies have demonstrated an implied role for serotonin 5-HT_{2C} receptors in the efficacy of SSRI to reduce compulsive drinking in SIP; thus, serotonin 5-HT_{2C} receptor agonists reduced compulsive drinking behaviour, whereas this effect was attenuated by treatment with serotonin 5-HT_{2C} receptor antagonists (Martin et al. 1998, 2002; Rosenzweig-Lipson et al. 2007). Although, recent studies have associated the alteration in the serotonin 5-HT2A receptor subtype with a high compulsivity in OCD, its implication in a compulsive phenotype population of rats, such as HD selected by SIP, has not been explored.

2. Objectives and hypothesis

The aim of the present Doctoral Thesis was to study whether in a population with a vulnerability to compulsive drinking, selected by the animal model of compulsive behavior SIP, there were also other alterations that are present in psychopathologies with comorbidity in compulsive symptomatology such as schizophrenia or OCD. The alterations explored were selective attention deficit, behavioural inflexibility, and the existence of myelin alterations. We also explored the neurochemical and neuroanatomical mechanisms that could be due under this vulnerability to compulsivity. For that purpose, we investigated the role of serotonin alterations in compulsive drinking on SIP and the participation of specific serotonin receptors in its modulation. Therefore, in the present Doctoral Thesis, we selected two specific populations with differences in compulsive behavior, high drinkers (HD) and low drinkers (LD) animals by SIP procedure to assess the following specific aims:

1. Evaluate the existence differences in selective attention and behavioural inflexibility between HD and LD rats selected by SIP through the behavioural animal models latent inhibition and reversal learning.

2. Investigate whether myelin abnormalities are present in HD rats through Myelin Basic Protein staining.

3. Explore the participation of the serotonergic system and strain dependent differences in compulsive drinking on SIP by chronic tryptophan depletion.

4. Explore the existence of neurochemical and neuroanatomical serotonin alterations after chronic tryptophan depletion in different brain areas.

5. Assess the function of serotonin 5-HT$_{2A/C}$ receptors in compulsive drinking on SIP.

The experimental scheduling is presented below:

Experimental series 1.   **Behavioural and biological markers of schizophrenia in high compulsive drinker rats selected by schedule-induced polydipsia (SIP)**
Previous SIP selection: HD vs LD
Evaluate selective attention through Latent Inhibition procedure
Evaluate behavioural inflexibility through Spatial Reversal Learning task
Evaluate myelin alterations by Myelin Basic Protein staining
Experimental series 2.   **Tryptophan depletion in compulsive behaviour in rats: strain dependent effects and associated neuromechanisms**
Previous SIP selection: HD vs LD
Test the effects of chronic tryptophan-free diet on SIP and locomotor activity
Investigate serotonergic alterations in different brain areas
Experimental series 3.   **Function of serotonin 5-HT2A/C receptors in compulsive behavior on SIP**
Previous SIP selection: HD vs LD
Evaluate the effect of SSRI, NRI and serotonin 5-HT2A/C receptor agonist on SIP
Assess the effect of serotonin 5-HT2A/C receptor antagonists on SIP
Test whether serotonin5-HT2A/C receptor antagonists block the effect of 5-HT2A agonist on SIP

Taking in account the information we have previously presented in the introduction section, we proposed the following hypothesis:

(1) The animals that present compulsive drinking on SIP (HD) might also show other behavioural and biological markers related to psychopathologies that have comorbidity of compulsivity symptoms. HD rats could show selective attention deficit by less latent inhibition effect, behavioural inflexibility on the spatial reversal learning task and myelin abnormalities.

(2) Chronic tryptophan depletion may induce compulsive drinking on SIP in both strain of rats, due to neurochemical and neuroanatomical changes in the serotonergic system.

(3) HD rats selected by compulsive drinking on SIP, could show reductions in their drinking on SIP after SSRI and atomoxetine administration. Furthermore, the activation of 5-HT2A/C receptors by the administration of the serotonin receptor agonist DOI might also reduce the compulsive drinking on SIP. This effect should be blocked by the co-administration of the 5-HT2A/C receptor antagonist ketanserin, M100907 and SB242084.

3. Series one:

Behavioural biomarkers of schizophrenia in high compulsive drinker rats selected by Schedule-induced polydipsia

In the present study we assessed the behavioural and biological markers of schizophrenia in compulsive rats selected by SIP through the Latent Inhibition paradigm and the behavioural inflexibility showed in a Spatial Reversal Learning task (Boulougouris and Robbins, 2010; Gray et al., 1991; Leeson et al., 2009; Morice, 1990; Murray et al., 2008; Pantelis et al., 1999; Waltz et al., 2013; Weiner 1990, 2003). We also analysed the existence of a myelin abnormalities in different brain areas that have shown to play a role in LI (Schiller and Weiner, 2014), by comparing the myelin basic protein (MBP) staining between HD and LD rats selected by SIP. Thus, these results could contribute to better characterize the HD rats as a compulsivity phenotype, helping to understand the comorbidity of these signs in the same individual and its contribution to vulnerability to schizophrenia.

3.1 Methods and materials

3.1.1 Subjects

61 male Wistar rats (Harlan Ibérica, Barcelona, Spain), weighing approximately 275–300 g at the start of the experiments, were housed three per cage (50×15×25 cm) at 22°C and under a 12 hour light–dark cycle (light off at 08:00 h), with food and water available ad libitum. After 10 days of habituation, rats were gradually reduced to 85% of their free-feeding body weight by controlled feeding and maintained at this level of deprivation throughout the experiment. Food was available by daily feedings of lab chow approximately 30 min after each experimental session. All testing was carried out between 09:00 and 14:00 h. All procedures were performed in accordance with Spanish Royal Decree 1201/2005 on the protection of experimental animals and the European Community Council Directives (86/609/EEC).

3.1.2 Experimental Procedures

3.1.2.1 Schedule-Induced Polydipsia (SIP) procedure

Two cohorts of rats were tested in thirteen operant SIP chambers (32×25×34 cm) (MED Associates, St. Albans, VT). SIP experimental procedure was based in a previous study (for review, Moreno and Flores, 2012). Scheduling and recording of the experimental events were controlled by computer with the program Med PC (Cibertec SA, Spain). Over two successive days, a water ingestion test was given (baseline). Sixty pellets (Noyes 45 mg dustless reward pellets; TSE Systems,

Germany) were placed together, and the amount of water consumed by each rat in 60 min was measured. After one day of adaptation, the animals were exposed to a fixed time 60-s (FT-60s) schedule of food pellet deliver in 60-min sessions. Water bottles containing 100 ml of fresh water were provided during each session. 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 of the two cohorts in each group LD and HD rats was n=15-16). The following measures were recorded for each rat: (a) total amount of water (millilitres) removed from the bottle, (b) total licks to the bottle and (c) total entries into the food magazine.

The order of training and screening was as follows: for the first cohort, SIP and Latent Inhibition were tested; for the second cohort of rats; SIP and Reversal Learning task were tested. Each task commenced at least 2 weeks after the previous one, and interference among tasks was unlikely because each one involved a different type of operant response for food reward.

3.1.2.2 Latent inhibition

After separation on HD and LD rats by SIP procedure, selective attention of the first cohort was tested by Latent Inhibition paradigm. Animals were formerly trained in lever press. Then, one group was preexposed to a tone and finally all animals were

tested in conditioned suppression of lever press (Hall and Channell, 1985; Killcross and Robbins, 1993).

Training sessions

Two 30 min sessions took place where each lever press was followed by the drop of one pellet of food in the magazine (Fixed Ratio 1), being 60 pellets the maximum that animals were allowed to earn. On the two following days, rats were exposed to a variable interval 30 s (VI30) schedule on 30 min and 40 min sessions, respectively. Thereafter, three 40 min sessions on VI60 were used.

Preexposure

LD and HDrats were divided intotwo subsequent groups according to preexposed condition (PRE) *versus* no-preexposed (NO-PRE) (n=8). All groups received a50 minsession of a VI60 schedule during thirteen days, but PRE groups received two presentations per session of a tone of 85 dB and 4 kHz and 90s of duration, the former after 10.5 min the rat enter in the chamber and the second after 39 min.

Conditioning

In the last four sessions of the experiment all the rats received a 50 minVI60 training session with two presentations of the tone immediately followed by a 0.5 mA shock of 0.5sduration. A suppression ratio was calculated for each session for each subject: $a/ a +b$, where a represents the total responses (lever presses) emitted in the presence of the tone and b the total responses (lever presses) emitted during the two

90 s periods immediately preceding each tone trial (Hall and Channell, 1985). Trained levers were counterbalanced between subjects.

3.1.2.3 Reversal Learning

After SIP procedure, the second cohort of HD and LD ratswas tested on behavioral inflexibility by Spatial Reversal Learning task. The animals were required to learn the initial spatial location of a reinforced lever and then to press the correct lever when the position was reversed (Boulougouris et al., 2007, 2008, 2009).

Pretraining

Rats were trained to enter in the magazine in order to trigger the presentation of the two levers and to press them for food pellets delivery. Right and left levers were trained separately in 15 min sessions, first under FR1 schedule with a criterion of 50 presses and after with FR3 schedule with a criterion of 150 presses, on two consecutive days. The subjects were required to enter in the magazine within the following 20 s in order to trigger the presentation of a single lever and make a press within 10 s to get a pellet of food, which lead to the retraction of the lever and the initiation of a 5 s intertrial interval (ITI). FR3 schedule was used instead FR1 with the purpose of prevent accidental correct responses and easy detection of the contingency.

Acquisition of spatial discrimination

This phase started with both levers present and consisted in a maximum of five 10-trials blocks in a 15 min session. Thus the subjects were required to complete nine correct trials in a block of ten to reach the criterion, by three consecutive presses in the reinforced lever and this criterion was maintained along the experiment. Each trial began with the switch on of the house light and a limited hold period of 20 s to enter in the magazine, which led to the presentation of the levers. Response interval was 10 s, and failure to respond in either of these periods resulted in the retraction of the levers and the start of the ITI. The reinforced lever was held constant in each subject and counterbalanced between subjects. This phase was repeated next days until the criterion was achieved.

Within session reversal learning

As long as reversal learning presupposes retention of previously acquired information, this stage began with a retention task of the previous one. Once the subjects achieved the criterion, the position of the reinforced lever was reversed. Rats were exposed to three more reversals, each one starting with a retention phase of the previous contingency.

Spatial discrimination

| Left lever | Right lever | 5x10-trial block |

Retention of spatial discrimination

| Left lever | Right lever | 5x10-trial block |

Within Session Reversal Learning

Initial phase

| Left lever | Right lever | 5x10-trial block |

Reversal phase

| Left lever | Right lever | 5x10-trial block |

Criterion: 9 correct trials/block

Adapted from Boulougouris, Castañé and Robbins, 2009

Main variables measured were: number of trials to criterion and number of incorrect responses. An incorrect trial meant three incorrect responses, thus, three presses in the incorrect lever. Errors were divided in perseverative: six or more consecutive presses in the wrong lever; and learning: those under six consecutive presses. Control measures were latency to collect the pellet of food, latency to respond to levers and omissions (for detailed description, see Boulougouris et al., 2007, 2008, 2009).

3.1.2.4 Myelin Basic Protein Immunohistochemistry

After termination of behavioural experiments, eight no preexposed rats from HD and LD groups of the first cohort (latent inhibition), were used to study the myelination, according with the method of Fuentes et al. (2007). For MBP inmunostaining, animals were deeply anesthetized by intraperitoneal injection of pentobarbital (180 mg/kg) and trans-cardially perfused with 4% paraformaldehyde

(PFA) in 0.1 phosphate buffer (PB), pH 7.4. Brains were placed in 30% sucrose/phosphate buffered solution (PBS) for 48 h at 4 ºC and then snap frozen in methylbutane and stored at -20 ºC. Serial coronal sections of 40 µm were cut with a cryostat (Leyca)and stored in -20 ºC in a 40% PB 0.1M, 30% glycerol and 30% ethylene glycol buffer. After permeabilization, the sections were incubated two overnights with anti-MBP Polyclonal Antibody (Chemicon, Barcelona, Spain) at a dilution of 1:1000. The sections were then incubated with goat anti-rabbit secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with avidin-biotin-peroxidase complex (Vector Laboratories) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution containing 0.02% 3, 3′ diaminobenzidinetetrahydroclhoride (DAB) and 0.03% H202 in Mm-TrisHCl (pH 7.6) for approximately 5 min. The sections were then washed three times and mounted onto gelatine-coated slides. The slides were air-dried two overnights at room temperature and cove rslips were mounted.

Different brain regions were selected according to the rat brain atlas of Paxinos and Watson (1998). according to the following stereotaxic anterior-posterior (AP) coordinates (Paxinos and Watson, 1998):in the centre of corpus callosum (AP-0.26 mm to -2.80 mm from bregma); in the corpus striatum AP+1.20 mm to +0.96 mm from bregma and in basolateral amygdala AP -1.80 to -3.30 mm from bregma. Quantification of myelinated fibres were analysed by Image J software (Image J ver. 1.47), using a defined threshold level and densities quantified as the average pixel

density in each group within frames of 0.25mm2, from three brain sections per animal. The results in the measure of each animal are expressed as the average density of both brain hemispheres.

3.2 Data analysis

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

Latent inhibition: For training sessions, statistical differences at VI30 and at VI60 were determined by Student's t-test with "group" (HD and LD) as the between-subject factor. Conditioning data were analysed by two-way repeated measures ANOVA, with "preexposure" (PRE and No-PRE) as between-subject factor and "session" (4 levels) as the within-subject factor.

Reversal learning: Data for each variable were analysed by two-way repeated measures ANOVA with "group" (HD and LD) as between-subject factor and either Retention phase without reversal occurring (4 levels: retention of spatial discrimination, retention of Reversals 1-3), or Retention phase preceding reversal (four levels: retention of spatial discrimination, retention of Reversals 1-3), or Reversal phase (4 levels: Reversals 1-4)as the within-subject factor.

Myelin Basic Protein Immunohistochemistry: Statistical differences in optical densities of myelinated fibres between HD and LD rats in the different brain areas were determined by Student's t-test with "group" (HD and LD) as the between-subject factor.

The results were expressed as the mean ± standard error of the mean (SEM). When appropriate, *post hoc* comparisons were made using the Newman-Keuls test. Statistical significance was set at $p<0.05$. All analyses were computed using Statistica software package (version 5.0).

3.3 Results

3.3.1 HD and LD selected by SIP

Figure 3.1 1and Table 3.1 show the mean of water intake, licks and magazine entries of HD and LD rats on SIP .HD rats increased water intake (interaction SIP session × group effects: group 1 $F_{19,570=}18.16$, p<0.0001; group 2 $F_{19,513}=9.29$, p<0.0001) and licks (interaction SIP session × group effect: group 1 $F_{19,570}=6.83$, p<0.0001; group 2 $F_{19,513}=14.03$, p<0.0001) compared the LD group on SIP. *Post hoc* analysis indicated a significant increase in water intake on SIP of HD compared LD rats, starting at session 3 in group 1 (p<0.01) and at session 5 in group 2(p<0.0001). Furthermore, compared to the 1st session, HD animals significantly increased their consumption of water at session 3in group 1 (p<0.05) and at session 4 in group 2 (p<0.01) reaching stable levels of water intake at session 11 in group 1and at session 10 in group 2. In addition, LD animals did not show a significant increase in their consumption of water across SIP sessions. In number of licks, HD showed a significant increase compared LD rats that started at session 2 in group 1 (p<0.01) and at session 1 in group 2 (p<0.01).There were no significant differences between the HD and LD rats in the magazine entries measure on SIP (Table 3.1).

Figure 3.1

The mean (±SEM) water intake in FT-60s across 20 sessions of SIP of group 1 (a), and group 2 (b). Statistical analyses indicate significant differences between HD and LD from that session onward (*asterisk*) *p<0.05*. Significant differences in the same group are indicated by number sign (#).
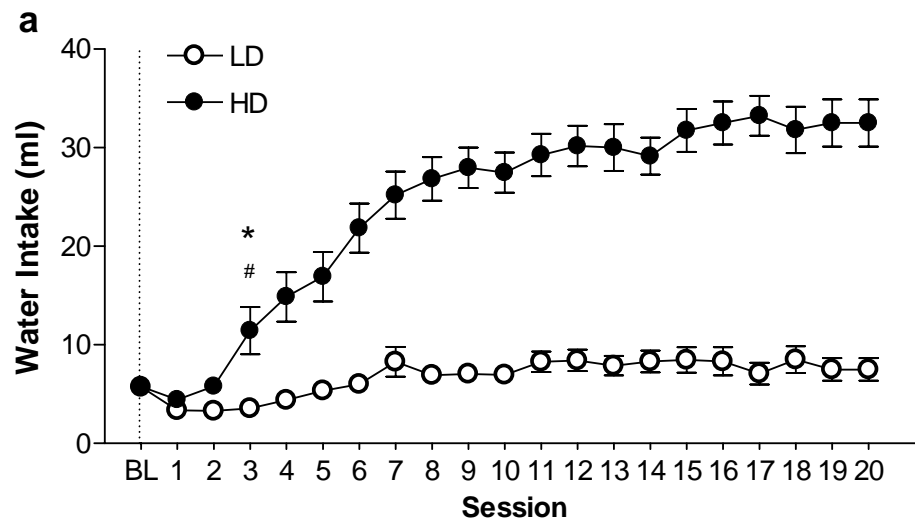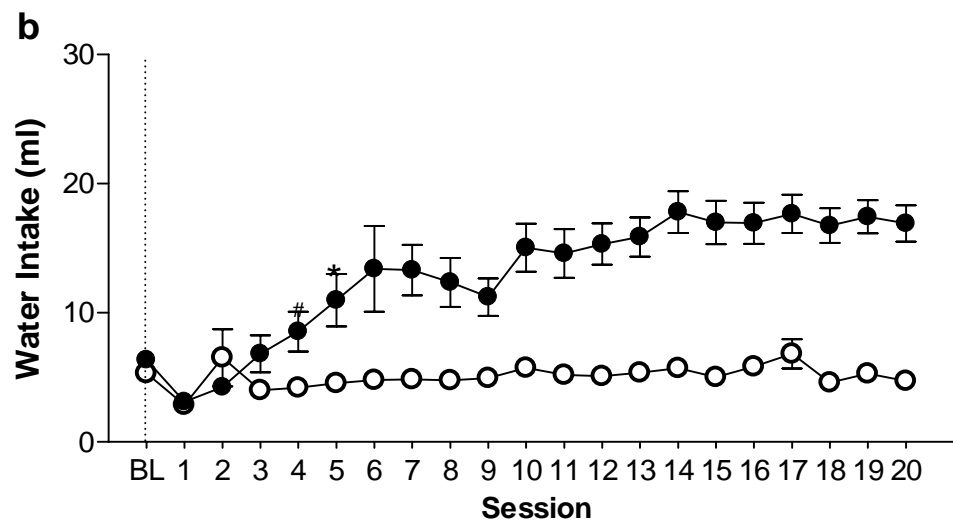
Table 3. 1

Number of licks and magazine entries of group 1 and group 2 during the last 5 sessions of SIP. Data are means ± SEM. ** p< 0.0001 indicates significant differences between HD and LD rats.

	Licks		Magazine entries	
	HD	LD	HD	LD
Group 1	6.143,5 ± 777,8**	1.554,8 ± 396,1	1.999,5 ± 298,6	2.153,9 ± 540,7
Group 2	4.141,6 ± 429,2**	721,2 ± 158,6	2.408,9 ± 550,9	2.641,3 ± 327,1

3.3.2 Latent inhibition

Training sessions

Figure 3.2 shows the means of lever pressing for HD and LD in VI 30 s and VI60 s reinforcement schedules. In VI60 s schedule, HD rats revealed a significant increase in lever pressing compared LD rats ($t(30)=-2.92$; $p<0.05$). No differences were observed in VI30between HD and LD rats ($t(30)= -1.15$, $p=0.25$).

Conditioning

Figure 3.3 shows the mean of suppression ratios of HD and LD rats on Latent Inhibition paradigm. In the LD group, the preexposure affected the conditioning phase showed by an increased suppression ratio response (interaction preexposure x session: $F_{3,42}=2.88$, $p<0.05$; preexposure effect: $F_{1,14}=18.12$, $p<0.001$; session effect: $F_{3,42}=6.63$, $p<0.001$). Post hoc analysis indicated that PRE rats had an increased response compared NO PRE rats from session 1 to 3($p<0.001$), showing a trend to still maintain significant differences at session 4 ($p=0.056$). In the HD group, there was a weaker preexposure effect revealed by less differences in the suppression ratios between PRE and NO PRE rats in the condition phase (interaction preexposure x session: $F_{3,42}=4.72$, $p<0.001$; session effect: $F_{3,42}=19.56$, $p<0.0001$; no preexposure effect: $F_{1,14}=1.86$, $p=0.19$). Post hoc analysis showed that the increased suppression ratio response of PRE rats compared NO PRE rats was only present at sessions 1 and 2 ($p<0.05$, $p<0.01$), but not at sessions 3 and 4 ($p=0.73$, $p=0.90$) on conditioning phase.

Figure 3.2

Lever presses for HD and HD rats during VI30 schedule session and last session of IV60 schedule in Latent Inhibition Experiment. *Open* symbols refer to LD group and *closed* symbols refer to HD group. Data are expressed as the mean ±SEM. **$p<0.01$ indicate significant differences in lever pressure versus control group.
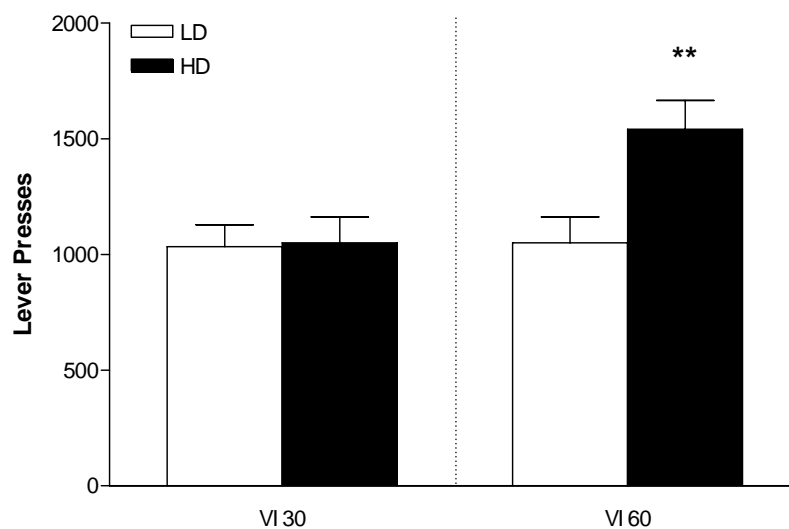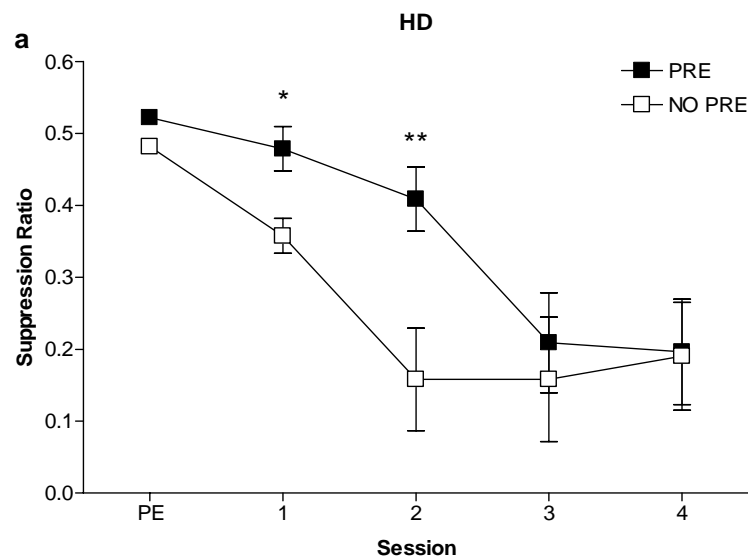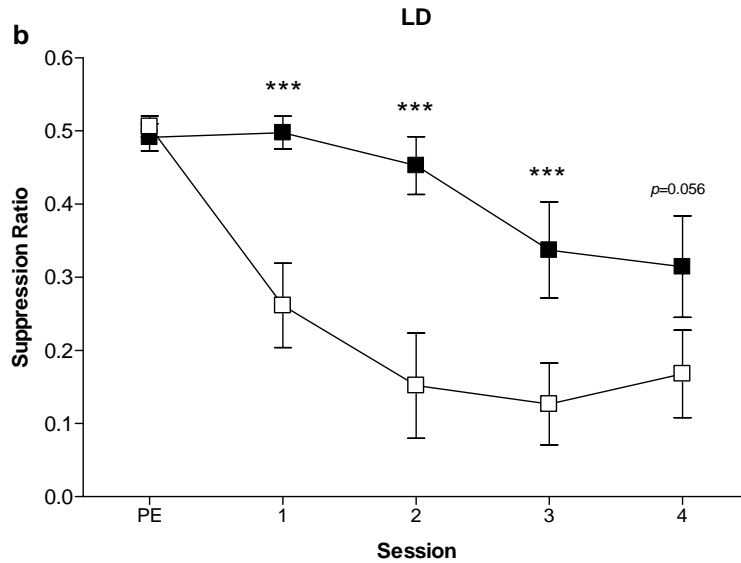
Figure 3.3

Suppression ratio for the last day of preexposure and the four sessions of conditioning for HD group (a) and LD group (b)in Latent Inhibition Experiment. Data are expressed as the mean ±SEM. *p<0.05, **p<0.01 and ***p<0.001 indicate significant differences in lever pressure versus control group.

b

LD

*(Figure: graph with y-axis labeled "Suppression Ratio" ranging from 0.0 to 0.6; x-axis labeled "Session" with values PE, 1, 2, 3, 4. Filled squares and open squares plotted; significance markers *** above sessions 1, 2, 3 and p=0.056 above session 4.)*

3.3.3 Reversal learning

Trials to criterion

Figure 3.4 shows the mean of the number of trials to criterion on the Reversal Learning task. During the Retention without reversal, no significant differences were found between HD and LD rats. In contrast, in the retention preceding reversal the HD had an increased number of trials to complete the criterion compared LD rats (interaction group x retention: $F_{3,81}=5.31$, p<0.05; group effect:$F_{1,27}=5.45$, p<0.05; retention phase effect: $F_{3,81}=9.63$, p<0.0001).*Post hoc* analysis revealed that HD had a significant increase of the trials to criterion in the retention of Reversal 1 compared to LD group (p<0.0001), these differences did not remain in the retention of reversals 2 and 3 (Figure 3.4a).During the Reversal, the HD had an increased number of trials to complete the criterion compared LD rats (interaction group x reversal:$F_{3,81}=3.81$, p<0.01; group effect:$F_{3,81}=30.65$, p<0.0001).*Post hoc* analysis revealed that HD rats

need significantly more trials to reach the criterion during Reversal 1 (p<0.01), and also in Reversal 2 by a trend to significant differences (p=0.09), compared to LD rats(Figure 3.4b).

Incorrect responses to criterion

Figure 3.5 shows the mean of the incorrect responses to criterion on the Reversal Learning task. During the Retention without reversal, ANOVA revealed a significant retention effect ($F_{3,81}$=2.88, *p*<0.05). In retention preceding reversal, HD had an increase in incorrect responses (interaction group x retention:$F_{3,81}$=2.96, *p*<0.05; retention effect:$F_{3,81}$=4.55, *p*<0.01). *Post hoc* analysis of retention preceding reversal indicated that HD rats made significantly more incorrect responses in Reversal 1 retention than LD rats (*p*<0.01) (Figure 3.5a).During the Reversal phase, HD showed an increased number of incorrect responses to criterion (interaction group x reversal:$F_{3,81}$= 2.91, *p*<0.05; session effect: $F_{3,81}$=56.85, *p*<0.0001; no group effect $F_{1,27}$=2.91, *p*=0.14). *Post hoc* analysis revealed that HD rats made significantly more incorrect responses in Reversal 1 (p<0.01), and also in Reversal 2 by a trend to significant differences (p=0.06), compared to LD rats (Figure 3.5b).

Perseverative and learning errors

Figure 3.6 shows the mean of perseverative and learning errors on the Reversal Learning task. . During, reversals, HD rats showed a significant increased number of perseverative errors compared LD rats (interaction group x session effect: $F_{3,78}$=2.55, *p*<0.01; session effect: $F_{3,78}$=55.26, *p*<0.001;no group effect $F_{1,26}$=1.82, *p*=0.18).*Post hoc*

analyses revealed that HD had a significant increased number of perseverative errors in Reversal 1 ($p<0.01$)compared to LD rats (Figure 6a). In contrast, during reversal no significant differences were observed in learning errors measure (Figure 3.6b).

Omissions and latencies to respond

No significant differences were found between HD and LD rats neither in omissions (Table 3.2) nor in latency to respond (Table 3.3) at any stage of the experiment.

Figure 3.4

Number of trials to criterion through (a) Reversal phase and the retention phase without reversal and retention phase preceding reversal (b) in Reversal Learning Experiment. Data are expressed as the mean ±SEM. ***$p<0.001$ and **$p<0.01$ indicates significant differences in number of trials to reach the criterion versus control group.
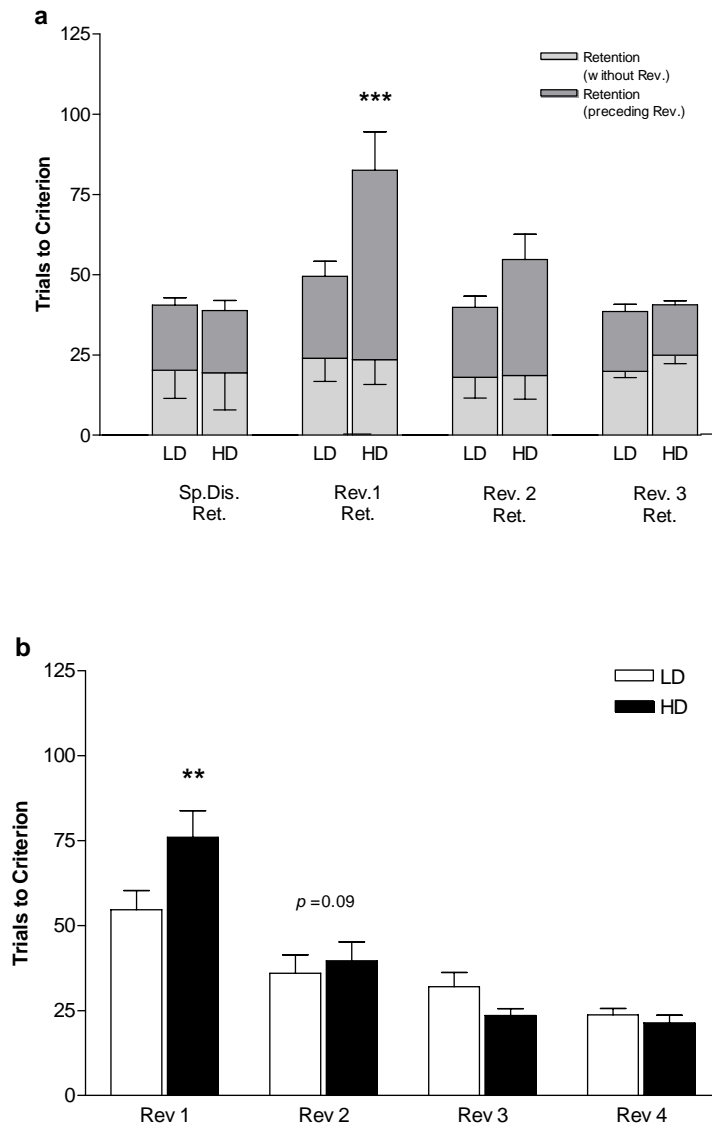
Figure 3.5

Number of incorrect responses to criterion through (a) Reversal phase and the retention phase without reversal and retention phase preceding reversal (b) in Reversal Learning Experiment. Data are expressed as the mean ±SEM. **p<0.01 indicates significant differences in number of incorrect responses to reach the criterion versus control group.
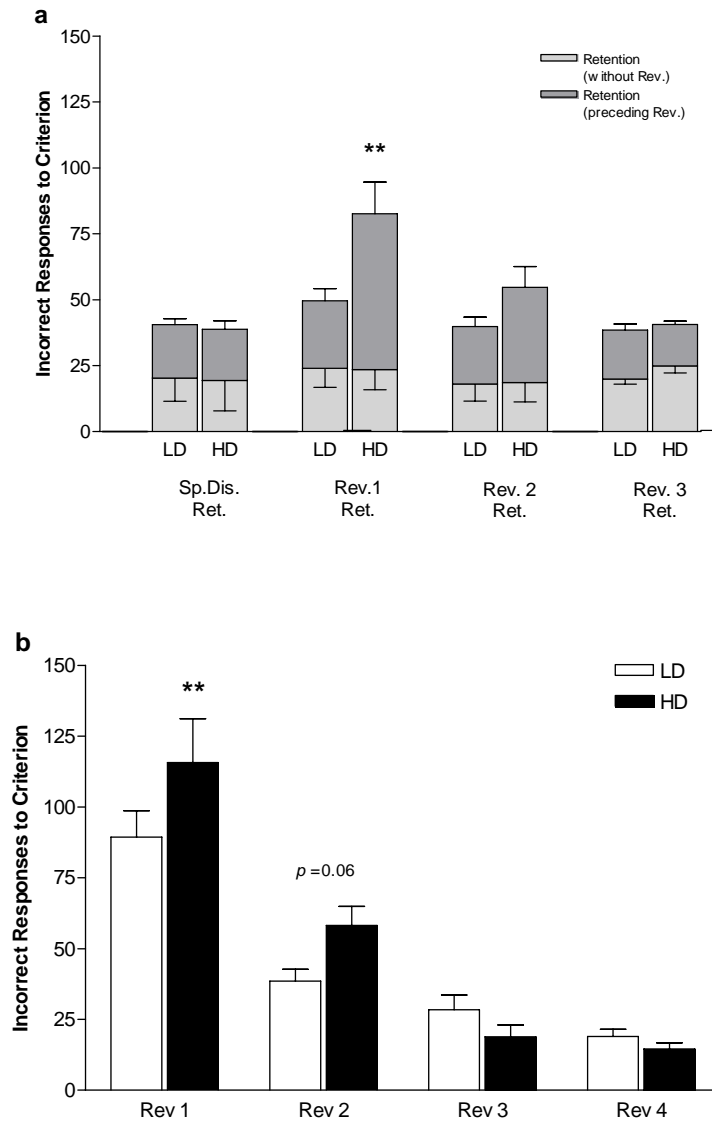
Figure 3.6

Mean error scores ±SEM of HD and LD groups in Reversal Learning Experiment: (a) perseveration (≤ 6 consecutive presses on the wrong lever), (b) learning (> 6 lever presses on the wrong lever). **$p<0.01$ indicates significant differences in number perseverative errors versus control group.

Table 3.2

Mean values ± SEM of Omissions during Retention (Collapsed Retention without Reversal and Retention Preceding Reversal) and Reversal phase of Reversal Learning Experiment.

	Retention of Spatial Discrimination	Retention of Reversal 1	Retention of Reversal 2	Retention of Reversal 3
HD	2,7 ± 2,4	4,3 ± 4,1	1,0 ± 0,1	0,0 ± 0,0
LD	0,4± 0,2	0,1 ± 0,1	0,1 ± 0,1	0,1 ± 0,1
	Reversal 1	Reversal 2	Reversal 3	Reversal 4
HD	1,1 ± 0,4	0,1 ± 0,1	0,1 ± 0,1	0,1 ± 0,1
LD	0,4 ± 0,2	0,1 ± 0,1	0,1 ± 0,1	0,1 ± 0,1

Table 3.3

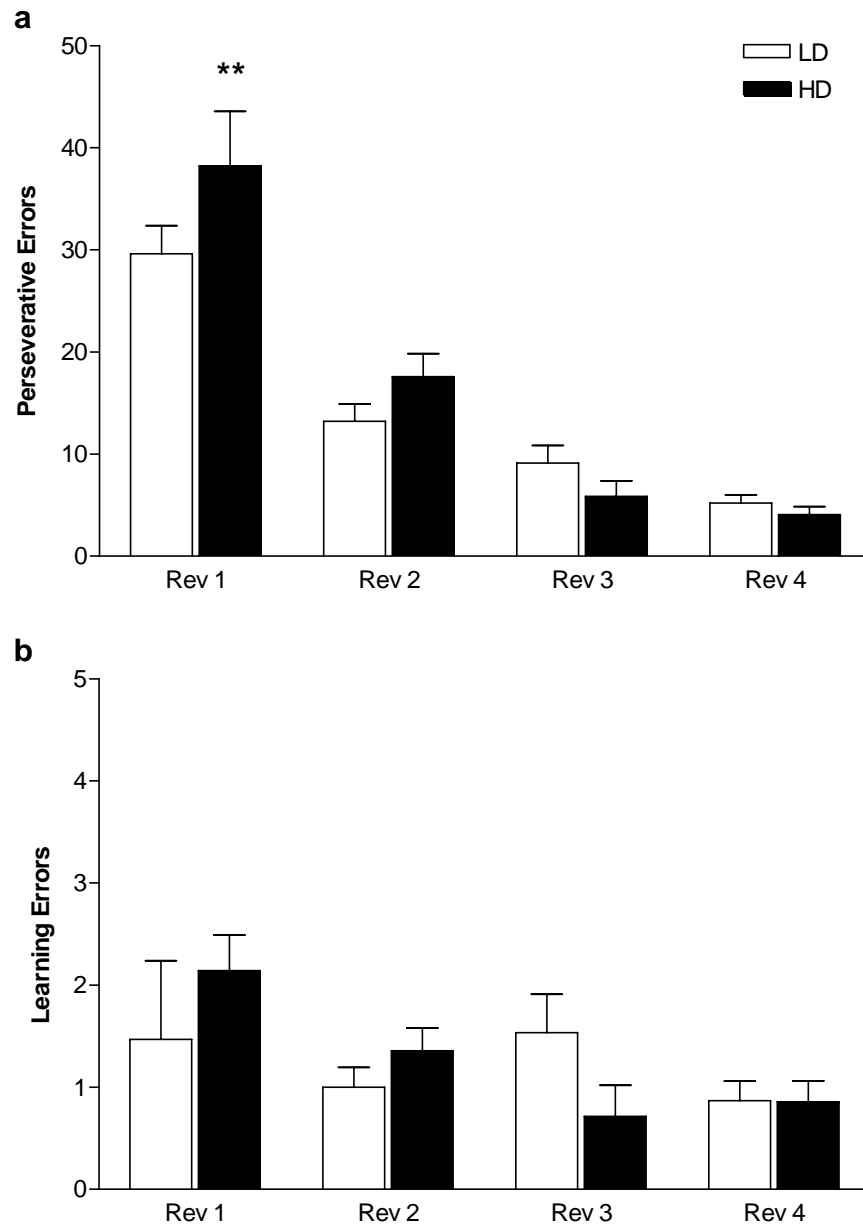Mean values ± SEM of Average Latencies to Respond during Retention (Collapsed Retention without Reversal and Retention Preceding Reversal) and Reversal phase of Reversal Learning Experiment.

	Retention of Spatial Discrimination	Retention of Reversal 1	Retention of Reversal 2	Retention of Reversal 3
HD	152,7 ± 13,9	129,9 ± 20,3	99,9 ± 11,4	65,4 ± 9,0
LD	112,1± 16,7	111,0 ± 10,8	100,7 ± 13,1	45,5 ± 5,7
	Reversal 1	Reversal 2	Reversal 3	Reversal 4
HD	139,3 ± 18,9	100,1 ± 19,5	77,6 ± 8,8	61,1 ± 7,7
LD	97,8 ± 12,2	71,4 ± 8,4	71,1 ± 8,6	57,2 ± 9,2

3.3.4 Myelination in HD and LD rats

The analyses of MBP revealed significant differences in optical densities of myelinated fibers between HD and LD rats (Figure 3.7). HD rats showed a reduced

myelination within the corpus callosum (t(35)=-3.73, p<0.001), striatum (t(15)=-2.51, p<0.05) and basolateral amygdala (t(62)=-3.44, p<0.001) compared LD rats.

Figure 3.7

Expression of myelin basic protein (MBP) in corpus callosum, striatum and basolateral amygdala in HD and LD rats. Data are expressed as the mean ±SEM. Statistical differences were determined by Student's t-test. *p<0.05 and***p<0.001indicate significant differences in myelination between HD and LD rats.
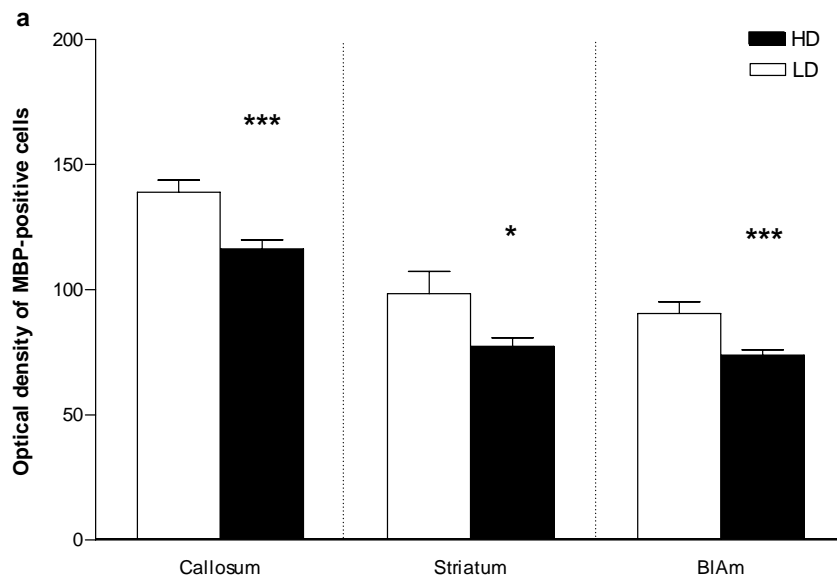
3.4 Discussion

In the present study, we demonstrated that an animal model of compulsivity, HD rats selected by SIP, showed the existence of other behaviours and biological markers related to schizophrenia and OCD: vulnerability to exaggerated habit formation responses, deficit in selective attention, behavioural inflexibility and reduced myelination. The HD rats, characterized by a compulsive drinking behaviour on SIP, showed increased lever pressing responses during VI60 training, less Latent Inhibition effect showed by a weaker effect of preexposure to stimuli in a conditioned emotional response task, and increased behavioural inflexibility on Spatial Reversal Learning task compared to LD rats. Moreover, HD rats showed less myelination in the centre of the corpus callosum, striatum and basolateral amygdala compared LD rats. These findings support the validity of high compulsive rats selected by SIP, as an animal phenotype characterized with behaviours and biomarkers of schizophrenia similar to those observed in human populations.

3.4.1 Latent Inhibition

In the present study, during the training previous to pre-exposure phase, we found that HD compulsive rats selected by SIP showed significantly increased lever presses than LD rats under VI60 s schedule. Previous studies posit the idea that training under intervals may induce habit formation responses, thus, whereas ratio schedules produce goal-directed actions (A-O), interval schedules tend to generate stimulus-response habits (Dickinson et al., 1983). Furthermore, more recent conceptualizations have defined habit formation as the mechanism through which actions that have been rewarded many times in the past are enabled to be "stamped

in", so they can be rendered automatic in the future (Gillan et al., 2015). Lever pressing is a response used in different animal models of compulsivity (for review see Boulougouris et al., 2009b) such as Reward Omission Procedure (Derusso et al., 2010; Yin et al., 2005) and Post-Training Signal Attenuation model (PTSA; Joel and Avisar, 2001; Joel et al., 2004).Our results in VI60 s schedule are coherent with the studies cited above, since HD rats keep pressing without earning more pellets. Thus, the present results point towards to HD as a vulnerable population to pathological habit formation responses.

In our study, HD rats had a weaker latent inhibition effect compared LD rats. In the HD rats, showed that the preexposed group only showed differences at the session 1 and 2 compared no preexposed group, while in the LD group the LI effect was maintained until the session 4. The disruption of latent inhibition effect through different procedures has been used as a model of schizophrenia (Weiner, 2003). Lesion studies in rats have demonstrated the implication of different brain structures in this phenomenon , such the nucleus accumbens shell (Gal et al., 2005; Nelson et al., 2011), basolateral amygdala, entorhinal cortex (Coutureau et al., 1999, 2001) and ventral hippocampus (Ouhaz et al., 2014). Moreover, the pharmacological manipulation of NMDA receptors known as an animal model of schizophrenia also induced LI disruption (Pouzet et al., 2004). Likewise, recent experiments have shown that the administration of MK-801 in rats, a NMDA receptor antagonist, increased water intake on SIP (Hawken et al., 2011; 2013). The idea that a compulsive trait could be also accompanied with an abnormal latent inhibition have also been

observed in human studies with contradictory results, two studies found enhanced LI in OCD patients (Swerdlow et al., 1999; Kaplan et al., 2006) whereas other showed deficits of LI in a visual task though (Lee and Telch, 2010).

3.4.2 Reversal learning

In our study, HD compulsive rats selected by SIP showed behavioural inflexibility as reflected by their significant poorer performance in all measures of Spatial Reversal Learning compared to LD rats. Thus, during Reversal 1, HD rats needed more trials to reach the criterion and had more incorrect responses than LD rats. HD had an increased significant number of perseverative errors compared LD rats. In contrast, there were no differences in the number of learning errors between HD and LD rats, which support the notion that the differences observed are due to an inflexible behaviour rather than learning problem. The relation between compulsive behaviours, thus behavioural inflexibility and repetitive drinking behaviour on SIP could be established comparing the following studies. First, animal lesion studies have previously demonstrated the implication of the orbitofrontal cortex (OFC) in the impaired reversal learning (Boulougouris and Robbins, 2009; Boulougouris et al., 2007; Clarke et al., 2008; Reading et al., 1991); moreover, in OCD patients and their clinically unaffected relatives, there is an abnormal reduced activation of OFC during reversal learning (Chamberlain et al., 2008); thus, according to these results recent c-fos studies have also implicated an abnormal activation of OFC and prefrontal areas in compulsive drinking on SIP (Gregory et al., 2015; Merchan et al., unpublished). Second, the systemic administration of D2/D3 receptors agonist

quinpirole that impaired reversal learning (Boulougouris et al., 2009a), have also shown to increase non-regulatory water intake in rats, the contrafreeloading a proposed model of psychotic polydipsia (Millela et al., 2010; Schepisi et al., 2014; De Carolis et al., 2011). Third, the serotonin depletion have shown to increase the behavioral inflexibility (Clarke et al., 2004, 2007), as well as the low tryptophan diet has shown to increase compulsive drinking on HD rats on SIP (Merchan et al., 2015, unpublished). Finally, in a recent study Barlow et al., (2015) found that high perseverative rats in a spatial-discrimination serial reversal learning showed reduced 5-HT2A receptor binding in the OFC and tryptophan hydroxylase in the dorsal raphe nucleus, suggesting that the serotonergic tone might be an endophenotype that predisposes to behavioural inflexibility and other forms of compulsive behaviour. Similarly, HD compulsive rats on SIP have shown a reduced 5-HT2A receptor binding and alterations on 5-HT tone compared LD rats (Moreno et al., 2012; Mora et al., unpublished).

3.4.3 Myelination in HD compulsive rats

In this study we found less myelination in corpus callosum, striatum and basolateral amygdala of HD rats selected by SIP. Reduced myelination in corpus callosum implies alterations in interhemispheric connection and is related to reduced leftward dominance for language, longer illness duration and severity of symptoms in schizophrenia and first episode of bipolar disorder in patients with psychosis (Whitford et al., 2010; Lu et al., 2011; Holleran et al., 2014; Leroux et al., 2015). In experimental studies, chronic treatment with the NMDA receptor antagonist MK-

801 in mice have shown demyelination of corpus callosum with schizophrenia-like behaviours including hyperlocomotor activity and anxiety (Xiu et al., 2014; 2015). This pharmacological model of schizophrenia has also shown to increase compulsive drinking in rats on SIP (Hawken et al., 2011; 2013). Moreover, animal models of schizophrenia based on maternal immune activation have also shown a reduction of myelinated fibers in the in the BLA amygdala, as well as long-lasting prepulse inhibition (PPI) deficits, locomotor hyperactivity and working memory deficits (Wischhof et al., 2015). Furthermore, schizophrenic patients have also shown reduced oligodendrocyte density in basolateral amygdala (Williams et al., 2013), pointing towards myelin abnormalities according with the results of the present study.

4. Series two:

Tryptophan depletion in compulsive behaviour in rats: strain dependent effects and associated neuromechanisms

In the present study, we screened Wistar and Lister Hooded rats for high and low drinking rates during SIP. Thus, we identified HD and LD rats in Wistar rats, but only LD in the Lister Hooded strain. Then, we produced a dysfunction of the 5-HT system in the brain by the administration of a TRP-free diet and evaluated the effect of chronic TRP depletion on SIP and open field test in both strains of rats. Later, brain monoamine levels and 5-HT_{2A} and 5-HT_{1A} receptors density were measured. Figure 1 resumes the experimental procedure

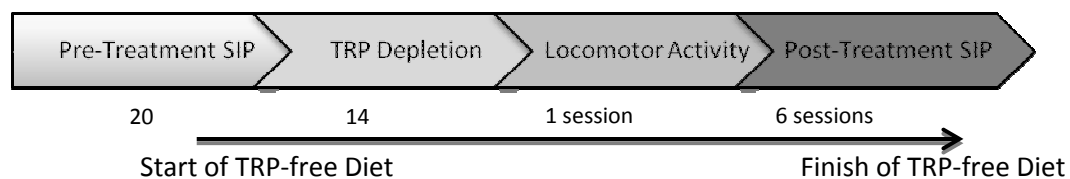| Pre-Treatment SIP	TRP Depletion	Locomotor Activity	Post-Treatment SIP
20	14	1 session	6 sessions
Start of TRP-free Diet			Finish of TRP-free Diet

Figure 4.1 Experimental procedure illustrated in a timetable.

4.1 Methods and materials

4.1.1 Subjects

Two strains of rats were used for this experiment, 20 adult male Lister Hooded rats from Charles River (Barcelona, Spain) and 30 adult male Wistar rats from Harlan Iberica (Barcelona, Spain).

Lister Hooded rats and Wistar rats weighed 294-406grams and 302-415 grams at the start of the experiment, respectively. The animals were housed three/cage or two/cage (50x15x25 cm) at 22ºC with 08:00-20:00 hours light-dark cycle, with food and water available ad libitum. Before SIP training and after 10 days of habituation to the vivarium conditions, rats were weighed and handled daily. They were gradually reduced to 80%-85% of their free-feeding body weight by controlled feeding and then maintained at this level of deprivation throughout the experiment. Food was made available by daily feeding of lab chow approximately 30 minutes after each experimental session. Water was always available in the home cages.

Rats were assigned to each experimental group taking into consideration the amount of water consumed in the previous experimental schedule-induced polydipsia, in order to match all groups. Wistar rats were split as following: High Drinkers receiving a TRP-free diet (n=7), High Drinker receiving a control diet (n=7), Low Drinker with TRP-free diet (n=7) and Low Drinker with control diet (n=7). Lister Hooded rats, as they did not show differences in SIP (water intake and licks), were

divided into two groups depending on the diet: One group (n=8) received a TRP-free diet, while the other group (n=9) received a control diet. Once the animals started the specific diets, they were housed in cages individually (50x15x25 cm) to control body weight and measure water consumption in home cages.

Two Wistar rats and one Lister hooded rat were not included in the statistical analysis due to the inadequacy of its drinking behaviour (spilling water from the water bottle during the SIP sessions). During the previous SIP, two Lister Hooded rats showed high levels of drinking and were discarded. Possibly, SIP acquisition can be developed in approximately 10% of this strain population.

All procedures were performed in accordance with Spanish Royal Decree 1201/2005 in the protection of experimental animals and with the European Community Council Directives (86/609/EEC).

4.1.2 Experimental Procedures

The experiment was developed in 2 phases. One previous phase consisted in screening the acquisition of schedule-induced polydipsia in Lister Hooded and Wistar rats. Once rats were identified as High Drinker and Low Drinkers by SIP, they were divided in groups depending on the given diet.

After 14 days of TRP-depletion by diet, rats were exposed to different behavioural tasks. The order of presentation was as follows: one session of open-field test and 6 sessions of schedule-induced polydipsia.

We conducted the tests in ten standard operant-conditioning chambers (MED Associates) 32-cm long×25-cm wide×34-cm high, with stainless-steel grid floors. A detailed description of the apparatus has been provided previously for the SIP and the 5-CSRT task (López-Grancha et al., 2008; Moreno et al., 2012). The scheduling and recording of experimental events were controlled by a Med PC computer and commercial software (Cibertec SA, Spain).

4.1.2.1 Post-treatment Schedule-induced Polydipsia.

After an initial SIP procedure to separate rats HD and LD populations, groups of animals were formed depending of the given diet. Afterwards, they were exposed to 14 days of TRP depletion diet. Then, the animals were exposed again to a FT60-s schedule of food pellet presentation during 60-min sessions. Water bottles with fresh water were available.

4.1.2.2 Spontaneous Lomocotor Activity

The test was an open-field test, performed in 8 plexiglas activity cages (measuring 39×39×15 cm) equipped with photocell beams (16x16x16) interfaced to a microcomputer Versa Max Animal Activity Monitoring System (AccuScan Instruments Inc., USA). Spontaneous Locomotor activity was measured as the number of photocell beam breaks due to the movements of the animals. Tryptophan depleted and non-depleted rats were tested for their locomotor response to a novel environment in the activity cages. Rats were not habituated to the activity cages prior to this test. The floor and walls of the open field was cleaned with a 10%

alcohol solution between trials to prevent transmission of olfactory cues. Spontaneous locomotor behaviour was quantified in 5-min blocks over a 60-min period following placement into the test cage. We measured total distance, counted as number of centimetres travelled by the animal (indicator of ambulatory activity).

4.1.2.3 Tryptophan depletion diet

The TRP-free diet (TD08126, Harlan Laboratories S.A., Barcelona, Spain) had a standard nutritional value, but with the complete lack of TRP. The control groups were fed a similar diet, containing a standard amount of TRP (1,8 g/kg diet) (TD99366, Harlan Laboratories S.A., Barcelona Spain).

The present experiment accomplishes not only a nutritional-deprivation approach, but also a food-restriction schedule. During the chronic TRP-free diet exposure, the amount of food (in grams) per rat given was between 10-14 g, amount of food sufficient to remain at 80%-85% body weight. Treneer and Bernstein (1981) and Tanke et al. (2008) reported a reduction of food consumption in TRP depleted rats. For this reason, a complete reporting of body weight during TRP-free diet administration was made as a control measure.

4.1.2.4 Brain analysis

The day after the post-treatment SIP, the rats were rapidly decapitated using a guillotine. Brains were quickly removed, frozen and stored at –80°C.The cerebral hemispheres were separated and each half was used either for measuring

monoamines analyses or for measuring receptor binding. The hemispheres were counterbalanced.

Brain Monoamines Analyses

For brain tissue preparation, the samples were thawed sufficiently to allow dissection of the prefrontal cortex, striatum, nucleus accumbens, hippocampus and amygdala. These were weighed and homogenized in 0.4 N perchloric acid with 0.1 metabisulfite, 0.01% EDTA and 1 ng/ml cysteine. The homogenates were centrifuged at 15,000 rpm for 20 min at 4°C, and supernatants were collected and frozen at –80°C until biochemical analyses for determining the levels of norepinephrine (NE), dopamine (DA), serotonin (5-HT) and 5-hydroxy-3-indolacetic acid (5-HIAA). These were measured using reverse-phase high-performance liquid chromatography with electrochemical detection (+0.7 V). The mobile phase, containing 100 mM KH_2PO_4, 0.1 mM Na_2-EDTA, 2.06 mM PICB8 and 16% methanol, adjusted to pH 2.65 with orthophosphoric acid, was delivered at 1 ml/ min. Monoamines were separated on a 5-mm particle size column at 30°C (Phenomenex C25 10×0.46 cm, Micron Analítica SA, Spain).

Autoradiography

To determine $5\text{-}HT_{1A}R$ and $5\text{-}HT_{2A}R$ binding in Wistar and Lister Hooded rats, brains were cut in 10-μm sections and mounted on Super Frost slides and stored at –80 °C. The $5\text{-}HT_{2A}$ autoradiography protocol was modified from (López-Gimenez et al., 1997) and performed using ^{3}H-MDL100907 [R(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-

fluorphenyl)-ethyl]-4-piperidin-methanol] (specific activity, 2.8 TBq/mmol, Novandi Chemistry, Sweden; and non-specific binding was determined using 10 μM ketanserin tartrate (3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]-ethyl]-2,4[1H,3H] quinazolinedione tartrate) (Tocris Cookson Ltd., Bristol, UK). For 5-HT_{1A}autoradiography we used ^{3}H-WAY100635 (specific activity, 2.9 TBq/mmol, Novandi Chemistry) and measured non-specific binding with 10 μM 5-HT (Sigma–Aldrich, Copenhagen, Denmark). Briefly, sections were allowed to thaw for 1 h at room temperature (RT) and then pre-incubated at with 50 mMTris–HCl (Sigma), pH 7.4 containing 0.01%ascorbic acid (Sigma) and 10 μM pargyline hydrochloride (N-methyl-N-2-propynylbenzylamine hydrochloride) (Research Biochemicals International, MA, USA) for 30 min at RT under constant gentle shaking. Sections were then incubated for 60 min at RT using the same buffer containing 2 nM of ^{3}H-MDL100907 (1.5 nM of ^{3}H-WAY100635 for 5-HT_{1A} binding). Following incubation, slides were washed 2 × 2 min in ice-cold 50 mMTris–HCl, pH 7.4, and 1 × 20 s in ice-cold dH$_2$O, dried for 1 h under a gentle stream of air.

All sections were placed at 4ºC in a fixator containing paraformaldehyde vapor and then put in an exicator box for 3 h before slides were together with ^{3}H-microscales (GE Healthcare, UK) exposed to a BAS-TR2040 Imaging Plate (Science Imaging Scandinavia AB, Nacka, Sweden) for 3–14 days at 4 °C. Finally, the imaging plate was scanned on a CR-35 scanner (Raytest, Straubenhardt, Germany) and specific and non-specific binding was determined in the frontal cortex,

striatum and hippocampus using AIDA 5.0 software (Raytest) and expressed as fmol/mg tissue equivalents (TE).

4.2 Data analysis

Data analyses of the different strains were made separately, due to the complexity of groups. Analyses of variance ANOVA were made with 2 between-subject factors for Wistar strain data, group (HD and LD) and treatment (T+ and T-) and one between-subject factor for Lister Hooded strain data (treatment, T+ and T-). The within-subject factors were sessions on SIP, bins on the locomotor activity, brain monoamine data and receptor binding.

Pearson's correlation analysis was used to assess the possible relationship between water intake and total licks (mean 3 last days of SIP) and body weight. Analysis of body weight during the chronic TRP depletion was assessed by repeated measures ANOVA.

When appropriate, post hoc comparisons were made using the Neuman-Keuls test. Significance level was set at $p \leq 0.05$. All statistics are two- tailed. Statistical analyses were performed using STATA Release 8.0 (Stata Corporation, College Station, TX, USA) and SPSS v20.0 for Windows (IBM Corporation, Armonk, NY, USA). Data are expressed as mean $\pm$ SEM.

4.3 Results

4.3.1 Pre-treatment Schedule-induced Polydipsia

Figure 4.3 shows the mean total licks, water intake and total magazine entries in WHD, WLD and LH rats on the Pre-Treatment SIP FT-60s schedule of food presentation. During the baseline period, WHD, WLD and LH drank a mean of 6.35±0.32, 5.18±0.24, and 2.8±0.3 ml, respectively. In the experimental phase, the mean total licks for WHD, WLD and LH rats during the first 3 days of the experiment were 674.57±146.36, 250.93±61.73 and 567.67±85.76, respectively. During the last 5 days of SIP, mean total licks for these groups were 4196.03±422.26, 606.30±117.51 and 707.59±85.25 for WHD, WLD and LH, respectively (Fig 4.3a). This SIP acquisition was also observed by the increase in water intake. During the first 3 days, WHD, WLD and LH rats drank a mean of 4.97±0.75, 4.45±0.82 and 3.99±0.63, respectively. During the last 5 days, average water intake was 16.61±1.37, 5.29±0.45 and 3.93±0.33 ml for WHD, WLD and LH, respectively (Fig. 3c). ANOVA revealed significant differences on SIP acquisition between HD and LD Wistar rats in total licks (group effect $F_{1,26}=71.5$, $p< 0.000$) and water intake (group effect $F_{1,26}=44.18$; $p<0.000$). SIP session effects were significant in both measures: total licks ($F_{19,494}=23.6$; $p<0.000$) and water intake ($F_{19,494}=13.03$; $p<0.000$). Interaction between sessions and group was also significant in total licks ($F_{19,494}=15.5$; $p<0.000$) and water intake ($F_{19,494}=9.24$; $p<0.000$). Post hoc analysis indicated that the FT-60s schedule of food delivery induced different drinking rates across the 20 test sessions in both

groups. Differences in total licks between WHD and WLD animals were evident from session 3 (p=0.031) and from session 3 in water intake (p=0.039). HD animals significantly increased their consumption of water from session 3 (p=0.002) to the end of training, reaching stable levels from session 10. LD animals did not show a significant increase in their consumption of water across SIP sessions. No interaction effect was found in magazine entries ($F_{19,494}$=0.56; p=0.933) (Fig. 4.3e). A main effect of session ($F_{19,494}$=5.76; p<0.000) emerged for the magazine entries measure, but no group effect was observed ($F_{1,26}$=0.083; p=0.775).

No significant differences were found between LH and WLD rats in water intake (strain effect $F_{1,29}$=1.76; p=0.195), total licks (strain effect $F_{1,29}$=2.26; p=0.143) and magazine entries (strain effect $F_{1,29}$=1.56; p=0.696). There was no correlation of mean total licks (5 last sessions of SIP) and body weight neither Wistar (Pearson's r=-0.195; p=0.320) nor Lister Hooded (Pearson's r=-0.009; p=0.973).

4.3.2 Post-treatment Schedule-induced Polydipsia

Figure 4.3 shows the effects of the chronic TRP depletion on Wistar and Lister hooded rats on SIP. Differences between HD and LD Wistar rats remained stable in water intake (group effect $F_{1,24}$=55.33; p<0.000) and total licks (group effect $F_{1,24}$=32.44; p<0.000). Wistar HD T- rats increased the total number of licks over the days (see Fig. 4.3b; group x treatment x session effect $F_{5,120}$=2.46; p=0.037), but no significant effect was observed in water intake (see Fig. 4.3d; group x treatment x session effect $F_{5,120}$=1.06; p=0.387). The increase of licks cannot be explained by

differences on body weight (Pearson's r=-0.13; p=0.093). Post-hoc analysis indicated

that the differences between WHD T+ and WHD T- occur from session 3 (p=0.007).

WHD T- animals significantly increased their licks rate from session 3 (p=0.021). An

increase of total licks was observed in WHD T+ (p=0.020) and WLD T- (p=0.002) on

session 6, but these groups remain statistically different from each other (p=0.040).

Lister Hooded groups of rats did not show differences in water intake (Fig. 4.3d;

treatment x session effect $F_{5,75}$=0.353; p=0.879) and total number of licks (Fig. 4.3b;

treatment x session effect $F_{5,75}$=1.013; p=0.416). No correlation was observed between

body weight and licks (Pearson's r=0.150; p=0.133). Total number of magazine entries

were not different between groups of Wistar (group x treatment x session: $F_{5,120}$=1.02;

p=0.410) and Lister Hooded rats (group x treatment x session: $F_{5,75}$=2.076; p=0.078)

(Fig. 4.3f).

Figure 4.3

The mean (±SEM) total licks (panel a-b), water intake (panel c-d) and total magazine entries (panel e-f) in FT 60s across 20 sessions of Pre-Treatment SIP and 6 sessions of Post-Treatment SIP for both Wistar and Lister Hooded rats. Rats are grouped in the Pre-treatment SIP as: High Drinker Wistar rats (WHD), Low Drinkers Wistar rats (WLD) and Lister Hooded rats (LH). Wistar rats are grouped in the Post-Treatment SIP as: TRP non-depleted High Drinkers (WHD T+), TRP depleted High Drinkers (WHD T-), TRP non-depleted Low Drinkers (WLD T+), TRP depleted Low Drinkers (WLD T-). Lister Hooded rats are grouped in the Post-Treatment SIP as: TRP non-depleted (LH T+) and TRP depleted rats (LH T-). (*) Statistical analyses indicate significant differences between HD T+ and HD T-. (#) Statistical analyses indicate significant differences between WHD and WLD from that session onward.

Figure 4.3
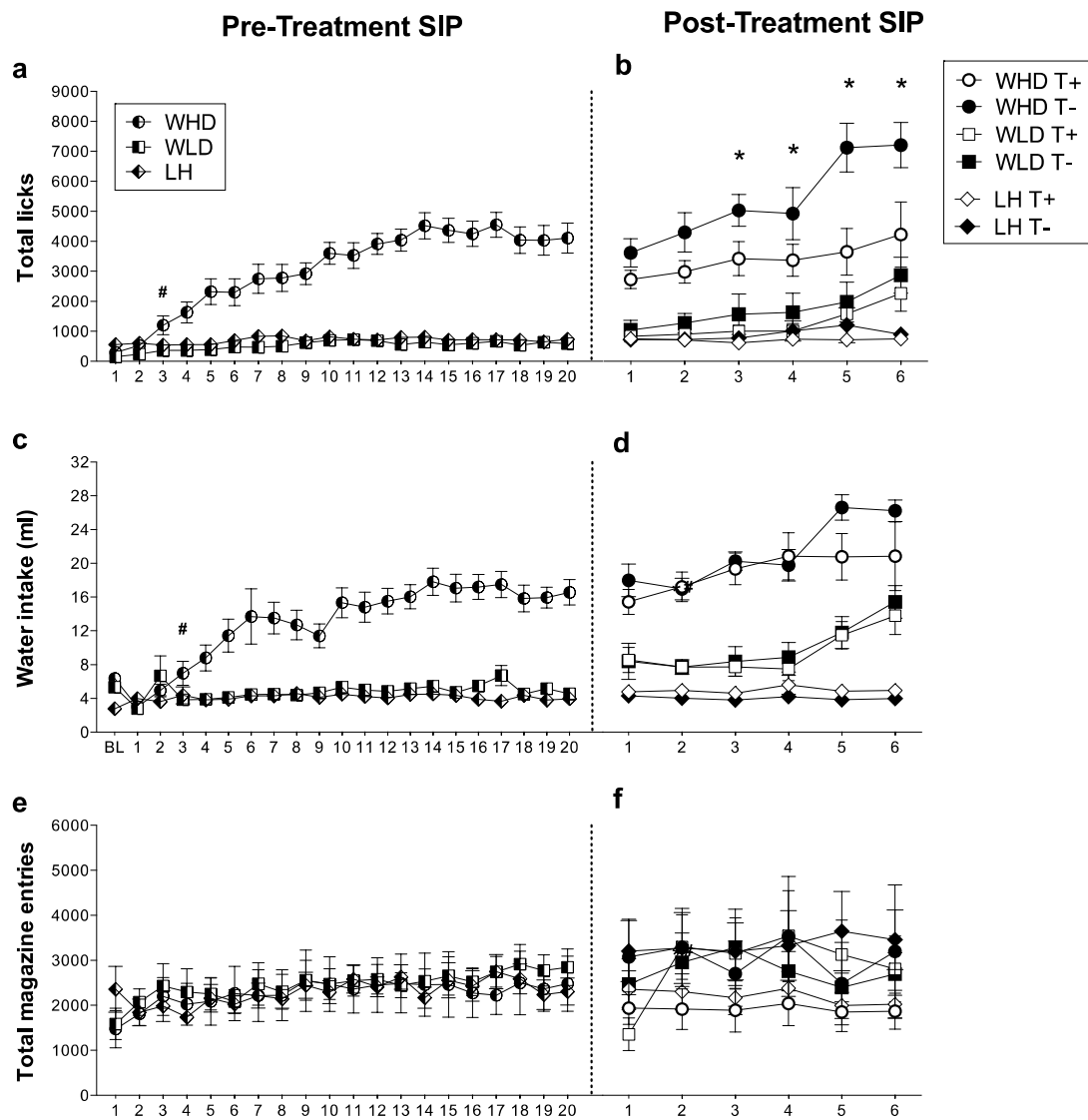
4.3.3 Spontaneous Locomotor Activity

Figure 4.4 shows locomotor response to novelty measured as total distance in 4 bins of 15 min for Wistar and Lister Hooded rats. No differences were found in locomotor response to novelty measured in groups of Wistar rats (see Fig 4.4.a; group x treatment x bins $F_{3,72}=0.78$; $p=0.508$). Besides TRP depletion did not affect locomotor response to novelty in Wistar rats (treatment x bins $F_{3,72}=1.30$; $p=0.280$), a treatment effect was observed in Lister Hooded rats (treatment x bins $F_{3,45}=3.08$; $p=0.037$). Post hoc analyses revealed that differences between T+ and T- were in the first 15 minutes ($p=0.015$) and the second 15 minutes ($p=0.008$) of the 60-min session (Fig. 4.4b). Wistar ($F_{3,72}=79.91$; $p<0.000$) and Lister Hooded rats ($F_{3,45}=81.16$; $p<0.000$) decreased the activity over the session significantly. An effect of treatment was observed in Lister Hooded ($F_{1,15}=4.62$; $p=0.048$) but not in Wistar rats ($F_{1,24}=1.40$; $p=0.248$).

Figure 4.4

Total distance in 4 blocks of 15 min for Wistar (a) and Lister Hooded rats (b). Wistar rats are grouped as: TRP non-depleted High Drinkers (HD T+), TRP depleted High Drinkers (HD T-), TRP non-depleted Low Drinkers (LD T+) and TRP depleted Low Drinkers (LD T-). Lister Hooded rats are grouped as: TRP non-depleted (T+) and TRP depleted rats (T-). Data are means ±SEM; (*) Statistical analyses indicate significant differences between T+ and T-.
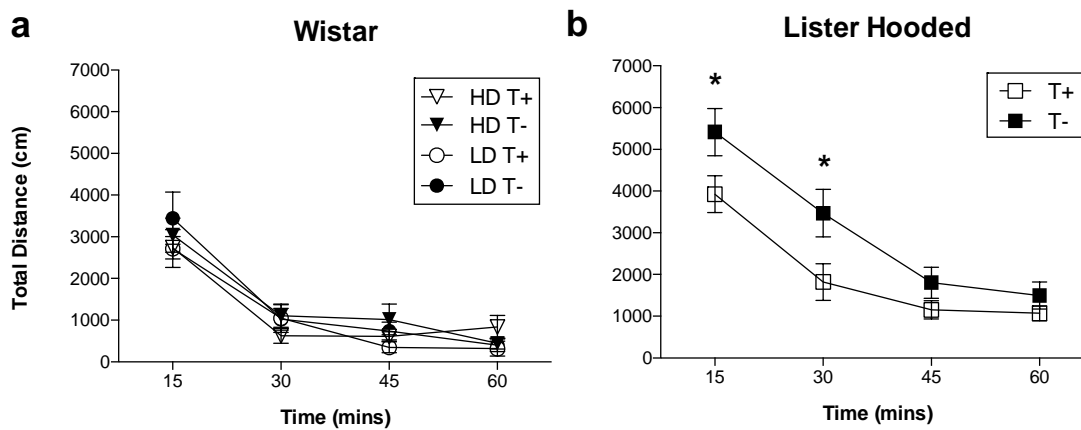
4.3.4 Monoamine concentration levels

Chronic TRP depletion by diet reduced significantly 5-HT, 5-HIAA and 5-HIAA/5-HT turnover ratio in PFC, amygdala and hippocampus in both strains of rats. No interaction effect of group and treatment was found in monoamine concentration levels in the different areas for Wistar rats. In the depleted group of Wistar rats, 5-HT levels were reduced in PFC ($F_{1,23}$=20.86; p<0.000) and hippocampus ($F_{1,24}$=5.89; p<0.023) (see Table 1), and there was a tendency toward significance in amygdala ($F_{1,24}$=3.92; p=0.059). 5-HIAA levels were decreased in PFC ($F_{1,23}$=29.52; p<0.000), striatum ($F_{1,22}$=4.79; p=0.040), amygdala ($F_{1,24}$=29.79; p<0.000) and hippocampus ($F_{1,24}$=19.837; p<0.000). In addition, a decreased 5-HIAA/5-HT turnover ratio in all areas was found: PFC ($F_{1,23}$=16.13; p<0.001), striatum ($F_{1,22}$=51.90; p<0.000), amygdala ($F_{1,24}$)=52.97; p<0.000), nucleus accumbens ($F_{1,19}$=19.67; p<0.000) and hippocampus ($F_{1,24}$=64.45; p<0.000). No significant changes in levels of 5-HT and 5-HIAA were found in nucleus accumbens. NE and DA were not significantly affected in any cerebral areas (Table 4.1 and 4.2).

Table 1

Monoamine concentration levels (picomole/milligramme of tissue) in the prefrontal cortex, striatum, amygdala, nucleus accumbens and hippocampus in T+ and T- for Wistar rats. (n=11-14). Data are mean ± SEM. *PFC* prefrontal cortex, *NAc* nucleus accumbens, *Amyg:* amygdala, *Striat:* striatum, *Hippo:* Hippocampus. Significant differences between T+ and T- (**p< 0.05, **p<0.01, a p=0.059)

		5-HT	5-HIAA	5-HIAA/5-HT ratio	NE	DA
PFC	T+	1.67±0.15	1.16±0.14	0.70±0.06	1.86±0.14	1.68±0.46
	T-	**0.86±0.09****	**0.36±0.03****	**0.44±0.03****	1.72±0.13	2.87±0.77
Striat	T+	3.56±0.81	4.13±0.88	1.17±0.07	0.14±0.03	130.64±27.47
	T-	3.08±0.47	**2.08±0.37***	**0.65±0.03****	0.22±0.08	146.89±23.26
Amyg	T+	2.93±0.32	2.36±0.22	0.83±0.04	1.10±0.21	18.89±3.57
	T-	**2.16±0.21**[a]	**1.01±0.09****	**0.48±0.02****	1.47±0.25	20.23±2.83
NAc	T+	2.78±0.75	2.50±0.69	0.90±0.02	2.50±0.55	41.04±13.00
	T-	2.61±1.22	1.45±0.59	**0.62±0.06****	5.73±2.58	44.67±20.58
Hippo	T+	1.23±0.15	1.70±0.22	1.38±0.03	1.97±0.20	1.11±0.19
	T-	**0.78±0.10***	**0.67±0.05****	**0.91±0.04****	1.56±0.11	1.72±0.25

Table 4.2

Monoamine concentration levels (picomole/milligramme of tissue) in the prefrontal cortex, striatum, amygdala, nucleus accumbens and hippocampus in T+ and T- for Lister Hooded rats. (n=4-9). Data are mean ± SEM. *PFC:* prefrontal cortex, *NAc:* nucleus accumbens, *Amyg:* amygdala, *Striat:* striatum, *Hippo:* Hippocampus. Significant differences between T+ and T- (*p< 0.05, **p<0.01)

		5-HT	5-HIAA	5-HIAA/5-HT ratio	NE	DA
PFC	T+	1.77±0.09	1.02±0.09	0.56±0.04	1.62±0.09	3.89±1.08
	T-	**0.99±0.11****	**0.04±0.07****	**0.35±0.07****	**1.31±0.08***	2.34±0.72
Striat	T+	2.20±0.27	1.98±0.31	0.88±0.04	0.25±0.07	79.35±11.61
	T-	**0.92±0.15****	**0.52±0.06****	**0.60±0.06****	0.22±0.08	83.19±7.21
Amyg	T+	2.81±0.32	2.12±0.21	0.78±0.05	1.38±0.28	12.94±2.56
	T-	**1.75±0.21***	**0.68±0.09****	**0.39±0.02****	1.13±0.34	20.18±3.28
NAc	T+	2.74±0.31	2.34±0.19	0.90±0.06	8.01±1.98	23.14±4.36
	T-	2.09±0.22	**1.01±0.15****	**0.48±0.02****	3.40±1.96	**48.99±9.52***
Hippo	T+	1,45±0.19	2.17±0.24	1.54±0.07	2.60±0.23	4.95±1.71
	T-	**0.79±0.07****	**0.64±0.07****	**0.81±0.06****	3.05±0.62	9.45±3.46

4.3.5 Serotonin receptor binding

Table 4.3 shows mean ±SEM ^{3}H-MDL100907 and ^{3}H-WAY100635 binding for groups of depleted and non-depleted Wistar and Lister Hooded groups of rats. For the 5-HT$_{2A}$ receptor in Wistar rats, there was a group x treatment interaction in striatum for ^{3}H-MDL100907 binding ($F_{1,23}$=8.648; p=0.007) (see Fig. 4.5a). Post hoc analyses revealed a reduction of 5-HT$_{2A}$ receptor density in depleted HD rats compared to non-depleted rats (p= 0.014). Although the statistical analysis did not find statistical differences on 5-HT$_{2A}$ receptor in frontal cortex (interaction effect $F_{1,24}$=0.990; p=0.330), they were reduced by 10%. In Lister Hooded rats no statistical differences in 5-HT$_{2A}$ density were found in neither frontal cortex ($F_{1,16}$=0,117; p=0,737) nor striatum ($F_{1,16}$=0.066; p=0.801).

The 5-HT$_{1A}$ receptor density in the frontal cortex showed a reduction in depleted Lister Hooded rats in terms of ^{3}H-WAY100635 binding ($F_{1,16}$=19.091; p=0.001) (see Fig. 4.5b), but no differences were found in striatum ($F_{1,16}$=1,80; p=0,677) and hippocampus ($F_{1,16}$=2.157; p=0.163). However, depleted groups of Wistar rats did not show any interaction effect of group x treatment in frontal cortex ($F_{1,24}$=0.240; p=0.629), striatum ($F_{1,23}$=0.138; p=0.714) and hippocampus ($F_{1,25}$=1.672; p=0.209) for 5-HT$_{1A}$ density.

Table 4.3

^{3}H-MDL100907 and ^{3}H-WAY100635 binding (fmol/mg TE) in the frontal cortex, striatum and hippocampus in all groups of Wistar and Lister Hooded rats. Data are mean ± SEM. *FC* frontal cortex, *Striat* striatum, *Hippo* Hippocampus. Significant differences between T+ and T- (*$p<$ 0.05, **$p<$ 0.01)

		Wistar				Lister Hooded	
		HD		LD			
		T+	T-	T+	T-	T+	T-
FC	**5-HT$_{2A}$**	73.16±4.68	65.44 ±2.87	73.90±4.86	76.39±7.17	102.70±4.59	105.7 ±7.85
	5-HT$_{1A}$	27.13 ±0.95	29.07 ±1.84	26.57±2.07	26.49±2.90	45.44 ±1.57	**37.07 ±1.00****
Striat	**5-HT$_{2A}$**	19.13 ±0.75	**16.41 ±0.49***	16.91±0.73	18.34±0.80	25.51 ±0.80	25.23 ±0.70
	5-HT$_{1A}$	10.48 ±0.33	10.38 ±0.41	10.03±0.30	9.68±0.28	14.75±0.26	14.41±0.79
Hippo	**5-HT$_{1A}$**	121.23±4.02	116.6±3.88	111.43±7.42	117.54±8.69	110.73 ±7.29	96.36 ±6.33

Figure 4.5

The mean (±SEM) 5-HT$_{2A}$ receptor binding of Striatum slices in the Wistar rats (a) and 5-HT$_{1A}$ receptor binding of Frontal Cortex slices in the Lister Hooded rats (b). 5-HT$_{2A}$ receptor binding was detected by [^{3}H]MDL100907 and 5-HT$_{1A}$ binding was detected by [^{3}H]WAY100635. Wistar rats are grouped as: TRP non-depleted High Drinkers (HD T+), TRP depleted High Drinkers (HD T-), TRP non-depleted Low Drinkers (LD T+) and TRP depleted Low Drinkers (LD T-). Lister Hooded rats are grouped as: TRP non-depleted (T+) and TRP depleted rats (T-). (*)Statistical analyses indicate significant differences between T+ and T- (*$p<$ 0.05, **$p<$ 0.01).
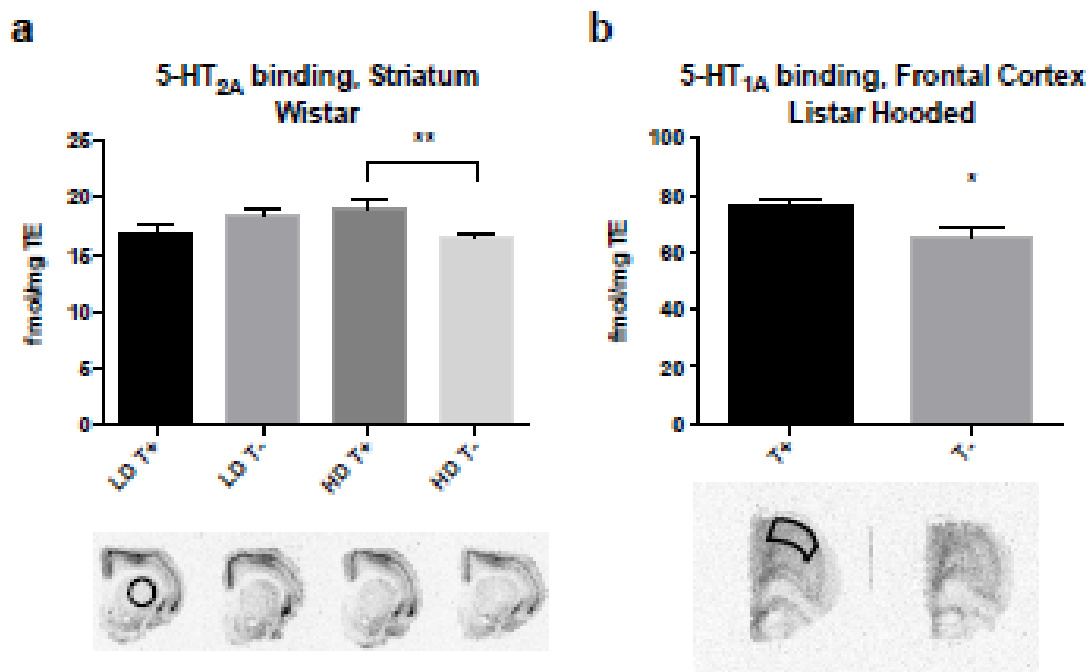
4.3.6 Control measures

Previous to the TRP-free diet exposure, Wistar rats drank in home cages during 24 hours a mean of 19.65±1.77 and 18.65±1.00 for HD and LD, respectively. No statistical differences were found between both groups (group effect $F_{1,27}=0.24$; $p=0.628$). Lister Hooded rats drank a mean of 16.12±1.03. After TRP-free diet treatment, HD T+, HD T, LD T+ and LD T- Wistar rats drank a mean of 24.64±3.17, 23.07±3.36, 20.36±3.09 and 18.29±1.55, respectively. No effect of 5-HT depletion was observed (group x treatment effect $F_{1,24}=0.008$; $p=0.932$). Lister Hooded rats drank a mean of 16.59±1.00 and 10.12±1.15 for T+ and T-, respectively. One way ANOVA revealed that T- group drank significantly less than T+ ($F_{1,16}=18.05$; $p=0.001$).

Figure 4.2 shows body weight during 14 days of tryptophan depletion diet and 6 days of post-treatment Schedule-induced Polydipsia in Wistar and Lister Hooded rats. No differences between T+ and T-groups was found neither in Wistar (treatment effect $F_{1,24}=0.09$; $p=0.761$) nor Lister Hooded rats (treatment effect $F_{1,15}=0.496$; $p=0.492$).

Figure 4.2

Body weight during 14 days of TRP depletion by diet and 6 days of post-treatment Schedule-induced Polydipsia for Wistar (a) and Lister Hooded rats (b). Wistar rats are grouped as: TRP non-depleted High Drinkers (HD T+), TRP depleted High Drinkers (HD T-), TRP non-depleted Low Drinkers (LD T+) and TRP depleted Low Drinkers (LD T-). Lister Hooded rats are grouped as: TRP non-depleted (T+) and TRP depleted rats (T-). Data are means ±SEM.
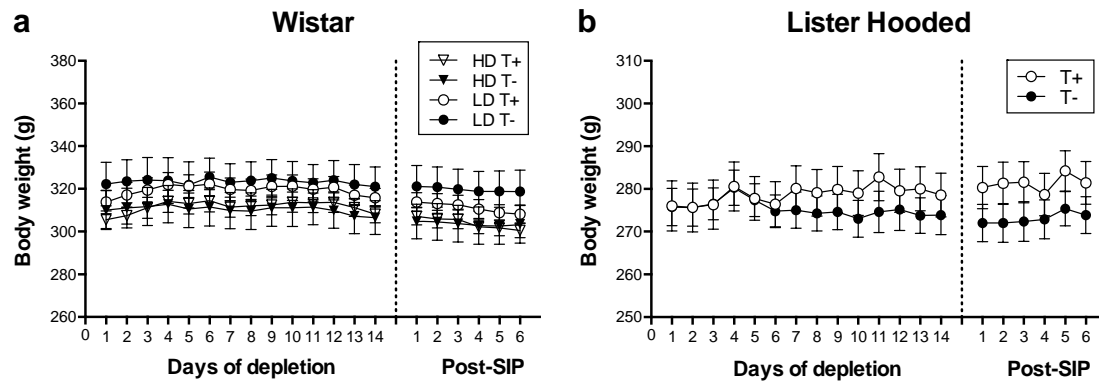
4.4 Discussion

The present study has shown the effects of chronic TRP-free diet administration in two strains of rats: Wistar and Lister Hooded. Monoamine measures confirmed that 5-HT, 5-HIAA and 5-HIAA/5-HT ratio was depleted in different brain regions in Wistar and Lister Hooded rats. We examined between-strain differences in SIP and we found in the Wistar strain two groups of rats based on their drinking behaviour, HD and LD, while Lister Hooded strain did not show acquisition of compulsive drinking. After a chronic exposure to TRP-free diet, TRP depleted HD group of Wistar rats increased compulsive drinking based on the licks rate, but no changes on drinking behaviour were observed in either LD Wistar or Lister Hooded rats. On the other hand, TRP depletion affected spontaneous locomotor activity only in Lister Hooded rats by showing more reactivity to a novel environment, while Wistar rats were unaffected. $5-HT_{2A}$ receptor binding was significantly lower in striatum for depleted HD Wistar rats compared to non-depleted HD Wistar rats, while depleted Lister Hooded rats showed a lower binding of the $5-HT_{1A}$ receptor in the frontal cortex. These results will be further discussed.

4.4.1 Effect of chronic TRP depletion on monoaminergic concentration levels

Chronic TRP depletion was effective in reducing the levels of 5-HT and 5-HIAA as well as the 5-HIAA/5-HT turnover ratio in prefrontal cortex and hippocampus in both strains of rats. Previous studies found similar reductions of 5-HT levels and its metabolite (Cahir et al., 2007; Franklin et al., 2012; Koot et al., 2012). However,

significant reductions of 5-HT were not found in all brain areas, such as nucleus accumbens, and there were strain-differences in 5-HT depletion. Lister Hooded rats seemed to be more affected by the depletion showing significant decreases in 5-HT, 5-HIAA and 5-HIAA/5-HT turnover ratio in all brain areas except the nucleus accumbens. Also this strain shows changes in other monoamines such as dopamine, increased in the nucleus accumbens, and norepinephrine, decreased in the prefrontal cortex. Ardis et al. (2009) did not find alteration in NA and DA concentration levels by acute TRP depletion in frontal cortex, hippocampus and striatum, while we observe that chronic TRP depletion produces certain changes on those neurotransmitters. Koot et al. (2012) also reported a decrease in NE and DA concentration levels in PFC by chronic TRP depletion. Changes on dopamine are not surprising since the influencing role of 5-HT upon central dopaminergic system is well known (for review see Di Giovanni et al., 2010).

4.4.2 Acquisition of Schedule-induced Polydipsia and strain differences

In the SIP procedure, the exposure of the Wistar strain to FT-60s schedule of food delivery differentiated two populations based on the amount of drinking, High and Low drinkers. WHD rats shown an increased volume of water drunk and number of licks from session 3 compared to WLD. These results confirm previous studies in which consistent individual differences are found (Cardona et al., 2006, 2011; Dantzer et al., 1988a, b; López-Grancha et al., 2006, 2008; Mittleman and Valenstein, 1985; Mittleman et al., 1988a, b; Moreno et al., 2010, 2012; Pellón et al., 2011; Tazi et al.,

1988; for review, Moreno and Flores, 2012). However, Lister Hooded rats did not show acquisition of SIP. This is the first study that evaluates strain differences between Wistar and Lister Hooded in SIP acquisition and development of SIP drinking. Another strains commonly used such as Fischer 344 (F344) and Lewis (LEW) or Roman high- (RHA) and low-avoidance (RLA) rats have shown differences in SIP behaviour (De Carolis et al., 2003; Moreno et al., 2010; Stöhr et al., 2000).Thus, SIP seems to be sensitive to distinguish phenotypes of rats that have shown deficits on inhibitory control responses in different tasks of impulsivity/compulsivity or vulnerability to drugs, pointing towards a lack of inhibitory control as the main characteristic involved in the compulsive drinking on SIP (Moreno and Flores, 2012).

4.4.3 Effect of chronic TRP depletion on Schedule-induced Polydipsia and possible mechanisms

Tryptophan depletion produced an increase of licks in HD Wistar rats without affecting the amount of water drunk. Increase in the number of licks without an increase in water intake has been seen previously (Cardona et al., 2006). Cardona et al. (2006) found that acute administration of Chlorpyrifos (CPF) after 6 months produced an increase of licks in the HD rats. There are evidences about long-term effect of CPF intoxication in the serotonergic system (Chen et al., 2011; Moreno et al., 2008) by inducing tryptophan hydroxylase, the rate-limiting enzyme for 5-HT biosynthesis, and suppressing expression of 5-HT transporter genes (Slotkin and Seidler, 2008). The serotonin disruption may be underlying the increase of licks on SIP common in our study and the one of Cardona et al. (2006). From our SIP data, it

could be suggested that the increase in the number of licks without an increase in water intake could be due to motor impairments that involves the muscles responsible for fine movements of the mouth and/or tongue caused by chronic TRP depletion. However, depleted LD rats do not significantly increase licks compared to non-depleted LD rats .On the other side, the increase of licks is task-dependent, since groups differ from session 3 onwards and not from session 1.The increasing effect over the sessions on licks is also observed in Cardona et al. (2006), in which previous exposition to CPF increased licks in HD rats compared to LD rats in different sessions (the 5th, 10[th]and 14th to 20th).

The specific effect of the chronic TRP depletion increasing licks on HD Wistar rats but not on LD Wistar rats indicates a vulnerability of the HD group to compulsive symptoms and an implication of the serotonergic system mediating this vulnerability. Patients with psychopathologies from the impulsive-compulsive spectrum aggravate their symptoms when exposing to acute tryptophan depletion. For instance, ATD enhanced caloric intake, mood irritability (Weltzin et al., 1995) and desire to binge eat (Kaye et al., 2000; Bruce et al., 2009) in women with bulimia nervosa, and increased craving in alcoholic patients (Wedekind et al., 2010; Pierucci-Lagha et al., 2004; but Petrakis et al., 2002, 2001). In ADHD patients, ATD increased omissions in the continuous performance task (Zepf et al., 2010) and aggressive behaviour (Kötting et al., 2013; Zimmermann et al., 2012; Zepft et al., 2009, 2008; Stadler et al., 2007). Interestingly, ATD impaired go/no go performance (LeMarquand et al., 1999) and stop signal reaction time (Crean et al., 2002) in

healthy men with family history of alcoholism, and increased also commission errors in the Go/No Go task in an aggressive subgroup of people with ADHD (LeMarquand et al., 1998). These findings suggest that ATD may reveal vulnerable 5-HT systems in some populations at risk of impulse control disorders (Faulkner and Deakin, 2014). Therefore, 5-HT system seems to exert a role as a modulator of symptoms in disorders with behavioural "disinhibition".

In our study, we have found a reduction of striatal 5-HT$_{2A}$ receptor density in depleted HD Wistar rats compared to non-depleted HD Wistar rats, without statistical differences between LD Wistar and Lister hooded rats. Alterations of 5-HT$_{2A}$R in 5-HT depletion studies are controversial because some studies find an up-regulation of this receptor subtype in hippocampus and frontal cortex (Seeman et al., 1980; Heal et al., 1980), while other studies do not observe any difference (Blackshear et al., 1981; Conn and Sanders-Bush, 1986; Fischette et al. 1987). However, Licht et al. (2010) found that 5-HT$_{2A}$ receptor binding was decreased in the frontal and cingulated cortices after chronic paroxetine administration, and markedly reduced in several regions after 5-HT depletion. Barlow et al. (2015) had similar findings regarding 5-HT$_{2A}$ receptors reductions and low levels of 5-HT in perseverative rats in the reversal learning task, as well as the MAO-A and MAO-B enzymes gene expression. They suggest that decreased MAO activity in the DRN resulted in reduced 5-HT breakdown and consequently increased autoinhibition of 5-HT neurons by somatodendritic 5-HT receptors (Barlow et al., 2015; Liu et al, 2005).

The specific down-regulation of the striatal $5HT_{2A}$ receptor in only High Drinkers but no Low Drinkers rats under manipulation of the central 5-HT system reveals a specific role of the $5\text{-}HT_{2A}$ receptor system that may be underlying the increases of the compulsive drinking on SIP. Evidence from animal and human studies pointed out the key role of the $5\text{-}HT_{2A/C}$ receptors in compulsive symptoms (Fineberg et al., 2010, 2011). Activation of prefrontal $5\text{-}HT_{2A}$ receptors has been proposed to underpin the anticompulsive effect of SSRIs (Dannon et al., 2000; for a review, see El Mansari and Blier, 2006; Westenberg et al., 2007). Second generation antipsychotics may exacerbate compulsive behaviours in patients with schizophrenia, and it has been proposed that this occurs through potent $5\text{-}HT_{2A}$ antagonism (Poyurovsky et al., 2008). In fact, $5\text{-}HT_{2A/C}$ agonist DOI reduced compulsive drinking on SIP, and this reduction was blocked by $5\text{-}HT_{2A}$ receptor antagonists ketanserin and M100907, but not by the $5\text{-}HT_{2C}$ receptor antagonist SB242084, indicating that 5-HT2A mediates the anticompulsive effect on SIP (Navarro et al., 2015). Moreover, systemic administration of a $5\text{-}HT_{2A}$ receptor antagonist impaired spatial reversal learning increasing perseverative responses (Boulougouriset al., 2008).The alterations of $5\text{-}HT_{2A}$ receptor levels has also been seen in Roman high avoidance (RHA) rats (Klein et al., 2014), an inbred strain characterised by a compulsive drinking profile on SIP, impulsivity on the delay-discounting task and a poor inhibitory control in 5-CSRTT task (Moreno et al., 2010), and dogs with compulsive behaviours, showing a significantly lower $5\text{-}HT_{2A}$ receptor availability in the frontal and temporal cortices (Vermeire et al., 2012).In a recent study, Barlow et al. (2015) found that high levels of

perseveration were also associated with decreased 5-HT_{2A} receptor binding in the OFC. Neuroimaging studies have shown that 5-HT_{2A} receptors play a crucial role in the development of compulsive spectrum disorders in humans. PET studies in drug-naive OCD patients revealed reductions in serotonin 5-HT_{2A} receptor availability in the frontal cortex (Perani et al., 2008), whereas increases have been found in the caudate nucleus (Adams et al., 2005), with specific correlations between serotonin 5-HT_{2A} receptor availability in the orbitofrontal cortex, clinical severity and age of onset of the disorder (Perani et al., 2008; Simpson et al., 2011).

Little is known about the contribution of the striatal serotonin receptor subtypes in cognitive function. In the striatum, 5-HT receptors modulates the activity of dopamine, GABA and glutamate neurotransmission and output regions of the basal ganglia (Nicholson and Brotchie, 2002), suggesting a role of the 5-HT system in regulating action selection and motor control (Di Matteo et al., 2008). It is possible that down-regulation of striatal 5-HT_{2A} can produce changes in other neurotransmitter systems such as dopamine that may explain the increase of compulsive licking. However dopamine levels were not significantly different between depleted and non-depleted Wistar rats. More studies are needed to evaluate the role of the 5-HT_{2A} receptor in the impulsive-compulsive spectrum disorders.

4.4.4 Effect of chronic TRP depletion on Spontaneous Locomotor Activity and possible mechanisms

Chronic TRP depletion by diet produced in the Lister Hooded strain an increase of novelty reactivity not seen in the Wistar strain. No differences between HD and LD

Wistar rats were found, as previously reported by Moreno et al. (2010). Studies depleting 5-HT by administration of 5,7-DHT in Lister Hooded (Eagle et al., 2008) and by TRP-free diet in Wistar rats (Vergnes and Kempf, 1981) reported increase of reactivity to novelty and locomotor activity. Vergnes and Kempf (1981) fed Wistar rats with a TRP-free diet during 5 weeks that reduced 5-HT levels about 75%. Two weeks of exposure has been reported to reduce about 35-40% of 5-HT levels (Fadda, 2000). Therefore, the shorter exposure used in this study to TRP-free diet may not be enough for producing an effect in locomotor activity in the Wistar strain.

Another possibility that could explain the differences between Lister Hooded and Wistar rats on locomotor activity is the increase of DA levels in the nucleus accumbens produced by chronic TRP depletion. Meso-accumbens DA system is involved in novelty seeking behaviour (Bardo et al., 1996). Microinjections of DA and DA receptor agonists into the nucleus accumbens septi heightened locomotor activity (for review Ikemoto and Panksepp, 1999; Staton and Solomon, 1984; Johnels, 1982; Jackson et al., 1975; Pijnenburg et al.,1975, 1976; Pijnengurg and Van Rossum, 1973). Staton and Solomon (1984) found that microinjections of d-amphetamine into the nucleus accumbens produced enhanced activity without increases of stereotypes. Similarly, the increased DA in the striatum of LH rats may explain the higher locomotor activity, while this increase does not affect the compulsive drinking on SIP.

On the other hand, depleted Lister Hooded rats showed a down-regulation of prefrontal 5-HT$_{1A}$ receptor that has not been observed in depleted Wistar rats

groups. Based on different studies showing no effect of 5,7-DHT lesions modulating 5-HT$_{1A}$ receptor, it seems to be less susceptible to change than other 5-HT receptor subtypes (Verge et al., 1986; Frazer and Hensler, 1990; Berendsen et al., 1991; Hensler et al., 1991; Miquel et al., 1992). However, Kawai et al., (1994) proposed a down-regulation of 5-HT$_{1A}$ receptors as a homeostatic adaptive change in response to chronic TRP deprivation in frontal cortex. The increase of locomotor activity, and therefore a possible increase of impulsivity, is hardly related to the reduction of 5-HT$_{1A}$ receptors. Serotonin 1A receptor subtype is implicated in the 5-choice serial reaction time task in perseverative responses, but no in premature responding (for review see Carli and Invernizzi, 2014; Robbins, 2002), both measures identified as compulsive and impulsive behaviour respectively. Besides 5-HT$_{1A}$ seems to be more implicated in compulsivity than impulsivity so far, the reduction of 5-HT$_{1A}$ receptors in the depleted group of LH rats did not manifest in statistical differences in compulsive licking on SIP, indicating that this receptor subtype is not mediating this behaviour on SIP.

5. Series three:

Function of serotonin 5-HT2A/C receptors in compulsive behaviour on Schedule-induced polydipsia

We investigated HD and LD rats on SIP: first, we examined the potential differences in serotoninergic and noradrenergic neurotrasmission by testing the efficacy of the selective serotonin versus noradrenaline re-uptake inhibitors, citalopram and atomoxetine, in reducing compulsive drinking on SIP. Second, we assessed the contribution of the serotonin $5\text{-}HT_{2A/C}$ receptors in the reduction of compulsive drinking via the acute administration of the receptor agonist DOI on SIP. Third, we explored the potential different implications of the $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptor subtypes by assessing the dose effect and the blocking efficacy on DOI effects using different serotonin receptor antagonists: the serotonin 5-HT2A receptor antagonist ketanserin and the highly selective serotonin 5-HT2A receptor antagonist M100907, and the high selective serotonin $5\text{-}HT_{2C}$ receptor antagonist SB242084 on SIP. These results are discussed in terms of the role of $5\text{-}HT_{2A/C}$ receptors in a compulsive phenotype population of rats and their implication as candidate mechanisms of vulnerability to compulsive disorders, as observed in different psychiatric populations. Experimental sequence is showed below (Fig. 5.1).

Figure 5.1

SIP Procedure	Experiment 1 *Dose-response study*	Experiment 2 *Dose-response study*	Experiment 3 *Combined treatments*
Selection of HD and LD rats	Citalopram Atomoxetine	Ketanserin M100907 SB242084	DOI + Ketanserin DOI + M100907 DOI + SB 242084

5.1 Methods and materials

5.1.1 Subjects

A total of 57 male Wistar rats (Harlan Ibérica, Barcelona, Spain), weighing approximately 275–300 g at the start of the experiments, were used in this study. The animals were housed three rats/cage (50×15×25 cm) at 22°C, with a 12:12-h light–dark cycle (light off at 08:00 h) and food and water provided ad libitum. Before SIP training and after 10 days of habituation, 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 approximately 30 min after each experimental session. All testing was performed between 09:00 and 14:00 h. All procedures were performed according to the Spanish Royal Decree 1201/2005 on the protection of experimental animals and the European Community Council Directives (86/609/EEC).

Three cohorts of rats were tested in thirteen operant SIP chambers (32×25×34 cm) (MED Associates, St. Albans, VT). A detailed description of the apparatus has been previously provided (López-Grancha et al. 2008; Moreno et al. 2010). The scheduling and recording of the experimental events was controlled using a computer and commercial software Med PC (Cibertec SA, Spain). 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=9-10).The following measures were recorded for each rat: (a) total amount of water (millilitres) removed from the bottle, (b) total number of licks to the bottle and (c) total number of entries into the food magazine (see López-Grancha et al. 2008, for more details).

5.1.2 Experimental procedures

The behavioural effects of the acute systemic administration of different drugs were tested in three separated groups of HD and LD rats on SIP. All of the animals received the drug according to a fully randomised Latin-square design and separated by a minimum of 72 h between drug test sessions and fifteen days between different drug experiments (the animals continued performing SIP sessions during these days). The experimental sessions were on Tuesdays and Fridays, and baseline testing occurred on Mondays and Thursdays. On Wednesdays, the animals performed the task, but the results were not analysed. Experimental events are summarized in Figure 1.

5.1.3 Experiment 1

We investigated the effects of citalopram, a selective serotonin reuptake inhibitor; atomoxetine, a selective norepinephrine reuptake inhibitor; and the serotonin 5-$HT_{2A/C}$ receptor agonist DOI in HD and LD rats on SIP. In a previous study (Moreno et al. 2012), we showed that HD rats selected by SIP exhibited increased levels of

serotonin and norepinephrine activity in the amygdala compared with LD animals. Thus, we selected a range of different doses of the noradrenergic and serotoninergic drug to explore the potential differences between HD and LD on SIP behaviour. The effects of atomoxetine (1, 2, 3 and 5 mg/kg) and citalopram (0.3, 1 and 3 mg/kg) were investigated in groups 1 and 2, respectively. Furthermore, we investigated whether the effect of the SSRIs to reduce compulsive drinking on SIP could be mediated via serotonin 5-HT$_{2A/C}$ receptors by the administration of serotonin 5-HT$_{2A/C}$ receptors agonist DOI (0.1, 0.3 and 0.5 mg/kg) in group 3. The drug doses and the time to injection, approximately 30 min before behavioural testing, were chosen based on previous experiments (Bari et al. 2009; Hadamitzky et al. 2009; Robinson et al. 2008).

5.1.4 Experiment 2

We explored the effects of the serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors subtypes in compulsive drinking on SIP. Thus, we selected a range of different doses of serotonin 5-HT$_{2A/C}$ receptors antagonists to evaluate the potential differences between HD and LD on SIP behaviour. We assessed a dose-response on SIP in the following groups: serotonin 5-HT$_{2A}$ receptor antagonist ketanserin (0.3, 0.6, and 1 mg/kg) in group 3; highly selective serotonin 5-HT$_{2A}$ receptor antagonist M100907 (0.1, 0.5, 1 and 2 mg/kg) in group 2; and selective serotonin 5-HT$_{2C}$ receptor antagonist SB242084 (0.1, 0.5, 1 and 2 mg/kg) in group 1. The drug doses and the time to injection, approximately 30 min before behavioural testing, were chosen based on previous experiments (Fletcher et al. 2007; Martin et al. 2002; Winstanley et al. 2004).

5.1.5 Experiment 3

We assessed the implication of the serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors on the reduction in compulsive drinking produced by DOI (0.5 mg/kg) in HD rats on SIP. We tested the blocking efficacy of the combined treatment with ketanserin (0.5 and 1 mg/kg) in group 3, M100907 (1 mg/kg) in group 2 and SB242084 (1 mg/kg) in group 1. In the combined treatments, the rats received one injection of the antagonist drug or vehicle 2 min before a second injection of the agonist drug or saline; the drug doses and the time to injection, approximately 30 min before behavioural testing, were chosen based on the results of Experiments 1 and 2 as well as previous experiments (Hadamitzky et al. 2009; Koskinen et al. 2000; Martin et al. 2002).

5.1.6 Drugs

Citalopram hydrobromide (1-[3-(dimethylamino)propyl]-1-(4-fl-uorophenyl)-1,3-dihydro-5-isobenzofurancarbonitril-e hydrobromide) and atomoxetine hydrochloride ((R)-N-Methyl-γ-(2-methylphenoxy)-ben-zenepropanamine hydrochloride) were dissolved in 0.01 M phosphate buffer saline (PBS).M100907 ((±) 2,3-dimethoxyphenyl-1-[2–4-(piperidine)-methanol]) was dissolved in 0.9% saline, and the pH was adjusted to 6.25 using 0.1 MNaOH and 0.1 MHCl. SB242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl]) was prepared in 0.9% saline solution containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid and the pH was adjusted to 6.4 using 0.1 MNaOH. All of these drugs were purchased from Tocris Bioscience (Madrid, Spain) and administered

intraperitoneally (ip). DOI hydrochloride ((±)-2,5-dimethoxy-4-iodoamphetamine) and ketanserin tartrate (3-[2-[4-(4-Fluorobenzoyl)-1-piperid-inyl]ethyl]-2,4[1H,3H]-quinazolinedione tartrate) were dissolved in 0.9% saline. Both drugs were purchased from Sigma-Aldrich (Madrid, Spain) and injected subcutaneously. The injection volumes were 1 ml/kg for all drugs.

5.2 Data analysis

Behavioural data on SIP acquisition were analysed using two-way repeated measures analysis of variance (ANOVA), with "group" (HD and LD) as the between-subject factor and "sessions" (20 levels) as the within-subject factor. *Experiments 1 and 2*: The effects of different drugs in HD and LD on SIP were analysed using a two-way repeated measures ANOVA, with one between-subject factor, "group" (HD and LD), and one repeated within-subject factor, "drug" (different doses of drug and vehicle). *Experiment 3:* The effects of co-administered serotonin 5-HT$_{2A/C}$ receptors agonist and antagonists in HD and LD on SIP were analysed using a three-way repeated measures ANOVA, with one between-subject factor, "group" (HD and LD), and two within-subject factors, "agonist" DOI (saline and 0.5 mg/kg) and "antagonist" (ketanserin: vehicle, 0.5 and 1 mg/kg; M100907: vehicle and 1 mg/kg; SB242084: vehicle and 1 mg/kg). *Post hoc* comparisons were performed using the Newman–Keuls test. Statistical significance was set at $p<0.05$. All analyses were computed using the Statistica software package (version 5.0).

5.3 Results

## 5.3.1 HD and LD selected by with SIP

The mean water intake in HD and LD for each of the three groups during the acquisition and maintenance on SIP is shown in Figure 5.2. During the baseline period (BL), HD and LD rats drank a mean of 6.2±0.6 and 5.9±0.4 ml (group 1, see Figure 5.2A), 5.9±0.4 and 5.7±0.3 ml (group 2, see Figure 5.2B) and 5.3±0.3 and 4.6±0.3 ml (group 3, see Figure 5.2C), respectively. In the experimental phase, the mean intake of water for these groups over the last 5 days of the experiment was 31.3±2.5 and 8.4±1.6 (group 1), 32.0±3.0 and 9.3±1.8 ml (group 2) and 26.4±1.5 and 9.9±0.9 ml (group 3) for HD and LD, respectively (Figure 5.2). This SIP acquisition was also observed as an increase in the number of licks. The mean total licks for these groups averaged across the last 5 days of the experiment were 5571.8±1124.5 and 1735.3±499.9 (group 1), 6262.0±953.7 and 1569.9±524.1 (group 2) and 5171.8±621.9 and 1614.4±204.8 (group 3) for HD and LD, respectively (data not shown).

ANOVA revealed significant differences in the water intake according to the interaction between SIP acquisition sessions and group HD vs. LD (interaction SIP session x group effects: group 1 $F_{19,304}=13.97$, $p<0.0001$; group 2 $F_{19,323}=7.29$, $p<0.0001$; group 3 $F_{19,342}=8.87$, $p<0.0001$). These differences were also confirmed by the significant interaction observed in the total licks (interaction SIP session×group effect: group 1 $F_{19,304}=2.47$, $p<0.001$; group 2 $F_{19,323}=3.19$, $p<0.0001$; group 3 $F_{19,342}=7.15$,

$p<0.0001$). *Post hoc* analysis indicated that the FT-60s schedule of food delivery induced different drinking rates across the 20 test sessions in both groups. Differences between the HD and LD animals were evident in the water intake: a difference was observed starting at session 4 in groups 1 and 3 ($p<0.01$, $p<0.0001$) and at session 3 in group 2 ($p<0.01$). Furthermore, HD animals significantly increased their consumption of water at the end of training, i.e., at session 4 in groups 1 and 3 ($p<0.001$, $p<0.01$) and at session 3 in group 2 ($p<0.01$), reaching stable levels of water intake at session 11 in group 1, session 8 in group 2 and session 12 in group 3. In addition, LD animals did not show a significant increase in their consumption of water across SIP sessions. There were no significant differences between the HD and LD rats in the total magazine entries on SIP (data not shown).

Figure 5.2

The mean (±SEM) water intake in FT-60s across 20 sessions of SIP of group 1 (A), group 2 (B) and group 3 (C). Statistical analyses indicate significant differences between HD and LD from that session onward (*) .Significant differences from session 1 (#).
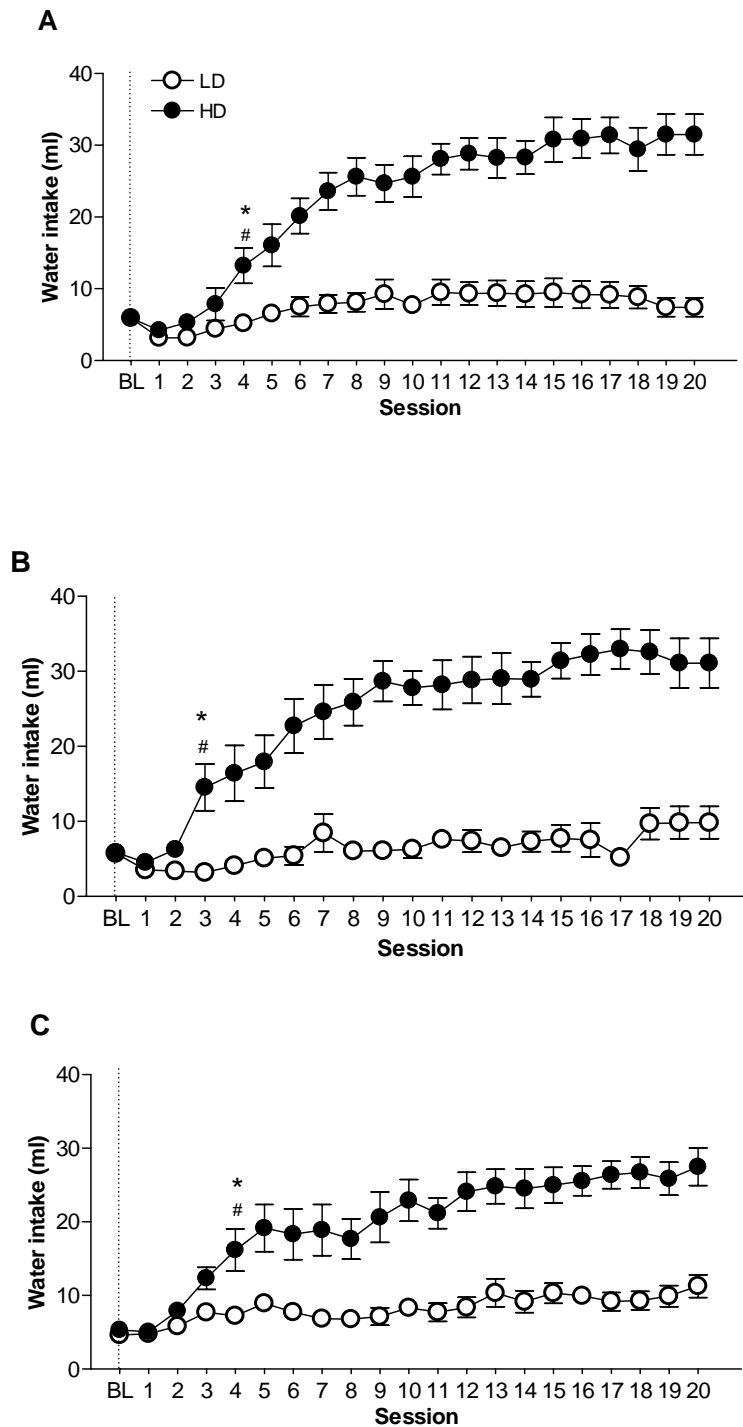
5.3.2 Experiment 1.

Effects of citalopram, atomoxetine and DOI on SIP

The effects of citalopram on water intake, total licks and total magazine entries on SIP are shown in Figure 5.3A and Table 5.1. Citalopram significantly reduced the compulsive water intake in HD rats compared to LD rats (group×drug interaction: $F_{3,51}$=4.67, p<0.01; group: $F_{1,17}$=55.68, p<0.0001; drug: $F_{3,51}$=14.66, p<0.0001). *Post hoc* analyses revealed that citalopram reduced the dose-dependent water intake in HD rats at the following doses: 0.3 mg/kg, 1 mg/kg (p<0.01) and 3 mg/kg (p<0.001), compared with vehicle. Differences between HD and LD rats remained significant at all doses tested (p<0.001). A repeated measures ANOVA also showed significant differences in the total licks following citalopram administration (drug effect: $F_{3,51}$=3.25, p<0.05; group: $F_{1,17}$=17.38, p<0.001), though without an interaction effect (group x drug: $F_{3,51}$=1.90, p=0.14). The *post hoc* comparison of the drug effect revealed a decrease in the total licks at the highest dose used, i.e., 3 mg/kg (p<0.05), compared with vehicle. There were no effects of citalopram observed in the total number of magazine entries.

The effects of atomoxetineon water intake, total licks and total magazine entries on SIP are shown in Figure 5.3B and Table 5.1. Atomoxetine did not produce any significant effects on water intake (drug: $F_{4,64}$=1.45, p=0.22) or the total licks (drug: $F_{4,64}$=0.56, p=0.69). Significant differences in both water intake (group: $F_{1,16}$=55.80, p<0.0001) and total licks (group: $F_{1,16}$=23.58, p<0.001) between HD and LD remained

at all doses tested . However, atomoxetine significantly reduced the magazine entries in both groups (drug: $F_{4,64}$=10.42, $p<0.0001$; group: $F_{1,16}$=0.24, p=0.63). A *post hoc* comparison of the drug effect revealed a decrease in the total magazine entries at the following doses: 1 mg/kg (p=0.05), 2 mg/kg (p=0.07), 3 mg/kg ($p<0.001$) and 5 mg/kg ($p<0.001$), compared with vehicle.

The effects of DOI on water intake, total licks and total magazine entries on SIP are shown in Figure 5.3C and Table 5.1. DOI significantly reduced water intake in HD on SIP (group×drug interaction: $F_{3,54}$=8.75, $p<0.0001$; group: $F_{1,18}$=22.49, $p<0.001$; drug: $F_{3,54}$=24.43, $p<0.0001$). *Post hoc* analyses revealed that DOI produced a dose-dependent reduction in water intake in the HD group at 0.1 mg/kg, 0.3 mg/kg and 0.5 mg/kg ($p<0.001$) compared with saline in the same group. In addition, DOI did not affect water intake in LD rats. The comparison between HD and LD revealed that the highest dose of DOI reduced the significant differences in water intake (saline, $p<0.001$; 0.1 mg/kg $p<0.001$, 0.3 mg/kg $p<0.05$ and 0.5 mg/kg p=0.22). Moreover, DOI also significantly reduced the total licks in HD rats compared with the LD group (group×drug interaction: $F_{3,54}$=8.98, $p<0.0001$; group: $F_{1,18}$=19.22, $p<0.001$; drug $F_{3,54}$=17.45, $p<0.0001$). *Post hoc* analyses confirmed that DOI produced a dose-dependent reduction in total licks in HD rats at 0.1 mg/kg ($p<0.05$), 0.3 mg/kg ($p<0.001$) and 0.5 mg/kg ($p<0.001$) compared with saline in the same group. In addition, DOI did not affect the total licks in LD rats. The comparison between HD and LD showed that DOI reduced the differences in total licks (saline, $p<0.001$; 0.1

mg/kg, *p*<0.001; 0.3 mg/kg, *p*<0.05; 0.5 mg/kg, *p*=0.21). No significant differences were found by the administration of DOI in magazine entries.

Figure 5.3

Effects of (A) the selective serotonin re-uptake inhibitor citalopram, (B) the selective norepinephrine re-uptake inhibitor atomoxetine and (C) the serotonin 5-HT$_{2A/C}$ receptor agonist DOI in water intake of HD and LD rats on SIP. Data are expressed as the mean ±SEM. * p<0.01 and ** p<0.001 indicate significant differences vs. vehicle administration in the same group of rats.
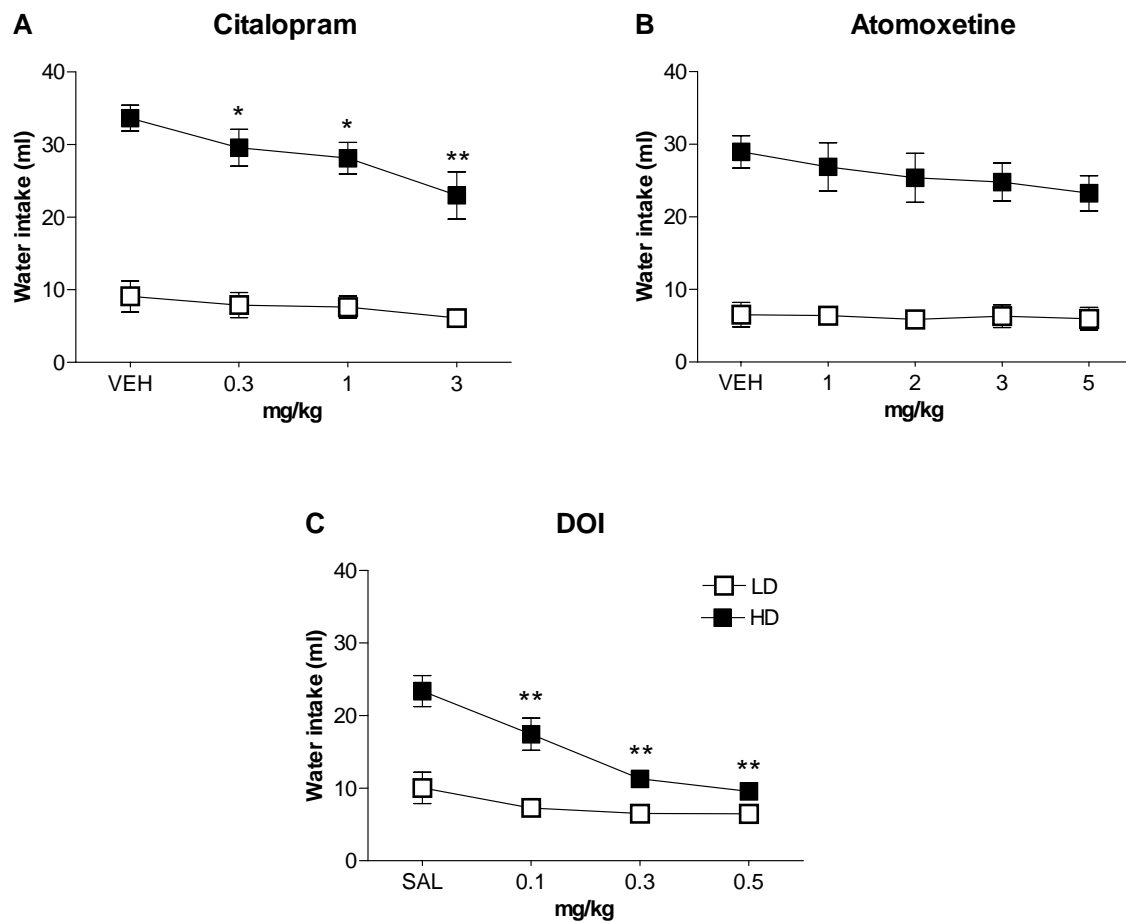
Table 5. 1

Effects of citalopram, atomoxetine and DOI on total licks and total magazine entries in HD and LD rats on SIP. Data are expressed as the mean ±SEM. * p<0.05 and ** p<0.001 indicate significant differences vs. vehicle administration in the same group of rats.

	Total licks		Total magazine entries	
	HD	**LD**	**HD**	**LD**
Ketanserin				
Vehicle	3.470,8 ± 805,7	1.566,3 ± 475,8	2.054,3 ± 559,0	2.091,9 ± 325,2
0.3 mg/kg	4.077,2 ± 1.057,5	1.611,4 ± 598,6	1.821,8 ± 501,9	2.220,2 ± 404,1
0.6 mg/kg	3.720,0 ± 1.105,2	1.477,0 ± 425,8	1.904,6 ± 520,2	1.989,0 ± 324,6
1 mg/kg	3.658,0 ± 1.024,3	1.240,8 ± 437,6	1.990,2 ± 593,9	2.057,6 ± 344,0
M100907				
Vehicle	6.882,6 ± 895,3	345,0 ± 117,6	1.496,4 ± 156,2	1.338,5 ± 410,0
0.1 mg/kg	7.461,6 ± 1.114,0	682,0 ± 163,0	1.531,4 ± 193,7	1.299,4 ± 340,1
0.5 mg/kg	7.392,2 ± 1.398,5	448,0 ± 183,6	1.237,3 ± 223,0	1.352,9 ± 440,8
1 mg/kg	6.666,7 ± 1.239,4	1.586,7 ± 522,5	1.739,5 ± 282,8	1.573,6 ± 553,5
2 mg/kg	8.047,0 ± 1.117,0	563,2 ± 197,2	1.124,2 ± 331,4	1.300,6 ± 475,3
SB242084				
Vehicle	6.335,1 ± 935,4	966,7 ± 264,2	2.319,8 ± 289,4	1.740,5 ± 441,2
0.1 mg/kg	6.243,3 ± 954,2	879,4 ± 207,2	2.483,4 ± 293,9	1.860,5 ± 451,1
0.5 mg/kg	5.722,0 ± 948,8	989,5 ± 365,8	2.606,4 ± 289,9	2.847,4 ± 834,4
1 mg/kg	7.290,9 ± 1.386,7	1.202,5 ± 415,1	1.987,0 ± 457,4	2,268.8 ± 541,6
2 mg/kg	7.409,1 ± 558,8	1.048,5 ± 469,8	2.212,8 ± 446,5	2.089,1 ± 479,8

5.3.3 Experiment 2.

Effects of serotonin 5-HT$_{2A/C}$ receptor antagonists on SIP

The effects of serotonin 5-HT$_{2A/C}$ receptor antagonist drugs on water intake and other behavioural measures on SIP are shown in Figure 5.4 and Table 5.2. The serotonin 5-HT$_{2A}$ receptor antagonist ketanserin did not produce a significant effect on water intake on SIP (Figure 5.4A), whereas the differences between HD and LD rats remained significant (group: $F_{1,17}=5.62$, $p<0.05$; drug: $F_{3,51}=2.39$, $p=0.08$; group×drug interaction: $F_{3,51}=0.76$, $p=0.52$). No significant effect was observed by ketanserin in the total licks (group: $F_{1.17}=5.09$, $p<0.05$; drug: $F_{3,51}=2.04$, $p=0.12$; group×drug interaction: $F_{3,51}=1.11$, $p=0.35$). Moreover, no significant differences were found in magazine entries (Table 5.2).

The highly selective serotonin 5-HT$_{2A}$ receptor antagonist M100907 did not produce a significant effect on water intake on SIP (Figure 5.4B), whereas the differences between HD and LD remained significant (group: $F_{1,17}=133.77$, $p<0.0001$; drug: $F_{4,68}=1.19$, $p=0.32$). There was also no effect in the total licks between HD and LD rats (group: $F_{1,17}=42.32$, $p<0.0001$; drug: $F_{4,68}=0.38$, $p=0.82$). Furthermore, M100907 did not affect magazine entries (Table 5.2).

The selective serotonin 5-HT$_{2C}$ receptor antagonist SB242084 significantly increased compulsive water intake in HD rats compared with LD rats (group×drug interaction: $F_{4,64}=4.09$, $p<0.01$; group: $F_{1,16}=70.25$, $p<0.0001$; drug: $F_{4,64}=4.54$, $p<0.01$). *Post hoc*

analyses revealed that SB242084 increased water intake in HD rats at the following doses: 1 mg/kg ($p<0.001$) and 2 mg/kg ($p<0.05$) compared with vehicle in the same group (Figure 5.4C). No significant differences were found following SB242084 administration in water intake in LD rats. The differences observed between HD and LD rats remained significant at all doses tested ($p<0.001$). SB242084 also increased the total licks (drug effect: $F_{4,64}=2.44$, $p<0.05$), but without a group×drug interaction effect. However, the group differences between HD and LD rats remained significant for the total licks ($F_{1,16}=34.78$, $p<0.0001$). No significant differences were found in the magazine entries (Table 5.2).

Figure 5.4

Effects of (A) the serotonin 5-HT$_{2A}$receptor antagonist ketanserin, (B) the high selective serotonin 5-HT$_{2A}$ receptor antagonist M100907 and (C) the selective serotonin 5-HT$_{2C}$ receptor antagonist SB242084 in water intake of HD and LD rats on SIP. Data are expressed as the mean ±SEM. * p<0.05 and ** p<0.001 indicate significant differences vs. vehicle administration in the same group of rats.
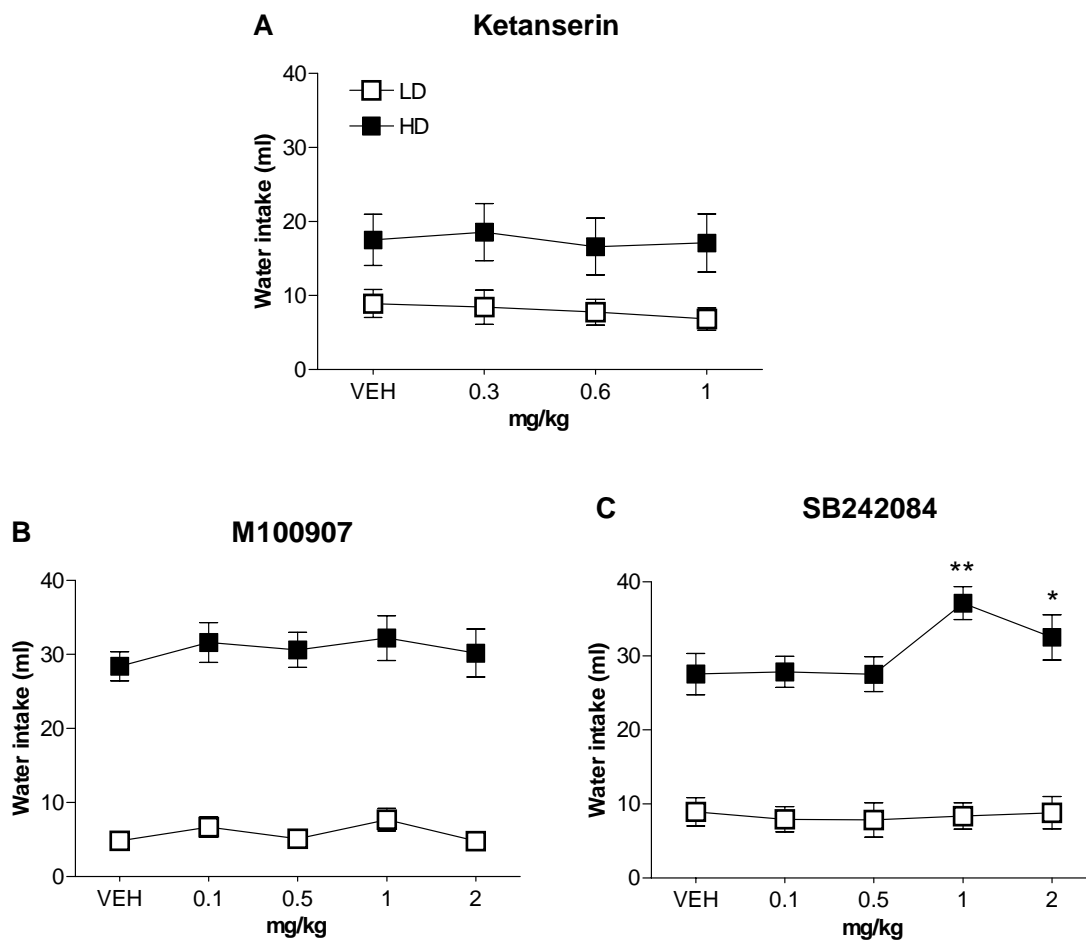
Table 5.2

Effects of the 5-HT$_{2A}$ receptor antagonist ketanserin, the highly selective 5-HT$_{2A}$receptor antagonist M100907 and the selective 5-HT$_{2C}$ receptor antagonist SB242084 in total licks and total magazine entries in HD and LD rats on SIP. Data are expressed as the mean ±SEM.

	Total licks		Total magazine entries	
	HD	LD	HD	LD
Ketanserin				
Vehicle	3.470,8 ± 805,7	1.566,3 ± 475,8	2.054,3 ± 559,0	2.091,9 ± 325,2
0.3 mg/kg	4.077,2 ± 1.057,5	1.611,4 ± 598,6	1.821,8 ± 501,9	2.220,2 ± 404,1
0.6 mg/kg	3.720,0 ± 1.105,2	1.477,0 ± 425,8	1.904,6 ± 520,2	1.989,0 ± 324,6
1 mg/kg	3.658,0 ± 1.024,3	1.240,8 ± 437,6	1.990,2 ± 593,9	2.057,6 ± 344,0
M100907				
Vehicle	6.882,6 ± 895,3	345,0 ± 117,6	1.496,4 ± 156,2	1.338,5 ± 410,0
0.1 mg/kg	7.461,6 ± 1.114,0	682,0 ± 163,0	1.531,4 ± 193,7	1.299,4 ± 340,1
0.5 mg/kg	7.392,2 ± 1.398,5	448,0 ± 183,6	1.237,3 ± 223,0	1.352,9 ± 440,8
1 mg/kg	6.666,7 ± 1.239,4	1.586,7 ± 522,5	1.739,5 ± 282,8	1.573,6 ± 553,5
2 mg/kg	8.047,0 ± 1.117,0	563,2 ± 197,2	1.124,2 ± 331,4	1.300,6 ± 475,3
SB242084				
Vehicle	6.335,1 ± 935,4	966,7 ± 264,2	2.319,8 ± 289,4	1.740,5 ± 441,2
0.1 mg/kg	6.243,3 ± 954,2	879,4 ± 207,2	2.483,4 ± 293,9	1.860,5 ± 451,1
0.5 mg/kg	5.722,0 ± 948,8	989,5 ± 365,8	2.606,4 ± 289,9	2.847,4 ± 834,4
1 mg/kg	7.290,9 ± 1.386,7	1.202,5 ± 415,1	1.987,0 ± 457,4	2,268.8 ± 541,6
2 mg/kg	7.409,1 ± 558,8	1.048,5 ± 469,8	2.212,8 ± 446,5	2.089,1 ± 479,8

5.3.4 Experiment 3

Selective implication of serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in compulsive drinking of HD rats on SIP

The effects of serotonergic 5-HT$_{2A/C}$ receptor antagonists on DOI (0.5 mg/kg) administration in HD and LD rats on SIP are shown in Figure 5.5 and Table 5.3. Co-administration of the selective serotonin 5-HT$_{2A}$ receptor antagonist ketanserin (0.5 and 1 mg/kg) effectively blocked the reduction in water intake produced by the serotonin 5-HT$_{2A/C}$ receptor agonist DOI (0.5 mg/kg) in HD rats (group×ketanserin×DOI effect: $F_{2,36}=3.52$, $p<0.05$; Figure 5.5A). *Post hoc* analyses showed that vehicle-DOI (0.5 mg/kg) significantly reduced water intake in HD rats ($p<0.001$) compared with vehicle-saline of the same group; this effect was blocked when DOI was combined with the highest dose of ketanserin 1 mg/kg ($p<0.001$) compared with vehicle-DOI group (Figure 5.5A). The same effects were observed for total licks (group×ketanserin×DOI effect: $F_{2,36}=5.93$, $p<0.01$). *Post hoc* analyses showed that vehicle-DOI (0.5 mg/kg) significantly reduced total licks in HD rats ($p<0.001$) compared with vehicle-saline of the same group; this effect was blocked when DOI was combined with the highest dose of ketanserin 1 mg/kg ($p<0.001$) compared with the vehicle-DOI group. No significant differences were found in the magazine entries (Table 5.3).

Co-administration of the highly selective serotonin $5\text{-}HT_{2A}$ receptor antagonist M100907 (1 mg/kg) effectively blocked the reduction in water intake produced by the serotonin $5\text{-}HT_{2A/C}$ receptors agonist DOI (0.5 mg/kg) in HD rats (group×M100907×DOI effect: $F_{1,17}=7.04$, $p<0.01$). *Post hoc* tests demonstrated that vehicle-DOI (0.5 mg/kg) significantly reduced water intake in HD rats ($p<0.001$) compared with vehicle-saline of the same group; this effect was blocked when DOI was combined with M100907 ($p<0.001$) vs. the vehicle-DOI group (Figure 5.5B). The same effects were observed for total licks (group×M100907×DOI effect: $F_{1,17}=4.26$, $p<0.05$). *Post hoc* analyses showed that vehicle-DOI (0.5 mg/kg) significantly reduced total licks in HD rats ($p<0.001$) compared with. vehicle-saline of the same group; this effect was blocked when DOI was combined with M100907 ($p<0.001$) vs. vehicle-DOI group. No significant differences were found for M100907 or DOI administration in either water intake or total licks in LD rats. No significant differences were found in the magazine entries in both groups (Table 5.3).

Co-administration of the selective serotonin $5\text{-}HT_{2C}$ receptor antagonistSB242084 (1 mg/kg) did not block the reduction in water intake produced by the serotonin $5\text{-}HT_{2A/C}$ receptors agonist DOI (0.5 mg/kg) in HD rats (group×SB242084×DOI interaction: $F_{1,16}=0.03$, $p=0.86$). However, the results from experiment 1 were replicated, and DOI (0.5 mg/kg) significantly reduced the water intake in HD rats (group×DOI interaction: $F_{1,16}=21.81$, $p<0.001$). In addition, the results from experiment 2 revealed that SB242084 (1 mg/kg) significantly increased the water intake in HD rats (group×SB242084: $F_{1,16}=15.21$, $p<0.001$), and the group differences

between HD and LD rats remained significant for water intake ($F_{1,16}=77.48$, $p<0.0001$) (Figure 5.5C). The same effects were found for the total number of licks (group×SB242084×DOI interaction: $F_{1,16}=1.36$, $p=0.25$; group×DOI interaction effect: $F_{1,16}=37.34$; $p<0.0001$; group×SB242084: $F_{1,16}=5.51$, $p<0.05$; group: $F_{1,16}=86.66$, $p<0.0001$). No significant differences were found in the magazine entries (Table 5.3).

Figure 5.5

Effects of the combined administration of the serotonin $5\text{-HT}_{2A/C}$ receptor agonist DOI (0.5 mg/kg) with (A) the serotonin 5-HT_{2A} receptor antagonist ketanserin (0.5 and 1 mg/kg), (B) the high selective serotonin 5-HT_{2A} receptor antagonist M100907 (1 mg/kg) and (C) the selective serotonin 5-HT_{2C} receptor antagonist SB242084 (1 mg/kg) in water intake of HD and LD rats on SIP. Data are expressed as the mean±SEM. ** $p < 0.001$ indicates significant differences vs. vehicle-saline administration in the same group.## $p < 0.001$ indicate significant differences vs. vehicle-DOI administration in the same group.
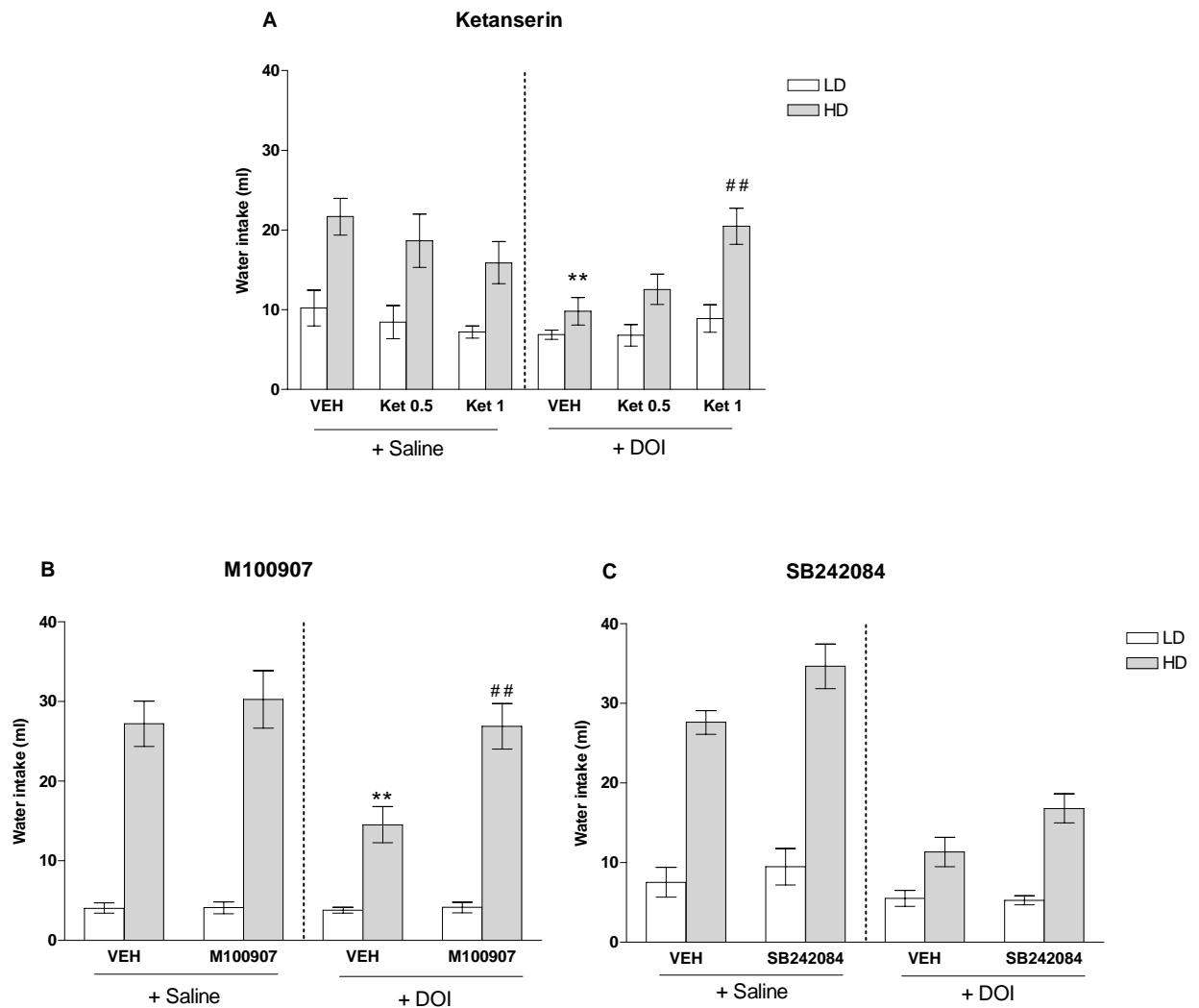
Table 5.3

Effects of the combined administration of the serotonin $5\text{-}HT2_{A/C}$ receptor agonist DOI (0.5 mg/kg) with (A) the serotonin $5\text{-}HT_{2A}$ receptor antagonist ketanserin (0.5 and 1 mg/kg), (B) the high selective serotonin $5\text{-}HT_{2A}$ receptor antagonist M100907 (1 mg/kg) and (C) the selective serotonin $5\text{-}HT_{2C}$ receptor antagonist SB242084 (1 mg/kg) in total licks and total magazine entries of HD and LD rats on SIP. Data expressed as the mean ±SEM. ** $p < 0.001$ indicate significant differences vs. vehicle-saline administration in the same group of rats. ## $p < 0.001$ significant differences vs. vehicle-DOI administration in the same group.

	Total licks		Total magazine entries	
	HD	**LD**	**HD**	**LD**
Ketanserin - DOI				
Veh + Sal	5.112,8 ± 979,7	1.220,7 ± 232,4	1.753,0 ± 275,9	1.874,0 ± 260,6
Ket 0.5 mg/kg + Sal	4.054,8 ± 1.134,0	1.549,6 ± 516,2	2.247,3 ± 821,0	1.683,9 ± 315,9
Ket 1 mg/kg + Sal	3.920,4 ± 1.500,7	1.518,2 ± 437,1	1.645,2 ± 554,6	2.341,6 ± 579,7
Veh + DOI	2.093,1 ± 297,5**	1.068,5 ± 122,4	2.256,8 ± 251,4	2.143,7 ± 198,8
Ket 0.5 mg/kg + DOI	2.740,1 ± 615,3	1.123,7 ± 367,8	2.150,6 ± 520,0	1.857,6 ± 269,3
Ket 1 mg/kg + DOI	4.907,0 ± 814,9##	1.313,6 ± 237,0	1.759,4 ± 269,2	2.642,2 ± 429,5
M100907 - DOI				
Veh + Sal	6.442,3 ± 1.023,7	399,2 ± 127,8	1.335,7 ± 269,5	1.474,1 ± 496,2
M100907 + Sal	8.753,6 ± 1.309,5	536,6 ± 194,5	870,6 ± 212,3	1.303,9 ± 494,1
Veh + DOI	3.126,4 ± 644,6**	283,3 ± 88,7	1.900,4 ± 252.9	2.009,3 ± 187,2
M100907 + DOI	7.513,2 ± 1.140,5##	483,4 ± 159,6	1.014,1 ± 206,3	1.244,2 ± 478,8
SB242084 - DOI				
Veh + Sal	5.264,0 ± 851,9	736,6 ± 246,2	2.191,9 ± 334,5	1.917,0 ± 463,2
SB242084 + Sal	7.685,5 ± 616,4	783,9 ± 204,2	2.020,1 ± 418,6	1.997,3 ± 552,7
Veh + DOI	2.756,4 ± 650,2	632,7 ± 200,4	2.340,7 ± 196,9	1.956,7 ± 202,1
SB242084 + DOI	3.679,7 ± 545,1	856,8 ± 168,9	2.984,9 ± 254,8	2.159,0 ± 453,3

5.4 Discussion

The present study investigated serotonin $5\text{-HT}_{2A/C}$ receptor function associated with the presumed trait of high compulsivity in rats (Moreno et al. 2012; Moreno and Flores, 2012). We found that in HD rats, which are characterised by excessive and persistent compulsive drinking on SIP, the systemic administration of citalopram and the serotonin 5-HT2A/C receptor agonist DOI reduced dose-dependent compulsive drinking, without affecting LD rat behaviour. However, atomoxetine did not affect compulsive drinking on SIP. Furthermore, the reduction of compulsive drinking produced by DOI in HD rats was reversed by the administration of the serotonin 5-HT_{2A} receptor antagonist ketanserin and the highly selective receptor antagonist M100907, but not by the serotonin 5-HT_{2C} receptor antagonist SB242084 that only increased compulsive drinking in HD rats on SIP when administered alone. These results suggest that serotonin 5-HT_{2A} receptors may have a relevant participation in the inhibition of compulsive behaviour exhibited by HD rats on SIP, pointing towards the implication of serotoninergic mechanisms in vulnerability to compulsive disorders.

5.6.1 Serotoninergic mechanisms underlying compulsive drinking on SIP

We found that the serotonin reuptake inhibitor citalopram (0.3, 1 and 3 mg/kg) reduced dose-dependent compulsive water intake in HD rats on SIP, which is consistent with previous reports describing the dose-dependent decrease of SIP

behaviour in non-selected rats by acute and also by chronic systemic administration of fluoxetine and others SSRIs (Martin et al. 1998, 2002; Platt et al. 2008; Woods et al. 1993). The present data add to the accumulating evidence that compulsive drinking is mediated via serotoninergic mechanisms (for review see Moreno and Flores 2012; Platt et al. 2008) and extend previous findings in the characterisation of the monoaminergic mechanisms implicated in compulsive HD rats selected by SIP. HD compared to LD rats have shown differences in the efficacy of D-amphetamine in reducing water intake (López-Grancha et al. 2008), an altered serotoninergic activity in the amygdala (Moreno et al. 2012) and divergences in brain dopamine D_1 and D_2 receptors (Pellón et al. 2011). Consistent with these findings, we propose that citalopram effectively reduces compulsive water intake in HD by an enhancement of the 5-HT tone and the subsequent stimulation of key serotonin receptors, such as 5-HT_{2A} or 5-HT_{2C}, which might be altered in compulsive HD rats.

In the present study, we found no changes in compulsive water intake or licks on SIP by the acute administration of the selective noradrenalin reuptake inhibitor atomoxetine at any of doses tested (1, 2, 3 and 5 mg/kg). However, we found significantly reduced magazine entries in both groups. Atomoxetine is an effective drug for reducing hyperactivity and distinct forms of impulsivity (Economidou et al. 2012; Robinson et al. 2008; Tanaka et al. 2013). In the contrary ,excessive drinking on SIP seems not to be related to hyperactivity measured by spontaneous locomotor activity (Moreno et al. 2012) nor by behavioural mechanisms related to impulsive choice measured by delay-discounting task (Íbias and Pellón, 2014), although SIP is a

phenomenon that could be maintained by a delayed reinforcement (Killeen and Pellón 2013). On the other hand, atomoxetine can also affect the motivation to perform a task, as shown by an increase in omission errors on the 5-CSRT task (Baarendse and Vanderschuren 2012). These effects of atomoxetine could be associated with the observed reduction in the magazine entries in the LD and HD groups on SIP, because the magazine entries could be considered as a SIP component associated with hyperactive or motivational behaviours and can be interpreted as a variable to control the normal activity of the animal in the procedure.

The present findings involving the effect of citalopram vs. atomoxetine on compulsive drinking on SIP are consistent with the effects observed using similar compounds in humans. Thus, citalopram and other SSRIs has demonstrated efficacy for the treatment of OCD (for a review, see Zohar et al. 2000); while antidepressants that selectively block the uptake of norepinephrine have less clinical efficacy in its treatment (for a review, see Westenberg et al., 2007).

5.6.2 Role of 5-HT$_{2A/C}$ receptors in compulsive drinking on SIP

The serotonin 5-HT$_{2A/C}$ receptor agonist DOI (at doses of 0.1, 0.3 and 0.5 mg/kg) reduced dose-dependent compulsive drinking in HD rats, which eliminated the significant differences with the LD group on SIP. This effect was observed in water intake and licks and did not affect the magazine entries, discarding any other potential side effects of the drug on animal activity. The present finding supports the

key role of serotonin 5-HT$_{2A/C}$ receptors in compulsive behaviours, which is consistent with previous results on the implication of the serotonin 5-HT$_{2C}$ receptor on SIP. The serotonin 5-HT$_{2C}$ receptor agonist WAY-163909 at doses of 3 and 5 mg/kg (Rosenzweig-Lipson et al. 2007) and Ro 60-0175 at doses of 0.3, 1 and 3 mg/kg decreased compulsive water intake on SIP in non-selected rats (Martin et al. 1998, 2002). However, the DOI compound exhibits a potent and moderate selectivity in the stimulation of 5-HT$_{2A}$ receptors (Knight et al. 2004; Rueter et al. 2000), and the use of lower doses, compared with previous studies, highlight the potential vital participation of serotonin 5-HT$_{2A}$ receptors on compulsive behaviours displayed on SIP.

When exploring the participation of serotonin 5-HT$_{2A/C}$ receptors, the administration of different doses of the serotonin 5-HT$_{2A}$ receptor antagonist ketanserin (0.3, 0.6, and 1 mg/kg) and the highly selective antagonist M100907 (0.1, 0.5, 1 and 2 mg/kg) revealed no changes in the different behavioural parameters on SIP, and the differences between the HD and LD groups were maintained. However, in a combined treatment with the serotonin 5-HT$_{2A/C}$ receptor agonist DOI, both serotonin 5-HT$_{2A}$ receptor antagonists at a dose of 1 mg/kg fully reversed the reduction on compulsive drinking produced by DOI in HD, without affecting magazine entries or LD behaviour on SIP. These results highlight the involvement of serotonin 5-HT$_{2A}$ receptors in the inhibition of compulsive drinking behaviour produced by DOI in HD rats on SIP. Previous studies have shown the role of the activation of serotonin 5-HT$_{2A}$ receptors in the neurochemical effects of DOI, in

which its activation is crucial to the modulation of dopamine transmission in the medial prefrontal cortex, ventral tegmental area and nucleus accumbens (Bortolozzi et al. 2005; Gobert and Millan, 1999; Kuroki et al., 2003). Thus, modulating dopaminergic transmission in these brain areas by activating $5\text{-}HT_{2A}$ receptors could underlie the reduction in compulsive drinking produced by DOI in HD rats on SIP. In these neurochemical studies (Bortolozzi et al. 2005; Gobert and Millan, 1999; Kuroki et al. 2003), the effects of DOI were reversed by M100907. Interestingly, they also showed that the administration of M100907 had no effect by itself on basal dopamine release, which is similar to the lack of behavioural effect observed in the present study after M100907 and ketanserin administration on SIP.

In contrast, the participation of $5\text{-}HT_{2C}$ receptors in compulsive drinking on SIP was confirmed in a different manner, namely, an increase in compulsive drinking in HD rats after the administration of the selective receptor antagonist SB242084 at doses of 1 and 2 mg/kg on SIP. Using the same compound, SB242084,in non-selected rats on SIP previous studies have reported contradictory effects; whereas only one study reported no changes in water intake by 1 mg/kg (Rosenzweig-Lipson et al. 2007). Furthermore, another study showed a dose-response increase in water intake at 0.1, 0.3 and 1 mg/kg (Martin et al. 2002). Nevertheless, both studies reported the efficacy of the selective $5\text{-}HT_{2C}$ receptor antagonist SB242084 at 1 mg/kg and 0.3 mg/kg to block the decrease in water intake produced by the $5\text{-}HT_{2C}$ receptor agonists, WAY-163909 (Rosenzweig-Lipson et al. 2007) and Ro 60-0175 (Martin et al. 1998) on SIP, respectively, but in non-selected rats. However, in the present study, blockade of the

5-HT$_{2C}$ receptors through the administration of the selective antagonist SB242084 at 1 mg/kg was not sufficiently effective to reverse the reduction of compulsive water intake produced by DOI in HD rats on SIP. A potential explanation for the lower efficacy of SB241084 to block DOI effects compared with previous studies using 5-HT$_{2C}$ agonists (Rosenzweig-Lipson et al. 2007; Martin et al. 1998) could be that the potent effect of DOI in reducing compulsive water intake in HD on SIP might be mediated by a higher affinity to 5-HT$_{2A}$ receptors (Knight et al. 2004), indicating the involvement of 5-HT$_{2A}$ receptors in the inhibition of compulsive drinking behaviour produced by DOI in HD rats on SIP.

Another explanation for the lower efficacy of SB242084 in contrast to M100907 and ketanserin in blocking the reduction of compulsive drinking produced by DOI in HD rats on SIP could be the different functional roles attributed to 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, as revealed by the contradictory effects observed when studying the effects of its antagonists in tasks that measure inhibitory control behaviours. For example, while the serotonin 5-HT$_{2A}$ receptor antagonist M100907 increased the perseverative responses in a spatial reversal learning task, the 5-HT$_{2C}$ receptor antagonist SB242084 decreased these responses (Boulougouris et al. 2008; Boulougouris and Robbins 2010). In addition, M100907 and ketanserin decreased the impulsive responses on the 5-CSRT test, while SB242084 increased these responses (Winstanley et al. 2004, Fletcher et al. 2007). Finally, a recent study also showed the efficacy of SB242084 in preventing the potentiation of a compulsive response in water contrafreeloading test, which is induced through dopaminergic activation

(Gross-Isseroff et al. 2004; Schepisi et al. 2013). Some authors have also argued that the different effects observed by both antagonists could also be dependent on the endogenous state of the serotoninergic system (Winstanley et al. 2004). Thus, the different effects observed in the present study that were due to the administration of the serotonin receptor antagonists M100907, ketanserin and SB242084 should be considered because of the altered serotonin basal levels observed in HD rats selected by SIP (Moreno et al. 2012).

5.6.3 Role of serotonin 5-HT$_{2A}$ receptor in a compulsive phenotype: HD rats on SIP

The novelty of the present study is the implication of the activation of the serotonin 5-HT$_{2A}$ receptors in reducing compulsive drinking in HD rats on SIP. This could be related to the proposed hypothesis regarding the role of postsynaptic 5-HT$_{2A}$ receptor stimulation as the mechanism for the efficacy of SSRIs to reduce compulsivity in OCD (Dannon et al. 2000; for a review, see El Mansari and Blier 2006; Westenberg et al. 2007). Experimental studies have demonstrated that the inhibitory effect of serotonin could be mediated via the 5-HT$_2$ receptors because the preferential 5-HT$_{2A}$ receptor agonist DOI and the serotonin 5-HT$_{2C}$ receptor agonist mCPP have the same effect in rats that were chronically treated with the SSRI paroxetine (El Mansari and Blier 2006). The anticompulsive activity produced by the activation of 5-HT$_{2A}$ receptors is also supported by the beneficial activity of some hallucinogens, which have potent agonistic properties on 5-HT$_{2A}$, such as LSD and psilocybin in OCD (for review Delgado and Moreno, 1998a,b). In the same sense,

atypical antipsychotics at high doses, which have a potent serotonin 5-HT$_{2A}$ receptor antagonism, may exacerbate compulsive symptoms (for a review, see El Mansari and Blier 2006), while its administration at low doses has demonstrated a therapeutic effect of enhancing the effects of SSRIs in resistant OCD patients (for a review, see Bokor and Anderson 2014; Erzegovesi et al. 2005; McDougle et al. 2000), thus suggesting a key role of serotonin 5-HT$_{2A}$ receptors in determining compulsive symptoms.

Moreover, neuroimaging studies have shown that 5-HT$_{2A}$ receptors play a crucial role in the development of compulsive spectrum disorders in humans. Positron emission tomography (PET) studies in drug-naive OCD patients revealed reductions in serotonin 5-HT$_{2A}$ receptor availability in the frontal cortex (Perani et al. 2008), whereas increases have been found in the caudate nucleus (Adams et al. 2005), with specific correlations between serotonin 5-HT$_{2A}$ receptor availability in the orbitofrontal cortex, clinical severity and age of onset of the disorder (Perani et al. 2008; Simpson et al. 2011). Interestingly, neuroimaging studies in dogs with compulsive behaviours have revealed significantly lower 5-HT$_{2A}$ receptor availability in the frontal and temporal cortices (Vermeire et al. 2012). Consistent with these results and the hypothesis of an altered 5-HT$_{2A}$ function in high compulsive HD rats selected by SIP, a recent study in selected Roman High Avoidance RHA rats, which were characterised by a compulsive drinking profile on SIP and an enhanced impulsive choice and activity on delay discounting and the 5-CSRT task, respectively (Moreno et al. 2010), have shown alterations in the prefrontal serotonin 5-HT$_{2A}$

receptor brain binding (Klein et al. 2013). Thus, the 5-HT$_{2A}$ receptor has emerging relevance in emotion-based actions, and it appears to play a different role according to the nature of the inhibitory control behaviour, which can be either impulsivity or compulsivity (for a review, see Aznar and Klein 2013). Future studies are therefore required to explore these hypotheses.

6. Conclusions

According to the results obtained the conclusions of the present Doctoral thesis are:

1.   In the present study, High Drinker rats selected by high compulsive behavior on SIP showed reduced latent inhibition effect, behavioural inflexibility and reduced brain myelination compared Low Drinker rats, which are well known behavioural and biological markers of schizophrenia.

  1.1.   HD rats, selected by SIP, showed selective attention deficit, in an animal model of schizophrenia, by less Latent inhibition effect in the preexposed condition to a stimuli compared to Low drinker (LD) animals.

  1.2.   HD rats showed behavioural inflexibility in a Spatial reversal learning task, evidenced by an increase in the number of trials to criterion, incorrect responses and perseverative responses in the reversal condition compared to LD animals.

  1.3.   HD rats had less myelination in corpus callosum, striatum and basolateral amygdala compared to LD animals.

2.   Chronic tryptophan depletion by diet demonstrated the implication of serotoninergic mechanisms in the vulnerability to compulsive behaviour on SIP.

  2.1.   Chronic tryptophan depletion by diet increased compulsive licking behaviour in HD rats on SIP, compared LD and Lister Hooded strain rats.

Thus, pointing towards a relevant role of genetic strain differences on SIP acquisition.

2.2.   The increase in compulsive licking behaviour in HD Wistar rats with Tryptophan depletion diet on SIP was also accompanied by a decrement in the serotonin neurochemical levels in prefrontal cortex, striatum, nucleus accumbens, amygdala and Hippocampus, and a decrease of 5-HT2A receptors in the striatum. However, no differences were observed in the other monoamines, dopamine and noradrenaline, by tryptophan depletion diet on Wistar rats.

2.3.   Chronic tryptophan depletion by diet increased locomotor response to habituation in an Open-field in Lister Hooded rats compared HD and LD Wistar rats. This effect could be due to the observed increase in the neurochemical level of dopamine in the nucleus accumbens, as well as, the decrease of the serotonin 5-HT1A receptors in the prefrontal cortex by tryptophan depletion diet on Lister Hooded rats.

3.   The pharmacological manipulation of serotoninergic mechanisms revealed the implication of 5-HT2A receptors in the compulsive behaviour of HD rats on SIP (Series 3).

3.1.   The SSRI citalopram and the activation of the serotonin 5-HT2A receptors by DOI agonist reduced dose-dependent compulsive behaviour on

SIP in HD rats, without affecting LD behaviour. In contrast, the noradrenaline re-uptake inhibitor Atomoxetine did not induce any effects on compulsive drinking on SIP.

3.2.   According to previous publications, the blockade of the serotonin 5-HT2C receptors by the antagonist SB242084 increased water intake in HD rats on SIP (Series 3).However, the blockade of the serotonin 5-HT2A receptors by the antagonists ketanserin and M100907 did not affect SIP behaviour.

3.3.   The crucial role of 5-HT2A receptors in the compulsive behaviour on SIP was demonstrated by the combination of the serotonin 5-HT2A versus 5-HT2C receptor antagonists with the serotonin 5-HT2A/C receptor agonist DOI. Thus, the serotonin 5-HT2A receptor antagonists ketanserin and M100907 blocked the DOI induced reduction on compulsive drinking on SIP, whereas the serotonin 5-HT2C receptor antagonist SB242084 did not.

Our data confirms previous findings that proposed SIP as an animal model of schizophrenia, and extends the knowledge about the implication of genetic strain differences, serotoninergic mechanisms and specifically the role of serotonin 5-HT2A receptor in the compulsive phenotype of HD rats selected by SIP. Future studies on the compulsive phenotype of HD rats selected by SIP can contribute to disentangle

possible explanations to the comorbidity of different compulsive behaviours and schizophrenia-like deficits in the same individual, helping to understand the mechanism of vulnerability to compulsive spectrum disorders and new therapy targets for its treatment.

References

Adams, K.H., Hansen, E.S., Pinborg, L.H., Hasselbalch, S.G., Svarer, C., Holm, S., Bolwig, T.G. Knudsen, G.M. 2005. Patients with obsessive-compulsive disorder have increased 5-HT2A receptor binding in the caudate nuclei. Int. J. Neuropsychopharmacol. 8:391–401. http://dx.doi.org/10.1017/S1461145705005055

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). American Psychiatric Press, Washington DC.

Angoa-Pérez M, Kane MJ, Briggs DI, Sykes CE, Shah MM, Francescutti DM, Rosenberg DR, Thomas DM, Kuhn DM (2012) Genetic depletion of brain 5HT reveals a common molecular pathway mediating compulsivity and impulsivity. J Neurochem. 121(6):974-84. doi: 10.1111/j.1471-4159.2012.07739.x.

Ardis, T.C., Cahir, M., Elliott, J.J., Bell, R., Reynolds, G.P. et al. 2009. Effect of acute tryptophan depletion on noradrenaline and dopamine in the rat brain. Journal of Psychopharmacoly,23, 51–55.

Atmaca, M., Onalan, E., Yildirim, H., Yuce, H., Koc, M., Korkmaz, S., 2010. The association of myelin oligodendrocyte glycoprotein gene and white matter volume in obsessive-compulsive disorder. J. Affect. Disord. 124 (3), 309-313.

Aznar S, Klein AB (2013) Regulating Prefrontal Cortex Activation: An Emerging Role for the 5-HT2A Serotonin Receptor in the Modulation of Emotion-Based Actions? Mol Neurobiol. doi 10.1007/s12035-013-8472-0

Baarendse, P.J., Vanderschuren, L.J. 2012. Dissociable effects of monoamine reuptake inhibitors on distinct forms of impulsive behavior in rats. Psychopharmacology (Berl). 219 (2), 313-26. doi: 10.1007/s00213-011-2576-x

Barak, S., Weiner, I., 2010. Dissociating scopolamine-induced disrupted and persistent latent inhibition: stage-dependent effects of glycine and physostigmine. Psychopharmacol. 209,175–184.

Bardo, M.T., Donohew, R.L., Harrington, N.G. 1996. Psychobiology of novelty-seeking and drug-seeking behavior, Behavioural Brain Research, 77, 23–43.

Bari, A., Eagle, D.M., Mar, A.C., Robinson, E.S., Robbins, T.W. 2009. Dissociable effects of noradrenaline, dopamine, and serotonin uptake blockade on stop task performance in rats. Psychopharmacology (Berl). 205 (2), 273-83.

Bari, A., Theobald, D. E., Caprioli, D., Mar, A. C., Aidoo-Micah, A., Dalley, J. W. Robbins, T. W. 2010. Serotonin modulates sensitivity to reward and negative feedback in a probabilistic reversal learning task in rats. Neuropsychopharmacology, 35, 1290-1301.

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. 40 (7), 1619-30.

Bartzokis, G., 2012. Neuroglialpharmacology: myelination as a shared mechanism of action of psychotropic treatments. Neuropharmacology. 62 (7), 2137-2153.

Begré, S., Koenig, T., 2008. Cerebral disconnectivity: an early event in schizophrenia. Neuroscientist. 14 (1), 19-45.

Berendsen, H.H.G., Broekkamp, C.L.E., Van Delft, A.M.L. 1991. Depletion of brain serotonin differently affects behaviour induced by 5-HT1A, 5-HT1C and 5-HT2 receptor activation in rats. Behav. Neural. Biol, 55, 214-226

Bernstein, H.G., Ernst, T.S., Lendeckel, U., Dobrowolny, H., Steiner, J., Bogerts, B., 2010a. What protects patients with schizophrenia from developing Alzheimer pathology? In: Ritsner, M.S. (Ed.), Brain protection in schizophrenia, mood and cognitive disorders. Springer, New York, pp. 45–66.

Bernstein, H.G., Steiner, J., Guest, P.C., Dobrowolny, H., Bogerts, B., 2015. Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. Schizophr. Res. 161 (1), 4-18.

Bigelow, L.B., Nasrallah, H.A., Rauscher, F.P., 1983. Corpus callosum thickness in chronic schizophrenia. Br. J. Psychiatry. 142, 284-287.

Bizot, J., Le Bihan, C., Puech, A.J., Hamon, M., Thiebot, M. 1999. Serotonin and tolerance to delay of reward in rats.Psychopharmacology. 146, 400–412.

Blackshear, M.A., Steranka, L.R., Sanders-Bush, E. 1981. Multiple serotonin receptors: Regional distribution and effect of raphe lesions. Eur. J. Pharmacol. 76, 325–334.

Bogerts, B., Meertz, E., Schönfeldt-Bausch, R., 1985. Basal ganglia and limbic system pathology in schizophrenia. A morphometric study of brain volume and shrinkage. Arch. Gen. Psychiatry. 42 (8), 784-791.

Bokor, G., Anderson, P.D. 2014. Obsessive-Compulsive Disorder. J. Pharm. Pract. [Epub ahead of print] PMID: 24576790

Bortolon, C., Raffard, S., 2015. Self-reported psychotic-like experiences in individuals with obsessive-compulsive disorder versus schizophrenia patients: characteristics and moderation role of trait anxiety. Compr. Psychiatry. 57:97-105.

Bortolozzi, A., Díaaz-Mataix, L., Scorza, M.C., Celada, P., Artigas, F. 2005. The activation of 5-HT2A receptors in prefrontal cortex enhances dopaminergic activity. J. Neurochem. 95, 1597–1607.

Boulougouris, V., Chamberlain, S.R., Robbins, T.W., 2009. Cross-species models of OCD spectrum disorders. Psychiatry Res. 170 (1), 15-21.

Boulougouris, V., Dalley, J.W., Robbins, T.W., 2007. Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat. Behav. Brain Res. 179 (2), 219-28.

Boulougouris, V., Glennon, J.C., Robbins, T.W., 2008. Dissociable effects of selective 5-HT2A and 5-HT2C receptor antagonists on serial spatial reversal learning in rats. Neuropsychopharmacology 33 (8), 2007-2019.

Boulougouris, V., Robbins, T.W. 2009. Pre-surgical training ameliorates orbitofrontal-mediated impairments in spatial reversal learning. Behav. Brain Res. 197(2):469-75.

Boulougouris, V., Robbins, T.W., 2010. Enhancement of spatial reversal learning by 5-HT2C receptor antagonism is neuroanatomically specific. J. Neurosci. 30 (3), 930-938.

Brewer, J. A., Potenza, M. N. 2008. The Neurobiology and Genetics of Impulse Control Disorders: Relationships to Drug Addictions. Biochemical Pharmacology, 75(1), 63–75.

Brown, C.M., Fletcher, P.J., Coscina, D.V. 1998. Acute amino acid loads that deplete brain serotonin fail to alter behavior. Pharmacology Biochemical and Behavior, 59, 115-21. http://doi.org/10.1016/S0091-3057 (97)00381-X

Bruce, K.R., Steiger, H., Young, S.N., Kin, N.M., Israël, M., Lévesque, M. 2009. Impact of acute tryptophan depletion on mood and eating-related urges in bulimic and nonbulimic women. Journal of psychiatry and neuroscience, 34, 376-382.

Cahir, M., Ardis, T., Reynolds, G.P., Cooper, S.J. 2007. Acute and chronic tryptophan depletion differentially regulate central 5-HT1A and 5-HT2A receptor binding in the rat. Psychopharmacology, 190, 497-506. http://doi.org/10.1007/s00213-006-0635-5

Cardona, D., López-Grancha, M., López-Crespo, G., Nieto-Escámez, F., Sánchez-Santed, F. and Flores, P. 2006. Vulnerability of Long-Term Neurotoxicity of Chlorpyrifos: Effect on Schedule-Induced Polydipsia and a Delay Discounting Task. Psychopharmacology, 189, 47-57.

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, 128-137.

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. http://doi.org/10.3389/fncir.2014.00058

Carli, M., Samanin, R. 2000. The 5-HT(1A) receptor agonist 8-OH- DPAT reduces rats' accuracy of attentional performance and enhances impulsive responding in a five-choice serial reaction time task: role of presynaptic 5-HT(1A) receptors. Psychopharmacology (Berl), 149, 259–268. http://doi.org/10.1007/s002139900368

Carpenter, W.T. Jr., Kirkpatrick, B.1988. The heterogeneity of the long-term course of schizophrenia. Schizophr. Bull. 14 (4), 645-652.

Cederlöf M., Lichtenstein, P., Larsson, H., Boman, M., Rück, C., Landén, M., Mataix-Cols, D., 2014. Obsessive-Compulsive Disorder, Psychosis, and Bipolarity: A Longitudinal Cohort and Multigenerational Family Study. Schizophr Bull.pii: sbu169. [Epub ahead of print].

Chamberlain, S.R., Menzies, L., Hampshire, A., Suckling, J., Fineberg, N.A., del Campo, N., Aitken, M., Craig, K., Owen, A.M., Bullmore, E.T. ,Robbins, T.W., Sahakian, B.J., 2008. Orbitofrontal dysfunction in patients with obsessive-compulsive disorder and their unaffected relatives. Science. 321 (5887), 421-422.

Chang, W.C., Hui, C.L., Tang, J.Y., Wong, G.H., Chan, S.K., Lee, E.H., Chen, E.Y., 2013. Impacts of duration of untreated psychosis on cognition and negative symptoms in first-episode schizophrenia: a 3-year prospective follow-up study. Psychol. Med. 43 (9), 1883-1893.

Clarke, H.F., Dalley, J.W., Crofts, H.S., Robbins, T.W., Roberts, A.C., 2004. Cognitive inflexibility after prefrontal serotonin depletion. Science. 304 (5672), 878-880.

Clarke, H.F., Walker, S.C., Dalley, J.W., Robbins, T.W., Roberts, A.C., 2007. Cognitive inflexibility after prefrontal serotonin depletion is behaviorally and neurochemically specific. Cereb. Cortex. 17 (1), 18-27.

Clarke, H.F., Robbins, T.W., Roberts, A.C., 2008. Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. J. Neurosci. 28 (43), 10972-10982.

Conn, P.J., Sanders-Bush, E. 1986. Regulation of serotonin-stimulated phosphoinositide hydrolysis: Regulation to the serotonin 5-HT2 binding site. J. Neurosci. 6, 3669–3675.

Coutureau, E., Blundell, P.J., Killcross, S., 2001. Basolateral amygdala lesions disrupt latent inhibition in rats. Brain. Res. Bull. 56 (1), 49-53.

Coutureau, E., Galani, R., Gosselin, O., Majchrzak, M., Di Scala, G., 1999. Entorhinal but not hippocampal or subicular lesions disrupt latent inhibition in rats. Neurobiol. Learn. Mem. 72 (3), 143-157.

Crean, J., Richards, J.B., de Wit, H. 2002. Effect of tryptophan depletion on impulsive behavior in men with or without a family history of alcoholism. Behavioural Brain Research, 136, 349–357.

Crow, T.J., 1980. Molecular pathology of schizophrenia: more than one disease process? Br. Med. J. 280 (6207), 66-68.

Dannon, P.N., Sasson, Y., Hirschmann, S., Iancu, I., Grunhaus, L.J., Zohar, J. 2000. Pindolol augmentation in treatment-resistant obsessive compulsive disorder: a double-blind placebo controlled trial. Eur. Neuropsychopharmacol. 3, 165–169.

Dantzer, R., Terlouw, C., Mormède, P. & Le Moal, M. (1988a) Schedule-induced polydipsia experience decreases plasma corticosterone levels but increases plasma prolactin levels. Physiology and Behavior, 43, 275–279.

Dantzer, R., Terlouw, C., Tazi, A., Koolhaas, J.M., Bohus, B., Koob, G.F. & Le Moal, M. (1988b) The propensity for schedule-induced polydipsia is related to differences in conditioned avoidance behaviour and in defense reactions in a defeat test. Physiology and Behavior, 43, 269–273.

De Carolis, L., Schepisi, C., Milella, M.S., Nencini, P. 2011. Clomipramine, but not haloperidol or aripiprazole, inhibits quinpirole-induced water contrafreeloading, a putative animal model of compulsive behavior. Psychopharmacology (Berl). 218 (4), 749-59. doi: 10.1007/s00213-011-2372-7.

De Leon, J., Tracy, J.I., McCann, E., McGrory, A. 2002. Polydipsia and schizophrenia in a psychiatric hospital: a replication study. Schizophr. Res. 57 (2-3), 293-301

De Leon, J., Verghese, C., Tracy, J.I., Jossiasen, R.C., Simpson, G.M. 1994. Polydipsia and water intoxication in psychiatric patients: a review of the epidemiological literature. Biol. Psychiatry 35, 408-419

De Russo, A.L., Fan, D., Gupta, J., Shelest, O., Costa, R.M., Yin, H.H., 2010. Instrumental uncertainty as a determinant of behavior under interval schedules of reinforcement. Front. Integr. Neurosci. 28, 4. pii: 17.

Delgado, P.L., Moreno, F.A. 1998. Hallucinogens, serotonin and obsessive-compulsive disorder. J. Psychoactive Drugs 30 (4), 359-66

Delgado, P.L., Moreno, F.A. 1998. Different roles for serotonin in anti-obsessional drug action and the pathophysiology of obsessive-compulsive disorder. Br. J. Psychiatry Suppl. (35), 21-5

Dickinson, A., Nicholas, D.J., Adams, C.D., 1983. The effect of the instrumental training contingency in susceptibility to reinforcer devaluation. Q. J. Exp. Psychol. B35, 35-51.

Didriksen, M., Christensen, A.V. 1993. The attenuation of schedule-induced polydipsia by dopamine blockers is not an expression of extrapyramidal side effect liability. Behav. Pharmacol. 4 (5), 517-522

Di Giovanni, G., Esposito, E., Di Matteo, V. 2010. Role of serotonin in central dopamine dysfunction. CNS Neuroscience and Therapeutics. 16, 179-194. http://doi.org/10.1111/j.1755-5949.2010.00135.x

Di Matteo, V., Pierucci, M., Esposito, E., Crescimanno, G., Benigno, A., Di Giovanni, G. 2008. Serotonin modulation of the basal ganglia circuitry: therapeutic implication for Parkinson's disease and other motor disorders. Prog. Brain. Res. 172, 423–463. http://doi.org/10.1016/S0079-6123(08)00921-7

Dundas, B., Harris, M., Narasimhan, M. 2007. Psychogenic polydipsia review: etiology, differential, and treatment. Curr. Psychiatry Rep. 9 (3), 236-41

Dwork, A.J., Mancevski, B., Rosoklija, G. 2007. White matter and cognitive function in schizophrenia. Int. J. Neuropsychopharmacol. 10 (4), 513-536.

Eagle, D.M. Baunez, C. 2010. Is there an inhibitory-response-control system in the rat? Evidence from anatomical and pharmacological studies of behavioral inhibition. Neuroscience and Biobehavioral Reviews, 34 (1-3), 50–72.

Economidou, D., Theobald, D.E., Robbins, T.W., Everitt, B.J., Dalley, J.W. 2012. Norepinephrine and dopamine modulate impulsivity on the five-choice serial reaction time task through opponent actions in the shell and core sub-regions of the

nucleus accumbens. Neuropsychopharmacology 37 (9), 2057-66. doi: 10.1038/npp.2012.53ttp://doi.org/10.1016/j.neubiorev.2009.07.003

El Mansari, M., Blier, P. 2006. Mechanisms of action of current and potential pharmacotherapies of obsessive-compulsive disorder. Prog. Neuropsychopharmacol. Biol. Psychiatry 30, 362–373.

Erzegovesi, S., Guglielmo, E., Siliprandi, F., Bellodi, L. 2005. Low-dose risperidone augmentation of fluvoxamine treatment in obsessive-compulsive disorder: a double-blind, placebo-controlled study. Eur. Neuropsychopharmacol. 15 (1), 69-74.

Evenson, R.C., Jos, C.J., Mallya, A.R. 1987. Prevalence of polydipsia among public psychiatric patients. Psychol. Rep. 60, 803–7

Fadda, F., Cocco, S. Stancampiano, R. 2000. A physiological method to selectively decrease brain serotonin release. Brain Research Protocols. 5, 219-222. http://doi.org/10.1016/S1385-299X(00)00016-7

Falk JL (1961) Production of polydipsia in normal rats by an intermittent food schedule. Science 133:195-196. doi: 10.1126/science.133.13447.195

Falk JL (1966b) The motivational properties of schedule-induced polydipsia. J Exp Anal Behav 9:19-25. doi: 10.1901/jeab.1966.9-19

Falk JL. 1971The nature and determinants of adjunctive behavior. Physiol Behav. 1971 May;6(5):577-88.

Fan, Q., Yan, X., Wang, J., Chen, Y., Wang, X., Li, C., Tan, L., You, C., Zhang, T., Zuo, S., Xu, D., Chen, K., Finlayson-Burden, J.M., Xiao, Z., 2012. Abnormalities of white matter microstructure in unmedicated obsessive-compulsive disorder and changes after medication. PLoS One. 7 (4), e35889.

Faulkner, P., Deakin, J.F. 2014. The role of serotonin in reward, punishment and behavioural inhibition in humans: insights from studies with acute tryptophan depletion. Neurosci Biobehav Rev. 46 Pt 3:365-78. doi: 10.1016/j.neubiorev.2014.07.024.

Federspiel, A., Begré, S., Kiefer, C., Schroth, G., Strik, W.K., Dierks, T., 2006. Alterations of white matter connectivity in first episode schizophrenia. Neurobiol. Dis. 22 (3), 702-709.

Fernstrom, J.D. 1983. Role of precursor availability in control of monoamine biosynthesis in brain. Physiological Reviews, 63,484–546http://doi.org/10.1093/brain/awu178

Fields, R.D. 2008. White matter in learning, cognition and psychiatric disorders. Trends Neurosci. 31(7), 361-70. doi: 10.1016/j.tins.2008.04.001. Epub 2008 Jun 5.

Fineberg NA, Brown A, Reghunandanan S, Pampaloni I. (2012) Evidence-based pharmacotherapy of obsessive-compulsive disorder. Int J Neuropsychopharmacol. 2012 Sep;15(8):1173-91. doi: 10.1017/S1461145711001829.

Fineberg, N.A., Chamberlain, S.R., Goudriaan, A.E., Stein, D.J., Vanderschuren, L.J., Gillan, C.M., Shekar, S., Gor- wood, P.A., 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, 69-89. http://dx.doi.org/10.1017/S1092852913000801

Fineberg, N.A., Chamberlain, S.R., Hollander, E., Boulougouris, V., Robbins, T.W. 2011. Translational approaches to obsessive-compulsive disorder: from animal models to clinical treatment. Br J Pharmacol. (4):1044-61. doi: 10.1111/j.1476-5381.2011.01422.x.

Fineberg NA, Potenza MN, Chamberlain SR, Berlin HA, Menzies L, Bechara A, Sahakian BJ, Robbins TW, Bullmore ET, Hollander E (2010) Probing compulsive and

impulsive behaviors, from animal models to endophenotypes: a narrative review. Neuropsychopharmacology 35(3):591-604. doi: 10.1038/npp.2009.185.

Fletcher, P.J., Tampakeras, M., Sinyard, J., Higgins, G.A. 2007. Opposing effects of 5-HT(2A) and 5-HT(2C) receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. Psychopharmacology (Berl) 195 (2), 223-34

Franklin, M., Bermudez, I., Murck, H., Singewald, N., Gaburro, S. 2012. Sub-chronic dietary tryptophan depletion - An animal model of depression with improved face and good construct validity. Journal of Psychiatric Research, 46, 239-246 http://doi.org/10.1016/j.jpsychires.2011.10.003

Franklin, M., Craven, R.D., Dowling, B., Campling, G., Elliott, J.M. Cowen, P.J. 1999. Effect of a long-term low tryptophan diet on the prolactin responses to the 5-HT1A and 5-HT2C agonists, 8-OH-DPAT and mCPP in the male rat. Journal of Psychopharmacology, 13, 58-63. http://doi.org/10.1177/026988119901300107

Fratta, W., Biggio, G. Gessa, G.L. 1977. Homosexual mounting behavior induced in male rats and rabbits by a tryptophan-free diet. Life Sciences, 21, 379–384. http://dx.doi.org/10.1016/0024-3205 (77)90518-5

Frazer, A., Hensler, J.G. 1990. 5-HT1A-mediated responses: Effects of treatments that modify serotonergic neurotransmission. Ann. N.Y. Acad. Sci. 600, 460-474

Fuentes, S., Colomina, M.T., Vicens, P., Franco-Pons, N., Domingo, J.L., 2007. Concurrent exposure to perfluorooctane sulfonate and restraint stress during pregnancy in mice: effects on postnatal development and behavior of the offspring. Toxicol. Sci. 98 (2), 589-598.

Gessa, G.L., Biggio, G., Fadda, F., Corsini, G.U. Tagliamonte, A. 1974. Effect of the oral administration of tryptophan-free amino acid mixtures on serum tryptophan

and serotonin metabolism. Journal of Neurochemistry, 22, 869–870http://doi.org/10.1111/j.1471-4159.1974.tb04308.x

Gillan, C.M., Otto, A.R., Phelps, E.A., Daw, N.D., 2015. Model-based learning protects against forming habits. Cogn. Affect. Behav. Neurosci. Mar 24. [Epub ahead of print] PMID: 25801925.

Gilpin NW, Badia-Elder NE, Elder RL, Stewart RB (2008) Schedule-induced polydipsia in lines of rats selectively bred for high and low ethanol preference. Behav Genet 38(5):515-24. doi: 10.1007/s10519-008-9224-1

Gobert, A., Millan, M.J. 1999. Serotonin (5-HT)2A receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. Neuropharmacology 38 (2), 315-7

Gray, J.A., Feldon, J., Rawlins, J. N. P., Hemsley, D.R., Smith A.D., 1991. The neuropsychology of schizophrenia. Behav. Brain Sci., 14, 1-84

Gregory, J.G., Hawken, E.R., Banasikowski, T.J., Dumont, E.C., Beninger, R.J., 2015. A response strategy predicts acquisition of schedule-induced polydipsia in rats. Prog. Neuropsychopharmacol. Biol. Psychiatry. 61, 37-43.

Gross-Isseroff, R., Cohen, R., Sasson, Y., Voet, H., Zohar, J. 2004. Serotonergic dissection of obsessive compulsive symptoms: a challenge study with m-chlorophenylpiperazine and sumatriptan. Neuropsychobiology 50 (3), 200-5

Guloksuz, S., van Nierop, M., Lieb, R., van Winkel, R., Wittchen, H.U., van Os, J., 2015. Evidence that the presence of psychosis in non-psychotic disorder is environment-dependent and mediated by severity of non-psychotic psychopathology. Psychol. Med. 1-13.

Gur, R.E., Loughead, J., Kohler, C.G., Elliott, M.A., Lesko, K., Ruparel, K., Wolf, D.H., Bilker, W.B., Gur, R.C., 2007. Limbic activation associated with misidentification of

fearful faces and flat affect in schizophrenia. Arch. Gen. Psychiatry. 64 (12), 1356-1366.

Hadamitzky, M., Feja, M., Becker, T., Koch, M. 2009. Effects of acute systemic administration of serotonin 2A/C receptor ligands in a delay-based decision-making task in rats. Behav. Pharmacol. 20 (5-6), 415-23. doi: 10.1097/FBP.0b013e3283305e11

Hadamitzky, M., Koch, M. 2009. Effects of acute intra-cerebral administration of the 5-HT(2A/C) receptor ligands DOI and ketanserin on impulse control in rats. Behav. Brain. Res. 1, 204 (1), 88-92. doi: 10.1016/j.bbr.2009.05.021

Häfner, H., Nowotny, B., Löffler, W., an der Heiden, W., Maurer, K., 1995. When and how does schizophrenia produce social deficits? Eur. Arch. Psychiatry. Clin. Neurosci. 246 (1), 17-28

Hall, G., Channell, S., 1985. Latent inhibition and conditioning after preexposure to the training context. Lear. and Motiv. 16, 381-397.

Harrison, A.A., Everitt, B.J. Robbins, T.W. 1997a. Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. Psychopharmacology (Berl), 133, 329–342. http://doi.org/10.1007/s002130050410

Harrison, A.A., Everitt, B.J. Robbins, T.W. 1997b. Doubly dissociable effects of median- and dorsal-raphe lesions on the performance of the five-choice serial reaction time test of attention in rats. Behavioural Brain Research, 89, 135–149. http://doi.org/10.1016/S0166-4328 (97)00053-3

Harrison, A.A., Everitt, B.J. Robbins, T.W. 1999. Central serotonin depletion impairs both the acquisition and performance of a symmetrically reinforced go/no-go conditional visual discrimination. Behavioural Brain Research, 100, 99–112. http://doi.org/10.1016/S0166-4328(98)00117-X

Harvey, P.D., Pappadopulos, E., Lombardo, I., Kremer, C.M.., 2009. Reduction of functional disability with atypical antipsychotic treatment: a randomized long term comparison of ziprasidone and haloperidol. Schizophr. Res. 115 (1), 24-29.

Hawken, E.R., Beninger, R.J. 2013. The amphetamine sensitization model of schizophrenia symptoms and its effects on schedule-induced polydipsia in the rat. Psychopharmacology (Berl); e-pub ahead of print 16 November 2013. doi: 10.1007/s00213-013-3345-9.

Hawken, E.R., Delva, N.J., Reynolds, J.N., Beninger, R.J. 2011. Increased schedule-induced polydipsia in the rat following subchronic treatment with MK-801. Schizophr Res 125(1):93-8. doi: 10.1016/j.schres.2010.07.022

Heal, D.J., Philpot, J., Molyneux, S.G., Metz, A. 1985. Intracerebroventricular administration of 5,7-DHT to mice increases both head twitch response and the number of cortical 5-HT2 receptors. Neuropharmacology, 24, 1201–1205.

Hensler, J.G., Kovachich, G.B., Frazer, A. 1991. A quantitative autoradiographic study of serotonin1A receptor regulation. Effects of 5,7-dihydroxytryptamine and antidepressant treatments. Neuropsychopharmacology, 4, 131-144

Hofer, A., Bodner, T., Kaufmann, A., Kemmler, G., Mattarei, U., Pfaffenberger, N.M., Rettenbacher, M.A., Trebo, E., Yalcin, N., Fleischhacker, W.W., 2011. Symptomatic remission and neurocognitive functioning in patients with schizophrenia. Psychol. Med. 41, 2131-2139.

Hoffman, R.E., Hawkins, K.A., Gucorguieva, R., Boutros, N.N., Rachid, F., Carroll, K., Krystal, J.H., 2003. Transcranial magnetic stimulation of left temporoparietal cortex and medication-resistant auditory hallucinations. Arch. Gen. Psychiatry. 60 (1), 49-56.

Hollander, E. Wong, C.M. 1995. Obsessive-compulsive spectrum disorders. Journal of Clinical Psychiatry, 56, Suppl 4:3-6; discussion 53-5

Holleran, L., Ahmed, M., Anderson-Schmidt, H., McFarland, J., Emsell, L., Leemans, A., Scanlon, C., Dockery, P., McCarthy, P., Barker, G.J., McDonald, C., Cannon, D.M., 2014. Altered interhemispheric and temporal lobe white matter microstructural organization in severe chronic schizophrenia. Neuropsychopharmacology. 39 (4), 944-954.

Hull, J.W., Smith, T.E., Anthony, D.T., Goodman, M.S., Kentros, M.K., Sepe, O., Yanulis, M.A., Li, C., 1997. Patterns of symptom change: A longitudinal analysis. Schizophr. Res. 24, 17-19.

Ibias, J., Pellón, R. 2014. Different relations between schedule-induced polydipsia and impulsive behaviour in the Spontaneously Hypertensive Rat and in high impulsive Wistar rats: questioning the role of impulsivity in adjunctive behaviour. Behav. Brain Res. (1) 271, 184-94. doi: 10.1016/j.bbr.2014.06.010. Epub 2014 Jun 13.

Ikemoto, S., Panksepp, J. 1999. The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Research Reviews, 31, 6–41

Insel, T. R., 2010. Rethinking schizophrenia. Nature. 468, 187-193.

Jackson, D.M., Anden, N., Dahlstrom, A. 1975. A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain.Psychopharmacologia. 45,139–149.

Jensen, J., Willeit, M., Zipursky, R.B., Savina, I., Smith, A.J., Menon, M., Crawley, A.P., Kapur, S., 2008. The formation of abnormal associations in schizophrenia: neural and behavioral evidence. Neuropsychopharmacology. 33 (3), 473-479.

Joel D, Ben-Amir E, Doljansky J, Flaisher S. 2004. 'Compulsive' lever-pressing in rats is attenuated by the serotonin re-uptake inhibitors paroxetine and fluvoxamine but not by the tricyclic antidepressant desipramine or the anxiolytic diazepam. Behav Pharmacol. 2004 May;15(3):241-52.

Joel, D., 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 (1), 77-87.

Johnels, B. 1982. Locomotor hypokinesia in the reserpine-treated rat: drug effects from the corpus striatum and nucleus accumbens. Pharmacology Biochemistry and Behavior. 17, 283–289.

Kane, JM., Honigfeld, G, Singer, J, Meltzer, H. 1988. Clozapine in treatment-resistant schizophrenics. Psychopharmacol Bull. 24 (1), 62-7

Kantak, K.M., Hegstrand, L.R., Whitman, J. Eichelman, B. 1980. Effects of dietary supplements and a tryptophan-free diet on aggressive behavior in rats. Pharmacology Biochemistry and Behavior, 12, 173-179. http://doi.org/10.1016/0091-3057 (80)90351-2

Kaplan, O., Dar, R., Rosenthal, L., Hermesh, H., Fux, M., Lubow, R.E., 2006. Obsessive-compulsive disorder patients display enhanced latent inhibition on a visual search task. Behav. Res. Ther. 44 (8), 1137-1145.

Kapur, S. 2003. Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. Am. J. Psychiatry. 160 (1), 13-23.

Kaye, W.H., Gendall, K.A., Fernstrom, M.H., Fernstrom, J.D., McConaha, C.W., Weltzin, T.E. 2000. Effects of acute tryptophan depletion on mood in bulimia nervosa. Biological Psychiatry, 47, 151-7.

Kawai, K., Yokota, N., Yamawaki, S. 1994. Effect of chronic tryptophan depletion on the circadian rhythm of wheel-running activity in rats. Physiol. Behav. 55, 1005–1013

Killcross, A.S., Dickinson, A., Robbins, T.W. 1994. Amphetamine-induced disruptions of latent inhibition are reinforcer mediated: implications for animal

models of schizophrenic attentional dysfunction. Psychopharmacology (Berl). 115 (1-2),185-95.

Killcross, A.S., Dickinson, A., Robbins, T.W. 1994. Effects of the neuroleptic alpha-flupenthixol on latent inhibition in aversively- and appetitively-motivated paradigms: evidence for dopamine-reinforcer interactions. Psychopharmacology (Berl). 115 (1-2), 196-205.

Killcross, A.S., Robbins, T.W., 1993. Differential effects of intra-accumbens and systemic amphetamine on latent inhibition using an on-baseline, within-subject conditioned suppression paradigm. Psychopharmacology (Berl) 110 (4), 479-489.

Killeen, P.R., Pellón, R. 2013. Adjunctive behaviors are operants. Learn. Behav. 41 (1), 1-24. doi: 10.3758/s13420-012-0095-1.

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. doi:10.1016/j.neuroscience.2013.12.063

Knapman, A., Heinzmann, J. M., Holsboer, F., Landgraf, R., Touma, C., 2010. Modeling psychotic and cognitive symptoms of affective disorders: Disrupted latent inhibition and reversal learning deficits in highly stress reactive mice. Neurobiology of Learning and Memory, 94, 145–152.

Knight, A.R., Misra, A., Quirk, K., Benwell, K., Revell, D., Kennett, G., Bickerdike, M. 2004. Pharmacological characterisation of the agonist radioligand binding site of 5-HT2A, 5-HT2B and 5-HT2C receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 370, 114–123 doi: 10.1007/s00210-004-0951-4.

Koot, S. ,Zoratto, F. ,Cassano, T., Colangeli, R., Laviola, G.,van den Bos, R., Adriani, W. 2012. Compromised decision-making and increased gambling proneness

following dietary serotonin depletion in rats. Neuropharmacology, 62, 1640-1650. http://doi.org/10.1016/j.neuropharm.2011.11.002

Koskinen, .T, Ruotsalainen, S., Puumala, T., Lappalainen, R., Koivisto, E., Männistö, P.T., Sirviö, J. 2000. Activation of 5-HT2A receptors impairs response control of rats in a five-choice serial reaction time task. Neuropharmacology. 39 (3), 471-81.

Koskinen, T., Ruotsalainen, S., Sirviö, J. 2000. The 5-HT(2) receptor activation enhances impulsive responding without increasing motor activity in rats. Pharmacol. Biochem. Behav. 66 (4), 729-38.

Kötting, W.F., Bubenzer, S., Helmbold, K., Eisert, A., Gaber, T.J., Zepf, F.D. 2013. Effects of tryptophan depletion on reactive aggression and aggressive decision-making in young people with ADHD. Acta Psychiatr. Scand. 128 (2), 114-23. doi: 10.1111/acps.12001.

Kuroki, T., Meltzer, H.Y., Ichikawa, J. 2003. 5-HT2A receptor stimulation by DOI, a 5-HT2A/2C receptor agonist, potentiates amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. Brain Res. 2003; 972:216-221

Lapiz-Bluhm, M.D., Soto-Pina, A.E., Hensler, J.G., Morilak, D.A. 2009. Chronic intermittent cold stress and serotonin depletion induce deficits of reversal learning in an attentional set-shifting test in rats. Psychopharmacology, 202, 329–341. http://doi.org/10.1007/s00213-008-1224-6

Lee, H.J., Telch, M.J., 2010. Differences in latent inhibition as a function of the autogenous-reactive OCD subtype. Behav. Res. Ther. 48 (7), 571-579.

Leeson, V.C., Robbins, T.W., Matheson, E., Hutton, S.B., Ron, M.A., Barnes, T.R., Joyce, E.M., 2009. Discrimination learning, reversal, and set-shifting in first-episode schizophrenia: stability over six years and specific associations with medication type and disorganization syndrome. Biol. Psychiatry. 66 (6), 586-593.

LeMarquand, D.G., Benkelfat, C., Pihl, R.O., Palmour, R.M., Young, S.N. 1999. Behavioral disinhibition induced by tryptophan depletion in nonalcoholic young

men with multigenerational family histories of paternal alcoholism. The American Journal of Psychiatry, 156, 1771-9.

LeMarquand, D.G., Pihl, R.O., Young, S.N., Tremblay, R.E., Séguin, J.R., Palmour, R.M., Benkelfat, C. 1998. Tryptophan depletion, executive functions, and disinhibition in aggressive, adolescent males. Neuropsychopharmacology. 19 (4), 333-41.

Leroux, E., Delcroix, N., Dollfus, S., 2015. Left-hemisphere lateralization for language and interhemispheric fiber tracking in patients with schizophrenia. Schizophr. Res. pii: S0920-9964(15)00165-6. doi: 10.1016/j.schres.2015.03.028.

Levin, S., Yurgelun-Todd, D., Craft, S., 1989. Contributions of clinical neuropsychology to the study of schizophrenia. J. Abnorm. Psychol. 98 (4), 341-356.

Licht, C.L., Knudsen, G.M., Sharp, T. 2010. Effects of the 5-HT(4) receptor agonist RS67333 and paroxetine on hippocampal extracellular 5-HT levels. Neurosci. Lett. 476 (2), 58-61. doi: 10.1016/j.neulet.2010.04.002. Epub 2010 Apr 8.

Lieben, C.K., Blokland, A.,Westerink, B. Deutz, N.E. 2004. Acute tryptophan and serotonin depletion using an optimized tryptophan-free protein-carbo- hydrate mixture in the adult rat. Neurochemistry International, 44, 9–16. http://doi.org/10.1016/S0197-0186 (03)00102-5

Lindenmayer, J.P., Khan, A., 2012. Psychopathology, in: Lieberman, J.A., Stroup, T.S., Perkins, D.O. Essentials of Schizophrenia. American Psychiatric Publishing, Inc., Washington D.C., pp. 11-56.

Liu, R.J., Lambe, E.K., Aghajanian, G.K. 2005. Somatodendritic autoreceptor regulation of serotonergic neurons: dependence on L-tryptophan hydroxylase-activating kinases. Eur. J. Neurosci. 21, 945–958.

Lopez-Gimenez, J.F., Mengod, G., Palacios, J.M. &Vilaro, M.T. 1997. Selective visualization of rat brain 5-HT2A receptors by autoradiography with [3H]MDL

100,907. Naunyn. Schmiedebergs. Arch. Pharmacol. 356. 446–454 http://doi.org/10.1007/PL00005075

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 (Berl). 197 (3), 487-98. doi: 10.1007/s00213-007-1059-6

López-Grancha, M., López-Crespo, G., Venero, C., Cañadas, F., Sánchez-Santed, F., Sandi, C., Flores, P. 2006. Differences in corticosterone level due to inter-food interval length: implications for schedule-induced polydipsia. Hormones and Behaviour, 49, 166-172.

Lu, L.H., Zhou, X.J., Keedy, S.K., Reilly, J.L., Sweeney, J.A., 2011. White matter microstructure in untreated first episode bipolar disorder with psychosis: comparison with schizophrenia. Bipolar. Disord. 13 (7-8), 604-613.

Lubow, R.E., 1989. Latent inhibition and conditioned attention theory. Cambridge, England: Cambridge University Press.

Lubow, R.E., 2005. Construct validity of the animal latent inhibition model of selective attention deficits in schizophrenia. Schizophr. Bull. 31 (1), 139-153.

Lubow, R. E., Weiner, I., eds. 2010. Latent Inhibition: Cognition, Neuroscience and Applications to Schizophrenia. Cambridge University Press.

Martin, J.R., Bös, M., Jenck, F., Moreau, J., Mutel, V., Sleight, A.J., Wichmann, J., Andrews, J.S., Berendsen, H.H., Broekkamp, C.L., Ruigt, G.S., Köhler, C., Delft, A.M. 1998. 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. J. Pharmacol. Exp. Ther. 286 (2), 913-24

Martin, J.R., Ballard, T.M., Higgins, G.A. 2002. Influence of the 5-HT2C receptor antagonist, SB-242084, in tests of anxiety. Pharmacol. Biochem. Behav. 71 (4), 615-25

Masaki, D., Yokoyama, C., Kinoshita, S., Tsuchida, H., Nakatomi, Y., Yoshimoto, K., Fukui, K. 2006. Relationship between limbic and cortical 5-HT neurotransmission and acquisition and reversal learning in a go/no-go task in rats. Psychopharmacology, 189, 249–258. http://doi.org/10.1007/s00213-006-0559-0

McDougle, C.J., Epperson, C.N., Pelton, G.H., Wasylink, S., Price, L.H. 2000. A double-blind, placebo-controlled study of risperidone addition in serotonin reuptake inhibitor-refractory obsessive-compulsive disorder. Arch. Gen. Psychiatry 57 (8), 794-801.

Menzies, L., Williams, G.B., Chamberlain, S.R., Ooi, C., Fineberg, N., Suckling, J., Sahakian, B.J., Robbins, T.W., Bullmore, E.T. 2008. White matter abnormalities in patients with obsessive-compulsive disorder and their first-degree relatives. Am. J. Psychiatry. 165(10), 1308-15. doi: 10.1176/appi.ajp.2008.07101677. Epub 2008 Jun 2.

Milella M.S., Passarelli, F., De Carolis, L., Schepisi, C., Nativio, P., Scaccianoce, S., Nencini, P. 2010. Opposite roles of dopamine and orexin in quinpirole-induced excessive drinking: a rat model of psychotic polydipsia. Psychopharmacology (Berl). 211 (3), 355-66. doi: 10.1007/s00213-010-1909-5.

Miquel, M.C., Doucet, E., Riad, M., Adrien, J., Verge, D., Hamon, M. 1992. Effect of the selective lesion of serotoninergic neurons on the regional distribution of 5-HT1A receptor mRNA in the rat brain. Mol. Brain. Res. 14: 357-362

Mittleman, G., Call, S.B., Cockroft, J.L., Goldowitz, D., Matthews, D.B., Blaha, C.D. 2011. Dopamine dynamics associated with, and resulting from, schedule-induced alcohol self-administration: analyses in dopamine transporter knockout mice. Alcohol 45(4), 325-39. doi: 10.1016/j.alcohol.2010.12.006.

Mittleman, G., Jones, G.H., Robbins, T.W. 1988a. The relationship between schedule-induced polydipsia and pituitary-adrenal activity: pharmacological and behavioral manipulations. Behav. Brain. Res. 28 (3), 315-24

Mittleman, G., Jones, G.H., Robbins, T.W. 1988b. Effects of diazepam, FG 7142, and RO 15-1788 on schedule-induced polydipsia and the temporal control of behavior. Psychopharmacology (Berl) 94 (1), 103-9

Mittleman, G., Rosner, A.L., Schaub, C.L. 1994. Polydipsia and dopamine: behavioral effects of dopamine D1 and D2 receptor agonists and antagonists. J. Pharmacol. Exp. Ther. 271(2), 638-50

Mittleman, G., Van Brunt, C.L., Matthews, D.B. 2003. Schedule-induced ethanol self-administration in DBA/2J and C57BL/6J mice. Alcohol. Clin. Exp. Res. 27 (6), 918-25

Mittleman, G., Valenstein, E.S. 1985. Individual differences in non-regulatory ingestive behavior and catecholamine systems.Brain Research. 348, 112–117.

Mobini, S., Chiang, T.J., Al-Ruwaitea, A.S., Ho, M.Y., Bradshaw, C.M., Szabadi, E. 2000. Effect of central 5-hydroxytryptamine depletion on inter-temporal choice: a quantitative analysis. Psychopharmacology, 149, 313–318. http://doi.org/10.1007/s002130000385

Moreno, M., Cardona, D., Gómez, M.J., Sánchez-Santed, F., Tobeña, A., Fernández-Teruel, A., Flores, P. 2010. Impulsivity Characterization in the Roman High- and Low-Avoidance Rat Strains: Behavioral and Neurochemical Differences. Neuropsychopharmacology, 35, 1198-208. http://dx.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 (Berl) 219 (2), 647-59. doi: 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 (Berl) 219 (2), 661-72. doi: 10.1007/s00213-011-2575

Morice, R. 1990. Cognitive inflexibility and pre-frontal dysfunction in schizophrenia and mania. Br J Psychiatry. 157:50-4.

Mulert, C., Kirsch, V., Whitford, T.J., Alvarado, J., Pelavin, P., McCarley, R.W., Kubicki, M., Salisbury, D.F., Shenton, M.E., 2012. Hearing voices: a role of interhemispheric auditory connectivity?. World. J. Biol. Psychiatry. 13 (2), 153-158.

Murray, G.K., Cheng, F., Clark, L., Barnett, J.H., Blackwell, A.D., Fletcher, P.C., Robbins, T.W., Bullmore, E.T., Jones, P.B. 2008. Reinforcement and reversal learning in first-episode psychosis. Schizophr. Bull. 34 (5), 848-55. doi: 10.1093/schbul/sbn078. Epub 2008 Jul 15.

Navarro, S.V., Gutiérrez-Ferre, V., Flores, P., Moreno, M. 2015. Activation of serotonin 5-HT2A receptors inhibits high compulsive drinking on schedule-induced polydipsia. Psychopharmacology (Berl), 232 (4), 683-97. http://doi.org/10.1007/s00213-014-3699-7.

Nicholson, S.L., Brotchie, J.M. 2002. 5-hydroxytryptamine (5-HT, serotonin) and Parkinson's disease - oportunities for novel therapeutics to reduce the problems of levodopa therapy. Eur. J. Neurol. 9 (supple. 3), 1-6.

Nielsen, M.Ø., Rostrup, E., Wulff, S., Bak, N., Lublin, H., Kapur, S., Glenthøj, B., 2012. Alterations of the brain reward system in antipsychotic naïve schizophrenia patients. Biol. Psychiatry. 71 (10), 898-905.

Nuechterlein KH, Pashler HE, Subotnik KL. 2006. Translating basic attentional paradigms to schizophrenia research: reconsidering the nature of the deficits. Dev Psychopathol. 18(3):831-51.

Oades RD, Daniels R, Rascher W (1998) Plasma neuropeptide-Y levels, monoamine metabolism, electrolyte excretion and drinking behaviour in children with attention-deficit hyperactivity disorder. Psychiatry Res 80(2):177-86

Ouhaz, Z., Ba-M'hamed, S., Bennis, M., 2014. Haloperidol treatment at pre-exposure phase reduces the disturbance of latent inhibition in rats with neonatal ventral hippocampus lesions. C. R. Biol. 337 (10), 561-570.

Palaniyappan, L., Liddle, P.F., 2012. Does the salience network play a cardinal role in psychosis? An emerging hypothesis of insular dysfunction. J. Psychiatry. Neurosci. 37 (1), 17-27.

Pantelis C, Barber FZ, Barnes TR, Nelson HE, Owen AM, Robbins TW. 1999. Comparison of set-shifting ability in patients with chronic schizophrenia and frontal lobe damage. Schizophr Res. 37 (3), 251-70.

Pearce, J. M. J., Hall, G., 1980. A model for pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. Psychological Review, 87(6), 532-532-552.

Pellón, R., Ruíz, A., Moreno, M., Claro, F., Ambrosio, E., Flores, P. 2011. Individual differences in schedule-induced polydipsia: neuroanatomical dopamine divergences. Behav. Brain. Res. 217(1):195-201. doi: 10.1016/j.bbr.2010.10.010

Pelloux, Y., Dilleen, R., Economidou, D., Theobald D., Everitt, B. J. 2012. Reduced forebrain serotonin transmission is causally involved in the development of compulsive cocaine seeking in rats. Neuropsychopharmacology, 37, 2505–2514. http://doi.org/10.1038/npp.2012.111

Peng, Z.W., Xu, T., Miao, G.D., He, Q.H., Zhao, Q., Dazzan, P., Chan, R.C., 2012. Neurological soft signs in obsessive-compulsive disorder: the effect of co-morbid psychosis and evidence for familiality. Prog.Neuropsychopharmacol. Biol. Psychiatry. 39 (1), 200-205.

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 42:306–314. http://doi.org/10.1016/j.neuroimage.2008.04.233

Peters, B.D., Machielsen, M.W., Hoen, W.P., Caan, M.W., Malhotra, A.K., Szeszko, P.R., Duran, M., Olabarriaga, S.D., de Haan, L., 2013. Polyunsaturated fatty acid concentration predicts myelin integrity in early-phase psychosis. Schizophr. Bull. 39 (4), 830-838.

Petrakis, I.L., Buonopane, A., O'Malley, S., Cermik, O., Trevisan, L., Boutros, N.N., Limoncelli, D., Krystal, J.H. 2002. The effect of tryptophan depletion on alcohol self-administration in non-treatment-seeking alcoholic individuals. Alcohol Clin. Exp. Res. 26 (7), 969-75.

Petrakis, I.L., Trevisan, L., Boutros, N.N., Limoncelli, D., Cooney, N.L., Krystal, J.H. 2001. Effect of tryptophan depletion on alcohol cue-induced craving in abstinent alcoholic patients. Alcohol Clin Exp Res. 25 (8), 1151-5.

Pierucci-Lagha, A., Feinn, R., Modesto-Lowe, V., Swift, R., Nellissery, M., Covault, J., Kranzler, H.R. 2004. Effects of rapid tryptophan depletion on mood and urge to drink in patients with co-morbid major depression and alcohol dependence. Psychopharmacology (Berl), 171, 340-8.

Pijnenburg, A.J.J., Honig, W.M.M., Van Rossum, J.M. 1975. Inhibition of D-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. Psychopharmacology. 41, 87–95.

Pijnenburg, A.J.J., Honig, W.M.M., Van Rossum, J.M. 1976. Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. European Journal of Pharmacology. 35, 45–58.

Pijnenburg, A.J.J., Van Rossum, J.M. 1973. Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. Journal of Pharmacy and Pharmacology, 25, 1003–1005.

Platt, B., Beyer, C.E., Schechter, L.E., Rosenzweig-Lipson, S. 2008. Schedule-induced polydipsia: a rat model of obsessive-compulsive disorder. Curr. Protoc. Neurosci. 43:9.27.1-9. doi: 10.1002/0471142301.ns0927s43

Pouzet, B., Zhang, W.N., Weiner, I., Feldon, J., Yee, B.K.., 2004. Latent inhibition is spared by N-methyl-D-aspartate (NMDA)-induced ventral hippocampal lesions, but is attenuated following local activation of the ventral hippocampus by intracerebral NMDA infusion. Neuroscience. 124 (1), 183-194.

Poyurovsky, M., Faragian, S., Shabeta, A., Kosov, A. 2008. Comparison of clinical characteristics, co-morbidity and pharmacotherapy in adolescent schizophrenia patients with and without obsessive-compulsive disorder. Psychiatry. Res. 159, 133–139.

Reading, P.J., Dunnett, S.B., Robbins, T.W., 1991. Dissociable roles of the ventral, medial and lateral striatum on the acquisition and performance of a complex visual stimulus-response habit. Behav. Brain. Res. 45 (2), 147-161.

Remijnse, P.L., Nielen, M.M., van Balkom, A.J., Hendriks, G.J., Hoogendijk, W.J., Uylings, H.B., Veltman, D.J. 2009. Differential frontal-striatal and paralimbic activity during reversal learning in major depressive disorder and obsessive-compulsive disorder. Psychol Med. 2009 Sep;39(9):1503-18. doi: 10.1017/S0033291708005072. Epub 2009 Jan 27.

Robbins, T. 2007. Shifting and stopping: fronto-striatal substrates, neurochemical modulation and clinical implications. Philosophical Transactions of the Royal Society B: Biological Sciences, 362(1481), 917–932. http://doi.org/10.1098/rstb.2007.2097

Robbins, T.W., Crockett, M.J. 2010. Role of central serotonin in impulsivity and compulsivity: comparative studies in experimental animals and humans. In: Müller C and Jacobs B (eds), Handbook of Behavioral Neuroscience, Elsevier, 2010, Vol 21, pp 415-42

Robinson, E.S., Eagle, D.M., Mar, A.C., Bari, A., Banerjee, G., Jiang, X., Dalley, J.W., Robbins, T.W. 2008. Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. Neuropsychopharmacology. 33(5):1028-37

Rosenheck, R., Cramer, J., Xu, W., Thomas, J., Henderson, W., Frisman, L., Fye, C., Charney, D. 1997. A comparison of clozapine and haloperidol in hospitalized patients with refractory schizophrenia. Department of Veterans Affairs Cooperative Study Group on Clozapine in Refractory Schizophrenia. N Engl J Med. 337(12):809-15

Rosenthal, R., Bigelow, L.B., 1972. Quantitative brain measurements in chronic schizophrenia. Br. J. Psychiatry. 121 (562), 259-264

Rosenzweig-Lipson S, Sabb A, Stack G, Mitchell P, Lucki I, Malberg JE, Grauer S, Brennan J, Cryan JF, Sukoff Rizzo SJ, Dunlop J, Barrett JE, Marquis KL. 2007. Antidepressant-like effects of the novel, selective, 5-HT2C receptor agonist WAY-163909 in rodents. Psychopharmacology (Berl) 192(2), 159-70

Rueter, L.E., Tecott, L.H., Blier, P. 2000. In vivo electrophysiological examination of 5-HT2 responses in 5-HT2C receptor mutant mice. Naunyn Schmiedebergs Arch. Pharmacol. 361 (5), 484-91

Ruscio, A.M., Stein, D.J., Chiu, W.T., Kessler, R.C. 2008. The epidemiology of obsessive-compulsive disorder in the National Comorbidity Survey Replication. Mol. Psychiatry. 5 (1):53-63. doi: 10.1038/mp.2008.94

Rygula, R., Clarke, H.F., Cardinal, R.N., Cockcroft, G.J., Xia, J., Dalley, J.W., Robbins, T.W., Roberts, A.C. 2014. Role of central serotonin in anticipation of rewarding and punishing outcomes: effects of selective amygdala or orbitofrontal 5-HT depletion. Cereb. Cortex (online) http://doi.org/10.1093/cercor/bhu102

Schechter, L.E., Lin, Q., Smith, D.L., Zhang, G., Shan, Q., Platt, B., Brandt, M.R., Dawson, L.A., Cole, D., Bernotas, R., Robichaud, A., Rosenzweig-Lipson, S., Beyer, C.E. 2008. Neuropharmacological profile of novel and selective 5-HT6 receptors

agonists: WAY-181187 and WAY-208466. Neuropharmacology 33:1323-1335. doi:10.1038/sj.npp.1301503

Schepisi, C., De Carolis, L., Nencini, P. 2013. Effects of the 5HT2C antagonist SB242084 on the pramipexole-induced potentiation of water contrafreeloading, a putative animal model of compulsive behavior. Psychopharmacology (Berl) 227 (1), 55-66. doi: 10.1007/s00213-012-2938-z

Schepisi, C., De Carolis, L., Nencini, P. 2014. Effects of the 5HT2C antagonist SB242084 on the pramipexole-induced potentiation of water contrafreeloading, a putative animal model of compulsive behavior. Psychopharmacology 227, 1, 55-66.

Schlagenhauf, F., Huys, Q.J., Deserno, L., Rapp, M.A., Beck, A., Heinze, H.J., Dolan, R., Heinz, A., 2014. Striatal dysfunction during reversal learning in unmedicated schizophrenia patients.Neuroimage. 89, 171-180.

Schmitt, A., Hasan, A., Gruber, O., Falkai, P., 2011. Schizophrenia as a disorder of disconnectivity. Eur. Arch. Psychiatry. Clin. Neurosci. 261 Suppl. 2, pp.150-154.

Seeman, P., Westman, K., Coscina, D., Warsh, J.J. 1980. Serotonin receptors in hippocampus and frontal cortex. Eur. J. Pharmacol. 66, 179–191.

Shepherd, A.M., Laurens, K.R., Matheson, S.L., Carr, V.J., Green, M.J. 2012. Systematic meta-review and quality assessment of the structural brain alterations inschizophrenia. Neurosci. Biobehav. Rev. 36 (4), 1342-1356.

Simpson, H.B., Slifstein, M., Bender, J. J., Xu, X., Hackett, E., Maher, M.J. Abi-Dargham, A. 2011. Serotonin 2A receptors in obsessive-compulsive disorder: a positron emission tomography study with [11C]MDL100907. Biol. Psychiatry. 70:897–904. http://doi.org/10.1016/j.biopsych.2011.06.023

Skodol, A.E., Oldham, J.M. (1996) Phenomenology, differential diagnosis, and comorbidity of impulsive-compulsive spectrum of disorders. In: Oldham JM,

Hollander E, Skodol AE (eds) Impulsivity and compulsivity. American Psychiatric Press, Inc., Washington DC, pp 1.36

Slotkin, T.A., Seidler, F.J. 2008. Developmental neurotoxicants target neurodifferentiation into the serotonin phenotype: Chlorpyrifos, diazinon, dieldrin and divalent nickel. Toxicology and Applied Pharmacology, 233, 211-9.

Stadler, C., Zepf, F.D., Demisch, L., Schmitt, M., Landgraf, M., Poustka, F. 2007. Influence of rapid tryptophan depletion on laboratory-provoked aggression in children with ADHD. Neuropsychobiology. 56 (2-3), 104-10. doi: 10.1159/000112951. Epub 2008 Jan 10.

Stancampiano, R., Cocco, S., Melis, F., Cugusi, C., Sarais, L., Fadda, F. 1997b. The decrease of serotonin release induced by a tryptophan-free amino acid diet does not affect spatial and passive avoidance learning. Brain Research, 762, 269-74.http://dx.doi.org/10.1016/S0006-8993 (97)00506-4

Stancampiano, R., Melis, F., Sarais, L., Cocco, S., Cugusi, C., Fadda, F. 1997a. Acute administration of a tryptophan-free amino acid mixture decreases 5-HT release in rat hippocampus in vivo. American Journal of Physiology, 272, 991-994.http://doi.org/10.1177/0269881108089597

Staton, D.M., Solomon, P.R. 1984. Microinjections of D-amphetamine into the nucleus accumbens and caudate–putamen differentially affect stereotypy and locomotion in the rat. Physiological Psychology 12, 159 – 162.

Stewart, S.E., Platko, J., Fagerness, J., Birns, J., Jenike, E., Smoller, J.W., Perlis, R., Leboyer, M., Delorme, R., Chabane, N., Rauch, S.L., Jenike, M.A., Pauls, D.L., 2007. A genetic family-based association study of OLIG2 in obsessive-compulsive disorder. Arch. Gen. Psychiatry. 64 (2), 209-214.

Stöhr, T., Szuran, T., Welzl, H., Pliska, V., Feldon, J., Pryce, C.R. 2000. Lewis/Fischer rat strain differences in endocrine and behavioral responses to environmental challenge. Pharmacology Biochemistry and Behavior, 67, 809- 819.

Swerdlow, N.R., Hartston, H.J., Hartman, P.L., 1999. Enhanced visual latent inhibition in obsessive-compulsive disorder. Biol. Psychiatry. 45 (4), 482-488.

Szeszko PR, Ardekani BA, Ashtari M, Malhotra AK, Robinson DG, Bilder RM, Lim KO. 2005. White matter abnormalities in obsessive-compulsive disorder: a diffusion tensor imaging study. Arch Gen Psychiatry. 2005 Jul;62(7):782-90.

Takahashi, N., Sakurai, T., Davis, K.L., Buxbaum, J.D. 2011. Linking oligodendrocyte and myelin dysfunction to neurocircuitry abnormalities in schizophrenia. Prog Neurobiol. 2011 Jan;93(1):13-24. doi: 10.1016/j.pneurobio.2010.09.004. Epub 2010 Oct 13.

Tanaka, Y., Rohde, L.A., Jin, L., Feldman, P.D., Upadhyaya, H.P. 2013. A meta-analysis of the consistency of atomoxetine treatment effects in pediatric patients with attention-deficit/hyperactivity disorder from 15 clinical trials across four geographic regions. J. Child Adolesc. Psychopharmacol. 23 (4), 262-70. doi: 10.1089/cap.2012.0049

Tanke, M.A., Alserda, E., Doornbos, B., van der Most, P.J., Goeman, K., Postema, F. Korf, J. 2008. Low tryptophan diet increases stress-sensitivity, but does not affect habituation in rats. Neurochemistry International, 52, 272-281. http://dx.doi.org/10.1016/j.neuint.2007.05.022

Tazi, A., Dantzer, R., Le Moal, M. 1988. Schedule-induced polydipsia experience decreases locomotor response to amphetamine. Brain Research, 445, 211–215.

Toscano, C.A., Kamevama, M., García-Mijares, M., Silva, M.T., Santarem, E.M. 2008. Relationship between ethanol and sucrose sel-administration and schedule-induced polydipsia. Pharmacol. Biochem. Behav. 90(4), 586-9. doi: 0.1016/j.pbb.2008.04.019

Treneer, C.M., Bernstein, I.L. 1981. Learned aversions in rats fed a tryptophan-free diet. Physiology & Behaviour, 27 (5-27), 757-760. http://dx.doi.org/10.1016/0031-9384(81)90038-X

Uchida, S., Kitamoto, A., Umeeda, H., Nakagawa, N., Masushige, S. Kida, S. 2005. Chronic reduction in dietary tryptophan leads to changes in the emotional response to stress in mice. Journal of Nutritional Science and Vitaminology, 51, 175–181. http://doi.org/10.3177/jnsv.51.175

Van der Plasse, G., Feenstra, M.G. 2008. Serial reversal learning and acute tryptophan depletion. Behavioural Brain Research, 186, 23–31. http://doi.org/10.1016/j.bbr.2007.07.017

van Westen M, Rietveld E, Figee M, Denys D. 2015. Clinical Outcome and Mechanisms of Deep Brain Stimulation for Obsessive-Compulsive Disorder. Curr Behav Neurosci Rep. 2015;2(2):41-48.

Verge, D., Daval, G., Marcinkiewiczm, M., Patey, A., Mestikawy, S., Gozlan, H., Hamon, M. 1986. Quantitative autoradiography of multiple 5-HT1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. J. Neurosci. 6, 3474–3482

Verghese C, de Leon J, Josiassen RC (1996) Problems and progress in the diagnosis and treatment of polydipsia and hyponatremia. Schizophr Bull. 22:455–64

Vergnes, M. Kempf, E. 1981. Tryptophan deprivation: effects on mouse-killing and reactivity in the rat. Pharmacology Biochemistry and Behavior, 14, Suppl. 1:19-23.

Vermeire, S., Audenaert, K., De Meester, R., Vandermeulen, E., Waelbers, T., De Spiegeleer, B., Eersels, J., Dobbeleir, A., Peremans, K. 2012. Serotonin 2A receptor, serotonin transporter and dopamine transporter alterations in dogs with compulsive behaviour as a promising model for human obsessive-compulsive disorder. Psychiatry Res. 1, 78–87. doi:10.1016/j.pscychresns.2011.06.006

Wagner, G., Koch, K., Schachtzabel, C., Schultz, C.C., Gaser, C., Reichenbach, J.R., Sauer, H., Bär, K.J., Schlösser, R.G., 2013. Structural basis of the fronto-thalamic dysconnectivity in schizophrenia: A combined DCM-VBM study. Neuroimage. Clin. 3, 95-105.

Wallace, A., Pehrson, A.L, Sánchez, C., Morilaz, D.A. 2014. Vortioxetine restores reversal learning impaired by 5-HT depletion or chronic intermittent cold stress in rats. Int. J. Neuropsychopharmacol, 23, 1-12. http://doi.org/10.1017/S1461145714000571

Walterfang, M., Velakoulis, D., Whitford, T.J., Pantelis, C., 2011. Understanding aberrant white matter development in schizophrenia: an avenue for therapy? Expert. Rev. Neurother. 11 (7), 971-987.

Walterfang, M., Wood, S.J., Velakoulis, D., Pantelis, C., 2006. Neuropathological, neurogenetic and neuroimaging evidence for white matter pathology in schizophrenia. Neurosci. Biobehav. Rev. 30 (7), 918-948.

Waltz, J.A., Kasanova, Z., Ross, T.J., Salmeron, B.J., McMahon, R.P., Gold, J.M., Stein, E.A. 2013. The roles of reward, default, and executive control networks in set-shifting impairments in schizophrenia. PLoS One. 2013; 8(2):e57257. doi: 10.1371/journal.pone.0057257. Epub 2013 Feb 27.

Wayner, M.J. 2002. Craving for alcohol in the rat: adjunctive behavior and the lateral hypothalamus. Pharmacol. Biochem. Behav. 73(1), 27-43

Wedekind D, Herchenhein T, Kirchhainer J, Bandelow B, Falkai P, Engel K, Malchow B, Havemann-Reinecke U. 2010. Serotonergic function, substance craving, and psychopathology in detoxified alcohol-addicted males undergoing tryptophan depletion. J. Psychiatr. Res 44 (16), 1163-9.

Weiner, I., 2003. The "two-headed" latent inhibition model of schizophrenia: modeling positive and negative symptoms and their treatment. Psychopharmacology (Berl). 169 (3-4), 257-297.

Weiner, I., Arad, M., 2009. Using the pharmacology of latent inhibition to model domains of pathology in schizophrenia and their treatment. Behav. Brain Res. 204, 369–386.

Weltzin, T.E., Fernstrom, M.H., Fernstrom, J.D., Neuberger, S.K., Kaye, W.H. 1995. Acute tryptophan depletion and increased food intake and irritability in bulimia nervosa. America Journal of Psychiatry, 152, 1668-71.

Westenberg, H.G., Fineberg, N.A., Denys, D. 2007. Neurobiology of obsessive-compulsive disorder: serotonin and beyond. CNS Spectr, 12,14–27.

Whitford, T.J., Kubicki, M., Schneiderman, J.S., O'Donnell, L.J., King, R., Alvarado, J.L., Khan, U., Markant, D., Nestor, P.G., Niznikiewicz, M., McCarley, R.W., Westin, C.F., Shenton, M.E., 2010. Corpus callosum abnormalities and their association with psychotic symptoms in patients withschizophrenia. Biol. Psychiatry. 68 (1), 70-77.

Williams, M.R., Harb, H., Pearce, R.K., Hirsch, S.R., Maier, M., 2013. Oligodendrocyte density is changed in the basolateral amygdala in schizophreniabut not depression. Schizophr. Res. 147 (2-3), 402-403.

Winstanley, C.A., Theobald, D.E., Dalley, J.W., Glennon, J.C., Robbins, T.W. 2004. 5-HT2A and 2C receptor antagonists have opposing effect on measure of impulsivity: interaction with global 5-HT depletion. Psychopharmacology (Berl), 176, 376-385. http://doi.org/10.1007/s00213-004-1884-9

Wischhof, L., Irrsack, E., Osorio, C., Koch, M., 2015. Prenatal LPS-exposure--a neurodevelopmental rat model of schizophrenia--differentially affects cognitive functions, myelination and parvalbumin expression in male and female offspring. Prog Neuropsychopharmacol. Biol. Psychiatry. 57, 17-30.

Wogar, M.A., Bradshaw, C.M., Szabadi, E. 1993. Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on choice between delayed reinforcers. Psychopharmacology, 111, 239–243. http://doi.org/10.1007/bf02245530

Woods- Kettelberger, A., Kongsamut, S., Smith, C.P., Winslow, J.T, Corbett, R. 1997. Selective serotonin re-uptake inhibitors decrease schedule-induced polydipsia in rats: a potential model for obsessive compulsive disorder. Psychopharmacology (Berl) 112, 195-198. doi: 10.1007/BF02244910

Woods, A., Smith, C., Szewczak, M., Dunn, R.W., Cornfeldt, M., Corbett, R. 1993. Selective serotonin re-uptake inhibitors decrease schedule-induced polydipsia in rats: a potential model for obsessive compulsive disorder. Psychopharmacology (Berl) 112 (2-3), 195-8

Worbe, Y., Marrakchi-Kacem, L., Lecomte, S., Valabregue, R., Poupon, F., Guevara, P., Tucholka, A., Mangin, J.F., Vidailhet, M., Lehericy, S., Hartmann, A., Poupon, C., 2015. Altered structural connectivity of cortico-striato-pallido-thalamic networks in Gilles de la Tourette syndrome. Brain. 138 (Pt 2), 472-482

Xiu, Y., Kong, X.R., Zhang, L., Qiu, X., Chao, F.L., Peng, C., Gao, Y., Huang, C.X., Wang, S.R., Tang, Y., 2014. White matter injuries induced by MK-801 in a mouse model of schizophrenia based on NMDA antagonism. Anat. Rec. (Hoboken). 297 (8), 1498-1507.

Xiu, Y., Kong, X.R., Zhang, L., Qiu, X., Gao, Y., Huang, C.X., Chao, F.L., Wang, S.R., Tang, Y., 2015. The myelinated fiber loss in the corpus callosum of mouse model of schizophrenia induced by MK-801. J. Psychiatr. Res. pii: S0022-3956(15)00045-X.

Yin, H.H., Ostlund, S.B., Knowlton, B.J., Balleine, B.W., 2005. The role of the dorsomedial striatum in instrumental conditioning. Eur. J. Neurosci. 22 (2), 513-523.

Young, A. M., Moran, P.M. y Joseph, M. H., 2005. The role of dopamine in conditioning and latent inhibition: what, when, where and how? Neuroscience & Biobehavioral Reviews, 29, 6, 963–76.

Zai, G., Bezchlibnyk, Y.B., Richter, M.A., Arnold, P., Burroughs, E., Barr, C.L., Kennedy, J.L., 2004. Myelin oligodendrocyte glycoprotein (MOG) gene is associated

with obsessive-compulsive disorder. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 129b (1), 64-68.

Zepf, F.D., Gaber, T.J., Baurmann, D., Bubenzer, S., Konrad, K., Herpertz-Dahlmann, B., Stadler, C., Poustka, F., Wöckel L. 2010. Serotonergic neurotransmission and lapses of attention in children and adolescents with attention deficit hyperactivity disorder: availability of tryptophan influences attentional performance. Int. J. Neuropsychopharmacol. 13 (7), 933-41. doi: 10.1017/S146114571000012X.

Zepf, F.D., Holtmann, M., Stadler, C., Magnus, S., Wöckel, L., Poustka, F. 2009. Diminished central nervous 5-HT neurotransmission and mood self-ratings in children and adolescents with ADHD: no clear effect of rapid tryptophan depletion. Hum Psychopharmacol. 24 (2), 87-94. doi: 10.1002/hup.1002.

Zepf, F.D., Stadler, C., Demisch, L., Schmitt, M., Landgraf, M., Poustka, F. 2008. Serotonergic functioning and trait-impulsivity in attention-deficit/hyperactivity-disordered boys (ADHD): influence of rapid tryptophan depletion. Hum Psychopharmacol. 23 (1), 43-51.

Zimmermann, M., Grabemann, M., Mette, C., Abdel-Hamid, M., Uekermann, J., Kraemer, M., Wiltfang, J., Kis, B., Zepf FD. 2012. The effects of acute tryptophan depletion on reactive aggression in adults with attention-deficit/hyperactivity disorder (ADHD) and healthy controls. PLoS One. 7(3):e32023. doi: 10.1371/journal.pone.0032023. Epub 2012 Mar 14.

Zohar, J., Chopra, M., Sasson, Y., Amiaz, R., Amita,l D. 2000. Obsessive compulsive disorder: serotonin and beyond. World. J. Bio.l Psychiatry 1 (2), 92-100