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Dysfunction in striatal dopaminergic and oxytocinergic system on impulsive choice behavior

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RESUMEN (Español e Inglés):

Una medida clave de la elección impulsiva implica la preferencia por un refuerzo más pequeño pero entregado de inmediato frente a recompensa más grande asociada con un retraso en su entrega. Este tipo de conducta está presente en varios trastornos neuropsiquiátricos, que incluyen: trastornos de la personalidad, trastorno por déficit atencional e hiperactividad y adicción. En los últimos años se ha propuesto que todas estas patologías comparten una alteración en la transmisión dopaminérgica mesencefálica, pero los mecanismos fisiológicos que acompañan la expresión de la elección impulsiva no están completamente aclarados. Además, hallazgos recientes sugieren que oxitocina (OXT), puede modular varios comportamientos relacionados con el sistema dopaminérgico y que existe una modulación bidireccional entre estos sistemas. El presente estudio tuvo como objetivo investigar: (1) La dinámica dopaminérgica basal en el estriado dorsolateral (SDL) presente en las diferencias individuales de las conductas de elección impulsiva. (2) El rol del sistema oxitocinérgico sobre la transmisión dopaminérgica nigro-estriatal y su modulación en las conductas de elección impulsiva.

Los resultados mostraron que: (1) La liberación de dopamina (DA) en el SDL es mayor en animales altamente impulsivos que en animales menos impulsivos, a pesar de que los niveles extracelulares de DA y la actividad del transportador de DA (DAT) no varían entre los grupos. (2) La activación bilateral de OXT-R en substancia nigra pars compacta (SNpc), induce una disminución en la conducta impulsiva, principalmente en animales clasificados previamente como altamente impulsivos. Este hallazgo muestra que la liberación de DA en el SDL está aumentada en las ratas clasificadas como altamente impulsivas, lo que sugiere que la vía nigro-estriatal hiperactiva contribuye a la elección impulsiva. Por otro lado, la activación del OXT-R en la SNpc influye en la toma de decisiones relacionadas con el retraso, mediante cambios sutiles en la transmisión dopaminérgica estriatal. Estos resultados proponen una nueva línea de investigación sobre los mecanismos neurobiológicos que subyacen a la posible interacción entre los sistemas de DA y OXT, y su rol en conductas de elección impulsiva presentes en patologías asociadas a una alteración de estos dos sistemas en la vía nigro-estriatal.

A key measure of impulsive choice involves a preference for smaller but immediately delivered reinforcements versus larger rewards associated with delayed delivery. This behavior is present in several neuropsychiatric disorders, including personality disorders, attention deficit disorder, hyperactivity, and addiction. In recent years it has been proposed that all of these pathologies share an alteration in mesencephalic dopaminergic transmission, but the physiological mechanisms that accompany the expression of impulsive choice are not fully clarified. Moreover, recent findings suggest that oxytocin (OXT) can modulate several behaviors related to the dopaminergic system and a bidirectional modulation between them. The present study aimed to investigate: (1) The basal dopaminergic dynamics in the dorsolateral striatum (DLS) present in individual differences of impulsive choice behaviors. (2) The role of the oxytocinergic system on nigro-striatal dopaminergic transmission and its modulation in impulsive choice behaviors.

The results showed that: (1) Dopamine (DA) release in DLS is higher in highly impulsive animals (HI) than in low impulsive animals (LI). However, DA extracellular (DA_{ext}) $_{ext}$ levels and transporter activity (DAT) do not vary between groups. (2) The bilateral activation of OXT-R in substantia nigra pars compacta (SNpc) induces a decrease in impulsive behavior, in animals previously classified as HI. Moreover, the OXT-R activation in SNpc induces a modest increase in DA_{ext} in DLS. This finding shows that DA release is augmented in the DLS of rats classified as HI, suggesting that hyper-activity nigro-striatal pathway contributes to impulsive choice. On the other hand, the activation of the OXT-R in the SNpc influences delay-related decision making by subtle changes in the striatal dopamine transmission. These results propose a new line of research on the neurobiological mechanisms underlying the possible interaction between the OXT and DA systems and their role in impulsive choice behaviors present in pathologies associated with altering these two systems in the nigrostriatal pathway.

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La presente tesis está dedicada a todas las personas que de una u otra forma estuvieron y son parte de mi crecimiento como persona y como científica.

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List of abbreviations

aCSF: Artificial cerebrospinal fluid

ADHD: Attention deficit hyperactive disorder

CNS: Central Nervous System

D2-R: Dopamine receptor, type 2

D1-R: Dopamine receptor, type 1

DA: Dopamine

DAG: Dyacylglicerol

DAT: Dopamine transporter

DLS: Dorso lateral Striatum

HPLC: High-performance liquid chromatography

IP3: Inositol Triphosphate

LY368,899: Potent, non-peptide oxytocin receptor antagonist

NAcb: Nucleus Accumbens

NAcbC: Nucleus Accumbens Core

nM: Nanomolar

OFC: Orbitofrontal Cortex

OXT: Oxytocin

OXT-R: Oxytocin receptor

PCA: Ácido perclórico

PFA: Paraformaldehyde

PFC: Prefrontal Cortex

PKC: Protein kinase C

PLC: Phospholipase C

PND: Postnatal day

PVN: Paraventricular Nucleus

Sec: Seconds

SN: Substantia nigra

SNpc: Substantia nigra pars compacta

SON: Supraoptic Nucleus

VGCCs: Voltage-gated calcium channel

VMAT: Vesicular monoamine transporter

VTA: Ventral tegmental area

WAY267464: Potent, non-peptide oxytocin receptor agonist

µl: Microlitre

µM: Micromolar

1 Introduction

1.1 Impulsivity

Impulsivity is a multifactorial construct that can be adaptive or maladaptive depending on the consequences resulting from it (Eysenck and Eysenck, 1977; Amorim Neto and True, 2011; Strickland and Johnson, 2020). Maladaptive impulsivity, in particular, has been classically described as the predisposition to inappropriate, badly planned decisions with negative consequences. This behavior is subdivided into two broad categories: impulsive choice and impulsive action (Winstanley et al., 2010). Impulsive action refers to a failure in inhibiting an inappropriate response to a stimulus (Schachar et al., 2007; Eagle et al., 2008). On the other hand, impulsive choice is characterized by impulsive decision-making caused by poor consideration of future behavioral consequences (e.g., preferences in choosing a small reward delivered immediately over larger rewards delivered with a delay) (Evenden and Ryan, 1996; Cardinal et al., 2001; Dalley et al., 2011; Hamilton et al., 2015). It is proposed that both types of impulsive behaviors are characterized by high individual variability, which is determined by environmental, social and physiological factors (Arce & Santisteban, 2006; Hamilton et al., 2015; C. Liu et al., 2016). The focus of our research is on impulsive choice, as it has been observed in several neuropsychiatric disorders, including personality disorders (Perry & Körner, 2011), mood disorders (Lombardo et al., 2012), attention deficit hyperactivity disorder (ADHD) (Avila et al., 2004) and addiction (Ersche et al., 2012), pointing to its possible role in the pathogenesis of these neuropathologies (Renda et al., 2014; Rung et al.,2018).

1.2 Neuroanatomical circuits

Research in humans and animals have associated impulsive choice with functional integration of the orbitofrontal cortex (OFC) and the nucleus accumbens core (NAcbC) (Dinn et al., 2001; Winstanley et al.,2004), proposing that changes in the subcortical-cortical connections are related to this behavior. Additionally, it can be interpreted that changes in patterns of functionality linked to a decrease in dopaminergic system activity contribute to the induction of high levels of impulsive choice (Brooks & Berns, 2013; Kravitz et al., 2015; Sellitto et al., 2011). Moreover, studies using functional magnetic resonance imaging (fMRI) showed that dysregulation in functional connectivity between cortical and putamen regions was associated with high impulsivity levels in subjects (Carmona et al., 2009; Wilbertz et al., 2012). This change in functionality has been corroborated in studies using the Delay Discounting Task (DDT), where the subject or animal must choose between a small reinforcer delivered immediately or a larger reinforcer delivered after a delay. These studies showed that the selection of large reinforcement is associated with an increase in putamen activity (Wittmann et al., 2007).

Interestingly, animals studies have suggested that prefrontal cortex (PFC) and NAcb contribute differentially to decision making, giving further insight into the dynamic interactions between the prefrontal and striatal systems (St. Onge et al., 2012). The subcortical circuit provides a visceral and intuitive control toward options that have larger rewards, in comparison with the PFC, that appears to have a supervisory role, monitoring the frequency of rewarded actions over time. This could be associated with fluctuations in tonic dopamine (DA) level, facilitating the implementation of decision policies (St. Onge

et al., 2012; St Onge et al., 2012b). Following this, newer studies have started to reveal that the dorsolateral striatum (DLS) or the putamen in humans, conventionally known for its participation in motor functions (Kobayashi et al., 2013), has also been associated with cognitive processes (Balleine et al., 2007; Hariri et al., 2006) hypothesizing that there exists a connection between drug-seeking habits and impulsive behaviors, and that they have a common biological substrate which is changes in the striatal DA levels (Besson et al., 2013; Jentsch & Taylor, 1999). Using DDT, lesions, and pharmacology techniques in animal models, it has been proposed that high level of impulsivity is associated with a decrease in DA level in the DLS. For example, Tedford., et al (2015) showed that the bilateral retrograde lesion with 6-hydroxydopamine (6-OHDA) in dopaminergic terminals in the DLS induces an increase in impulsivity choice during DDT, which is in concordance with studies with humans that showed that DA in DLS participates and is necessary for decision-making (Hong et al., 2013; McHugh et al., 2013). This dopaminergic hypofunction could be associated with an increase in dopamine transporter (DAT) activity, which is mainly mediated by presynaptic mechanisms related with D_2-R presynaptic activation, playing a key role in the regulation of DA neuronal activity and the control of synthesis, release, and uptake of DA (Baarendse & Vanderschuren, 2012). In contrast, the report of Magnard et al., 2018 showed that anterograde bilateral lesion with 6-OHDA in substantia nigra pars compacta (SNpc), does not induce changes in the impulsive choice behavior during DDT performance. This is interesting because both studies focus on the role of DLS and dopaminergic system in impulsive choice. However, to date, the changes in the endogenous DA dynamic into the nigrostriatal pathway contributing to impulsive choice behavior are unknown.

1.3 Monoamine neurotransmitter systems

Prior research has suggested that monoamine dysfunctional signaling, particularly in the dopaminergic and noradrenergic systems, contribute to the generation of impulsive behaviors (Humpston et al., 2013; Yates et al., 2014). Studies using radioligands with positron emission tomography (PET) in humans have identified a decrease in the availability of D_2 and/or D_3 receptors in striatal regions in impulsive subjects (Ghahremani et al., 2012; B. Lee et al., 2009). Moreover, studies in animal models using *in situ* hybridization have shown significant differences in mRNA expression for DA receptors in striatal regions (Simon et al., 2013). Finally, it has been proposed that high levels of impulsivity during DDT (linked to salience and experience to a known reinforcement) are associated with decreased levels of DA, probably mediated by presynaptic mechanisms. It should be noted that these mechanisms have not been well determined (see, for instance: Costa et al., 2014; Eubig et al., 2014; Smith et al., 2016; Tedford et al., 2015).

1.4 Dopamine system

The DA system has been classically linked to reward behavior, pleasure, euphoria, motor control, and impulsivity. DA is synthesized in two nuclei, the ventral tegmental area (VTA) and SNpc (C. R. Lee & Tepper, 2009; Roeper et al.,2013). The system consists of dopaminergic neurons, receptors expressed both at a pre- and a postsynaptic level and a membrane transporter, which contribute to the control and the balance of dopaminergic activity in several regions of the central nervous system (CNS) (Missale et al., 1998). DA

receptors share a high degree of homology. These are G protein-coupled metabotropic receptors, with seven transmembrane domains, but have different pharmacological properties: receptors belonging to the D_1 family are formed by D_1 and D_5 receptors and activate a family of proteins Gs linked to the activation of intracellular cascades mediated by adenylate cyclase. They contribute to the intracellular production of the second messenger cyclic adenosine monophosphate (cAMP) (Hernández-López et al., 1997; Missale et al., 1998; Surmeier et al., 2007). Additionally, these receptors are expressed exclusively in postsynaptic neurons; for example in medium spiny neurons (MSN) in the DLS (Centonze et al., 2003). On the other hand, D_2 family receptors including D_2, D_3, and D_4, are coupled to G_i protein, which is characterized by inhibiting adenylate cyclase (Fazio et al., 2011; Sokoloff et al., 2006). The role of D_2 and D_3 receptors are more complex than D_1 receptors, as they are expressed both at a pre- and a postsynaptic level, and are associated to the control of DA release mediated by activation of the presynaptic receptors (Barton et al., 1991; Missale et al., 1998). Another protein that helps regulating the levels of this neurotransmitter is the dopamine transporter (DAT), which is a membrane protein that is expressed pre-synaptically. Its function is to control the intensity of DA-mediated signaling by recapturing the neurotransmitter released by presynaptic neurons (Zhang et al., 2009). DAT belongs to a family of transporters which depends on the activity of the Na^+/Cl^- pump (Kanner, 1994; Zhang et al., 2009). There is evidence to postulate a functional interaction between the activation of presynaptic D_2 receptors and increased expression of DAT in the cell membranes, contributing to improved clearance of DA in the synaptic space (Bolan et al., 2007).

1.5 Oxytocinergic system

OXT is a neuropeptide which is synthesized in a group of neurons located in the paraventricular (PVN) and supraoptic (SON) nucleus of the hypothalamus. Axons of these neurons project and move the OXT to the anterior pituitary, where it is finally released into the bloodstream as a hormone which functions in both central and peripheral systems (Loup et al., 1991; Zink & Meyer-Lindenberg, 2012). OXT projections have been described to reach extra-hypothalamic regions, like the prefrontal cortex, VTA, NAcb, hippocampus, amygdala, and SN, the latter being one of our regions of interest (Dogterom et al., 1978; Mai, Berger, & Sofroniew, 1993). This neuropeptide is classically known for its actions in peripheral targets, for example, its role in uterine contraction and milk ejection during childbirth. In turn, at the central level, OXT has a role both as a neurotransmitter and/or as a neuromodulator, acting mainly through dendritic release (Leng & Ludwig, 2008; Neumann et al., 2012) regulating cognitive processes and complex social behaviors (Insel, 2010). Moreover, there is a growing number of studies showing that signaling pathways mediated by OXT are altered in neuropsychiatric disorders associated with several social deficits (Aspé-Sánchez et al., 2015; Meyer-Lindenberg et al., 2011).

There are two types of oxytocin receptors (OXT-R) located both in the central and the peripheral systems. They are a family of metabotropic receptors, which have seven transmembrane domains that acts through protein-coupled type G_q or G_i (Ebstein et al.,2012; Wilkie et al.,1991). The OXT-R is expressed in different areas of the CNS. For example, the OXT-R coupled to protein G_q is expressed in the SN, VTA, NAcb, anterior

cingulate cortex, central and basolateral amygdala, medial preoptic area, anterior and ventromedial hypothalamus, among others (Boccia et al., 2013; Rault et al., 2013; Skuse, Gallagher, & Frith, 2009) while the OXT-R type G_i is abundantly expressed in the spinal cord, the optic chiasm, frontal and occipital cortices, caudate and putamen (Milligan, 1993). When OXT binds to the OXT-R type G_q, it initiates an intracellular pathway mediated by phospholipase C (PLC), generating inositol triphosphate (IP3) and 1,2 diacilglicerol (DAG). IP3 mobilizes calcium from intracellular stores and DAG activates protein kinase C (PKC) which contributes to phosphorylation protein. The activation of these receptors results in a depolarization induced by decrease inward rectifying K^+ (IRK) currents (Berrada et al., 2000; Gravati et al., 2010; Thibonnier & Schork, 1995). On the other hand, the OXT-R G_i inhibits adenylate cyclase activity, in turn decreasing the concentration of cAMP, which activates phosphatidylinositol-4,5-biphosphate 3-kinase (PI3K) and increases IRK currents (Garibay et al., 1991; Gravati et al., 2010).

1.6 Role of dopamine and oxytocin on neuropsychiatric disorders

New research is proposing the use of neuropeptides such as oxytocin (OXT), as a neuromodulator of DA transmission. Studies also support its potential role as a therapeutic target for neuropsychiatric diseases associated with diminished social behavior (Baskerville & Douglas, 2010; Love, 2014; McGregor & Bowen, 2012; Tops et al., 2014), and as a treatment for neuropsychiatric disorders, such as depression, autism, addiction and others (McGregor & Bowen, 2012; Meyer-Lindenberg et al., 2011; Peters et al.,2013).

Most studies on OXT and its neuromodulatory role have involved models of addiction, in which it has been shown that increased oxytocinergic tone (the peripheral or central application), decreases drug seeking and attenuates anxiety levels (Bowen et al., 2011; McGregor & Bowen, 2012). Most studies on OXT and its neuromodulatory role have involved models of addiction. It has been shown that increased oxytocinergic tone (the peripheral or central application) decreases drug seeking and attenuates anxiety levels (Bowen et al., 2011; McGregor & Bowen, 2012). On the other hand, resents studies showed that the intranasal OXT administration moderated impulsive behavior in subjects with a single nucleotide polymorphism (SNP) rs2254298 genotype (Bozorgmehr et al., 2020). In line with these, the intracerebroventricular administration of OXT reduces risky decisions in animal models (Tapp et al., 2020). Nevertheless, the neurobiological mechanism that underlies the role of the OXT in impulsive behavior not fully understood.

1.7 Interaction between oxytocinergic and dopaminergic systems

It is known that there is a bidirectional modulation between dopaminergic and oxytocinergic systems (Moos & Richard, 1982; Shahrok et al., 2010). It has also been shown that DA is capable of modulating the oxytocinergic pathway (Moos & Richard, 1982). For example, at a neurophysiological level, it has been found that the effect of intraventricular injections of DA induced an increase in the electrical activity of oxytocinergic neurons in PVN (Moos and Richard 1982). It has also been shown that these hypothalamic neurons express both DA and OXT receptors and form heterodimers in

hypothalamic and striatal regions (Baskerville & Douglas, 2008; Romero-Fernandez, et al., 2013). Finally, it has been proposed that both dopaminergic and oxytocinergic pathways converge in the PFC, striatum and tegmental areas (Succu et al., 2007; Chang et al., 2014). Additionally, a dysfunction between dopaminergic and oxytocinergic transmission is present in a variety of neuropsychiatric disorders, such as schizophrenia, autism, ADHD and addiction (Baskerville & Douglas, 2010; Park et al., 2010; Rosenfeld, Lieberman, & Jarskog, 2011) which are disorders associated with impulsive behavior.

In recent years, there has been an increasing interest in the use of OXT as a new treatment for many neuropsychiatric disorders (McGregor & Bowen, 2012; Meyer-Lindenberg et al., 2011; Peters et al., 2013), but its role in impulsive choice (a feature present in several of these disorders) is not fully understood. There is evidence that the oxytocinergic system is able to activate mesocorticolimbic and nigrostriatal pathways (Y. Liu & Wang, 2003; Young & Wang, 2004, Xiao et al, 2017). Similarly, it has been showed that the central and peripheral OXT administration attenuates withdrawal effects induced by morphine, ethanol, and cocaine, preventing the generation of tolerance and stereotypical behaviors caused by repeated use of these drugs in animals models of addiction (Bentzley, Jhou, & Aston-Jones, 2014; Kovács et al., 1998; Peters et al., 2013). Clinical studies using fMRI techniques have shown that the administration of OXT induces an increase in the activity of VTA (Schott et al., 2008; Chang et al., 2014). Additionally, it has been shown in preclinical studies, that OXT perfusion in the VTA induces an increase in DA levels in the NAcb (Shahrokh et al., 2010). The mechanisms underlying modulation offered by OXT, and its role in the regulation of rewarding properties of drugs of abuse are not clear yet. Clinical experiments using single-photon emission computed tomography (SPECT)

and genotyping methods have indicated that there exists a correlation between OXT-R gene polymorphism (rs53576) and striatal DAT availability (Chang et al., 2014). Using SPECT, Love et al. 2012 showed that humans with anxiety phenotypes have a variation in the OXT-R gene associated with the presence of various polymorphisms, for example, the rs53576, that could be influencing the striatal DA activity, increasing the evidence of the interactions between oxytocinergic and dopaminergic systems.

Concluding, this research is presented in two chapters:

The first chapter aims to evaluate the striatal dopaminergic dynamic in impulsive choice in animals using the DDT paradigm. We particularly want to test the contribution of the DA basal dynamic in DLS, and how it is correlated with low or high impulsive behavior. For this, we used the quantitative neuro-chemical technique: no-net-flux microdialysis *in vivo* (see review Justice, 1993).

The second chapter aims to evaluate the role of the oxytocinergic system activation on the nigrostriatal dopaminergic signaling and its contribution to individual differences in impulsive choice behavior. For this, *in vivo* microdialysis was performed to study the effect of the perfusion of WAY267464 in SNpc on DA extracellular levels in DLS. In complementary studies, adult male rats were well-trained in the DDT. On separate test days, they received microinfusions of the OXT-R agonist (WAY267464) in the SNpc.

2 Hypothesis

General Hypothesis:

High impulsive choice level is associated with a decrease in the dopaminergic and oxytocinergic activity in the nigrostriatal pathway.

Specific Hypothesis:

2.1 Chapter 1:

Animals with high levels of impulsive choice have decreased levels of DA associated with increased DAT activity in DLS.

2.2 Chapter 2:

The activation of OXT-R in SNpc induces a decrease in impulsive choice behavior during DDT in high impulsive animals.

3 Objectives

General Objective:

Evaluate the contribution of dopaminergic and oxytocinergic systems in nigrostriatal pathway in the individual differences present in impulsive choice in rats.

Specific Objective:

3.1 Chapter 1:

1. To evaluate and classify the individual differences present in impulsive choice during delay discounting task.
2. To correlate the different impulsive choice levels in rats during DDT and the DA basal dynamic parameters (DA_{ext}, Ed, DA release) using No-Net-Flux microdialysis.

3.2 Chapter 2:

1. To evaluate the effect of bilateral administration of non-peptide specific OXT-R agonist (WAY267464) in SNpc on impulsive choice during DDT.

2. To determine the effect of administration of non-peptide specific OXT-R agonist (WAY267464) in SNpc on DA extracellular levels in DLS and SNpc.

4 Chapter 1: High impulsive choice is accompanied by an increase in dopamine release in rat dorsolateral striatum

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Highlights

- Rats were classified as high impulsive and low impulsive according to the AUC.
- High impulsive rats showed an increased DA release in dorsolateral striatum.
- A negative correlation was observed between impulsive levels and DA release.

4.1 Abstract

Dopamine neurotransmission has been consistently associated with individual differences in impulsive choice. Clinical and preclinical evidence suggests that low striatal dopamine D_2 signaling predisposes to engage in impulsive behaviors. Although dopamine D_2 signaling controls dopamine (DA) extracellular levels, the relationship between striatal dopamine extracellular levels and impulsive choice remains poorly understood. Using quantitative microdialysis, we investigated whether extracellular DA levels in rat dorsolateral striatum (DLS) correlates with preference for an immediate small reward or for a delayed larger reward. Rats were tested in a delay-discounting task and classified as high impulsive (HI) or low impulsive (LI) according to the area under the discounting curve (AUC). No-net flux microdialysis experiments, assessing basal DA release, DA-uptake, and DA extracellular concentration (DA C_{ext}), were carried out in dorsolateral striatum (DLS) of urethane-anesthetized rats. Rats classified as HI showed a higher DA release compared with LI rats. Differences in DLS DA-uptake and DA C_{ext} were non-significant. Importantly, a significant negative correlation was observed between AUC and DA release, indicating that the lower the AUC, the higher the DLS DA release. This finding shows that DA release is augmented in the DLS of rats classified as HI, suggesting that a hyper-activated nigro-striatal pathway contributes to impulsive choice.

Keywords: Delay discounting task; impulsive choice; dorsolateral striatum; dopamine; No-net flux microdialysis.

Abbreviations: DDT, delay discounting task; DA C_{ext}, dopamine extracellular concentration; DLS, dorsolateral striatum; HI, high impulsive; LI, Low impulsive.

4.2 Introduction

Impulsivity is a construct characterized by unreflective decision making, premature behavior, and deficits in delaying gratification associated with an increased salience of the rewarding properties of the stimulus [1], [2]. High levels of impulsive behavior are key symptoms of attention-deficit hyperactivity disorder (ADHD), mania within bipolar disorder, problematic gambling, and substance use disorders [3]–[6]. Impulsivity has been proposed to comprise different dimensions, namely, impulsive action, and impulsive choice. Impulsive action is associated with a deficit in motor inhibition, whereas the impulsive choice, also known as impulsive decision-making, is associated with a deficit in delayed reward (for a review, see Dalley and Robbins [7] and Strickland & Johnson [8]).

Impulsive choice is a trait showing important individual differences, that is, characteristics that distinguish one organism from another and that are relatively stable over time and consistent across situations [9]. A common measure of impulsive choice is the delay discounting task (DDT), in which an excessive preference for a small immediate reward over a larger delayed reward, at the expense of more rewards earned per unit of time, is considered as an expression of potential maladaptive impulsive choice [10]–[12].

Clinical and preclinical evidence supports the notion that variability in dopamine (DA) neurotransmission function is a major contributor to individual differences observed in impulsive choice [13], [14]. For instance, genetic association studies provide evidence that the 10-variable number of tandem repeats (VNRT) polymorphism on the dopamine transporter gene (*DAT1*), related to hyperactive dopamine transporter (DAT) [15]–[18], is

believed to be a high risk allele associated to impulsive behaviors [19], [20]. Coincidently, the DAT over-expression in rat nucleus accumbens (NAc) is accompanied by increased preference for a small reward in DDT [21]. These experiments suggested that an increase in DAT expression is associated with high impulsive choice.

Moreover, experiments using positron emission tomography (PET) showed that low dopamine D_2/D_3 receptor availability in the ventral tegmental area (VTA) and substantia nigra (SN) is associated with a highly impulsive phenotype [22]. Similarly, the knock-down of dopamine D_2 receptors in rat VTA induces preference for a smaller immediate reward indicating an increase in impulsive choice [23]. Decreased dopamine D_2/D_3 receptor availability in the dorsal striatum of methamphetamine users correlates with a high discounting rate of a delayed reward [24].

However, while it is known that an increased DAT activity and an attenuated nigro-striatal D_2 signaling could contribute to impulsive choice, the relationship between dorsal DA extracellular levels and impulsiveness needs further exploration. According to the inhibitory role of dopamine D_2 auto-receptor on DA release [25], an augmented amphetamine-stimulated DA release in human striatum is observed in impulsive subjects displaying low dopamine D_2/D_3 receptor availability [22]. Using functional magnetic resonance imaging (fMRI), Pine et al., [26] showed that an increased striatal activity induced by L-DOPA is observed in parallel with an enhanced preference for a smaller, immediate reward. These results suggest that an increased striatal DA release contributes to impulsive decision-making. However, the impulsivity associated with repeated exposure to psychostimulants has been associated with an attenuated striatal DA release

[27]. A blunted amphetamine-stimulated DA release in the dorsal striatum is associated with an impulsive choice of cocaine over money in cocaine users [28]. Insofar as psychostimulants users showed high levels of impulsivity, it has been suggested that a decreased striatal DA release observed in cocaine [29] and methamphetamine [30] abusers contribute to impulsive choice. In summary, low and high DA release could contribute to impulsive choice behavior, accordingly with the inverted-U shape pattern observed between DA signaling and cognitive function [31]–[33].

Nevertheless, preclinical approaches aimed to understand the mechanisms underlying the relationship between striatal DA extracellular levels and impulsive choice have only partially reproduced the imaging studies. In this context, evidence indicates that administration of the selective catecholamine uptake inhibitor 3,4-Methylenedioxypyrovalerone (MDPV) increase impulsive choice, accompanied by an increase in striatal DA turnover [34]. On the other hand, voltammetry experiments showed that rats classified as high impulsive in the DDT display a blunted ventral striatal DA release independent of the history of self-administered cocaine [35]. Similarly, a decrease in the electrically evoked tritiated DA release is observed in slices of NAc and mPFC of impulsive rats [36]. However, a recent amperometric study fails to show differences in ventral striatal DA release between rats classified as high and low impulsive in the DDT [37]. Moreover, studies assessing the role of the nigro-striatal dopaminergic pathway on impulsive choice fail to show conclusive results due to important methodological issues. Using intracranial self-stimulation as positive reinforcement in the delay discounting task, Tedford et al., [38] showed that lesion of the dopaminergic terminal in the dorsolateral striatum (DLS) by 6-hydroxydopamine (6-OHDA) is accompanied by an increase in

impulsive choice. However, using sucrose as a positive reinforcer, Magnard et al., [39] showed that (6-OHDA)-induced lesion of substantia nigra pars compacta (SNpc) is not accompanied by changes in impulsive choice. Because the dorsal striatal DA content was not measured in these studies, it remains unclear whether changes in DA concentration are associated with impulsive choice. The main goal of this research is to determine the relationship between delay discounting behavior and DA dynamics in rat DLS. Microdialysis no-net flux experiments were carried out to assess the contribution of DA release and DA-uptake on DLS DA extracellular concentration (DA C_{ext}) in rats classified as high or low impulsive in the DDT. Our results showed that an increased DLS DA release is positively correlated with impulsive levels, showing that high DA release is associated with higher levels of delay discounting

4.3 Experimental Procedures

4.3.1 Animals

Fifteen male Sprague Dawley rats, approximately postnatal day (PND) 25, were grown from the Animal Care Faculty of Biological Science, Pontificia Universidad Católica de Chile (Charles River; Wilmington, MA, USA; research resource identifiers: RGD_728193) under the strict supervision of a veterinarian. During behavioral test, rats were maintained in the Animal Care of the Department of Pharmacy, Pontificia Universidad Católica de Chile, using protocols and instructions approved by the veterinarian. Rats were housed in pairs in ventilated cages by an Air Handling Unit (Tecniplast, West Chester, PA) under controlled conditions including temperature (22±1°C), and humidity (50±10 %). The animals were additionally maintained under a 12-hour light-dark cycle (lights on 07:00-19:00 hrs). Experiments were conducted during the light period. Rats were handled for seven days before starting the experiments. Six days after arrival (PND 31), food access was restricted, and rats were weighed daily to maintain body weights to 90% of the free-feed body weight. We allowed the increase in the restricted body weight per week to let the growth associated with age. The rats started with an approximate weight of 75 gr and ended up weighing approximately 420 gr. The target weights were adjusted according to normal growth curves obtained from the animal supplier. Water was available ad libitum. All procedures were in strict accordance with the guidelines published in the "NIH Guide for the Care and Use of Laboratory Animals" (8^{th} ed) and principles presented in the "Guidelines for the Use of Animals in Neuroscience Research" by the Society for Neuroscience. Furthermore, all procedures were approved

by the ethics committee of the Pontificia Universidad Católica de Chile. Moreover, advice from a veterinarian was also obtained during the process.

4.3.2 Apparatus

Sessions were conducted in ventilated, sound-attenuating enclosures containing an operant conditioning chamber with an interior space of measures 31 × 24 × 21 cm (Med Associates, Inc.; St Albans, VT). The front door and the rear panel were made from clear polycarbonate, and both ends were made of aluminum panels. The right panel was equipped with two response levers, horizontally aligned 11.5 cm apart. Above each lever, there was a translucent circle trans-illuminated with white light, 2.5 cm in diameter. A pellet receptacle was centrally located between the two levers. 45 mg of food chocolate in form (F0299 chocolate dustless precision pellets, Bio-serv; Flemington, NJ) was delivered to this receptacle from an external food dispenser. The panel on the opposite side of the chamber was equipped with a house-light centrally located near the top of the chamber. Data was collected using MED-PC IV software and a PC-compatible interface (Med Associates, Inc; St Albans, VT).

4.3.3 Procedures

Rats were tested in the behavioral procedures as described below. All procedures were adapted from Eubig P et al., [40] and Tanno et al., [41].

4.3.3.1 Initial lever press training

Approximately after PND 35, a shaping program was used to train rats to begin pressing the response levers. Both levers were extended at the onset of the session. As described in Eubig P et al., [40] the delivery was programmed according to a 3-min fixed-time schedule, in which a food pellet was dispensed every 3 min. Also, one pellet was delivered with every lever press. Sessions finished after either 60 min elapsed, or 100 reinforcements were delivered. The criterion for advancement to the next phase of training was 100 lever presses, performed for three consecutive sessions.

4.3.3.2 Fixed ratio training

The second training phase began between PND 39-40. During this phase, only one lever was extended, and the corresponding cue light was illuminated. After five lever presses, the extended lever was retracted, and the opposite lever was extended. Every response resulted in the delivery of a food pellet (i.e. a fixed ratio 1 contingency). After 100 reinforcements across three consecutive sessions, rats continued to the next training phase.

4.3.3.3 Delay discounting training and testing

The DDT training phase began approximately after PND 45. The phase consisted of 40 trials for each session. Two levers with different reinforcers were presented and were always accompanied by an illuminated cue light: the lever press of the larger reinforcer (LR) resulted in 3 pellets and the small reinforcer (SR) lever resulted in 1 pellet. There

was no delay in the delivery of either the small or large reinforcers in this phase. The session finished after 40 lever presses between large or small reinforcers. The criterion for advancement to the next phase of training required an 80 percent of preference for the larger reinforcer.

The DDT testing adapted from Tanno et al., [41], began in darkness with the levers retracted (blackout). After 10 minutes of blackout, the house-light turned on, and the session started. One session consisted of five blocks comprising 12 trials each. The duration of the trials was of 60 seconds. The first two trials of each block were forced-choice trials in which the LR lever and the SR lever were alternately introduced. Ten free-choice trials followed the force trials in each block. At the beginning of the free-choice trial, the lights over the levers were turned on, and the levers appeared. If a lever was pressed, both levers were retracted. If the rat pressed the SR lever, one pellet was immediately delivered, and both levers were retracted. If the LR lever was chosen, the stimulus light blinked during the delay and, following the delay, three pellets were delivered. Delays to the LR were increased from 0s in block 1 to 4s in block 2, 8s in block 3, 16s in block 4, and 32s in block 5. Failure to choose a response within 20 s resulted in the levers retracting and the trial was considered as an omission (see figure 1). The rats were trained five times per week for approximately ten weeks (50 sessions) in this task until stable performance was achieved. (See Data Analysis section for more details).

4.3.4 Surgery and no-net-Flux Microdialysis

Surgery and no-net-flux microdialysis were performed at approximately PND 115-120, two hours after the last DDT session. Rats were anesthetized with urethane (1.5 g/kg i.p) which remained constant throughout the experiment due to its extended half-life [42] and were placed in a stereotaxic apparatus. The skull of each rat was exposed, and a hole was drilled targeting the DLS. A concentric microdialysis probe (CMA-11 Microdialysis, Holliston, MA) was implanted in the DLS using the following coordinates +1.2 AP, +3.6 ML relative to bregma and -4.8 DV from the dura [43]. Body temperature was maintained by a thermostatically controlled electric heating pad. The microdialysis probe was perfused for 40 minutes to allow equilibrium with Krebs– Ringer phosphate (KRP) buffer at a rate of 2 μL/min using a Harvard infusion pump (Harvard Apparatus, Holliston, MA). The composition of the KRP was 120 mM NaCl, 2.4 mM KCl, 1.2 mM CaCl2, 0.9 mM NaH2PO4, 1.4 mM Na2HPO4, and 0.2 mM of ascorbic acid (pH 7.4). The probe was randomly perfused with four different concentrations of DA: 0, 5, 20, and 40 nM in a KRP to determine extracellular DA concentration (DA C_{ext}), DA release, and extraction fraction (Ed), which is an indirect measure of DA-uptake [44], [45]. Three consecutive samples were collected every 5 minutes for each concentration of DA after a stabilization period of 30 minutes. Perfusion samples were collected in 2 μL of perchloric acid (0.2 N) and maintained at 4ºC, until analysis (see, section 2.4.2).

4.3.5 Histology

After microdialysis experiments, rats were decapitated under deep anesthesia (urethane 1.5 g/kg i.p.), and brains were extracted and cleaned with a saline solution (NaCl 0.9%). Brains were stored in 4% paraformaldehyde (PFA). At least two days before slicing, brains were cryoprotected using a 30% sucrose solution. For probe implantation, brains were frozen and coronally sliced in sections of 50 μm. Slices were stained with cresyl violet, and the probe placement was observed in a light microscope using the atlas of Paxinos & Watson for rats [41]. Only data coming from correct probe placements were considered for further analysis (see figure 2).

4.3.6 Analysis of dialysate dopamine

Ten ml of the dialysate was injected using a Rheodyne injector valve into an HPLC system (BASi America, West Lafayette, IN). The mobile phase contained 100 mM NaH2PO4, 1 mM EDTA, 1 mM octane-1-sulfonic acid sodium salt, and 3% acetonitrile (pH 3.0), and was pumped at a flow rate of 700 μL/min. The potential of the amperometric detector was set at 650 mV. Under these experimental conditions, the retention time for DA was 6 min.

4.3.7 Data Analysis

4.3.7.1 Delay discounting analysis

4.3.7.1.1 The logarithmic area under the curve (AUC_{log})

Delay-discounting curves were obtained by plotting the proportion of LR choices (the number of LR choices divided by the total number of successful trials) of each block versus each delay (sec) (see figure 3A). The area under the delay-discounting curve (AUC) was used as a measure of impulsive choice [46], [47]. The AUC was calculated using a log-transformation of delays (AUC_{log}) to avoid the disproportionate contribution at long delays in the delay-discounting curves [48]. Specifically, each delay was transformed to the logarithm of the delay divided by the bigger logged delay. To avoid the problem of calculating the logarithm of zero, a small amount (value=1) was added in each delay before the transformation. We use GraphPad Prism to calculate AUC_{log} (version 5, GraphPad Software, Inc.; La Jolla, CA). Higher AUC_{log} values indicate lower discounting by delay (i.e., preference for larger rewards, indicating lower impulsivity), while lower AUC_{log} values correspond to higher discounting by delay (i.e., preference for small and immediate rewards, indicating higher impulsivity) [46].

4.3.7.1.2 Behavioral Stability and Classification

To assess behavioral stability, we compared the AUC_{log} of five consecutive sessions (week 1) with the five following sessions (week 2). An animal was considered trained when the difference between weeks was not statistically significant. For each animal, a stable AUC_{log} value was calculated using the average of the last five sessions (Figure 3B). Rats were classified as low impulsive (LI) and high impulsive (HI) based on the median split of AUC_{log}. The minimum sample size was calculated based on a priori power analysis

[49]. The input parameters for behavioral experiments were the following: two-tails, alpha= 0.05, power= 0.95, and ratio N1/N2= 1. The effect size was based on preliminary DDT results, suggesting a minimum sample size of n= 7/group for the experiment considering a Cohen's d effect size of 2, using G*Power statistical analysis software Version 3.1.9.4 [50].

4.3.7.1.3 Omission and latencies

Latency was defined as the time since the levers were extended until the rat pressed one of them during free-choice trials in each block. An omission was considered as a failure to choose a response within 20 s after lever appearance. For each animal, we recorded the latency and the number of omissions in the 5 daily blocks in free-choice trials. Final latency and omission values were calculated averaging total latency and omissions of the last five sessions.

4.3.8 No-Net-Flux analysis

The amount of DA gained or lost from the probe during the no-net flux microdialysis (C_{in}-C_{out}) was calculated for each animal at each perfusion concentration (C_{in}:0, 5, 10, 20, 40 nM DA). The net change in DA was plotted against Cin (C_{in}-C_{out} vs. C_{in}) and subjected to linear regression (figure 4A). The DA C_{ext} concentration and Ed were assessed using linear regression. The point where no DA was gained or lost (C_{in}-C_{out} = 0) represents an estimate of DA C_{ext}. The Ed is the slope of the linear regression and has been shown to provide an indirect measure of the DA-uptake. Basal dialysate DA was calculated for each animal as

the average of the three basal samples (C_{in}=0). An unpaired t-test was used to assess the effect of the impulsivity level on basal dialysate DA levels, DA C_{ext}, and Ed [45], [51].

4.3.9 Statistical Analyses

All statistical analyses were carried out using Prism 5.0 GraphPad Software. Each group of data was analyzed with the nonlinear regression ROUT method (Q= 1%) [52], the outlier detected was removed from the statistical analysis and graphs (total outliers: 1). The resulting data were analyzed using an unpaired t-test when it was appropriate (Figure 4; all data are reported as mean ± SEM). Correlation analyses were performed using the Spearman correlation coefficient for non-parametric data (Figure 5).

4.4 Results

4.4.1 Individual differences in basal level of impulsive choice

Figure 3A shows the delay-discounting curve for all rats. According to an AUC_{log} median split, rats were classified as LI (n= 7) and HI (n= 7) (figure 3B LI: 0.81 ± 0.02; vs HI: 0.54 ± 0.05; p = 0.0002, according to unpaired t-test). Non-significant differences between HI and LI rats were observed in latency (LI: 1.74 ± 0.22 sec vs HI: 1.81 ± 0.01 sec, p = 0.785 according to unpaired t-test) and omissions (LI: 1.09 ± 0.64 vs HI: 1.81 ± 0.10, p = 0.322 according to unpaired t-test).

4.4.2 Animals classified as high impulsive in DDT showed higher DA C_{ext} in DLS

To compare the DLS DA dynamics between HI and LI rats, no-net-flux microdialysis experiments were carried out after DDT. Rats classified as HI showed a significantly higher DA release in DLS compared with LI rats (figure 4B LI: 0.43 ± 0.11 nM vs HI: 0.89 ± 0.14 nM, p = 0.022 according to unpaired t-test). The extraction fraction (Ed) showed no significant differences between HI and LI rats (figure 4C LI: 0.52 ± 0.05 vs HI: 0.46 ± 0.04, p = 0.331, according to unpaired t-test). Also, no significant differences were observed in DA extracellular concentration (C_{ext}) in DLS between rats classified as HI compared with LI rats (figure 4D LI: 1.13 ± 0.23 nM vs HI: 1.61 ± 0.28 nM;, p = 0.215 according to unpaired t-test). Additionally, using a third classification criterion the differences in the DLS DA release in HI rats compared with LI rats are maintained (LI: 0.34 ± 0.11, n=5 vs HI: 0.88 ± 0.20, n=5; p = 0.042).

4.4.3 Correlation between impulsive choice levels and DA dynamic in the DLS

In order to evaluate if there is an association between basal levels of impulsive choice and DA dynamics in the DLS, a correlation between AUC and the different neurochemical parameters associated with DA dynamics was carried out. As illustrated in figure 5A a significant negative correlation was observed between AUC and DLS DA release ($r = -0.69$, $p = 0.008$; $n=14$). The AUC and Ed in DLS showed non-significant correlation (figure 5B $r = 0.26$, $p = 0.374$; $n=14$). A non-significant correlation was also observed between AUC and DLS DA C_{ext} (figure 5C $r = -0.51$; $p = 0.064$; $n=14$).

4.5 Discussion

It has been consistently shown that variability in DA neurotransmission is a mechanism underpinning individual differences observed in impulsive choice, also named delay-related decision making [21], [23], [53]. Single unit recording experiments showed that DA neurons in the SNpc modify their activity depending on delay and reward magnitude [54], implying that DA neurotransmission in the dorsal striatum could contribute to information processing during delay-related decision making [55]. In the current study, we examined the relationship between impulsive choice and dopaminergic dynamics in the rat DLS. Rats were classified as high and low impulsive using a median split of AUC in discounting curves. Given that, this classification criterion could be considered problematic because some rats may have AUCs near the average score, we also analyzed our data using selected extremes values after a third classification. In this analysis, the differences in DLS DA extracellular levels are maintained between rats classified as HI (lower third) and LI (upper third). Thus, our findings seem to be robust to different classification criteria, in the absence of a consensual procedure of classification in the delay discounting literature. A higher DLS DA release was observed in HI rats compared with LI rats. Also, a significant negative correlation was observed between AUC and DA release, indicating that lower AUC is associated with higher DLS DA release. Notwithstanding of limitations of our study, these results suggest that an increased DLS DA signaling contribute to delay-related decision-making.

In line with our results, clinical evidence shows that basal striatal DA concentration appears to be relevant to predict both vulnerabilities to engage in impulsive behaviors as

well as to the response to pharmacological treatment. The impulsiveness observed in ADHD has been correlated with both an increase [26], [56] and a decrease in striatal DA signaling [57]. Consequently, the therapeutic effectiveness of psychostimulants for ADHD treatment has been associated with a decrease [56] and an increase in striatal DA release [58]. This apparent discrepancy in results related to dopaminergic signaling and impulsivity could be attributed to individual variability in striatal basal DA concentration [11], [31]. It has been proposed that the relation between basal DA concentration and neural reward processing during decision-making shows an inverted-U shape pattern: excessively low or high DA concentration could predispose to impulsive behavior [31], [32]. Then, an increase in striatal DA release induced by psychostimulant drugs would benefit subjects with low DA concentration but could worsen impulsivity in subjects with elevated DA concentration [27], [32]. Then, given that our study is limited by the relatively small sample size, the results could be showing the descending limb of the curve, i.e., the impulsive choice associated with an increase in DLS dopamine neurotransmission. The ascendent limb of the curve, necessary to observe the inverted-U curve, that could be appear after a pharmacological blockade of DA neurotransmission was not tested in this study [33].

The increased DA release observed in rats classified as HI could be explained by presynaptic and postsynaptic mechanisms. It has been consistently shown that an attenuated D_2 receptor activity contributes to impulsive choice. Critically, it has proposed that individual differences in D_2 receptor mRNA expression predicts different levels of impulsive choice [59]. Low D_2 receptor mRNA expression in the NAc is observed in male rats showing higher levels of delay discounting [59]. Interestingly, a low D_2 receptor

mRNA expression in the mPFC has been observed in male and female rats classified as high impulsive in the DDT [59], [60]. In the same line, a polymorphism in the D_2 receptor gene (C957T) showing a decreased striatal binding of D_2 receptor has been associated with a higher preference for the immediate reinforcement in the DDT during stress [61]. Furthermore, clinical and preclinical research has shown that prolonged exposure to methamphetamine, amphetamine, or cocaine reduced D_2 receptor expression in the dorsal striatum, leading to high levels of impulsive choice [24], [62]–[64]. Similarly, the specific down expression of D_2 receptors in rat dopaminergic mesencephalic neurons is accompanied by an increase in impulsive choice [23]. Then, it is possible to suggest that the higher DA release observed in HI rats is due to a loss of the inhibitory tonic control of D_2 receptors on DA extracellular concentration [25]. Together with the D_2 receptor, DAT activity also controls striatal DA release, keeping DA C_{ext} levels stabilized [65]–[67]. In addition to its important role in DA re-uptake [68], it was recently shown that DAT also promotes DA release in the dorsal striatum, contributing to local short-term plasticity mechanisms of DA release [69]. Importantly, it has been shown that an increase in striatal DAT activity underlies impulsivity in ADHD [70], and consequently, DAT blockade has been proposed as a therapeutic mechanism for the treatment of impulsivity in clinical [58], [71] and preclinical [72]–[74] studies. In line with these findings, striatal over-expression of DAT in rat ventral striatum increases impulsive choice [21]. However, attenuation of DAT expression and repeated exposure to DAT antagonist MDVP also induced an increase in impulsive choice [21], [34]. This inconsistency may be due to DAT activity complex effects on DA release [69], so the consequences of modifying the DAT expression on striatal DA C_{ext} are difficult to predict. In this context, our results showed

no significant differences in striatal DA Ed between HI and LI rats, suggesting that changes in DAT activity in DLS do not contribute to individual differences in delay-related decision making. Because the Ed is an indirect measure of DA-uptake [44], [75], this result therefore should be interpreted with caution, and other approaches [76] should be considered to better understand the role of dorsal striatum DAT to impulsive choice.

Regarding postsynaptic mechanisms, the increased DA release observed in the DLS of HI rats could be associated with changes in neuronal activity in the nigro-striatal pathway. Supporting this idea, the persistent impulsivity observed after repeated administration of MDPV is accompanied by an increase in striatal DOPAC/DA ratio, suggesting an increased nigro-striatal pathway neuronal activity [34], [77]. The neuronal activity of mesencephalic DA neurons controls striatal DA C_{ext} [78], [79]. An increase in DLS DA release is observed concomitantly with the increase in the firing rate and burst activity of DA neurons in SNpc [79]. Then, it is possible to suggest that higher neuronal activity in the dopaminergic neurons of SNpc supports the increased DA release in DLS observed in rats classified as HI, compared with LI rats. In fact, we recently showed that the increase in DLS DA release observed after adolescent exposure to cannabinoids is accompanied by an increase in population activity in SNpc [80]. It has been established that GABA and glutamate inputs control the excitability of mesencephalic dopaminergic neurons. While the number of spontaneously active DA neurons is regulated by GABA afferences, the burst pattern of DA neurons depends on glutamate afferences [78]. Our results suggest that an activated nigro-striatal pathway contributes to high delay discounting probably associated with an increase in population activity or bursting activity of DA neurons in

the SNpc [67]. Whether variability in GABA and glutamate levels in SNpc contribute to individual differences in delay-discounting behavior is unknown.

In summary, our results showed that DA neurotransmission in DLS contributes to individual differences in impulsive choice. The increased DLS DA release observed in HI rats could maintain activated postsynaptic DA receptor in dorsal striatum responsible for increasing delay aversion [81]. Further research is necessary to elucidate the role of nigral DA neurons during impulsive choice and the mechanisms contributing to the activated nigro-striatal pathway associated with a high discounting rate of a delayed reward.

4.6 Ackonwledments

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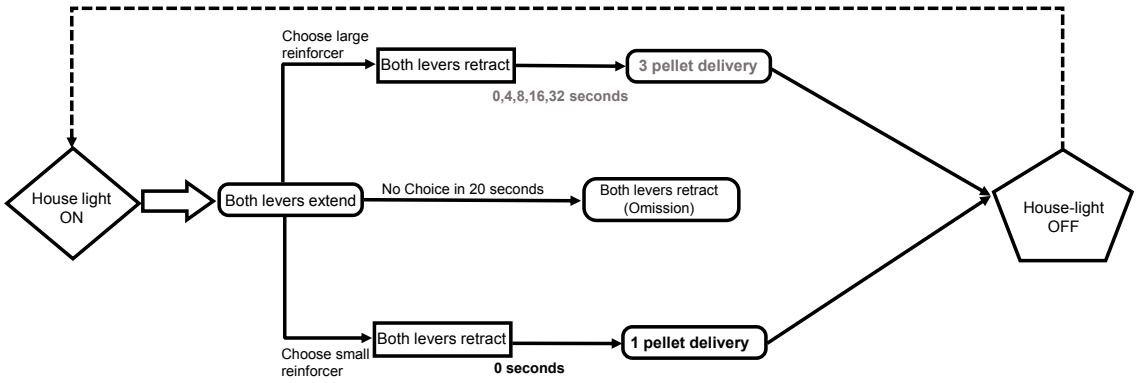

Figure 1: Delay discounting task design. Scheme of a single free-choice trial on the delay discounting task used in the present study.

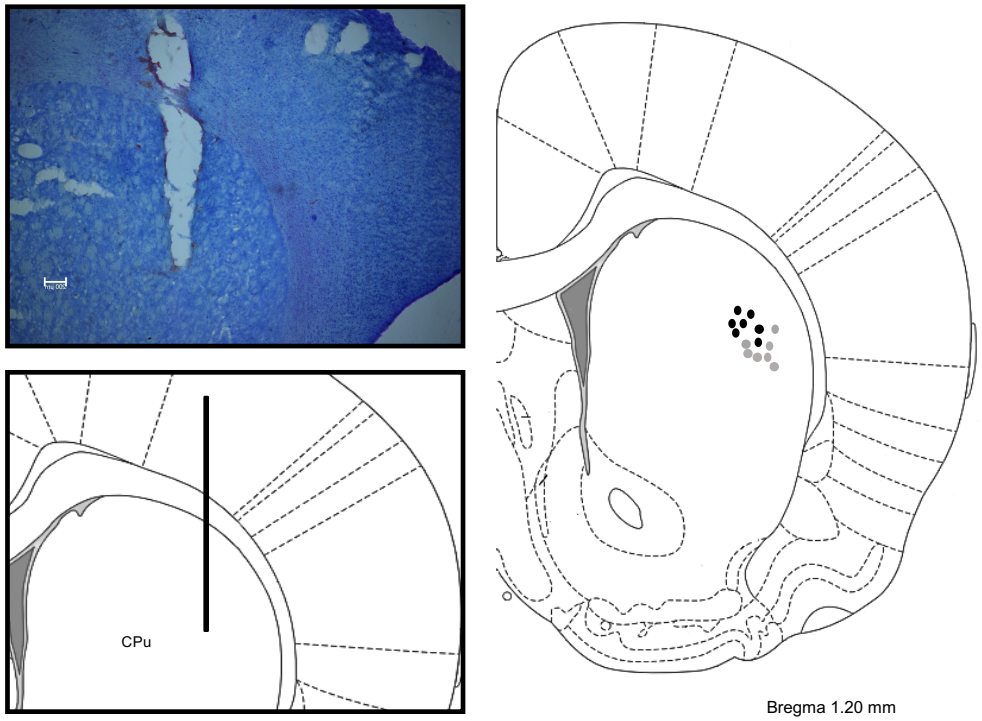

Figure 2: Anatomical placements of the microdialysis probe in DLS. Left, Photo of representative microdialysis probe placement in DLS, and map showing the microdialysis probe placement (black line) of the representative photo. **Right,** schematic, coronal section of the rat brain showing the placement of the microdialysis probe for LI (black dots) or HI (gray dots) rats in the No-net-Flux microdialysis experiment. Brain sections correspond to the atlas of Paxinos and Watson [41].

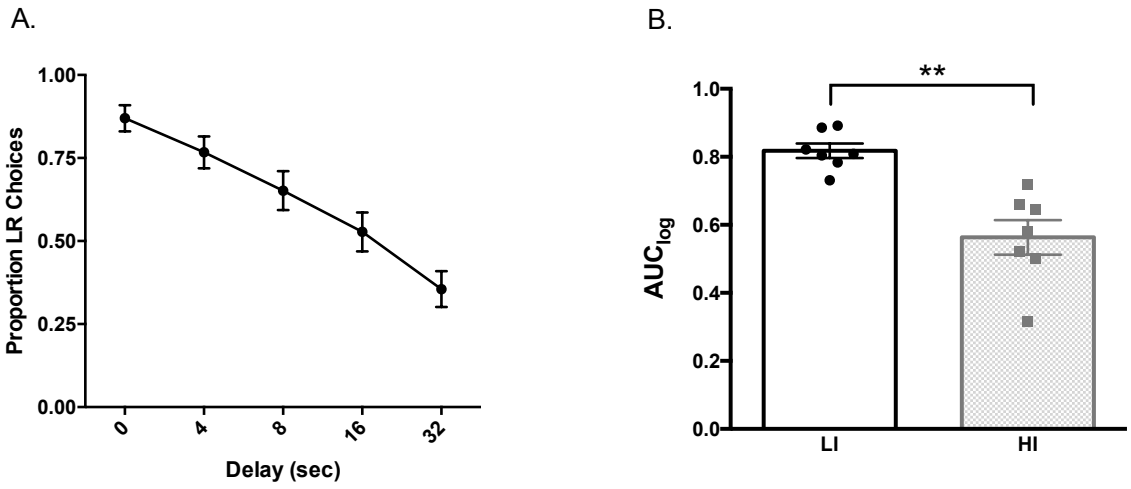

Figure 3: Individual differences in the basal level of impulsive choice. **(A)** Delayed-discounting curve for all animals (n=14) and **(B)** Rats classified as LI (n=7) show a higher AUC_{log} compared with rats classified as HI (n=7), ** represents $p< 0.05$ according to unpaired t-test.

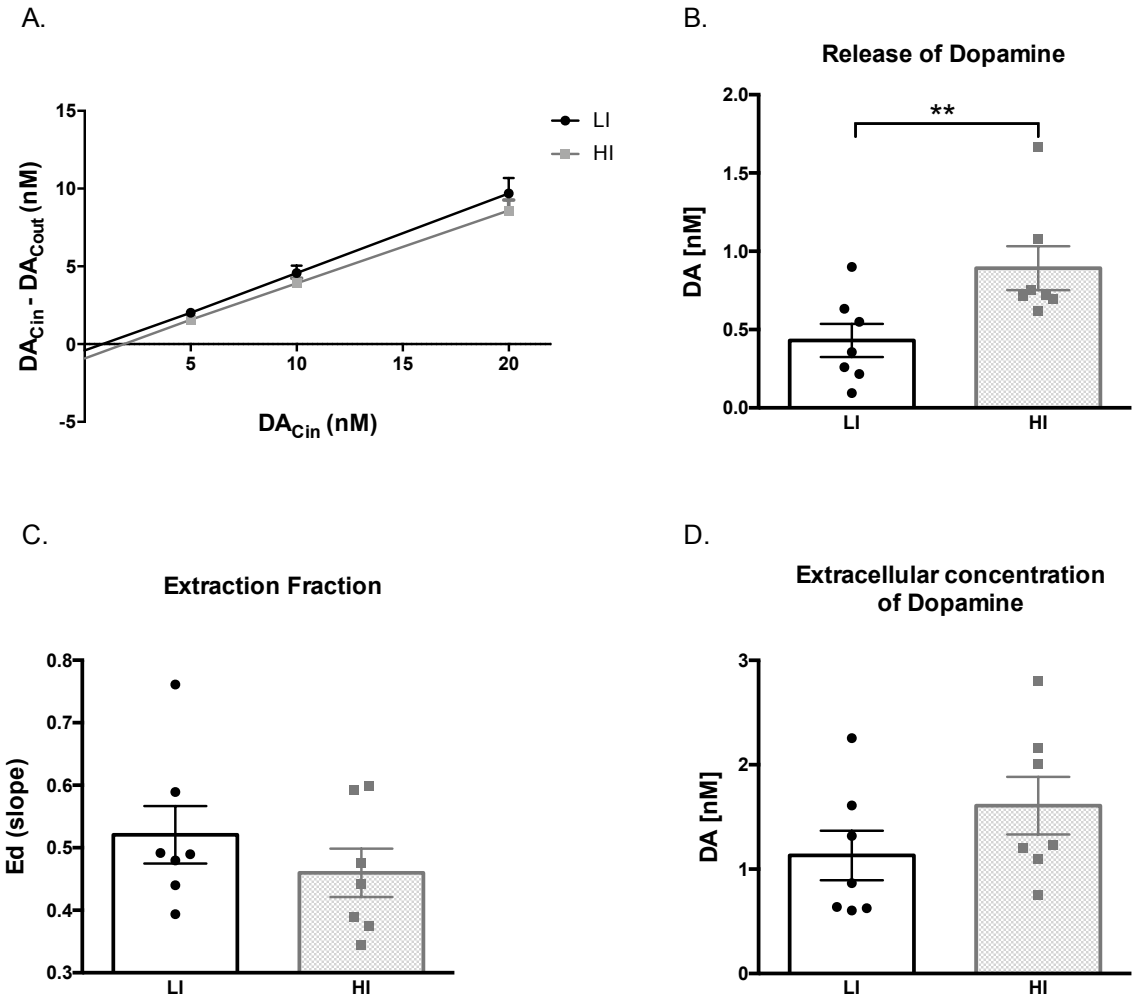

Figure 4: Animals classified as high impulsive in DDT showed higher DA release in DLS. In vivo No-net-flux microdialysis in anesthetized rats classified as HI (n= 7) and LI (n=7). **(A)** The plot of average gain or loss of DA **(**C_{in}-C_{out}) as a function of perfusate DA (C_{in}) and the linear regression of LI (black line) and HI (gray line) rats. The slope of the regression line represents the Ed. The point when DA is not perfused (C_{in}= 0) represents the DA release. When no DA is gained or lost from the perfusate (C_{in}-C_{out}= 0) represents an estimate of DA C_{ext}. **(B)** Bar graph of DA release, **(C)** Ed, and **(D)** DA C_{ext}. ** represents $p< 0.05$ according to an unpaired t-test.

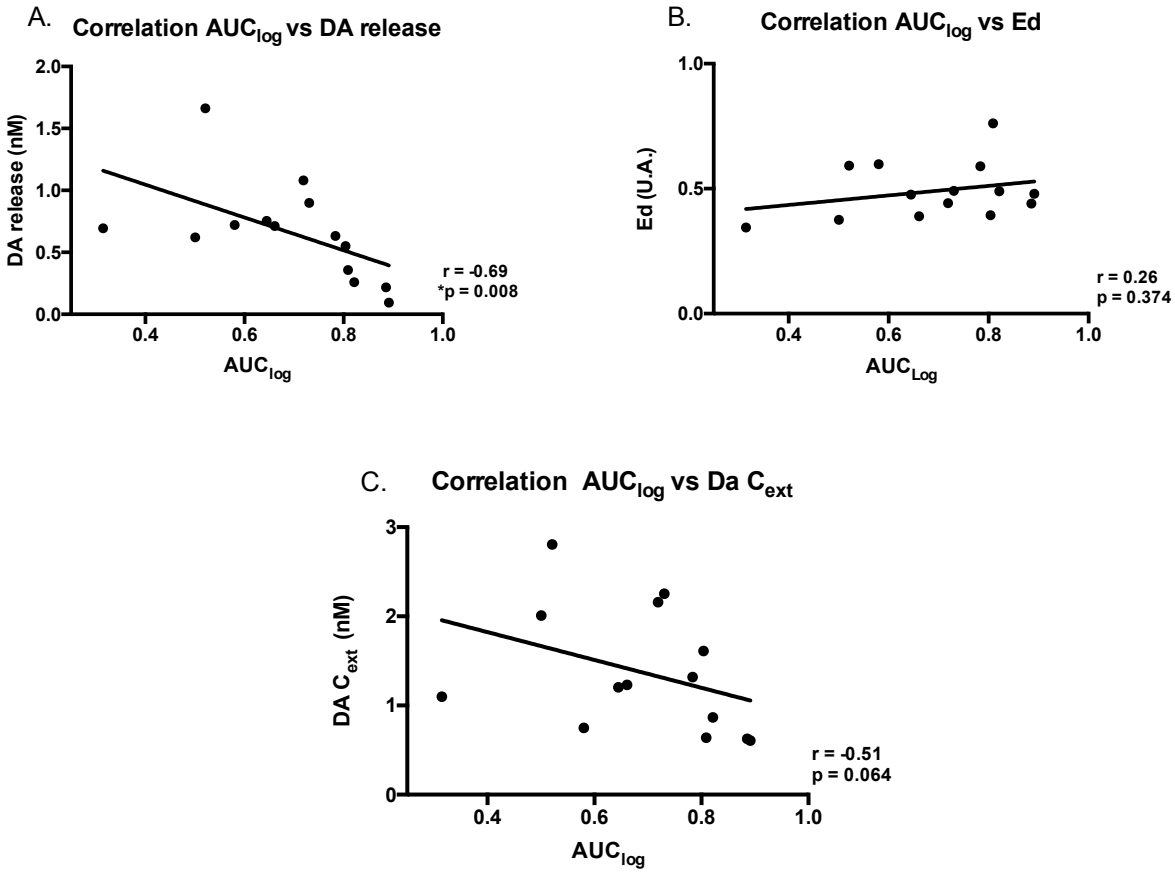

Figure 5: Correlation between impulsive choice level measured by AUC and basal dynamic of DA in DLS. **(A)** Correlation between AUC and DA release. A significant, negative correlation was observed (r= -0.69; *p=0.008 according to Spearman correlation; n= 14). **(B)** Correlation between AUC and Ed. A non-significant correlation was observed (r= 0.26; p= 0.374 according to Spearman correlation; n= 14). **(C)** Correlation between AUC and DA C_{ext}. A non-significant correlation was observed (r= -0.51; p= 0.064 according to Spearman correlation; n=14).

5 Chapter 2: Effect of OXT-R activation on the nigro-striatal pathway during delay discounting task, a study of impulsive choice behaviors

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Highlights

- The bilateral activation of OXT-R in SNpc, induces a decrease in impulsive choice in HI animal during DDT.
- A slight but significant DA_{ext} increase in DLS was observed post OXT-R activation in SNpc.
- The activation of OXT-R in SNpc, induce a non-significant change in the DA_{ext} in SNpc.

5.1 Abstract

Impulsive choice has been used as a predictor of several neuropsychiatric disorders, including personality disorders, attention deficit hyperactivity disorder (ADHD), and addiction. All these pathologies are linked to a dysfunction in dopaminergic transmission. We recently showed that dorsal striatal dopamine (DA) signaling contributes to individual differences in impulsive choice. Because oxytocin (OXT) modulates the activity of mesencephalic dopaminergic neurons, we wonder whether activation of the oxytocin receptor (OXT-R) in the substantia nigra pars compacta (SNpc) also contributes to modulate impulsive choice. To fully understand how the OXT system modulates the DA release from mesencephalic neurons, we also studied the consequences of the activation of OXT-R in the SNpc on DA extracellular (DA_{ext}) level in the nigro-striatal pathway. Firstly, Adult male rats were well-trained on a delay discounting task, where they chose between a smaller (1 pellet) reward delivered immediately, or a larger 4-pellet reward delivered after a delay (0-45 s). On separate test days, they received the microinfusion of the non-peptide agonist WAY267464 (at a dose of 3µg/0.5µl) in SNpc. These treatments decreased impulsive choice in high impulsive (HI) rats, displaying a greater preference for larger, delayed rewards. Secondly, using in vivo microdialysis revealed that the same agonist WAY267464 (5µM) perfusion in SNpc induces a slight increase in DA_{ext} level in the dorsolateral striatum (DLS) but does not induce a change in DA_{ext} level in SNpc. Together these findings suggest that activation of the OXT-R in the SNpc influences delay-related decision making by subtle changes in the striatal dopamine transmission.

Keywords: Delay discounting task; impulsive choice; dorsolateral striatum; dopamine; oxytocin.

Abbreviations: DDT, delay discounting task; DLS, dorsolateral striatum; HI, high impulsive; LI, Low impulsive; OXT-R, oxytocin receptor; SNpc, substantia nigra pars compacta.

5.2 Introduction

The dopaminergic and oxytocinergic systems alteration is present in a variety of neuropsychiatric disorders, such as schizophrenia, ADHD, and addiction [1–4], which are disorders that are related to a high level of impulsive behaviors [5–8]. We recently showed that DA transmission changes in DLS contribute to individual differences in impulsive choice; specifically, we find that DA release is augmented in the DLS of rats classified as HI, suggesting that a hyper-activated nigro-striatal pathway contributes to impulsive choice. This result is consistent with clinical and preclinical evidence that supports the notion that variability in DA neurotransmission function is a significant contributor to individual differences observed in impulsive choice [9,10]. Moreover, the clinical approach using neuroimaging techniques showed that low DA D_2/D_3 receptor availability in the ventral tegmental area (VTA) and substantia nigra (SN) is associated with a highly impulsive phenotype [11]. Preclinical evidence indicate that administration of the selective catecholamine uptake inhibitor 3,4-Methylenedioxypyrovalerone (MDPV) increase impulsive choice [12]. However, existing evidence proposes that low and high DA release could contribute to impulsive choice, accordingly to the inverted-U shape pattern observed between DA signaling and cognitive function [13–15]. Therefore, it is necessary to study the neurochemical and pharmacological performance at the impulsivity curve extremes. Approaches have been proposed that using psychostimulant drugs would decrease impulsive behavior in subjects with low DA concentration but could worsen impulsivity in subjects with elevated DA concentration [14,16]. Interestingly, in the DA and OXT interaction context, clinical studies showed a significant association between

basal plasma OXT and the Karolinska Scale of Personality (KSP) factor impulsiveness, suggesting positive correlation between impulsiveness and plasma OXT level male psychiatric outpatients [17]. On the other hand, resents studies showed that the intranasal OXT administration moderated impulsive behavior in subjects with a single nucleotide polymorphism (SNP) rs2254298 genotype [18]. Even so, the interaction of dopamine and OXT systems in impulsive behavior is still unknown.

OXT is a neuropeptide synthesized by neuron of hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON) and is transported along the axons projected into the neurohypophysis from where they are spread in the blood stream [19,20]. The principal role of OXT release into the blood is linked to parturition and lactation [21–23]. On the other hand, there are long-range axonal projection of OXT neurons in the hypothalamus to the extra-hypothalamic regions of the brain, such as the prefrontal cortex (PFC), nucleus accumbens (NAcb), lateral septum, amygdala, and SN [24–27]. In these extra-hypothalamic regions, OXT is capable of acting as a neurotransmitter and/or as a neuromodulator of cognitive processes and complex social behavior such as attachment, exploration, peer linking and social recognition [28,29], as well as in non-social behaviors, such as stress, anxiety, and aggressiveness [30]. It has been proven that the aforementioned prosocial behaviors can be also affected by a deep alteration in the dopaminergic pathways [31–33].

There is evidence of both the innervation and expression of OXT-R in different regions linked to the dopaminergic transmission, such as SN and VTA [26,34,35], which may be pointing to a regulatory role of OXT in different behaviors linked to the DA system

[35,36]. The OXT-R is a G-protein-coupled receptor (GPCRs), coupled to G_q, which active the phospholipase C (PLC) pathway to increase intracellular Ca^{2+} stores release [23]. It has also been shown that there exists a postsynaptic co-localization between the DA type D_2-R with the OXT-R in brain regions that receive dopaminergic projections as NAcb and the DLS. These receptor crosstalk and/or heteromeric receptor complex (D_2-R–OXT-R) could represent a molecular mechanism for OXT through positive allosteric regulation in receptor-receptor interaction, increases dopaminergic signaling, and contributes to the induce changes in social and emotional behavior [37,38].

It has also been shown that there exists a bidirectional modulation between dopaminergic and oxytocinergic systems [39–41]. Therefore, it has been established that both dopaminergic and oxytocinergic pathways converge in the PFC, the striatum, and the ventral tegmental areas [36,42,43]. In this context, the neurophysiological and behavioral approach has shown that DA intraventricular injections increase the frequency and amplitude of oxytocinergic neurons in PVN [39], in line with these, it has been shown that OXT release from PVN in VTA enhances the activity of a specific population of DA neurons that influence social interaction [36]. On the other hand, Xiao et al., [26] showed that OXT release in midbrain DA regions as VTA or SNpc exert a differential effect, where the OXT release in VTA induces an increase in the VTA-DA firing rate (FR). Moreover, the OXT release in the SNpc induces a heterogeneous effect, depending on the topographical location into SNpc, where the OXT release in lateral-SNpc induces a decrease in the SNpc-DA FR. These studies suggest that the action of OXT-VPN is essential for the reinforcing the component of social interaction via VTA DA neurons [36], and OXT-driven inhibition of DA neuron in SN can dampen non-social behavior,

where OXT infusion in SN decrease locomotor activity [35]. Nevertheless, the changes in the population neuronal activity in SNpc post OXT-R activation area associated with DA release changes in DLS, and their behavioral effect have not been studied.

Therefore, we directly investigated the role of OXT in the SNpc on impulsive choice behavior and on the modulation of nigro-striatal dopaminergic transmission. For behavioral studies, animals were well-trained on a delay discounting task (DDT), and on the test days, animals received bilateral microinfusions of the OXT-R agonist (WAY267464 dihydrochloride, at a dose of 3mg/0.5ml) in the SNpc. An in vivo microdialysis in adult male rats were performed to study the effect of the perfusion of WAY267464 in SNpc on DA extracellular (DA_{ext}) levels in the DLS and SNpc, respectively. Our result showed that bilateral activation of OXT-R in SNpc induces a decrease in impulsive choice in highly impulsive animals. Interestingly, the activation of OXT-R in SNpc induces a moderate increase in the DA_{ext} in SNpc, suggesting that activation of the OXT-R in the SNpc influences delay-related decision making by subtle changes in the striatal dopamine transmission.

5.3 Methodology

5.3.1 Animals

Adult male *Long-Evans* rats (Charles River Laboratories, St Constant Quebec, Canada) weighing between 275 to 300g at the start of the experiment were used in behavioral experiments. Rats were maintained with 14 g food per day and water *ad libitum*. All the animals were put in pairs in each of the houses, under a 12-hour light-dark cycle (lights were turned on at 8:30 AM) and controlled temperature (21±1°C).

Adult male *Sprague Dawley* rats (weighing between 300 and 400 g) were used in neurochemical experiments. They were housed in pairs in ventilated cages with an Air Handling Unit (Tecniplast, West Chester, PA) under controlled conditions of temperature (22±1°C), and humidity (50±10 %), and were maintained under 12-hour light-dark cycle (lights on between 07:00 AM and 5:00 PM). Experiments were conducted during the light period. Rats were handled for seven days before starting the experiments. The food and water access were *ad libitum*.

All procedures were in strict accordance with the guidelines published in the 8th edition of the NIH Guide for the Care and Use of Laboratory Animals as well as principles presented in the *Guidelines for the Use of Animals in Neuroscience* Research by the Society for Neuroscience. Furthermore, all procedures were approved by the ethics committee of the Pontificia Universidad Católica de Chile as well as by the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia.

5.3.2 Behavioral Testing

5.3.2.1 Apparatus

Sessions were conducted in ventilated, sound-attenuating enclosures containing an operant conditioning chamber with an interior space of measures 30.5×24×21 cm (Med-Associates, St. Albans, VT, USA). Each box has a house-light which is turned on at the beginning of the tests, two levers associated to another light in the chamber and a dispenser of food reinforcement (sucrose pellet, 45mg; Bioserv, Frenchtowm, NJ, USA) between them. All experimental data was recorded by an IBM personal computer connected to the chamber via an interface using the Med-PC-IV software (Med-Associates, St. Albans, VT, USA).

5.3.2.2 Procedures

Rats were tested in the behavioral procedures as described below. All procedures were adapted from Eubig P et al., [44].

5.3.2.2.1 Initial lever press training

Approximately after PND 35, a shaping program was used to train rats to begin pressing the response levers. Both levers were extended at the onset of the session. As described in Eubig P et al., [44] the delivery was programmed according to a 3-min fixed-time schedule, in which a food pellet was dispensed every 3 min. Also, one pellet was delivered with every lever press. Sessions finished after either 60 min elapsed, or 100 reinforcements were delivered. Criterion for advancement to the next phase of training was 100 lever presses, performed for three consecutive sessions.

5.3.2.2.2 Fixed ratio training

The second training phase began between PND 39-40. During this phase, only one lever was extended, and the corresponding cue light was illuminated. After five lever presses, the extended lever was retracted, and the opposite lever was extended. Every response resulted in the delivery of a food pellet (i.e. a fixed ratio 1 contingency). After 100 reinforcements across three consecutive sessions, rats continued to the next training phase.

5.3.2.2.3 Delay discounting training and testing

The DDT training phase began approximately after PND 45. The phase consisted of 40 trials for each session. Two levers with different reinforcers were presented and were always accompanied by an illuminated cue light: the lever press of the larger reinforcer (LR) resulted in 4 pellets and the small reinforcer (SR) lever resulted in 1 pellet. There was no delay in the delivery of either the small or large reinforcers in this phase. The session finished after 40 lever presses between large or small reinforcers. Criterion for advancement to the next phase of training required an 80 percent of preference for the larger reinforcer.

The DDT testing began in darkness with the levers retracted (blackout). After 10 minutes of blackout, the house-light turned on and the session started. One session consisted of five blocks comprising 12 trials each. The duration of the trials was of 60 seconds. The first two trials of each block were forced-choice trials in which the LR lever and the SR lever were alternately introduced. Ten free-choice trials followed the force trials in each block. At the beginning of the free-choice trial, the lights over the levers were turned on, and the levers appeared. If a lever was pressed, both levers were retracted. If the rat pressed

the SR lever, one pellet was immediately delivered, and both levers were retracted. If the LR lever was chosen, the stimulus light blinked during the delay and, following the delay, three pellets were delivered. Delays to the LR were increased from 0s in block 1 to 15s in block 2, 30s in block 3, 45s in block 4. Failure to choose a response within 20s resulted in the lever retracting and the trial was considered as an omission. The rats were trained five times per week for approximately ten weeks (50 sessions) in this task until stable performance was achieved. They then were subjected to bilateral implantation of guide cannula in SNpc.

5.3.2.3 Bilateral microinfusion in the SNpc

Rats were given a subanesthetic dose of ketamine (50 mg/kg) and xylazine (4 mg/kg) and were maintained on isoflurane for the whole duration of the surgery. Bilateral implantation of guide cannulas (22G) was performed on the SNpc, and these guides were fixed to the skull with stainless steel screws and dental cement. The following stereotaxic coordinates were used: -5.6 AP from bregma, ±2.1 ML and -8.1 DV, according to Paxinos and Watson [45]. Animals were allowed to recover for one week before resuming the behavioral test.

The microinfusions procedure, was adapted from Winstanley C.A [46]. Once the impulsive choice behavior was re-established post-surgery animals received a mock infusion prior to a training session to familiarize them with the procedure prior to the actual microinfusion test day (see, figure 1). Injectors were placed inside the guide cannula for 2 minutes, but no microinfusion was administrated. Rats were then placed in their home cage for 15 min until their training sessions started. The animals received their first

microinfusion 1 to 3 days after the mock injection. The animals trained to DDT received two bilateral microinfusions with either a saline or a non-peptide OXT-R agonist (WAY267464, 3μg/0.5μl), according to a counterbalanced design [25,47]. For all animals, the saline or drug was infused at a volume of 0.25 ul and the microinfusion was made using a dual channel microinfusion pump (Harvard Apparatus, Holliston, MA, USA).

5.3.3 Delay discounting analysis

5.3.3.1 The logarithmic area under curve (AUC_{log})

Delay-discounting curves were obtained by plotting the proportion of LR choices (the number of LR choices divided by the total number of successful trials) of each block versus each delay (sec). The area under the delay-discounting curve (AUC) was used as a measure of impulsive choice [48,49]. The AUC was calculated using a log-transformation of delays (AUC_{log}) to avoid the disproportionate contribution at long delays in the delay-discounting curves [50]. Specifically, each delay was transformed to the logarithm of the delay divided by the bigger logged delay. To avoid the problem of calculating the logarithm of zero, a small amount (value=1) was added in each delay before the transformation. We use GraphPad Prism to calculate AUC_{log} (version 5, GraphPad Software, Inc.; La Jolla, CA). Higher AUC_{log} values indicate lower discounting by delay (i.e., preference for larger rewards, indicating lower impulsivity), while lower AUC_{log} values correspond to higher discounting by delay (i.e., preference for small and immediate rewards, indicating higher impulsivity) [48].

5.3.3.2 Behavioral Stability and Classification

To assess behavioral stability, we compared the AUC_{log} of five consecutive sessions (week 1) with the five following sessions (week 2). An animal was considered trained when the difference between weeks was not statistically significant. For each animal, a stable AUC_{log} value was calculated using the average of the last five sessions. Rats were classified as low impulsive (LI) and high impulsive (HI) based on the median split of AUC_{log} (see figure 3A and B). The minimum sample size was calculated based on a priori power analysis [51]. The input parameters for behavioral experiments were the following: two-tails, alpha= 0.05, power= 0.80, and ratio N1/N2= 1. The effect size was based on preliminary DDT results, suggesting a minimum sample size of n= 4/group for the experiment considering a Cohen's d effect size of 2, using G*Power statistical analysis software Version 3.1.9.4 [52].

5.3.3.3 Omission and latencies

Latency was defined as the time since the levers were extended until the rat pressed one of them during free-choice trials in each block. An omission was considered as a failure to choose a response within 20s after lever appearance. For each animal, we recorded the latency and the number of omissions in the 4 daily blocks in free-choice trials. Final latency and omission values were calculated averaging total latency and omissions of the last five sessions.

5.3.4 Conventional Microdialysis

To study the effect of OXT-R activation in the nigro-striatal dopaminergic pathway induced by perfusion of a non-peptide OXT-R agonist (WAY267464), due to its chemical characteristics linked to the stability of the molecule and half-life. Altogether, it is an excellent candidate to cross the blood-brain barrier [53,54]. *In vivo* microdialysis in anesthetized animals was performed at approximately PND 60-75. Rats were deeply anesthetized with urethane (1.5 g/kg i.p.) and placed in a stereotaxic apparatus. Then, two microdialysis probes (CMA-11 Microdialysis, Holliston, MA, USA) were implanted into the brain: one in the DLS, using the following coordinates according to the atlas of Paxinos and Watson (2009): +1.4 AP, +3.2 ML, -4.8 DV; and the other in the SNpc, using the following coordinates: -5.0 AP, +2.1 ML y -8.1 DV. During the surgery, the body temperature of the animals was maintained and monitored.

5.3.4.1 Microdialysis Protocol

The microdialysis probe in the DLS was continuously perfused with a Krebs ringer phosphate solution (KRF; containing NaCl 120 mM; KCl 2.4 mM; $CaCl_2$ 1.2 mM; 0.9 mM NaH_2PO_4; Na_2HPO_4 1.4 mM, pH = 7.4) at a flow rate of 2 µl/min using a Hardvard infusion pump. The microdialysis probe in the SNpc was perfused with KRF during a stabilization period of 100 min, and three consecutive samples were collected every 5 min for determination of an average DA basal level in SNpc and DLS. After that, to corroborate the activation of the nigro-striatal pathway, two KRF potassium solutions (70 mM) were perfused in the SNpc for 5 minutes; only experiments where perfusion of K^+ in SNpc induced an < 20% increase in DA_{ext} levels in the DLS were used. Meanwhile, the collection of samples also continued every 5 minutes in the SNpc and DLS. After that,

three different concentrations of KFR/WAY267464 (5μM, 20μM y 50μM) were perfused` for 10 minutes [54]. Furthermore, the nonpeptide antagonist for OXT-R (LY369 100 mM) was also perfused (adapted doses from C.E king et al., [55]). Three consecutive samples were collected in each experimental condition. The collected perfusion samples were maintained on ice during the experiment and stored at −20°C until analysis.

5.3.4.2 Analysis of Dialysate Dopamine

Ten ml of the dialysate was injected using a Rheodyne injector valve into an HPLC system (BASi America, West Lafayette, IN). The mobile phase contained 100 mM NaH2PO4, 1 mM EDTA, 1 mM octane-1-sulfonic acid sodium salt, and 3% acetonitrile (pH 3.0), and was pumped at a flow rate of 700 μL/min. The potential of the amperometric detector was set at 650 mV. Under these experimental conditions, the retention time for DA was 6 min. Dialysate samples were analyzed by comparing the peak area and elution time with reference standards (ChromNAV 2.0 Jasco Co, ltd, Tokyo, Japan).

5.3.5 Histology

After the behavioral test and microdialysis experiments, rats were decapitated under deep anesthesia (urethane 1.5 g/kg i.p.), and brains were extracted and cleaned with a saline solution (NaCl 0.9%). Brains were stored in 4% paraformaldehyde (PFA). At least two days before slicing, brains were cryoprotected using a 30% sucrose solution. For probe implantation, brains were frozen and coronally sliced in sections of 50 μm. Slices were stained with cresyl violet and the probe placement was observed in a light microscope

using the atlas of Paxinos & Watson for rats [41]. Only data coming from correct probe placements was considered for further analysis (see figure 1 and 4).

5.3.6 Data Analysis

5.3.6.1 Statistical Analyses

All statistical analyses were carried out using Prism 5.0 GraphPad Software. Each group of data was analyzed with the nonlinear regression ROUT method (Q= 1%) [56] to identify outliers and exclude them from the statistical analysis and graphs. No outliers were found in these studies. Resultant data were analyzed using unpaired t-test, one and two-way ANOVA with Bonferroni post-test when appropriate (all data are reported as mean ± SEM).

5.4 Results

5.4.1 Effect of SNpc administration of WAY267464 on impulsive choice during delay discounting task

To assess the effect of OXT-R activation in DLS on impulsive choice during DDT, adult male *Long-Evans* rats were well-trained on a DDT. On separate test days, they received bilateral infusions of the OXT-R agonist (WAY267464) at a dose of 3µg/0.5µl in the SNpc (see experimental design in figure 1A). The bilateral infusion of WAY267464 in SNpc, which induces a decrease in impulsive choice, showed significant difference in the number of choices during the delays of 15, 30 and 45 seconds between saline and WAY267464 groups according to two-way ANOVA (n= 8; Bonferroni test: delay 15: Saline: 0.42± 0.07 vs WAY267464: 0.62± 0.07 p= 0.03; Delay 30: Saline: 0.25± 0.07 vs WAY267464: 0.47± 0.07 p= 0.01; Delay 45: Saline: 0.19± 0.07 vs WAY267464: 0.38± 0.07 p= 0.04, see figure 2A). The bilateral infusion of WAY267464 in the SNpc induced an increase in the area under the curve (AUC_{log}) pointing to a higher preference to the larger reinforcement (n= 8; t-test p= 0.02, see figure 2B). The response latency and omissions in control (saline perfusion) and agonist OXT-R (WAY267464) experimental conditions showed non-significant differences between the two experimental conditions (latency: n=8 t-test; p= 0.35, see figure 2C and omission: n=8; t-test; p= 0.41; see figure 2D). Finally, this experiment showed that bilateral OXT-R activation in SNpc induced a decrease in impulsive choice behavior during DDT.

5.4.2 The effect of bilateral OXT-R activation in SNpc depends on basal impulsivity

An exploratory analysis was performed from the previous results, where we used the behavioral classification using AUC_{log} values during DDT as previously described (see methodology). From these, two groups were formed: LI (n =4) and HI (n =4) (see figure 3A and B). The effect of OXT-R activation is dependent on basal impulsivity levels, can be observed in animals previously classified as LI or HI (see Figures 3C and 3E). The bilateral OXT-R activation in LI animals showed a non-significant difference in the number of large reinforcement choices during delays of 15, 30, and 45 sec observed between saline and WAY267464 condition (according to Two-Way ANOVA; n= 4; Bonferroni test: delay 15: Saline: 0.65± 0.09 vs WAY267464: 0.72± 0.09 p> 0.99; Delay 30: Saline: 0.45± 0.09 vs WAY267464: 0.66± 0.09 p= 0.16; Delay 45: Saline: 0.39± 0.09 vs WAY267464: 0.51± 0.09 p= 0.91, see figure 3C) and non-significant different in the AUC_{log} value between saline and WAY267464 conditions (n= 4; t-test p= 0.19, see figure 3D). In HI animals, the bilateral OXT-R activation induced a decrease in impulsive behavior, with significant differences for 15 seconds delay (according to Two- Way ANOVA, Saline: 0.17± 0.11 vs WAY267464: 0.52± 0.11 p= 0.05, see figure 3E) and a non-significant difference in the AUC different in the AUC_{log} value between saline and WAY267464 conditions (n= 4; t-test p= 0.05, see figure 3F).

5.4.3 Effect of non-peptide specific OXT-R agonist (WAY267464) administration in SNpc on DA_{ext} levels in DLS

To fully understand how the OXT system modulates the DA release from mesencephalic neurons, we also studied the consequences of the activation of OXT-R in the SNpc on DA_{ext} level in the nigro-striatal pathway. We perfused a non-peptide agonist WAY267464 in the SNpc and collected samples from the SNpc and the DLS (see figure 4). First, to corroborate the activation of the nigro-striatal pathway, we perfused a depolarizing stimulus of K^+ solution (70 mM) in SNpc and measured DA_{ext} levels in SNpc and DLS. The only experiments used were those where perfusion of K^+ in SNpc induced an > 20% increase in DA_{ext} levels in the DLS (according to paired t-test: DLS: p= 0.004 and SNpc: p= 0.038; n=5, see figure 5A and 5B). To verify the oxytocinergic endogenous tone in SNpc, we used an OXT-R antagonist LY369 (100 mM). The perfusion of OXT-R antagonist in SNpc did not produce any change in DA_{ext} levels in the DLS and SNpc (according to paired t-test: DLS: p= 0.64; SNpc: p= 0.65, n=5, see figure 6A). Subsequently, to study the effect of OXT-R activation, we perfused different concentrations of WAY267464 (5, 20, and 50 µM). The experiment showed that the effect of OXT-R activation in SNpc is dose-dependent, and low doses (5 µM) generated a moderated but significant effect, inducing a subtle increase in DA_{ext} levels in the DLS (according to paired t-test; p= 0.05; n= 5, see figure 6 B) but a non-significant change in SNpc (according to paired t-test; p= 0.64 n=5). On the other hand, high doses (20-50 µM) did not have a significant effect in both DLS (according to paired t-test; 20 µM p= 0.08; 50 µM p= 0.15; n=5, see figure 6B) and SNpc (according to paired t-test; 20 µM p= 0.42; 50 µM p= 0.25; n=5, see figure 6C).

5.5 Discussion

Various approaches have suggested that hypothalamic oxytocinergic projection modulates midbrain DA regions [26,36], but the neurochemical DA dynamic and behavioral consequences of this potential interaction are not yet completely known. Here, we study the activation of OXT-R in the SNpc during DDT and its contribution to impulsive choice behavior. Moreover, we examine the effect of OXT-R activation in SNpc on DA_{ext} level in the nigro-striatal pathway.

Our behavioral experiment indicated that the bilateral activation of OXT-R by microinfusion of WAY267464 induces a decrease in impulsivity reflected in an increase in the number of choices for the LR. Due to the individual differences present in impulsive choice, this research showed that in animals classified as HI, the bilateral activation of OXT-R in SNpc decreases impulsive behaviors compared to the LI group. The difference between groups suggests that the OXT-R activation in SNpc could induce a differential effect in the nigro-striatal pathway, which could depend on the DA basal dynamic that underlies individual differences present in impulsive choice behavior. These results are in line with previous results (see chapter 1), where individual differences in impulsive choice level during DDT are accompanied by a differential change of DA in DLS. The individual differences in impulsive choice behavior could be due to changes in the expression and activity of D_2-R presynaptic in DLS. Several clinical and preclinical research has shown that prolonged exposure to methamphetamine, amphetamine, or cocaine reduced D_2-R expression in the midbrain region, such as the SN, VTA, and striatum, leading to high levels of impulsive choice [11,57,58]. It is then possible to propose that the differences

observed in HI animals are due to a loss of the inhibitory tonic control of D_2-R [59]. This basal neurophysiological condition could be modulated differentially by the activation of OXT-R in the nigro-striatal pathway. Interestingly, Xiao et al., [26] showed that the OXT-R activation in SNpc induces a heterogeneous set of responses, dependently on topographic location into SNpc; the dorsolateral OXT-R activation induces a decrease in the activity of dorsolateral SNpc DA neurons via inhibitory circuits, but in the medial region of SNpc did not find a significant change in medial SNpc DA neurons. These different topographical effects are generated by the high density for OXT-R in the dorsolateral region than the medial region of SNpc. Hence, we suggest that the mechanism associated with the bilateral activation of OXT-R could be linked to a decrease in the dopaminergic activity due to the activation of GABAergic neurons that express OXT-R. In this context, Xiao et al., [26] identified a high co-localization of OXT-R in cell $Vgat^+$ (vesicular GABA transporter) in dorsolateral SNpc, compared to co-localization of the canonical marker of DA neurons, tyrosine hydroxylase (TH). In this sense, the increment in GABA signaling could be an indirect mechanism that provokes a hyperpolarization of membrane potential, causing a decrement in the DA FR in SNpc [26,60], producing a decrease of DA levels in the DLS [61,62]. This modulation could regulate the behavior by altering the tonic activity of DA striatal tone, helping to recover neurochemical homeostasis linked to impulsive behavior. Alternatively, we speculated that the decrease in impulsive choice level might be due to bilateral OXT-R activation in SNp dorsolateral, improving the somatodendritic D_2-R affinity for DA. It has been shown that an increase in the concentration of ligands for OXT-R induces an increase in the affinity of D_2-R for

DA [37,63–65], which induced changes in the dopaminergic activity during the presence of a salience stimulus, facilitating advantageous decision making [66,67].

In these behavioral experiments, a direct measure of locomotor activity and motivation was not collected. However, during DDT, the response latency and omissions were recorded, which could be used as indirect locomotor activity and motivation measures, respectively [68,69]. Therefore, we analyzed the changes in response latencies to assess whether the motor activity variations through the delay blocks were not related to the different levels of impulsive choice between experimental conditions. On the other hand, we analyzed the number of omissions to assess whether the variations in motivational behavior were not related to the experimental condition in the impulsive choice levels. Thus, these results indicate that the microinfusion of WAY267464 in SNpc only affect neurobiological mechanisms linked to decision making.

Exist evidence that OXT neuron from the PVN project to VTA and SNpc [26,70]. To identify if there is a tonic control of OXT, we used a selective non-peptide antagonist for the OXT-R receptor LY369 (100mM) in SNpc. This antagonist perfusion induced non-significant differences in the DA_{ext} level of the SNpc and the DLS, which suggests that no endogenous OXT tone exists in the SNpc. This finding is consistent with evidence that proposes that the dynamic of endogenous OXT release depends on the nature of the stimulus —for example, social interaction or parental care—promoting this OXT release[71,72]. On the other hand, our neurochemical approached shows that the activation of OXT-R by perfusion of WAY267464 in SNpc induces a non-significant difference in DA_{ext} level in SNpc. This result is in line with the heterogeneous set of

responses showed by Xiao et al., [26]. As presented above, the effect of oxytocinergic activity on SNpc is topographically dependent, reporting that the dorsolateral region of the SNpc is where the highest density and collocation of OXT-R is found in GABAergic neurons, and that the release of OXT or activation of OXT-R in dorsolateral region induces a decrease in the FR of the DA neurons of the SNpc. This finding could explain why we did not find a significant change in the somatodendritic release of DA in the SNpc, because the agonist's perfusion was done in more medial regions, making the activation of OXT-R difficult.

Interestingly, when analyzing the axonal DA release post-perfusion of WAY267464 (5μM), we were able to identify a modest increase in DA_{ext} levels. This subtle dose-dependent increase could be due to compensatory changes made to maintain homeostasis of the nigro-striatal pathway for its optimal functioning of the motor control, against the decrease of the FR of the DA neurons coming from the SNpc, and this compensatory mechanism is supported by numerous studies involving animal with varying striatal depletion [73–75]. In line with this evidences, it has been reported that the decrease of the FR, associated with the increase of the GABAergic activity in the SNpc, specifically by the activation of $GABA_A$ receptors in interneurons, can induce an increase of the axonal DA release in the DLS [76]. The SNpc perfusion of higher concentrations of WAY267464 (20-50μM, respectively) did not produce any significant changes in the DA_{ext} levels in the DLS as SNpc. These results could be related to the activation of the different variants of OXT-R (G_i), as it has been shown that the concentration necessary for the activation of the OXT-G_q receptor is much lower than for the OXT-G_i receptor [77,78]. Therefore, the use of high concentrations of the agonist could induce the activation of G_i-type receptors,

associated with a decrease in the neuronal excitability, generates a decrease in the probability of neurotransmitter release [79,80] but the expression of this variant in SNpc has not yet been shown. Another mechanism that could be participating is the desensitization and internalization of OXT-R, a mechanism that prevents neuronal hyperstimulation [81]. The repeated acute administration of OXT or agonist of OXT-R induces the rapid and marked desensitization of neuronal response in different brain areas [82,83].

In summary, we reported previously that the individual differences in impulsive choice behavior are associated with a change in the basal DA dynamic in the nigro-striatal pathway. The present research shows that bilateral activation of OXT-R in SNpc generates a differential effect that depends on the basal impulsive choice level; specifically, the activation de OXT-R in SNpc affects highly impulsive animals and induces a decrease in impulsive choice behavior. Interestingly, the activation of OXT-R in SNpc induces a moderate increase in DA_{ext} levels in the DLS, it is necessary to note that the neurochemical experiments did not differentiate between HI or LI animals. Regarding the effect of OXT-R activation on reducing impulsive behavior, further research is necessary to elucidate the role of the oxytocinergic transmission in the nigro-striatal pathway on DA levels during impulsive choice and the mechanisms underlying the low discounting rate of delayed reward.

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1. Figures

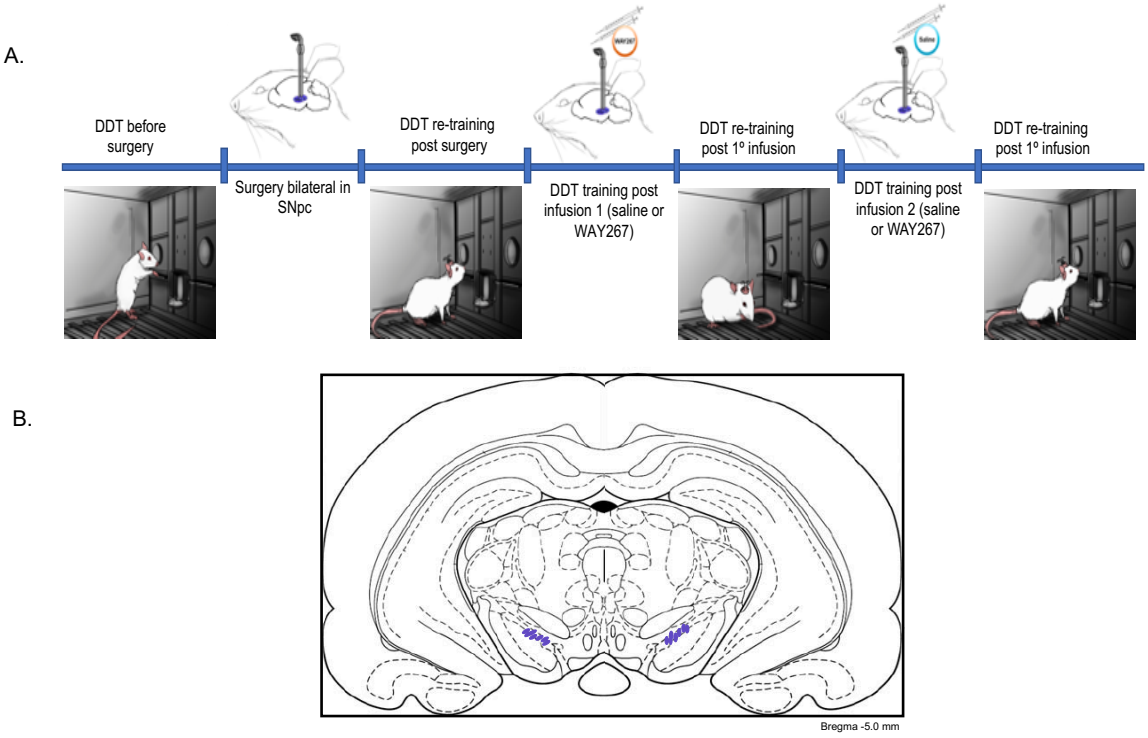


Figure 1: Experimental design of micro-infusion of WAY267 in SNpc and histology scheme. (A) Experimental design. **(B)** Histology. Schematic coronal sections of the rat brain showing the placements of the cannula for all rats in the experiment that evaluated the effect of OXT-R activation in SNpc on the behavior of impulsive choice. Rats received micro-infusion of OXT-R agonist (WAY267464) or vehicle (saline) into the SNpc. Brain sections correspond to the atlas of [44].

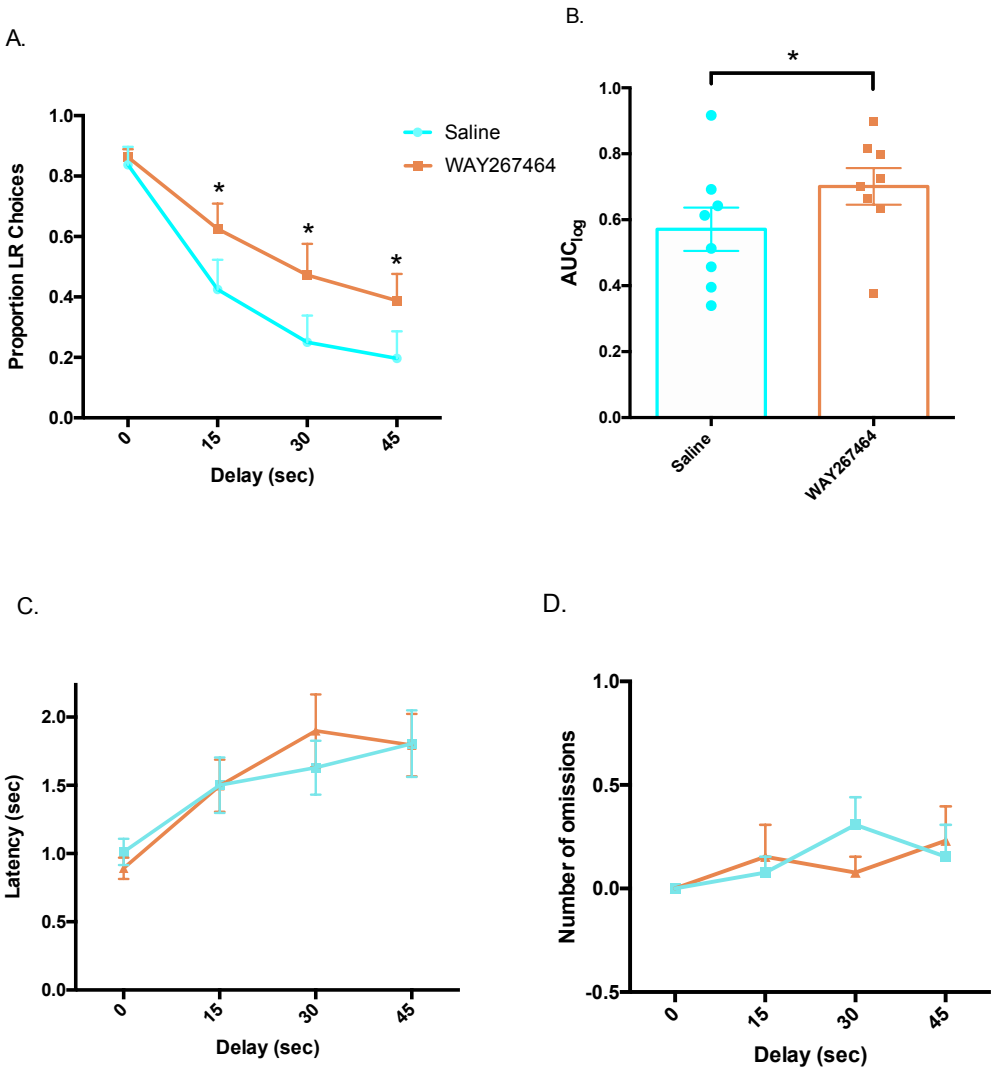


Figure 2: Effect of bilateral OXT-R activation in SNpc during DDT. (A) Delay-discounting curve of basal (saline microinfusion) condition and bilateral microinfusion of non-peptide agonists WAY267464 (3µg/0.5µl) in SNpc. Showed significant difference in the number of choices during the delays of 15, 30 and 45 seconds between saline and WAY267464 groups according to two-way ANOVA (n= 8; Bonferroni test: delay 15: Saline: 0.42± 0.07 vs WAY267464: 0.62± 0.07 p= 0.03; Delay 30: Saline: 0.25± 0.07 vs WAY267464: 0.47± 0.07 p= 0.01; Delay 45: Saline: 0.19± 0.07 vs WAY267464: 0.38± 0.07 p= 0.04). **(B)** The bilateral microinfusion of WAY267464 in the SNpc induced an increase in the area under the curve (AUC_{log}) pointing to a higher preference to the larger reinforcement (n= 8; t-test p= 0.02). **(C)** Graph of response latency during the DDT showed no differences between the two experimental conditions (n=8 t-test: p= 0.35). **(D)** Number of total omissions during free choice showed non-significant differences between the two experimental conditions (n=8; t-test; p= 0.41).

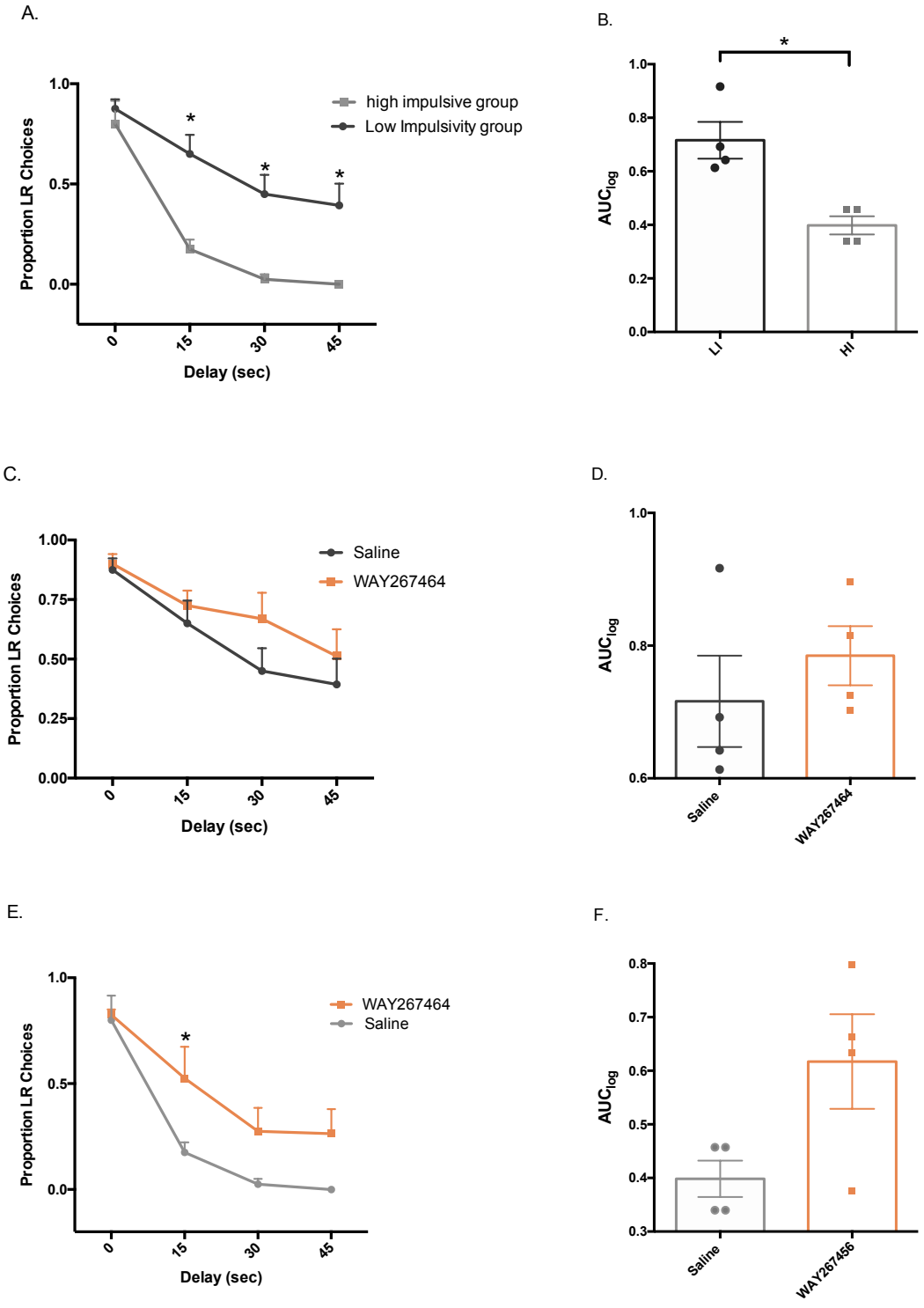


Figure 3: Differential effect of bilateral OXT-R activation in SNpc by microinjection of WAY267464 (3 ug/ul) and saline solution. **(A)** Delay discounting curve of HI and LI

animals as classified by the AUC_{log} criteria (LI=4; HI=4). Showed significant difference in the number of choices during the delays of 15, 30 and 45 seconds between HI and LI groups according to two-way ANOVA (n= 4/group; Bonferroni test: delay 15: LI: 0.65± 0.08 vs HI: 0.17± 0.08 p= 0.001; Delay 30: LI: 0.45± 0.08 vs HI: 0.02± 0.08 p= 0.002; Delay 45: LI: 0.39± 0.08 vs HI: 0.1± 0.08 p= 0.003). **(B)** HI animals showed an increase in the AUC_{log} pointing to a higher preference to the larger reinforcement compared to LI animals (n= 4/group; t-test p= 0.01). **(C)** Bilateral microinfusion of non-peptide agonists WAY267464 (3µg/0.5µl) in SNpc. For the LI group, there were non-significant differences in the number of large reinforcement choices during delays of 5, 30 and 45 seconds according to two-way ANOVA (n= 4; Bonferroni test: delay 15: Saline: 0.65± 0.09 vs WAY267464: 0.72± 0.09 p> 0.99; Delay 30: Saline: 0.45± 0.09 vs WAY267464: 0.66± 0.09 p= 0.16; Delay 45: Saline: 0.39± 0.09 vs WAY267464: 0.51± 0.09 p= 0.91). **(D)** The bilateral microinfusion of WAY267464 in the SNpc induced a non-significant difference in AUC_{log} pointing to a higher preference to the larger reinforcement (n= 4; t-test p= 0.19). **(E)** Bilateral microinfusion of non-peptide agonists WAY267464 (3µg/0.5µl) in SNpc. For the HI group, there were one significant difference in the number of large reinforcement choices during delay of 15sec and non-significant differences in 30 and 45 seconds according to two-way ANOVA (n= 4; Bonferroni test: delay 15: Saline: 0.17± 0.11 vs WAY267464: 0.52± 0.11 p= 0.05; Delay 30: Saline: 0.02± 0.11 vs WAY267464: 0.27± 0.11 p= 0.21; Delay 45: Saline: 0.0± 0.11 vs WAY267464: 0.26± 0.11 p= 0.16). **(F)** The bilateral microinfusion of WAY267464 in the SNpc induced a non-significant difference in AUC_{log} pointing to a higher preference to the larger reinforcement (n= 4; t-test p= 0.05).

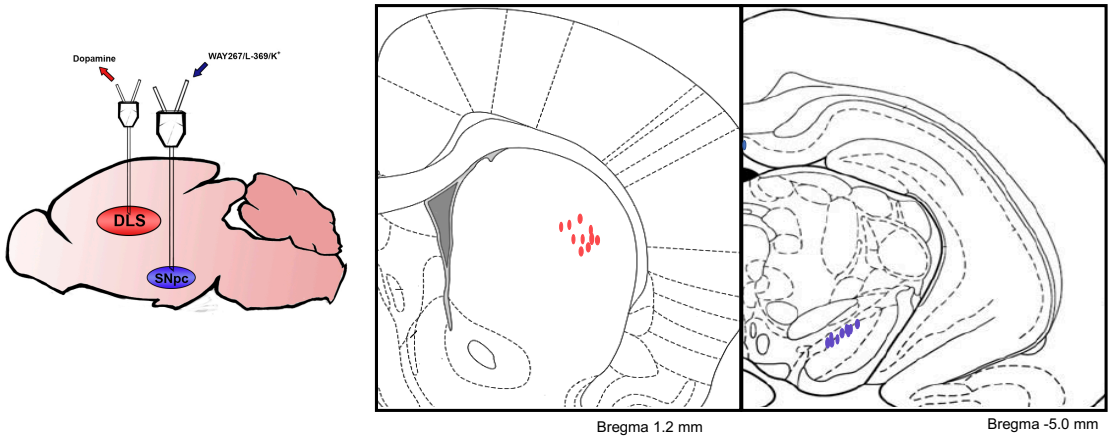


Figure 4: Representative scheme of probe locations during the microdialysis protocol. On the **Left**, the scheme shows two probes: one probe in SNpc in which different concentrations of WAY267464, K^+ solution and the OXT-R antagonist (LY369) were perfused. Another probe was inserted in the DLS to measure extracellular DA levels. On the **Right:** Histology. Schematic coronal sections of the rat brain showing the placements of the microdialysis cannula tips for all rats in the experiment analyzing the effect on DLS extracellular DA levels due to OXT-R activation in SNpc. Brain sections correspond to the atlas of Paxinos and Watson [45].

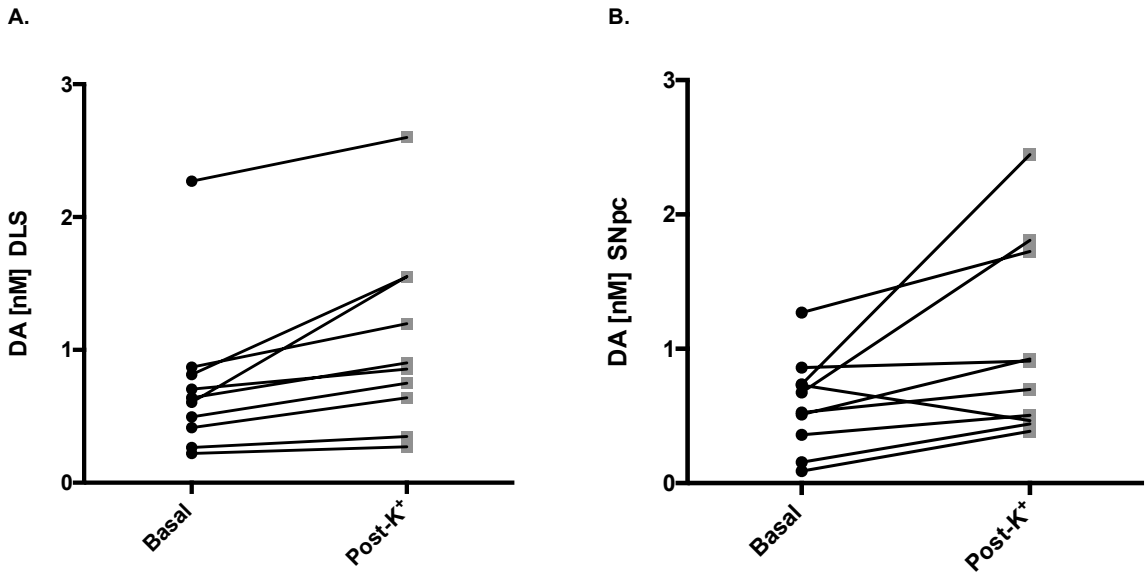


Figure 5: The effect of perfusing the SNpc with a high concentration of Potassium (70mM) on DA_{ext} in the nigro-striatal pathway. (A) and **(B)**, Perfusion with depolarizing potassium solutions (70 mM) for 5 min in SNp, >20% of increase DA_{ext} level in the DLS and SNpc, respectively (according to paired t-test: DLS: p= 0.004 and SNpc: p= 0.038; n=10).

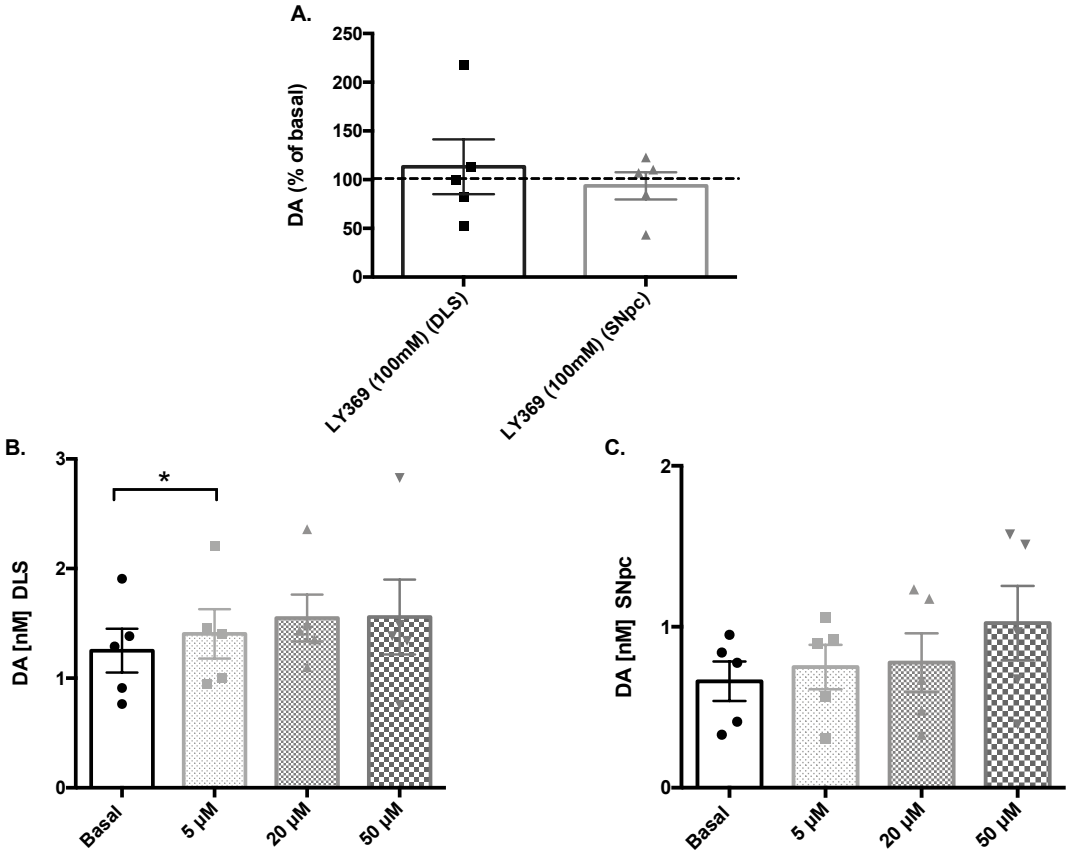


Figure 6: Effect of OXT-R activation in SNpc on DA_{ext} levels in DLS and SNpc. (A), showed that the perfusion of OXT-R antagonist LY369 (100mM) in SNpc, did not produce any change in DA_{ext} levels in DLS and SNpc (according to paired t-test: DLS, p= 0.64; SNpc, p= 0.65, n=5). **(B)** and **(C)** Perfusion of different concentrations of OXT-R agonist WAY267464 (5, 20, 50 mM) in the SNpc on DA_{ext} levels in DLS and SNpc, respectively. DA is expressed as nM concentration. **(B)** Results showed a moderated but significant increase in DLS DA_{ext} levels after infusion of WAY267464 (5 mM) (according to paired t-test; p= 0.05; n= 5). **(C)** results showed a non-significant change in DA_{ext} levels in SNpc after infusion of WAY267464 in the SNpc (according to paired t-test; 20 µM p= 0.42; 50 µM p= 0.25; n=5).

6 General Discussion

Evidence supports the notion that variability in dopamine (DA) neurotransmission function is a major contributor to individual differences observed in impulsive choice (Joutsa et al., 2015; Van Gaalen et al., 2006). Interestingly, various approaches have suggested that hypothalamic oxytocinergic projection modulates midbrain DA regions (Hung et al., 2017; Xiao et al., 2017), but the neurochemical DA dynamic and behavioral consequences of this potential interaction are not yet completely known. Here, we report that a higher DLS DA release was observed in HI rats than LI rats. Also, a significant negative correlation was observed between AUC and DA release. These results suggest that an increased DLS DA signaling contributes to delay-related decision-making. Subsequently, we showed that activation of the OXT-R in the SNpc induces a decreased impulsive choice in HI rats, displaying a greater preference for larger delayed rewards. Secondly, the OXT-R activation in SNpc induces a slight increase in DA_{ext} level in DLS but does not induce a change in DA_{ext} level in SNpc. This latter finding suggests that activation of the OXT-R in the SNpc influences delay-related decision making by subtle changes in the striatal dopamine transmission.

Our first general hypothesis was modified due to technical drawbacks that did not allow us to study one of the research's initial focuses associated with identifying oxytocinergic transmission variations. Specifically, studying changes in the expression of OXT-R in the SNpc present at impulsive choice behaviors; this could not be studied due to the low specificity of the existing antibodies for OXT-R used in the colorimetric immunohistochemistry (Yoshida et al., 2009). Therefore, labeling, identification, and quantification under these conditions made it impossible to obtain a reliable result. For

this reason, we reformulated the general hypothesis to fully evaluate it by the different experimental designs performed in this doctoral research. The new general hypothesis was formulated based on the finding by Tedford et al., (2015), where they associated high levels of impulsive choice with dopaminergic hypofunction. Moreover, evidence has showed that DA and OXT interaction (Hung et al., 2017; Xiao et al., 2017). Clinical studies showed a significant association between basal plasma OXT and the Karolinska Scale of Personality (KSP) factor impulsiveness, suggesting a positive correlation between impulsiveness and plasma OXT level in male psychiatric outpatients (Bendix et al., 2015).

The new general hypothesis was partially corroborated by the results obtained in chapters 1 and 2, showing that high DA release in DLS is associated with higher levels of impulsive choice and that increase in the oxytocinergic activity in the nigro-striatal pathway induce a decrease in the impulsive choice. In order to corroborate the specific hypothesis stated in chapter 1, our objective was to evaluate the relationship between delay discounting behavior and DA dynamics in the DLS in rats, but the hypothesis was not true; our results showed that a higher DLS DA release was observed in HI rats compared with LI rats. A significant negative correlation was observed between AUC and DA release, indicating that lower AUC is associated with higher DLS DA release. Notwithstanding the limitations of our study, these results suggest that an increased DLS DA signaling contributes to delay-related decision-making.

The increased DA release observed in rats classified as HI could be explained by a presynaptic mechanism associated with a loss of the inhibitory tonic control of D_2

receptors on DA extracellular concentration (Mayer et al., 1988); it has been proposed that individual differences in D_2 receptor mRNA expression predict different levels of impulsive choice (Simon et al., 2013). Furthermore, it could be explained by the postsynaptic mechanism associated with an increase in population activity or bursting activity of DA neurons in the SNpc that contribute to HI animals (Martinez et al., 2019). Our results showed that DA neurotransmission in DLS contributes to individual differences in impulsive choice. The increased DLS DA release observed in HI rats could maintain activated postsynaptic DA receptors in the dorsal striatum responsible for increasing delay aversion (Martinez et al., 2019).

On the other hand, the specific hypothesis studied in chapter 2 was fully corroborated. For this purpose, we evaluated the effect of the bilateral activation of OXT-R in SNpc in impulsive choice behavior. Our finding showed that bilateral microinfusion of WAY267464 induces a decrease in impulsivity. Due to the individual differences present in impulsive choice, this finding showed that in animals classified as HI, the bilateral activation of OXT-R in SNpc decreases impulsive behaviors compared to the LI group. Xiao et al. (2017), showed that the OXT-R activation in SNpc induces a heterogeneous set of responses, dependently on topographic location into SNpc; the dorsolateral OXT-R activation induces a decrease in the activity of dorsolateral SNpc DA neurons via inhibitory circuits, but in the medial region of SNpc did not find a significant change in medial SNpc DA neurons. In this sense, the mechanism underlying the effect exerted by OXT-R activation on impulsive behavior could be linked to an increment in GABA signaling, which could be an indirect mechanism that provokes a hyperpolarization of membrane potential, causing a decrement in the DA FR in SNpc (Lobb et al., 2010; Xiao

et al., 2017), producing a decrease of DA levels in the DLS (Waddington, 1980; Wood, 1982). This modulation could regulate the behavior by altering the tonic activity of DA striatal tone, helping to recover neurochemical homeostasis linked to decreased impulsive choice behavior. Another alternative mechanism could be associated with improving the somatodendritic D_2-R affinity for DA. It has been shown that an increase in the concentration of ligands for OXT-R induces an increase in the affinity of D_2-R for DA (Cragg & Greenfield, 1997; de la Mora et al., 2016; Romero-Fernandez et al., 2013; Stagkourakis et al., 2016), which induced changes in the dopaminergic activity during the presence of a salience stimulus, facilitating advantageous decision making (Bilder et al., 2015).

Various research has attributed an essential role to striatal regions involved in the inhibitory control of cognitive operations, in the positive reward choice (Ghahremani et al., 2012; Robinson et al., 2009), and in the habits and routines (Tricomi et al., 2009). In line with these, neuroimaging studies have proven that in response to a stimulus experimented as gratifying, it correlates with an increase of fast DA tone in ventral and dorsal striatal areas (Volkow et al., 2014). Two types of dopaminergic activity have been identified: low-frequency tonic firing (2–10 Hz), which determines basal extracellular DA level, and phasic firing patterns (≥20 Hz), which produce transient large-amplitude increases in DA level (Floresco et al., 2003; Grace & Bunney, 1984; Siciliano et al., 2015). The phasic activity of DA neurons has been observed during the expectation of rewards (Apicella et al., 1992; Schultz et al., 2015). At the same time, tonic DA modulates performance without altering learning (Beeler et al., 2010). Grace. A, (1991) proposed

that phasic and tonic DA signaling has a separate but complementary role in maintaining the homeostasis of circuits associated with the reward system.

Considering this evidence, we propose the following mechanism: (1) The animals classified as HI (showed more preferences for SR or "NOW" reinforcement) presented higher DA release in the DLS, possibly mediated by presynaptic mechanism (e.g., decrease in the D_2-R activity) or postsynaptic mechanism (e.g., increase in population activity or bursting activity of DA neurons in the SNpc) which could contribute to HI behavior (Ford, 2014; Marcott et al., 2014; Rice & Patel, 2015; Sulzer et al., 2016). The increase of DA release in DLS levels is associated with an increase in the tonic DA tone in HI groups, which could alter the phasic DA activity during the attention of salient stimulus, promoting the choice of SR or "NOW" reinforcement (see supplementary figure 1.B). It has been shown that the hyperactivity of the DA system may attenuate the firing of phasic burst (Stopper et al., 2014) and that diminished tonic DA tone promote an increase in burst firing of DA neurons would result in overstimulation via phasic dopamine release (Floresco et al., 2003). Therefore, it has been proposed that tonic DA release regulates the intensity of the phasic DA response. In this way, the DA tonic release would establish the background level of stimulation of dopamine receptors (Grace. A, 1991). (2) The animals classified as LI (showed more preferences for LR or "LATER" reinforcement) present normal DA release and normal tonic DA tone in the DLS than the HI group (see figure supplementary 1.C). Thus, the expression as an activity mediated by the presynaptic D_2-R receptors is intact, allowing homeostasis between the tonic and phasic DA activity, thus generating an optimum balance in the behaviors associated with the LR or "LATER" preference.

From the results finding in chapter 2, we suggest the following mechanism: (1) There exists a difference between tonic and phasic dopaminergic activity linked to decision making: our results suggest that the HI animals present an imbalance between the tonic-phasic DA tone, showing an alteration in the phasic tone in the presence of salience of stimulus inducing a disadvantages choice or preferences for "NOW" reinforcement (see supplementary figure 1.D). (2) The bilateral activation by the non-peptide agonists of OXT-R microinfusion in the SNpc induces a decrease in the levels of impulsive choice during DDT. As previously discussed, it has been proposed that the increase in DA levels due to OXT-R in SNpc could be facilitating the interaction between presynaptic D_2-R and DA. This mechanism, could balance the homeostasis in phasic striatal dopamine tone, facilitating de LR choice. On the other hand, in animals previously classified as LI, the bilateral activation of OXT-R in SNpc does not significantly alter their behavior. This mechanism could be explained because these LI animals balance the phasic and tonic DA tone, facilitating their flexibility when choosing between LR or SR during the behavioral test (Hikida et al., 2010; Yawata et al., 2012).

Even though theses result differs from previous results (see chapter 1), it was found that high impulsive choice level is associated to high DA basal level in DLS and the OXT-R activation in SNpc decreases impulsive in HI animals. However, low concentration of WAY267464 in SNpc induce a subtle DA increase in DLS. Our neurochemical study did not differentiate HI from LI rats. Future studies are needed to study OXT-R activation, as it may have opposite effects on the nigro-striatal pathway depending on neurochemical basal conditions. The OXT-R have different isomorphs that become activated depending on the concentration of its ligand. This means that they present a higher or lower affinity

for the ligand, depending on their concentration (Busnelli et al., 2012). When analyzing the doses that were used in the infusion and microdialysis, differences can be observed in the concentrations used. This is mainly due to methodologic differences linked to the use of the microdialysis probes, which have a membrane that allows fluid exchange but also interferes in the percentage of the real drug concentration perfused during these protocols. The microinfusion uses a guide cannula into which a microinjector is introduced which doesn't have any kind of membrane at its end, generating an expedited flow of the solution with the desired concentration ensured. Also, it is important to remember the topographical effect of OXT-R in dorsolateral SNpc showed by Xiao et al., (2017).

The present research opens new possibilities for investigation and determination of different mechanisms linked to the individual differences present in impulsive behaviors. It would be fascinating to measure the levels of DA in the DLS during DDT and verify the dynamic changes in basal DA levels during decision making. On the other hand, research on the interaction between the OXT and DA systems with neurochemical and behavioral correlations generates several open questions for future research: (1) What are the dopaminergic neurochemical dynamics during DDT in different impulsive choice behaviors? (2) What is the mechanism through which the activation of OXT-R in SNpc induces changes in DA levels in the DLS? (3) Is the behavioral effect of the bilateral activation of OXT-R in SNpc dose-dependent and mediated only by OXT-R? Further investigations are needed to answer these questions and have the possibility to contribute to our understanding of mechanism associated to dopamine nigro-striatal pathway during impulsive choice behaviors.

7 General Conclusions

1. High levels of impulsive choice behavior are associated to an increase in the release DA in the DLS in anesthetized condition, possibly mediated by presynaptic mechanism.

2. Pharmacological and behavioral data show that bilateral activation of OXT-R in the SNpc by microinfusion of WAY267465 induces a decrease in impulsive choice in high impulsive animals.

3. Neurochemical data shows that the activation of OXT-R through the perfusion of WAY267464 (5 μM) in anesthetized animals, induces a subtle increase in the DA levels in the DLS.

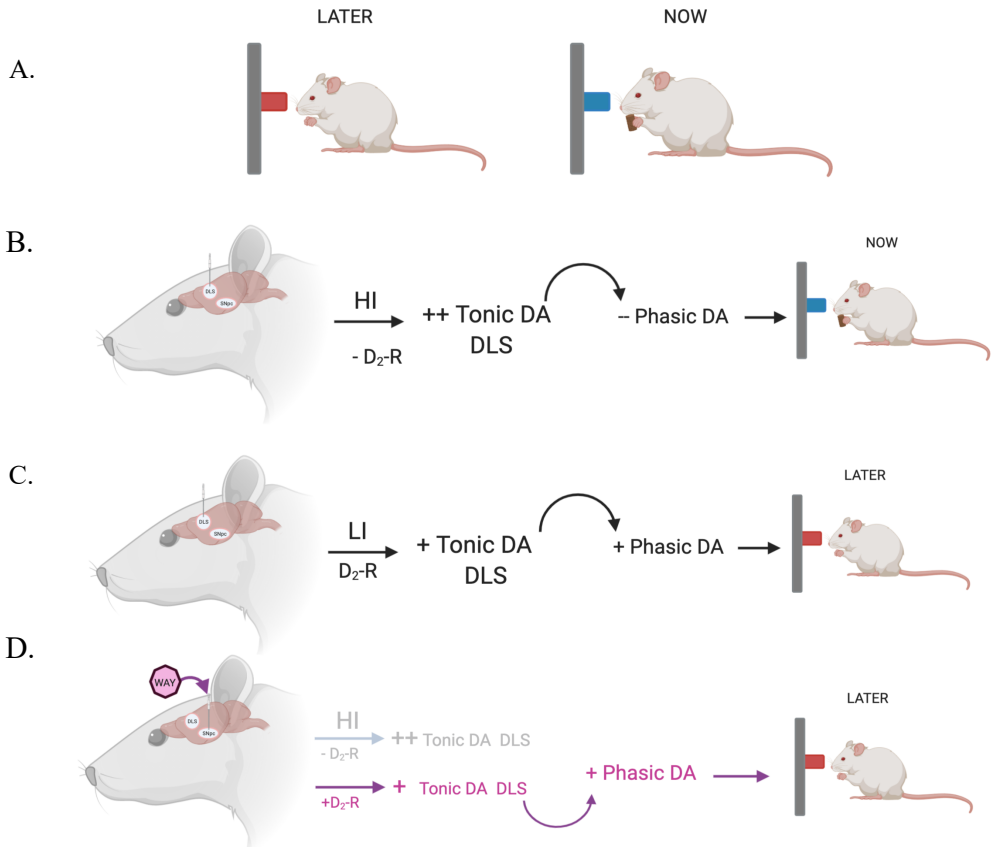
4. The perfusion of the antagonist OXT-R induced non-significant differences in the DA_{ext} level in the SNpc and DLS, which suggest that no endogenous OXT tone exists in the SNpc.

In summary, we reported that DA and OXT neurotransmission contributes to individual differences in impulsive choice. This finding suggests that HI animals have higher tonic levels of DA, so when a salient stimulus is presented, they release less phasic DA that induces a defective coding of a high-value reward. The activation of OXT-R in the SNpc stimulates the GABA signaling, this could be an indirect mechanism that was causing a decrement in the DA FR in SNpc, producing a decrease of DA levels in the DLS (Waddington, 1980; Wood, 1982). This modulation could regulate the behavior by

altering the tonic activity of DA striatal tone, helping to recover neurochemical homeostasis linked to impulsive behavior, facilitating the phasic activity against a salient stimulus, decreasing the impulsive behavior. Several questions remain to be answered. Future research should investigate the neurobiological mechanisms that underlie the dopaminergic and oxytocinergic systems modulation in the nigro-striatal pathway in impulsive choice behavior.

8 Supplementary information

8.1 Proposed Model



Supplementary figure 1: DA basal dynamic activity present in impulsive choice behavior and the effect of OXT-R activation in SNpc during DDT. (A) Different lever press in DDT, **Left**, lever press in red associated to large (LR) or "LATER" reinforcement. **Right**, lever press in blue associated to small (SR) or "NOW" reinforcement. **(B)** High impulsive rats have decreased presynaptic D_2-R activity and higher tonic DA levels in the DLS. Therefore, less phasic DA activity during a stimulus salience presentation, generating a wrong coding of the value reward (favoring SR or NOW choice). **(C)** Low impulsive animal has a normal presynaptic D_2-R activity and tonic DA level in DLS. Therefore, regular phasic DA activity during a stimulus salience presentation, generating a correct coding of the value reward (favoring LR or LATER choice). **(D)** Bilateral activation of OXT-R in the SNpc in HI animals, increase in presynaptic D_2-R affinity for DA and decrease of tonic DA activity, increased phasic DA activity during the presentation stimulus salience, decreasing impulsivity (favoring LATER behavior).

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