Division of Pharmacology and Pharmacotherapy
Faculty of Pharmacy
University of Helsinki Finland

and

Doctoral School in Health Sciences
Doctoral Programme in Drug Research

and

Finnish Institute for Health and Welfare
Finland

NEUROBIOLOGY OF GAMBLING DISORDER AND ALCOHOL USE DISORDER COMORBIDITY – PRECLINICAL STUDY

Ville Oinio

DOCTORAL DISSERTATION

To be presented for public discussion with the permission of the Faculty of Pharmacy of the University of Helsinki, in Hall 132, Psychologicum, on the 27th of October, 2022 at 12 o'clock.

Helsinki 2022

Supervisors

Docent Petteri Piepponen, PhD
Division of Pharmacology and Pharmacotherapy
Faculty of Pharmacy
University of Helsinki
Finland

Professor Emeritus Atso Raasmaja, PhD
Division of Pharmacology and Pharmacotherapy
Faculty of Pharmacy
University of Helsinki
Finland

Research Professor Emeritus Kalervo Kiianmaa, PhD
Finnish Institute for Health and Welfare
Finland

Reviewers

Professor Catharine Winstanley, PhD
Department of Psychology
University of British Columbia
Canada

Associate Professor Juho Joutsa, MD, PhD
Turku Brain and Mind Center
University of Turku
Finland

Opponent

Research Professor Emeritus Hannu Alho, MD, PhD
Finnish Institute for Health and Welfare
Finland

The Faculty of Pharmacy uses the Urkund system (plagiarism recognition) to examine all doctoral dissertations.

Dissertationes Scholae Doctoralis Ad Sanitatem Investigandam Universitatis Helsinkiensis

ISBN 978-951-51-8686-7 (softcover)
ISBN 978-951-51-8687-4 (PDF)
ISSN 2342-3161 (print)
ISSN 2342-317X (online)
Unigrafia
Helsinki 2022

To my wonderful wife and three stars of my life.

ABSTRACT

Gambling disorder is considered a behavioral addiction that combines reward with executive decision-making processes in a complex and fascinating way. Gambling disorder overlaps with various psychiatric diseases, and comorbidity with alcohol use disorder and other substance use disorders is significant in its etiology. As in drug addictions, the dopaminergic mechanisms play a significant role in the neurobiology of gambling disorder. Clinical studies with opioid antagonist naltrexone also suggest that the opioidergic mechanism may have a role in modulating gambling behavior, especially in the gambler subgroup with a family history of alcoholism. Currently, treatment of gambling disorder mostly relies on psychotherapeutic approaches, and no significant achievements in drug development have been made.

The main aim of the research was to validate a preclinical model of probability-based risky decision-making and investigate differences in dopaminergic and opioidergic mechanisms in decision-making behavior between AA (Alko Alcohol) and Wistar rats. The additional aim was to clarify the role of the nucleus accumbens in risky decision-making and develop a model to screen drug targets in gambling disorder. AA rats were used to represent a group of individuals with a genetic preference for alcohol use disorder, and a standard laboratory rat strain, Wistar, was chosen to represent the normal heterogenic population of gamblers. The decision-making of rats was studied in a probabilistic discounting task, which allows us to examine rats' behavior at different levels of uncertainty. In the task, rats went through operant lever pressing training where different sized sucrose rewards guided the lever choices. The probability of gaining rewards changed slowly to a level where choosing the smaller reward was the most profitable option. After training, the effects of dopaminergic and opioidergic drugs on decision-making behavior were studied.

In this research, we completed the aim to validate an animal model for studying the probability-based risky decision-making behavior and showed that D-amphetamine acts as a valid promoter for "gambling-like" behavior in rats. Results indicate that dopaminergic modulation of probability-based risky decision-making is pronounced in AA rats compared to Wistar. In the case of the opioidergic mechanisms, the results were ambiguous, and thus exact predictions of the relevance of opioids could not be made based on this study. The role of nucleus accumbens dopaminergic functions as a modulator of risky decisions was verified, but naltrexone's effect on reducing risky decisions failed to show any promising results, indicating that the role of the opioid antagonist in the pharmacotherapy of gambling disorder is focused more on decreasing the overall motivation to gamble than modulating the risky decision-making in gambling.

These studies create a platform for future studies aiming to point out the specific neurobiological mechanisms that control the behavior of the gambler subgroup with a genetic vulnerability to alcohol use disorder.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Oinio V, Bäckström P, Uhari-Väänänen J, Raasmaja A, Piepponen P, Kiianmaa K (2017) Dopaminergic modulation of reward-guided decision making in alcohol-preferring AA rats. Behav Brain Res 326:87-95.

II Oinio V, Sundström M, Bäckström P, Uhari-Väänänen J, Kiianmaa K, Raasmaja A, Piepponen P (2018) Amphetamine primes enhanced motivation toward uncertain choices in rats with genetic alcohol preference. Psychopharmacology (Berl) 235:5:1361-1370.

III Oinio V, Sundström M, Bäckström P, Uhari-Väänänen J, Kiianmaa K, Raasmaja A, Piepponen P (2021) The role of opioidergic system in modulating cost/benefit decision- making in alcohol-preferring AA rats and Wistar rats. Behav Pharmacol 32:220-228.

The publications are referred to in the text by their roman numerals. In addition to published research, some unpublished data is presented in this thesis.

ABBREVIATIONS

AA = Alko Alcohol
AMPH = D-amphetamine
ANA = Alko non-alcohol
DA = Dopamine
DSM-5 = Diagnostic and statistical manual of mental disorders, 5th ed.
fMRI = Functional Magnetic Resonance Imaging
GD = Gambling Disorder
IGT = Iowa Gambling Task
LL-lever = Large Lucky lever
Nacc = Nucleus Accumbens
NTRX = Naltrexone
PET = Positron Emission Tomography
RAC = Risk Aversion Criterion
rBT = rat betting task
RCC = Rational Choice Criterion
rGT = rat gambling task
RPE = Reward Prediction Error
SS-lever = Small Sure lever
VTA = Ventral Tegmental Area

1 INTRODUCTION

Gambling in different forms has been prevalent throughout human history, and new ways of gambling are constantly developed. However, all forms of gambling share the exact definition, which can be conceptualized as wagering of something of value, typically money, on an event with an uncertain outcome to win a larger reward than the wager. Recreational gambling, done voluntarily for fun and excitement, is a harmless activity for most people. However, for some individuals, gambling develops into a problematic behavior diagnosed as a gambling disorder (GD) which causes significant economic losses and distress in everyday life and can, in its most devastating form, lead to suicide. Numerous theories have been proposed concerning the etiology and regulation of neuronal processes that lead people to gamble, despite the negative consequences and awareness that monetary loss is inevitable in the long run ("the house always wins").

GD is considered as the prototypical example of behavioral addiction and its etiology and symptomology overlap with various psychiatric diseases (Nautiyal et al. 2017). Comorbidity with alcohol use disorder, nicotine addiction, and other substance use disorders is significant in GD etiology. The complex heterogeneity of GD makes understanding the neurobiological basis of gambling problems challenging (Grant et al. 2016). Moreover, to develop more effective pharmacotherapies, we need enough knowledge of how neurobiological processes must be targeted to achieve efficacy that would be enough to overcome this brain disease.

GD combines reward reinforcement with cognitive processes in a complex way and is characterized by an overstated preference for high and uncertain rewards (Brevers et al. 2014). To current knowledge, brain dopaminergic mechanisms play a key role in modulating gambling behavior by modulating cognitive and reward-related processes in GD. Based on drug addiction theories, opioidergic mechanisms are also hypothesized to contribute to GD (Potenza 2013a). However, the exact role of opioids has not been established so far.

Because of the ethical issues concerning drug development, preclinical models using laboratory animals are mandatory to study and understand the neuronal pathways' function modulating gambling behavior. Preclinical gambling research relies on different forms of Pavlovian self-administration and decision-making models focusing on specific aspects (e.g., risky decision-making) of gambling behaviors. Combining data from several models has produced data for a better understanding of this problematic behavior but has not yet been able to develop effective pharmacotherapies for GD. Much of this is because of the nature of addiction diseases and individual differences in these diseases' neurobiological processes.

This thesis focuses on preclinical modeling of probability-based risky decision-making behavior in GD and studies how familial alcohol preference impacts gamblers' behavior. As maladaptive risky decision-making can be

considered one of GD's hallmarks, the literature review is constructed to review the neurobiology of probability-based risky decision-making to the extent that it allows us to understand its linkage to GD. The leading topic of this thesis is to create a theoretical frame to answer the question, "Are individuals with a family background of alcoholism more prone to reinforcing effects of gambling than individuals without the burden of alcoholism in their genetics?" In this research, we aim to answer this question from the preclinical perspective.

2 REVIEW OF THE LITERATURE

2.1 GAMBLING DISORDER AS A BEHAVIORAL ADDICTION

"Gambling is a principle inherent in human nature"

– Edmund Burke

When discussing gambling, the fundamental question is what level of gambling can be considered problematic? Some may argue that all gambling is wrong, whereas some may not see any problems involved in gambling and consider it a behavior that the individual freely chooses. The free will of action is debated widely in the field of neuropsychology as well as in theoretical philosophy but could not be discussed in this thesis frame. However, we can discuss the stage when individuals lose the ability to control their actions when it comes to gambling and the neurobiological mechanisms controlling this uncontrolled behavior.

There is a thin red line between recreational gambling and disordered gambling, and this imaginary border is not sharp and should be considered a continuum. At the end of this continuum is gambling disorder (GD), which is characterized by persistent and recurrent gambling behavior that is problematic and impairs quality of life and is considered as a behavioral addiction (American Psychiatric Association 2013). GD shares the same key features as substance use disorders (SUD), the inability to stop despite the obvious harms that the behavior is causing and frequent relapses after periods of abstinence, in this case, periods without gambling. Different to drug addictions, in GD the object of addiction is not a drug but an activity. However, GD can be similar to drug addictions described as "the endpoint of a series of transitions from initial drug use - when a drug is voluntarily taken because it has to reinforce, often hedonic, effects - through the loss of control over this behavior, such that it becomes habitual and ultimately compulsive" (Everitt et al. 2008).

The important thing to underline is that the criteria for a behavior to be considered as an addiction is the inability to control the addictive behavior despite the obvious negative consequences that this behavior is causing to the addicted person or to the closest people surrounding them. This is the real core of all addictions.

2.1.1 DIAGNOSTIC CRITERIONS (DSM-5)

According to literature reviews, GD's lifetime prevalence varies across the continents, estimated at 0.2% to 5.3 % (Nautiyal et al. 2017). In western

societies, prevalence is 1-2% (Shaffer et al. 1999, Petry et al. 2005), which is also the situation in Finland (Salonen et al. 2020).

The classification of Gambling disorder was introduced in the newest version of the Diagnostic Statistical Manual (DSM-5) (APA 2013). In the previous version (APA 1994), the disorder was classified as "pathological gambling," and it is under a section of "impulse control disorders." In the DSM-5, GD is moved under "Substance use disorders," which suggests that GD shares clinical characteristics of substance use disorders and can be neurobiologically compared with other addictions (Linnet 2013, Yau and Potenza 2015). DMS-V also introduces the term behavioral addiction as a subsection, which under GD, emphasizes that addiction can be based purely on behavior rather than the use of external chemicals (Linnet 2013, Zack et al. 2020).

Based on the DSM-5, GD can be diagnosed if at least four out of nine clinical symptoms (table 1) are fulfilled during the past 12 months, and the gambling behavior cannot be explained by a manic episode (APA 2013).

Table 1. Diagnostic criteria of gambling disorder according to the DSM-5

1. Need to gamble with an increasing amount of money to achieve the desired excitement
2. Restless or irritable when trying to cut down or stop gambling
3. Repeated unsuccessful efforts to control, cut back on or stop gambling
4. Frequent thoughts about gambling (such as reliving past gambling experiences, planning the next gambling venture, thinking of ways to get money to gamble)
5. Often, gambling when feeling distressed
6. After losing money gambling, often returning to get even (referred to as "chasing" one's losses)
7. Lying to conceal gambling activity
8. Jeopardizing or losing a significant relationship, job or educational/career opportunity because of gambling
9. Relying on others to help with money problems caused by gambling

The GD is a comorbid disease whose etiology overlaps with several psychiatric disorders, like depression, personality disorders, and SUD (Blanco et al. 2015, Flórez et al. 2016, Cowlishaw et al. 2014, Zois et al. 2014). Based on clinical findings, it has been estimated that 60% of individuals diagnosed with GD are also diagnosed with SUD (Cunningham-Williams et al. 1998, Lorains et al. 2011), 73 % alcohol use disorder and 60% smoking (Petry et al. 2005). GD also shows strong hereditability, and studies have found that approximately 20% of the first-degree relatives of individuals with GD also have GD and individuals with a problem gambling parent are 3.3 times likely to have GD (Grant et al. 2016, Leeman and Potenza 2012, Hodgins et al. 2011).

2.1.2 GAMBLER SUBGROUPS

It is hypothesized that there are several subgroups or so-called "gambler genotypes." However, the "gambler phenotype" is primarily similar and is seen as extensive gambling without the ability to control one's actions. The "gambler genotype" comprises a broad spectrum of cognitive dysfunctions and disordered reward-based actions. These two can be emphasized differently across the gamblers resulting from different neurobiological systems interacting with each other (Khanbhai et al. 2017). This partly explains the finding that various forms of gambling selectively appeal to players with different motives (Griffiths 1995, Stewart et al. 2008, Granero et al. 2020)

Based on different psychological profiles and neurobiology, at least three gambler subgroups have been suggested (Blaszczynski and Nower 2002, Nower et al. 2013, Devos et al. 2020). Based on the "pathway model", the first group is *behaviorally conditioned* problem gamblers who typically gamble for recreational purposes, are prone to cognitive distortions, and are easily behaviorally conditioned to gambling. The second group, called *emotionally vulnerable* problem gamblers, consists of individuals with premorbid psychological distresses (e.g., mood disorders). Gambling is working via negative reinforcement for this subgroup and coping/escaping from negative feelings. In the third group, called *'antisocial impulsivist'* problem gamblers, heightened impulsivity and impaired executive control are the main drivers of gambling behavior. These gamblers typically exhibit early onset of gambling, a family history of problem gambling and comorbid alcohol and other substance use dependence (Blaszczynski and Nower 2002). Although the classification consists of three different subgroups, it is notable that the border between these groups is not sharp, and thus, individual psychological profiles can be mixed between different subgroups.

2.1.3 ALCOHOL USE DISORDER AND GAMBLING

Comorbidity with GD and alcohol use disorder is well documented (Slutske et al. 2000, Mann et al. 2017, Tackett et al. 2017), and its prevalence is five to six times higher in disordered gamblers versus the general population (Bischof et al. 2013, Flórez et al. 2016). Twin and adoption studies provide strong evidence of genetics in the etiology of alcoholism (heritability estimates of 40–60%) (Prescott and Kendler 1999, Prescott et al. 1999, Kampov-Polevoy et al. 2003) and additionally show that GD and alcohol use disorder have shared genetic vulnerability (Slutske et al. 2000, Slutske et al. 2013). There is also a positive association between gambling severity and alcohol consumption (French et al. 2008). According to Mann et al. (2017), 27.0 % of first-degree relatives of pathological gamblers suffer from alcohol dependence (7.4 % in the control group) and 8.3 % with pathological gambling (0.7% in the control group). GD and alcohol use disorder also show similar symptomatology in terms of craving, tolerance, withdrawal symptoms, and frequent relapses

(Ledgerwood and Petry 2006, de Castro et al. 2007, Blaszczynski et al. 2008, Kovács et al. 2017). Although clear correlations are observed with the comorbidities, it is challenging to show whether these are the cause of individual preference to gambling or symptoms of disordered gambling.

2.1.4 CURRENT STAGE OF GD PHARMACOTHERAPY

There have been several different pharmacotherapeutic approaches to GD concerning dopaminergic, opioidergic, serotonergic and glutamatergic drugs (Yip and Potenza 2014). Effects of these vary among patients, and no single drug has a formal indication for treating GD (Kraus et al. 2020). The most promising results are found with opioid antagonist naltrexone (NTRX) and nalmefene, which reduce gambling urges (Grant et al. 2006, Grant et al. 2008, Potenza 2013b). However, these have shown the best effects on the gambler subgroup with comorbidity with alcohol use disorder or a family history of alcoholism (Bullock and Potenza 2012, Grant and Chamberlain 2015). This subgroup typically responds effectively with opioid antagonist pharmacotherapy, indicating that the neurocircuitry controlling the gambling behavior of this subgroup is working in the opioid-dopaminergic level more effectively than in other subgroups (Kim et al. 2001, Grant et al. 2008, Potenza 2013b). The treatment response to opioid antagonists also strongly indicates that the gambling behavior in this subgroup is driven by the rewarding effects of gambling and thus closely resembles SUDs.

However, thus far, none (not even the opioid antagonist) have shown significant effects in the treatment of GD on their own, but only when combined with psychological support from cognitive psychotherapy, which is still the primary treatment approach in GD (Toneatto et al. 2009, Ribeiro et al. 2021).

2.2 NEUROBIOLOGY OF GAMBLING DISORDER

"Much of the reward comes not from winning but from the possibility of winning"

- Dr David Sack

It is postulated that gambling behavior can engage dopaminergic pathways in similar ways as drugs, even though no chemical agent enters the brain to instantiate the hijacking process (Zack et al. 2020). As in other addictions, positive reinforcement occurs at the initial stage of gambling through experiences that can be considered rewarding (Koob and Volkow 2016). After continually gambling, positive reinforcement slowly decreases, and the individual needs stronger experiences to achieve the same psychological reward caused at the initial stage (i.e. tolerance) (Fig. 1). This occurs due to homeostatic neuroadaptations in the dopaminergic neuronal functions at the

mesolimbic system (Robinson and Berridge 1993, Robinson and Berridge 2003). Individual factors define how effectively these neuroplastic changes occur, and it can be hypothesized that genetic factors play a crucial role in addiction vulnerability (Koob and Le Moal 1997). Due to the neuroplastic changes in the mesolimbic system, the basal dopaminergic activity from daily pleasure-causing activities cannot produce enough pleasure, and the individual becomes dysphoric. This negative psychological stage drives individuals to seek learned actions that produce enough pleasure (negative reinforcement). By doing this, the homeostatic stage in DA systems continues to adapt more, which leads to the endpoint of addiction where gambling is done to achieve the same level (or even less) of pleasure that was previously the basal level of the individual. Side by side with tolerance and positive reinforcement, when shifting to negative reinforcement a neurobiological process called sensitization occurs (Robinson and Berridge 2003). Due to sensitization, motivation ("wanting") to pursue a rewarding agent or activity progressively increases after repeated exposure to drug abuse or gambling.

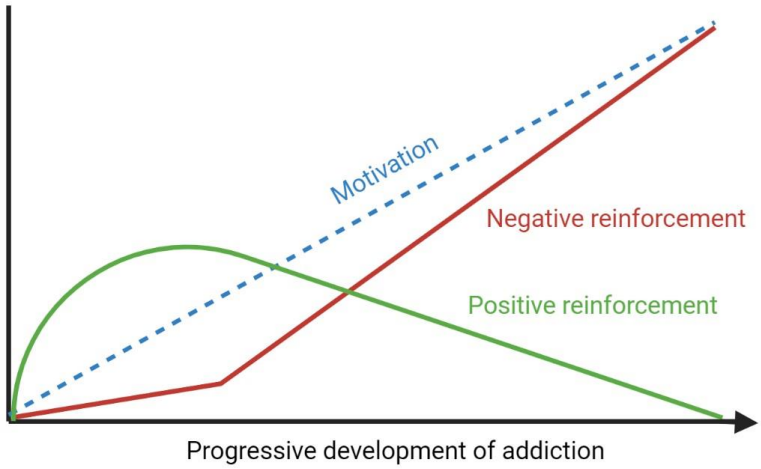

Fig 1. Motivation towards addictive substance or behavior is constantly increasing despite the positive effects decreasing. At the initial stage of addiction development, positive experience plays a key role in motivation. At the later stages, positive reinforcement gradually decreases, and the main motivation driver occurs due to negative reinforcement.

2.2.1 DOPAMINERGIC SYSTEM

The linkage of DA and gambling initiates from the clinical observation that DA agonists used in the pharmacotherapy of Parkinson's disease increased gambling activity among patients with no gambling problem history before starting DA agonist therapy (Avanzi et al. 2006, Weintraub et al. 2010, Voon et al. 2011, Djamshidian et al. 2011). It is shown that Parkinson's disease medication with D_2/D_3 agonists and/or L-Dopa produces an imbalance in

dopaminergic functions in mesocortical and mesolimbic pathways resulting in gambling behavior among patients (Merims and Giladi 2007, Djamshidian et al. 2010, Driver-Dunckley et al. 2013). These findings suggest that the increased gambling in Parkinson's patients is not due to the disease but to the DA pharmacotherapy used for the treatment. However, in humans, a DA receptor antagonist does not reduce risky behavior, and thus far, no dopaminergic pharmacotherapy has proven effective in GD treatment. These findings have indicated that the role of DA in gambling is not nearly as straightforward as it was thought at first.

There are two neuronal pathways that have been shown to modulate gambling behavior (Fig. 2). First is the mesolimbic pathway that projects from the ventral tegmental area (VTA) to the ventral striatum, where it modulates dopaminergic functions of the nucleus accumbens (Nacc). DA functions of Nacc play a major role in reinforcing the effects of drugs of abuse, like cocaine, AMPH, and alcohol (Wise 1998, Koob et al. 1998). This suggests that the Nacc also plays a key role in rewarding the effects of gambling.

The other mesocortical pathway that leads from VTA to the forebrain areas is the prefrontal and medial prefrontal cortex. The mesocortical pathway is responsible for the cognitive and conscious control over the mesolimbic (primate brain) areas. The dialog with these two pathways determines the individual's overall behavior in gambling as DA functions in prefrontal cortex signal changes in reward availability and DA functions in Nacc encode signals about reward rates, uncertainty, and choice (St Onge et al. 2012).

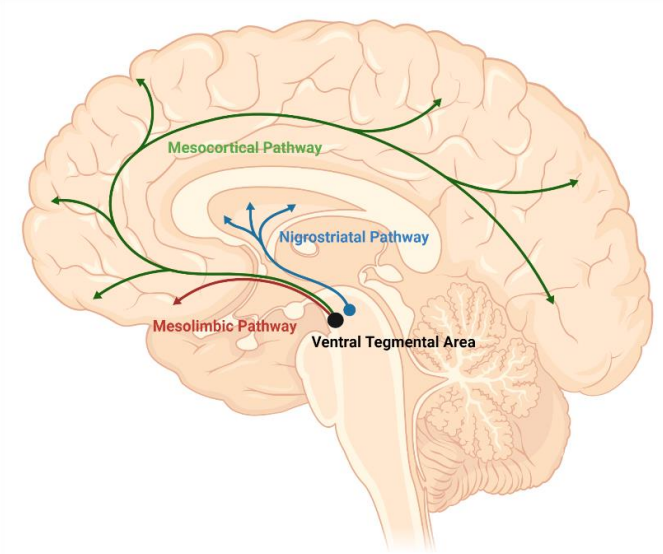

Figure 2. Brain dopaminergic pathways. A schematic representation of the main human brain dopaminergic pathways.

DA receptors belong to the family of seven transmembrane domain G-protein coupled receptors and are located in the pre- and postsynaptic sites of the synapse (Beaulieu et al. 2011. Baik 2013). Five types of DA receptors are found in the human brain based on their structural and pharmacological properties. These receptors are divided into two groups: D1-like receptors (D_1 and D_5), which stimulate intracellular cAMP levels, and D2-like receptors (D_2, D_3 and D_4), which inhibit intracellular cAMP levels (Beaulieu et al. 2011, Baik 2013). These two DA receptor types are present in mesolimbic dopamine areas, D_1 as a postsynaptic receptor, D_2 as postsynaptic receptors and presynaptic autoreceptor (Fig. 3). D_2 autoreceptors provide a negative feedback mechanism as somatodendritic D_2 autoreceptors reduce neuronal excitability and terminal D_2 autoreceptors decrease DA synthesis and inhibit impulse-dependent DA release (Beaulieu et al. 2011, Baik 2013).

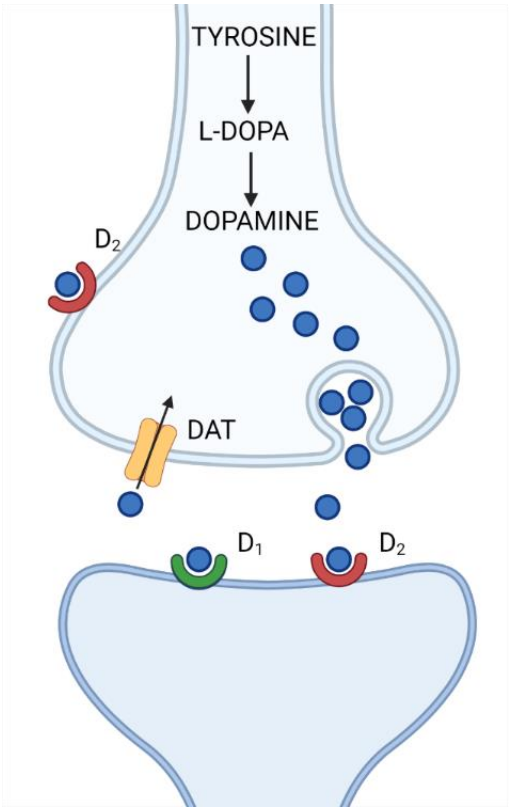

Figure 3. DA is released from the presynaptic terminal into the synaptic cleft, where it binds to D_1 or D_2 like receptors. Presynaptic D_2 autoreceptors act as negative feedback and regulate DA release from the presynaptic terminal. Dopamine transporter (DAT) removes DA from the synaptic cleft to the presynaptic nerve.

DA receptors in Nacc activate differently depending on the DA release, which can be either tonic or phasic (Floresco et al. 2003, Koob and Volkow 2016). Tonic DA release occurs in timescale seconds to minutes, whereas

phasic DA release refers to rapid "burst" firing of DA neurons in a timescale of ~300 ms (Schultz 1998). Relatively slow tonic firing mainly activates presynaptic DA D2 autoreceptors and thus attenuates phasic dopamine neurotransmission (Floresco et al. 2003). Phasic activity of DA neurons activates postsynaptic DA receptors, increasing the nerve cell's neuronal firing, which is suggested as a key component of reward processing and predicting the delivery of rewards (Floresco et al. 2003).

2.2.2 ENDOGENOUS OPIOID SYSTEM

From the gambling point of view, it is hypothesized that the dopaminergic mechanisms control the decisions made based on expected results. Additionally, opioids have an impact on the hedonic experience of the outcome of the results (Berridge 2007, Petrovic et al. 2008).

Endogenous opioid peptides are produced naturally within the body and have roles in the CNS, which involve modulation of pain responses, modulation of reward-reinforcement processes, and regulation of homeostatic functions such as food and water intake (Akil et al. 1998, Gilpin and Koob 2008, Koob 1992, Gianoulakis 2001). Endogenous opioids are composed of precursor molecules such as pro-opiomelanocortin, proenkephalin, and prodynorphin (Trigo et al. 2009), which form active endorphin peptides that mediate effects through opioid receptors known as μ, δ, and κ (Akil et al. 1998). All three opioid receptor types mediate activity through $G_{i/o}$ proteins (Trigo et al. 2009). Opioid receptors act directly through the G protein by inhibiting the formation of cyclic AMP and the function of voltage-sensitive calcium channels and opening potassium channels (Sarne et al. 1996, Law et al. 2000). Opioid receptors are located at both the pre- and postsynaptic terminals of neurons, and their activation results in cell membrane hyperpolarization (Chesselet 1984, Jiang and North 1992, Olive et al. 1997). In the presynaptic nerve terminal, activation of opioid receptors inhibits the release of neurotransmitters. At the postsynaptic end, activation of opioid receptors prevents the propagation of nerve impulses. β-endorphin and enkephalins have the highest affinity for μ and δ opioid receptors, while dynorphin has the highest affinity for κ opioid receptors (Corbett et al. 1982, Akil et al. 1984). In the CNS, endorphins and encephalins are mainly located in areas where opioid receptors can be shown to be abundant. Enkephalins, among others, are present in interneurons and mediate local effects, whereas β-endorphin is present in the pituitary-hypothalamic region, especially in the arch nucleus, where it is thought to regulate nervous system function of long nerve pathways and hormones.

Administration of exogenous opioids, such as morphine, activates the mesolimbic DA pathway mainly through the opioid receptors of the VTA and the Nacc, and the administration of opioids to the VTA causes self-administration of these substances in rats (Bozarth 1987, Wise 2002). One of the functions of endogenous opioids acting on opioid receptors is to release

reward pathway structures from GABAergic inhibition, which in turn results in activation of reward mechanisms (Trigo et al. 2009). In the VTA, opioids bind to opioid receptors located in GABAergic interneurons (Johnson and North 1992, Bonci and Williams 1997). This reduces the GABAergic inhibition of dopaminergic neurons from VTA to Nacc, resulting in increased DA being released from the terminal ends in Nacc.

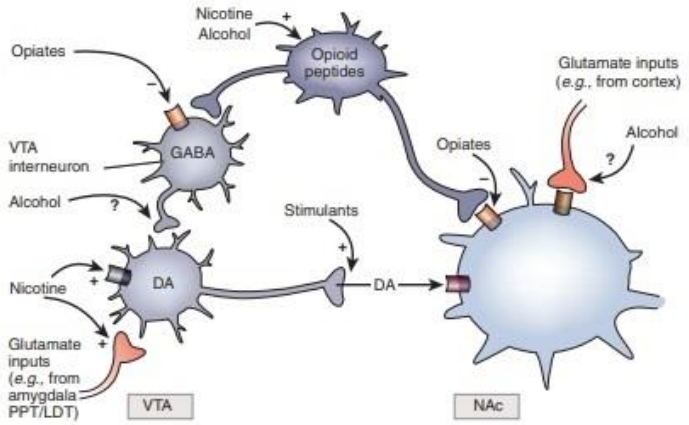

Figure 4. Function of opioid peptides. Picture adapted and modified from article Nestler 2005.

In addition to the VTA, opioids also regulate the amount of extracellular DA in the Nacc by directly acting on its opioid receptors (Trigo et al. 2009). Nacc has all three types of opioid receptors (μ, δ, κ) (Mansour et al. 1987, Sharif et al. 1989). Of these, activation of μ receptors causes an increase in DA concentration (Yoshida et al. 1999, Murakawa et al. 2004, Hirose et al. 2005, Okutsu et al. 2006). Activation of κ-receptors, in turn, causes a decrease in extracellular DA concentration by decreasing the presynaptic release of DA (Spanagel et al. 1992). Opioids have been shown to modulate the "liking" of rewards by direct modulation in the Nacc core and shell regions and ventral pallidum hotspots (Peciña 2008, Berridge and Kringelbach 2015). In rodents, "wanting," "liking," and reward learning can be modulated by manipulations of opioid receptors in, e.g., Nacc and ventral pallidum and basolateral parts of the amygdala (van Steenbergen et al. 2019).

The role of endogenous opioid peptides in GD is studied in PET and fMRI studies in humans (Mick et al. 2014, Mick et al. 2016, Majuri et al. 2017). The results indicate that individuals with GD do not differ from baseline levels of opioid receptors in key brain areas when compared to healthy volunteers. However, opioid release is shown to be blunted in the GD group after administration of dopaminergic drugs like cocaine or amphetamine, indicating dysfunction in the opioidergic system in individuals with GD (Mick et al. 2016).

2.2.3 DOPAMINE DERIVED REINFORCEMENT IN GAMBLING

"Usually a brain 'likes' the rewards that it 'wants'. But sometimes it may just 'want' them."

- Berridge et al. 2009

Based on the incentive-sensitization model by Terry E. Robinson and Kent C. Berridge, the reinforcing functions of dopaminergic processes in the mesolimbic system can be divided into the "wanting" (incentive motivation) and "liking" (hedonic impact). "Wanting" is associated with the anticipation of reward and "liking" is associated with outcome evaluation (Robinson and Berridge 1993, Robinson et al. 2003, Berridge 2003, Smith et al. 2005, Berridge 2007). Neurobiologically, "wanting" can be separated from the hedonic pleasure that the reward causes "liking." "Wanting" and "liking" are the central modulators of reinforcement in addiction and, thus, can also be hypothesized to modulate GD patients' behavior (Linnet 2014).

Clinical studies using slot machine task with problem gamblers have shown that the cue triggered "wanting" is stronger than "liking" (Davey et al. 2018). Moreover, it has been shown that and cue-triggered "wanting" is stronger in problem gamblers than non-problem gamblers overall, whereas "wanting" and "liking" do not differ between the groups. It is also shown that DA activation is increased in GD patients towards anticipated rewards (Fiorillo et al. 2003, Abler et al. 2006, Preuschoff et al. 2006, Linnet 2014), but blunted towards the outcome of the reward (Linnet 2014). The main message of these findings is that, as the incentive-sensitization model predicts, the "wanting" is dissociated from "liking," thus, the urge to gamble is present in problem gamblers despite the experienced pleasure from gambling. This partly explains why problem gamblers continue gambling despite experiencing negative results of losing (Linnet 2014). A similar phenomenon is observed in drug addicts, and it may lead to a situation where the addicted person pursues the drug ("wanting") even being aware the drug only causes low or no pleasure ("liking") (Robinson and Berridge 2003). "Wanting," especially cue triggered "wanting," also has a significant role in the relapse after gambling-free periods. Several cues (e.g., the sight of a slot machine or an advertisement of an online casino on a website) might trigger an urge to gamble ("wanting"). As in other addictions, the possible cues are constantly present in everyday life, creating a huge challenge to prevent relapses.

Preclinical studies of "chronic uncertainty" have also shown that long exposures to uncertainty events can produce neuroadaptations of the dopaminergic system, causing reinforced responses to dopaminergic drugs (Zack et al. 2014, Zeeb et al. 2017, Fugariu et al. 2020). These studies have shown the important factor of gambling evolving into an addiction: gambling is able to cause reinforcing effects that are not dependent on winning, indicating that gambling can be addictive alone.

2.2.4 REWARD PREDICTION ERROR

Maladaptive executive processes are profoundly linked to GD (Winstanley and Floresco 2016). These processes are modulated by changes in DA transmission, altering the prediction of rewards and causing difficulties in decision-making between different value choices (Schultz et al. 1997, Floresco 2016). Reward prediction refers to reward anticipation, while RPE refers to a neuronal mechanism of midbrain DA neurons that updates a stimulus' positive and negative reward predictions (Schultz et al. 1997, Linnet 2014). The primary function of RPE is that it codes learning via positive and negative prediction errors so that midbrain dopaminergic neurons are activated if the reward is better than expected and depressed if the reward is less than expected (Fig. 5). Reward that is equivalent as expected produces no changes in DA neuron activation. The primary function of RPE is that it enables an individual to learn from trial and error, which is valuable in typical day-to-day life (Schultz 2016). However, some behaviors (e.g., gambling and social media) are linked to enormous amounts of positive and negative reward predictions in short time windows producing phasic intermitted changes in DA neurons that the brains are not evolutionarily developed to handle. Similar to drug addictions, this continuous dopaminergic activation can produce neuronal adaptations in midbrain DA neurons that lead to addiction for some individuals (Schultz 2016).

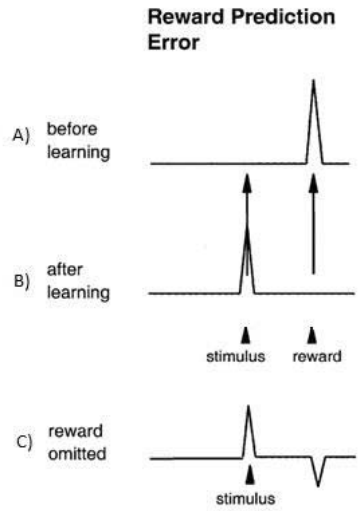

Figure 5. RPE increases DA firing in the human midbrain based on the previous outcomes A) The DA neuron is activated by the reward that is not predicted (+ Error) B) Predicted reward (stimulus) does not increase the DA firing (0 Error) C) Reward that is omitted despite prediction leads to reduction of DA firing (- Error) (Schultz 2016, figure adapted and modified from Suri & Schultz, 1998).

It has been shown in non-human primates that the waiting period before the outcome of choice-decision is known to create phasic increases of DA in the midbrain, and this increase of DA is strongest when the reward is big and the probability of gaining it is small (Schultz et al. 1997, Fiorillo et al. 2003).

Studies in humans also support the role of reward prediction and RPE in reinforcing gambling mechanisms. It has been shown in a PET study using [11C] raclopride that gambling releases DA in the striatal region during winning but also before winning, and the DA release correlates positively with the severity of gambling problems (Joutsa et al. 2012). Studies using fMRI had shown blood oxygen level activation in Nacc to be dependent on the reward probability (Abler et al. 2006). BOLD activation is higher when low probability options are rewarded and lower when high probability options are rewarded. RPE measurements also show sustained DA response toward stimuli when uncertainty is a maximum level (50%) and thus can explain the continued DA release and gambling despite losses in GD (Linnet 2013).

It is also notable that pre-existing brain states have been shown to impact risky decisions in humans (Huang et al. 2014), so it is theoretically possible that opioids may alter decisions via RPE by impacting the hedonic experience of the result outcomes.

2.3 DECISION-MAKING IN GAMBLING

"Remember this. The house doesn't beat the player. It just gives him the opportunity to beat himself."

– Nicholas Dandalos (a professional gambler)

As the dopaminergic theory of addictions plays a key role in understanding GD and allows us to create a neurobiological framework, numerous cognitive factors also modulate gambling behavior (Clark 2010, Clark 2014). Although these cognitive biases are usually viewed from a psychological perspective, these have a neurobiological basis that is mainly driven by dopaminergic neuronal functions and may impact the incentive-sensitization and RPE (and may also be driven by these). This makes understanding GD very challenging and not as straightforward as expected based on purely addiction-based theories. Adding to this, the individual personality factor that impacts vulnerability to reinforcing effects of gambling and risk-seeking tendencies predisposes that gambling behavior treatment must be personalized to be effective in each individual. Despite the complexity of gambling behavior (and also due to it), describing the most known cognitive biases is mandatory for creating a big picture of the modulatory aspects of gambling.

2.3.1 COGNITIVE BIASES IN GAMBLING

In this thesis, cognitive biases involved in gambling are described only briefly, excluding the probability-based risky decision-making, which is in the scope of the decision-making studies presented in this thesis. Cognitive biases are an important aspect of gambling because these distort the logical evaluation processes of winning probabilities. Some of these are used as effective conditioning factors, e.g., in Casinos and slot machines (Clark 2010). It has been shown that individuals with GD pose a variety of behavioral biases that predispose them to start gambling or keep them continually gambling (Toneatto et al. 1997). Here are listed some of the most well-known biases in gambling:

Personal Control, also called an *"illusion of control,"* illustrates a bias where a gambler is sure that they can have an impact on the outcomes of a gamble by practicing certain routines (like blowing the dice or choosing lottery numbers, etc.) (Clark 2010).

"Gambler's Fallacy" refers to a mistaken belief that if something happens more frequently, it will happen less frequently in the future, which quickly leads to a situation that resembles the *"illusion of control"* and makes a gambler very confident about the upcoming results leading to easier initiation to gamble more (Kovic and Kristiansen 2019). Casinos often exploit this bias in roulette tables where the previous results of red/black are shown in consecutive order.

"Near misses" is a bias where gamblers interpret near-miss as proof that they are mastering the game, which reinforces an *"illusion of control"* (Clark 2010). This reinforcing gambling mechanism is often seen in slot machines where different symbols are presented in fast space and reels stop just above the winning line. Feelings of almost winning keep the player in the mood that the game is going well (Griffiths 1991).

"Losses disguised as wins" is a bias that boosts the confidence that the game is going well, although players are continually losing money (Sharman et al. 2015). The textbook example is the slot machine game, which gives many "winnings" lower than the placed bet. The near-miss produces a similar neurobiological reaction in brains than actual winning, although the player is constantly losing.

"Hot hand" refers to a fallacy where gamblers think a winning streak is more likely to continue (Croson and Sundali 2005).

"Chasing losses" is probably the most devastating bias, leading to a situation where gamblers obsessively try to win back that previously lost money (Gainsbury et al. 2014).

2.3.2 PROBABILITY-BASED RISKY DECISION-MAKING IN GAMBLING

Gambling usually consists of several intermittent probability-based events where the player must make a choice of betting money or keeping the bet.

Although the probabilities of winning are not usually consciously known (unless, e.g., a professional poker player), the player is aware that there is always a risk of losing the bet. How the player handles this risk of losing depends on the individual tendency of risk-seeking / loss aversion. Because the decisions are also biased by cognitive distortions (described in chapter 2.3.1.), the probability-based risky decision-making during gambling is not straightforward as it would be based purely on an individual ability of probability estimations or risk-seeking / loss aversive tendencies of a player. People also do not evaluate their choices purely based on expected utility which was shown by the Prospect theory by Nobel prize winner Daniel Kahnemann (Kahneman and Tversky 1979). According to Kahnemann, most people avoid risky options when a guaranteed reward is simultaneously available, which results in an inverted-S-shaped curve in probability weighting (Fig. 6). People also usually overestimate low probabilities when facing probabilistic choices like a gamble. According to the prospect theory, the value function is steeper for losses than gains, and thus, people typically weigh losses larger than equivalent winnings.

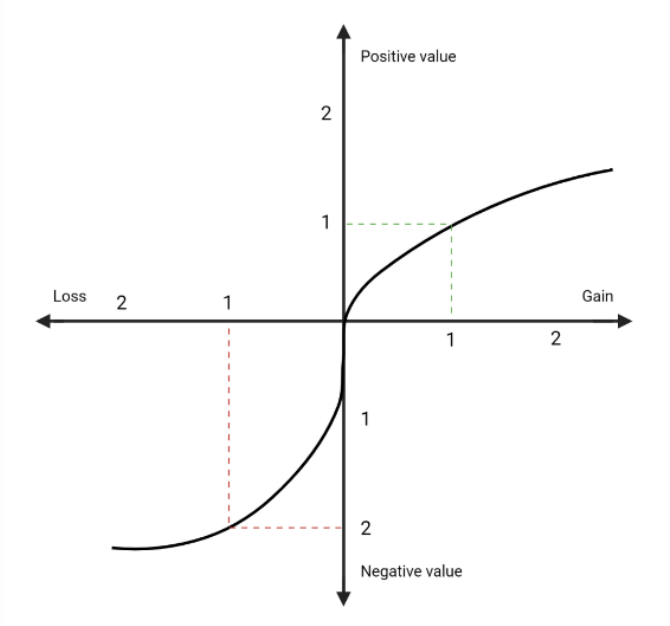

Figure 6. Inverted-S-shaped curve in the weighting of probabilistic outcomes in humans. The value function is steeper for losses than gains. Loss is psychologically weighted more than same sized win.

Gamblers typically favor high-risk opportunities, and excessive financial risk-taking is one of the hallmarks of gambling (Huettel et al. 2006, Linnet et al. 2012). Individuals with gambling problems show an elevation in probability weighting compared to healthy controls, reflecting an increased preference for

risk. They usually also overweight low winning probabilities and underweight high winning probabilities (Ojala et al. 2018). Linnet and colleagues (Linnet et al. 2012) found that experienced poker players, also pathological gamblers, had a larger error margin of probability estimation and played hands with lower winning probability than experienced non-pathological gamblers. Their data suggest that pathological players' cognitive deficits cannot be explained purely as a decision-making problem, i.e., pathological gambling poker players have intact probability estimation but are drawn toward risky gambles. Some of the pathological gamblers also reported that they often played low probability hands, even though they knew those were disadvantageous, but could not resist the temptation to see if they could win the hand.

DA is the main neurotransmitter to modulate probability-based risky decision-making. Clinical and preclinical studies have shown that an increase in DA transmission with AMPH robustly increases risky decision-making (St. Onge et al. 2009, Boileau et al. 2014). More specific studies have found that D_1 and D_2/D_3 DA receptor agonists increase risky decision-making, whereas antagonists reduce risky decision-making in rats (St. Onge et al. 2009, Zeeb et al. 2009, St Onge et al. 2010, Barrus and Winstanley 2016). Microinjection studies with rats suggest that especially D_1 and D_2/D_3 DA receptors in the Nacc have a major role in modulating probability-based risky decisions (Sommer and Hauber 2015, Mai et al. 2015).

Although preference for risky gambles might not be the main reason in the etiology of GD it is problematic because making more risky choices predispose the gambler to other cognitive biases (e.g., loss chasing) that will reinforce the overall gambling behavior and *vice versa*. Because of RPE changes, successful high-risk gambles also create large positive reinforcement effects in the mesolimbic system. Theoretically, these RPE differences can effectively re-organize dopaminergic functions of the mesolimbic system via neuroplastic changes.

2.4 PRECLINICAL MODELS OF PROBABILITY-BASED RISKY DECISION-MAKING

"All models are wrong, some models are useful."

- George Box

When studying gambling using animal models, the main question is how to model this extremely complex behavior with relatively simple operant protocols. As similar neurobiological processes modulate choice behaviors in humans and rats, a preference for the disadvantageous "high-risk, high-reward" options may reflect significant vulnerability for mental health problems (van den Bos et al. 2014, Winstanley and Clark 2016). There have been numerous preclinical models for studying gambling-related decision-making, and all are based, at least partly, on Pavlovian conditioning or operant

learning. One major drawback compared to drug addiction models is that no exact chemical in gambling models could be studied. The main difference compared to many Pavlovian conditioning models is that the unconditioned stimulus is probability-based rather than would be consistently associated with the conditioned stimulus. Zack and colleagues (Zack et al. 2020) described that the basic principle of slot machine gambling is similar to many Pavlovian conditioning models used in rodents and can be considered an instance of a standard operant learning paradigm comprising three events. *"First, a Discriminative Stimulus (i.e., the slot machine) signals reward availability if the operant response is performed. At this time, the gambler may configure his or her betting options (e.g., selecting the bet size). Second, the operant response is made (i.e., pressing the spin button). This initiates a few seconds of anticipation when the reels spin. Third, the reels stop to reveal whether or not the player has won (the rewarding outcome). Importantly, there is no contingency between the response and the occurrence of winning outcomes, which is determined by a random number generator at the moment the response is made.*" When considering this description, we can easily imagine that a gambler in front of a slot machine is similar to a rat in a Pavlovian operant chamber.

As all models measure specific decision-making behavior and have their pros and cons, none of these models can capture the whole gambling paradigm *per se*. Also, the face validity of preclinical models from a clinical perspective is challenging because gambling is affected by different cognitive functions combined with the reward of gambling outcomes. The face validity of preclinical models should thus be examined, not model by model, but more to combine the findings of several models. As the laboratory trials of this thesis focus on "probability-based risky decision-making," it is important to briefly review the specific approaches of other preclinical gambling models to understand the meaning of this research's findings fully.

The most widely used preclinical model focusing on gambling is conducted by using rat gambling tasks (rGT), which is based on a human analog named Iowa gambling task (IGT) developed by Bechara and Damasio (Bechara et al. 1994). The basic principle in rGT is the same as in IGT, and in this model, players/animals have four options to choose from. Two of these options can produce high immediate gains but are unprofitable in the long run. The other two produce lower immediate gains but are profitable in the long run. Players/animals do not know which options are which, and the model can effectively show what kind of strategy subjects choose, and they are able to change choosing behavior during the tasks. The IGT and rGT aim to model real-life or rational decision-making and capture many essential aspects of gambling-related decision-making (Brevers et al. 2013). Preclinical models are also developed for studying loss-chasing and near-misses (Winstanley et al. 2011, Cocker and Winstanley 2015).

2.4.1 PROBABILISTIC DISCOUNTING

Probability-based risky decision-making tasks are conducted in operant conditions (e.g., Skinner's box) where rats are trained to make choices based on different probabilities of gaining rewards (e.g., sucrose pellets or direct brain stimulation) by pressing operant levers (Cardinal and Howes 2005, Adriani and Laviola 2006, St. Onge and Floresco 2009). The levers are designated so that one of the levers always delivers a small reward (e.g., one sucrose pellet), and another lever delivers a larger reward (e.g., three or four sucrose pellets) at different probabilities. The probabilities are set in a way that at some probability levels, calculatory reward gain is higher by choosing the probability-based lever than choosing the smaller but sure lever. Some probabilities are disadvantageous, and therefore choosing the probability-based lever calculatory reward gain is lower than from the sure lever. In addition to the advantageous and disadvantageous probability levels, one level is set to be indifferent so that the calculatory reward gain is equal from both levers.

In traditional probabilistic discounting tasks, rats go through several "probability blocks" during the operant session in ascending or descending order, and during several sessions, these rats learn to adjust their decision-making based on the different probability levels. After the rats are trained, the model allows us to study the contribution of neurotransmitters to decision-making behavior using different pharmacological or optogenetic techniques. Thus far, the dopaminergic system is the most studied in these paradigms and has established DA as the main neurotransmitter in modulating probabilistic decision-making.

The advantage of the probability discounting task is that it produces a lot of data about the decision-making behavior and how the animals handle different levels of uncertainty. It has been robustly shown that rats execute similar decision-making patterns in these models as most humans do concerning the Prospect Theory of Kahnemann and Tversky (1979), which creates good face validity for these models. Although the probability discounting tasks do not replicate actual gambling *per se*, these models can produce essential data on how neurocircuits modulate the execution of gambling-related risky decisions.

2.4.2 BEHAVIORAL SENSITIZATION TO UNCERTAINTY EXPOSURE

As DA is considered the central neurobiological modulator in GD, many successful studies have captured DA's role in the conditioning to gamble (Zack et al. 2014, Zeeb et al. 2017, Fugariu et al. 2020). These studies have robustly shown that when rats are exposed to long periods of uncertainty in operant environments executing high amounts of choice based on probabilities that are close to slot machine gambling, rats are sensitized to the locomotor effects of

AMPH. This indicates that just being in a "gambling environment" is enough to change the dopaminergic systems based on neurons' neuroplasticity.

Studies using this kind of approach have shown that the long-term exposure to uncertainty sensitizes rats for locomotor effects of AMPH, indicating that the exposure to the gambling-like uncertainty is enough to produce neuroplastic changes in the mesolimbic dopamine system (Zack et al. 2014).

2.4.3 REINFORCERS IN OPERANT TESTING IN GD RESEARCH

For the apparent reason, money cannot be used as a reward in preclinical models that try to represent gambling behavior. One major limitation of preclinical GD models is that these models cannot replicate the risk of losing money, which is a crucial factor in gambling (Pettorruso et al. 2020). For this reason, we must rely on other reinforcers that motivate rats enough to accomplish the operant task and guide rats' decision-making. Most used reinforcers are palatable foods or liquids (e.g., sucrose), which have a high reward value for rats. Although appetitive rewards cannot produce similar money-like abstract rewards as present in humans, these can cause similar effects in rats' decision-making as money-paired rewards do in humans.

Palatable food, like sucrose, is a valid way to produce "liking" and "wanting" in rats (Castro and Berridge 2014). After training, rats willingly perform operant responses (e.g., pressing a lever) to obtain sucrose and learn robustly to favor a lever that delivers a higher amount of sucrose.

When using palatable food as a reinforcer, it must be acknowledged that satiety level can affect the motivation to pursue the sucrose rewards. For this reason, the basic principle is that the satiety level of rats is modulated by a restricted feeding method (e.g., rats are allowed to eat only 85% of their regular calories during the day) during the testing periods. Food restriction makes rats more motivated to pursue appetitive rewards but may have some drawbacks discussed in chapter 6.2.

Other reinforcers used in decision-making research are direct brain stimulations like optogenetics or intracranial self-administration. Because of the methodological differences, these are not in the scope of this thesis but can be reviewed, e.g., in studies of Tedford et al. 2014 and Orsini et al. 2017.

2.5 ALCOHOL-PREFERRING AND NON-PREFERRING RAT LINES

For studying alcohol-related gambling problems, alcohol-preferring/non-preferring rodent lines can be an excellent choice because these are bred by selective breeding based on their natural preference for alcohol. Based on the differences in natural alcohol preference, these rats can provide a valuable tool for identifying an integrative neurobiological factor in subgroups of gamblers with alcohol use disorder comorbidity. These rats can primarily be used to

identify differences in GD behaviors based on the familial risk of alcohol use disorder, which is shown to be a risk factor for GD.

One of the earliest alcohol-preferring/non-preferring rat strains are Alko alcohol (AA) and Alko non-alcohol (ANA) rats, which were produced by bidirectional breeding from Wistar and Sprague– Dawley rats in the Alko laboratory in 1968 based on voluntary 10% ethanol solution consumption of the rats (Eriksson 1968, Sinclair, Le and Kiianmaa 1989, Sinclair et al. 1992a). AA rats learn quickly to self-administer alcohol without any initiation or shaping and consume significantly higher amounts of ethanol solutions in different paradigms than ANA or Wistar (Hyytiä and Sinclair 1990, 1991). From the initiation of breeding in 1968 to this day, over 100 generations of these rats have been bred. Other rat lines bred by selective breeding are alcohol-preferring (P) rats /non-alcohol preferring (NP) rats (Li et al. 1991) and high-alcohol drinking (HAD) rats / low alcohol drinking (LAD) rats (Hansen and Spuhler 1984).

2.5.1 BEHAVIORAL AND NEUROBIOLOGICAL FEATURES OF AA RATS

Based on the findings between AA and ANA, alcohol preference of AA rats is hypothesized to occur due to abnormal function of opioidergic mechanisms (Hyytiä and Sinclair 1989, Koistinen et al. 2001), which are in light of the present knowledge closely related to DA functions (Berridge and Kringelbach 2015). Besides self-administrating high amounts of ethanol, AA rats have also shown differences in studies focused purely on opioidergic or dopaminergic mechanisms. A few of these studies are briefly presented here to give a few examples.

Research by Honkanen and colleagues (1999) reliably showed that cocaine-induced locomotor activity was comparable in AA and ANA and significantly higher than in Wistar, which indicates that dopaminergic activity in AA and ANA could be similar but more potent than in Wistar. AA rats drink more etonitazene and cocaine solutions voluntarily than alcohol naïve ANA or Wistar rats in two-bottle choice (tap water and drug solution) test (Hyytiä and Sinclair 1993), indicating that opioidergic and dopaminergic drugs are both more reinforcing for AA rats than ANA or Wistar. A study using microdialysis to investigate differences at baseline DA concentrations (nM) in Nacc of alcohol naïve AA, ANA, and Wistar rats showed that baseline DA concentrations in AA and Wistar were lower than in ANA (Katner and Weiss 2001). However, after a single i.p. injection of ethanol (1.5 g/kg of 20% ethanol), AA rats' percentual extracellular DA concentrations rose higher than in ANA or Wistar, indicating that ethanol promotes higher DA release in Nacc of AA than in ANA or Wistar.

3 AIMS OF THE STUDY

The knowledge of neurobiological factors that regulate GD has substantially increased in recent decades due to preclinical and clinical research. We can now predict which aspects of gambling are considered reinforcing and potentially dangerous, considering recreational gambling developing to GD. Despite this knowledge, no groundbreaking developments in GD's drug development have been made yet. The lack of effective pharmacotherapy for GD is partly due to the shortage of technological possibilities of targeting drugs to specific brain areas, but also the individual differences in neurobiological functions that modulate gambling behavior. As the "pathway model" predicts, GD patients cannot be considered one group, but moreover, several different groups of individuals with various neurobiological etiologies behind their problematic behavior. One of those groups consists of gamblers with a positive family background of alcohol use disorder. Clinical studies indicate that this subgroup responds to opioid antagonist pharmacotherapy better than other groups suggesting that the gambling behavior of this group is controlled by interactions of opioid- and dopaminergic mechanisms rather than only dopaminergic mechanisms.

Mainstream findings in preclinical studies of the neurobiology of gambling underlie the relevance of DA and are conducted using heterogenic rat strains. However, only a handful of preclinical studies have examined the role of opioids in gambling. To our knowledge, no preclinical studies have been trying to investigate the role of genetic alcohol preference in gambling behavior. The lack of literature on this subject is noteworthy and may be partly due to positive publication bias or lack of general interest. Whichever the reason may be, we consider it essential to address how genetic alcohol preference may impact gambling behavior. By examining this, we might be able to find neurobiological factors that lead to the development of individually based pharmacotherapies in the long run.

The research's scientific aim focused on the methodological aspects of preclinical behavioral pharmacology, examining differences in probability-based risky decision-making between AA and Wistar rats. The model presented in this thesis aims to replicate "human-like" decision-making in the probabilistic context, which allows us to examine how different neuropharmacological manipulations affect this behavior. The focus was not on making rats "gamble" but on studying how rational and risk-avoiding decision-making behavior could be imbalanced with dopaminergic and opioidergic drug manipulations. By conducting this, we can predict how these neurocircuits are imbalanced in the brains of individuals with GD.

The aims of the study were captured in four different publications:

1. To validate a preclinical model which reliably models similar decision-making behavior in probability-based risky decision-making that is observed in humans and validates the model by using AMPH as a risky decision-making promoter (I).

2. Investigate differences of dopaminergic and opioidergic mechanisms in decision-making behavior between AA and Wistar rats to achieve knowledge of how genetic alcohol preference impacts decision-making behavior (II, III).

3. To develop the model forward for studying screening drug targets to modulate dopamine-derived risky decision-making (IV, unpublished).

4 MATERIALS AND METHODS

4.1 ANIMALS

Male AA rats (University of Helsinki, Helsinki, Finland) and male Wistar rats (University of Helsinki) were used in the studies. At the beginning of the experiment, rats were 3 to 4 months old. On arrival, rats were given one week to acclimate to the environment. Food (regular chow SDS RM1 [E] SQC; Witham, Essex, England) and water were available *ad libitum* in the home cage. Rats were housed two to three per cage in a temperature and humidity-controlled room, with lights controlled on a reversed light/dark cycle of 12/12 h. All experiments were conducted in the dark phase of the light cycle. All testing was in accordance with the Animal Experiment Board of Finland (ESAVI/2073/04.10.03/2012 and ESAVI and ESAVI/5705/04.10.07/2013) and were conducted according to the 3R principles of the EU directive 2010/63/EU governing the care and use of experimental animals, and following local laws and regulations (Finnish Act on the Protection of Animals Used for Scientific or Educational Purposes (497/2013), Government Decree on the Protection of Animals Used for Scientific or Educational Purposes (564/2013)).

4.1.1 DISQUALIFYING ANA RATS

The research's initial aim was also to include the ANA rats in the behavioral testing. However, in the very early stage of the research we decided not to include ANA rats based on the finding that their motivation toward sucrose pellets was too low that perform reliable operant testing could have been conducted with these rats (Fig. 7).

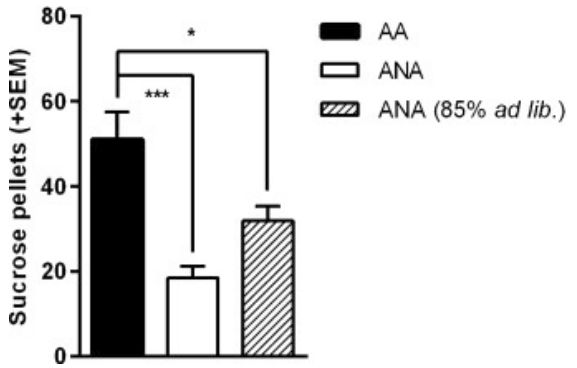

Figure 7. *Ad libitum* fed AA rats consume sucrose pellets (45 mg) in 60 min fixed ratio (one pellet received from either lever in 1 s intervals) training session significantly in higher amounts when compared to ANA rats after *ad libitum* feeding or after food restriction of 85% of free feeding (n = 16).

4.2 DRUGS

The D-amphetamine sulphate (AMPH, Sigma–Aldrich), the DA D_1-agonist SKF-81297 (Sigma–Aldrich), the $D_{2/3}$-agonist quinpirole (Sigma–Aldrich), the opioid agonist morphine (Yliopiston Apteekki), and opioid antagonist naltrexone (NTRX, Sigma-Aldrich) were used as test drugs. Drug doses were calculated as salt weights and dissolved in 0.9% saline. In study IV, AMPH was dissolved in Ringer solution. Saline (0.9%) was also used as a vehicle for injection in studies I, II, and III. In study IV, the Ringer solution was used as a vehicle. Each drug/vehicle test day was preceded by at least three drug-free days.

Table 2. Drug doses in the studies

Study	Drugs	Doses (mg/kg)
I	AMPH	0.1, 0.3, 1.0
	SKF-81297	0.1, 0.3, 0.5
	quinpirole	0.003, 0.010, 0.030
II	AMPH	0.3, 1.0
III	morphine	0.3, 1.0
	NTRX	0.3, 1.0
IV	AMPH	10 µg, 20 µg /side*
	NTRX	1.0

* Administered directly into Nacc

Drugs and vehicles were given in a Latin square design. Drug doses were administered as s.c. injections at a volume of 1 ml/kg 20 min prior to testing. In study IV, AMPH was administered bilaterally in a volume of 0.3µl at a rate of 0.3µl/min with a microinfusion pump (CMA, Stockholm, Sweden) and the NTRX (s.c.) was administered 20 minutes before AMPH microinjections.

In study II, we conducted an additional test after three injections to observe possible sensitization to AMPH effects. In this test, AMPH dose of 0.3 mg/kg was given three consecutive days, 20 min prior to placing rats into the operant chambers. The rats received one dose of AMPH 1.0 mg/kg 20 min prior to testing on the fourth day.

4.3 APPARATUS

Behavioral testing was conducted in operant chambers (30.5 × 24 × 21 cm; Med-Associates, St Albans, VT, USA) enclosed in sound-attenuating wooden boxes. The boxes were equipped with a fan that provided ventilation and masked extraneous noise. Each chamber was fitted with two retractable levers, one located on each side of a central food tray where a pellet dispenser

delivered sucrose reinforcement (45 mg; Opend, Denmark). Above each lever was a cue light. The chambers were illuminated by a single 100 mA house light located in the top center of the wall opposite the levers.

4.4 LEVER PRESS TRAINING

Three days before the first lever press training session, rats were placed in the operant chambers for 15 min each day with the chamber's food tray containing nine sucrose reward pellets. After this, the rats were returned to their home cage, and approximately 30 sucrose pellets were given per cage. This procedure was done to habituate rats to the operant chamber environment and the taste of sucrose.

After habituation days, the training period, which included three phases (A, B, and C), was initiated. In phase A, the rats were trained in forced-choice for 60 min so that only one lever was always present (left or right). By pressing the lever, rats received one sucrose pellet with a 3-s time-out during which the cue light was on. Phase A consisted of six training sessions, and the presented lever was changed each session.

In phase B, the rats were trained for 30 min so that only one lever was present (left or right) at the start of the session. Rats received one sucrose pellet for each press. After each press, the pressed lever retracted, and the other lever was presented after a 3-s time-out. During the time-out, the cue light was lit above the lever pressed and stayed on for 3 s. Phase B consisted of a total of six training sessions.

In the last training phase, C, the rats were trained in a free-choice task for 15 min so that both levers were presented at the same time. By pressing either one of the levers, the rat received one sucrose pellet, the cue light above the lever pressed lit for 15 s, both levers were retracted, and they were presented again after 15 s. Phase C consisted of a total of six training sessions.

All sessions were conducted in darkness. The house light was on after and before each session but was off during sessions.

4.5 RATIONAL DECISION-MAKING TASK

This task was modified from its original form based on the literature (Cardinal and Howes 2005, Adriani and Laviola 2006, St Onge and Floresco 2009). The rational choice task consisted of a total of 15 sessions, one session per day for five days a week. At the beginning of the session, rats were placed in the operant chambers, where the house light was on, and both levers were retracted. When the session started, the house light went off, and both levers were presented simultaneously. One lever was designated the SS-lever ("small/sure"), the other the LL-lever ("large/lucky"). Based on the literature and our findings (Floresco et al. 2008, Haluk and Floresco 2009, Stopper et al. 2013), levers were not randomized but were counterbalanced so that for

each rat, the LL-lever was designated to be the side that the rat did not spontaneously prefer during training phase C. Designated levers remained consistent throughout sessions for each rat.

The SS-lever's choice always delivered one pellet with a probability of 100%, and the choice of the LL-lever delivered three pellets with a probability of 100%. After pressing a lever, both levers retracted, and the cue light above the lever that had been pressed was lit, and one (SS lever) or three (LL lever) sucrose pellets were delivered to the food tray. Multiple pellets were delivered 0.5 s apart. After sucrose was delivered, the cue light remained on for another 15 s, after which both levers were presented again.

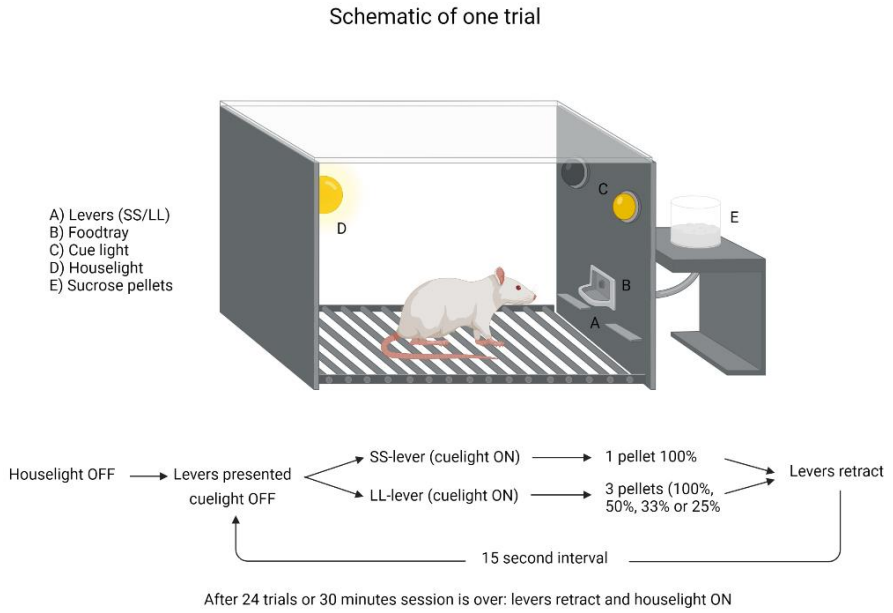

Figure 8. Schematic of one trial in session.

In one session, rats had free choice to press the levers at 15-s intervals. In study I, rats received daily sessions consisting of up to 60 trials, and session time was set to 15 min. Starting from study II, the duration of one session was set to 24 lever presses or 30 min (depending on which one was accomplished first), and session durations were registered from all sessions.

The criterion for rational choice behavior [rational choice criterion (RCC)] was set to LL-lever choice of ≥ 75% or three times the starting level (session 1). Either one of these criteria had to be achieved at least until the 15th session. Rats that achieved either one of these criteria were considered to behave rationally and proceeded to the probabilistic discounting task.

4.6 PROBABILISTIC DISCOUNTING TASK

This task was divided into three different probability levels. The probability of gaining three sucrose pellets by pressing the LL-lever decreased over time (50, 33, 25%), while the SS-lever always delivered one pellet with 100% probability. The probability of LL-lever changed between the sessions so that rats received five consecutive sessions with LL-lever probability of 50%, after which five consecutive sessions with LL-lever probability of 33%, and finally ten consecutive sessions with LL-lever probability of 25%, respectively. After the tenth session at the probability level of 25%, rats were given two saline injections (s.c.) to habituate the rats to the upcoming drug challenges.

During drug challenges, a stable baseline of operant behavior was required for three consecutive days between injections. The criterion for a stable baseline was achieved when the ± SEM in LL-lever choices of three previous baseline session averages was under 5.00. Each rat had its baseline values calculated separately, and each rat also received drugs based on their stability in baseline values.

In distinction to other studies, in study I, the probabilistic discounting with AMPH challenge was conducted at five different probability levels (100%, 50%, 33%, 25%, and 20%) and administering the drug at all probability levels. After this study I the protocol was changed only to examine the decision-making behavior at the most important probability levels, which are 100%, 50%, 33%, and 25%.

4.7 STEREOTAXIC SURGERY AND MICROINJECTIONS

In study IV, rats that fulfilled the risk aversion criterion (RAC) were chosen for stereotaxic operations. Guide cannulas were implanted for direct drug administration to the Nacc. Before the surgery, rats were injected with carprofen (Rimadyl®, 5 mg/kg s.c., Vericore, Dundee, UK) and were anesthetized with isoflurane (4% during induction for 5 min and then 2–2.5% for anesthesia maintenance). Rats were attached to a stereotaxic frame, and the guide cannulas were implanted bilaterally 2 mm above the Nacc (AP+1,6; ML±1,8; DV −5,0 from the dura, Paxinos and Watson 1998) and attached to the skull with four stainless steel screws and dental cement. Dummy stylets were placed inside the guide cannulas. At the end of the surgery, a 10 ml saline solution injection was administered to prevent dehydration during anesthesia. After surgery, the rats were returned to their home cage to recover for one week before the onset of the experiments and were administered carprofen for two days post-surgery.

After surgery and the recovery week, rats continued the 25% sessions and were habituated several times to the intracranial injection procedure by removing and placing back the dummy stylets from the guide cannulas right before placing the rat in the operant chamber. The microinjection testing was initiated after a stable baseline was achieved at the 25% probability. At first,

rats received a sham infusion, during which the injection needle was placed into the guide cannula, but no infusion was given. The tip of the injection needle extended 2 mm beyond the guide cannula shaft. After the sham infusion, the rats received a training vehicle infusion. After the infusions, the injection needle was left for 1 min to allow the spreading of the drug and to avoid leakage up the cannula track. Right after this, the rat was placed in the operant chamber, and the session was initiated. D-amphetamine and vehicle were administered in a Latin square design. Each drug/vehicle test day was preceded by at least three drug-free days, and a stable baseline of operant behavior was required before the next dosing.

4.8 HISTOLOGY

Coronal sections (100 μm) were cut from the 10% formalin-fixed brains to check the right guide cannulae placements verified from the rat brain atlas (Paxinos and Watson 1998). Rats were excluded from the study if the mark of the injection needle tip was outside the Nacc.

4.9 INITIATION OF DRUG CHALLENGES

The criterion to initiate the drug challenges (RAC) was set so that the LL-lever choice of rats had to be ≤ 50% at least after the 10th session at the probability level of 25%. In addition, completion of all the 24 lever presses for 30 min was required. During the drug challenges, rats went through one session each day at the probability level of 25%, and rats were given injections in the previously described manner.

4.10 SATIETY CONTROL

AMPH's effect on sucrose pellet eating was studied in a 30-min free sucrose pellet eating test after drug challenges. In this test, rats were placed in the operant chamber for 30 min (house light off) with the additional food cup placed in front of the central food tray. The food cup was filled with 10.0 g of sucrose pellets. After 30 min, rats were removed, and the remaining sucrose pellets were weighed to calculate sucrose pellet consumption. Sucrose consumption was calculated in grams per kilogram for each rat. Effects of AMPH doses (0.3 and 1.0 mg/kg) were examined, and saline was given as a vehicle. Drugs and vehicles were given s.c. in Latin square design 20 min prior to the satiety test with 3-day injection-free time between each dose.

4.11 STATISTICAL ANALYSIS

Data were analyzed with SPSS version 22.0 (Study I, II) and SPSS version 25.0 (Study III, IV). Data were collected on all rational choice behavior,

probabilistic discounting, drug challenges, lever pressing activity, session duration, and satiety control. In study I, statistical analysis of the rational choice behavior test data was conducted using a repeated-measures two-way ANOVA [session, group and session × group interaction]. Statistical analysis of the data from the probabilistic discounting task, number of lever responses and satiety control were conducted using a repeated-measures ANOVA in a within-subject manner. A paired two-tailed t-test with Holm–Bonferroni method was used to detect differences between vehicle and drug treatments if a significant main effect was found. Data from every probability level (100%, 50%, 33%, 25% and 20% for AMPH group; 100% and 25% for SKF-81297 and quinpirole groups) was analyzed separately to show the effects of drugs at each probability level.

In study II, data from rational choice behavior and probabilistic discounting were analyzed with two-way ANOVAs between AA and Wistar rat groups. Data from other experiments were analyzed by within-subjects repeated measures ANOVAs followed by Bonferroni's test as a post hoc test.

In study III, effects on drug, strain, or drug × strain interaction, all data were analyzed by two-way repeated-measures ANOVA. If a significant main effect was detected, additional statistical testing was conducted by one-way ANOVA with repeated measures to observe any effects within each group. Bonferroni's test was used as a post hoc test. Within-group comparisons in all studies (when conducted) were analyzed with repeated measures ANOVA or paired t-test, depending on the sample type.

LL-lever choices in the drug challenges task were compared to the vehicle (LL-lever choice (%) = percentage of LL-lever choices of total lever responses. In addition to the drug-vehicle comparison, the effects of drugs on LL-lever choices were always compared to the baseline average of the three preceding days. A criterion for significance in all tests was set at $p < 0.05$, but $p<0.01$ and $p<0.001$ are presented if found.

5 RESULTS

5.1 BASELINE DECISION-MAKING OF AA AND WISTAR RATS

To observe the baseline decision-making of AA and Wistar rats, we compared the decision-making of all rats used in studies II and III. A total of 34/39 AA and 34/40 Wistar rats fulfilled the RCC and RAC. AA and Wistar rats showed similar decision-making behavior during the rational choice and probabilistic discounting tasks (Fig. 9). A two-way ANOVA revealed significant main effects of sessions [$F(4, 330)=140.48$, $p < 0.001$], but no strain × session interaction was detected [$F(4, 330)=2.01$, $p= 0.08$]. Data presented in Fig. 9 represent only the behavior of rats that fulfilled both criteria (RCC and RAC) demanded to proceed to the drug challenges.

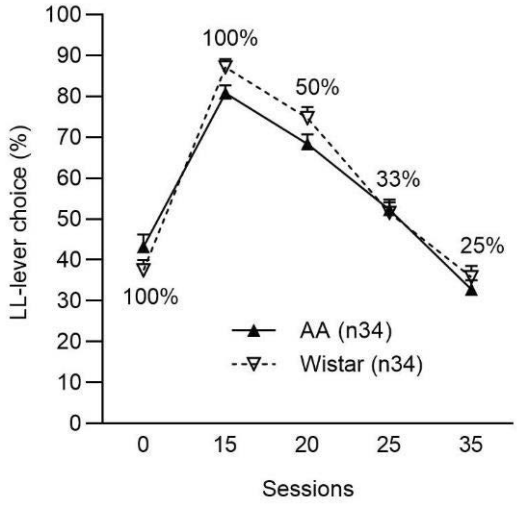

Figure 9. Session 1 represents lever choices at the beginning of the study, and Session 15 represents the rational choice behavior of Wistar and AA rats that fulfilled the criteria set for proceeding to drug challenges. Session 20 represents lever choices at the last session at LL-lever probability level 50%, Session 25 at 33%, and Session 35 at 25%. [LL-lever choice (%) = percentage of LL-lever choices of total lever responses, RCC = Rational Choice Criterion, RAC = Risk Aversion Criterion, ±SEM].

To observe individual group baselines, see studies II and III.

5.2 DOPAMINERGIC MODULATION OF PROBABILITY-BASED RISKY DECISION-MAKING IN AA RATS (I) – (VALIDATING THE MODEL)

At the 100% probability level, AMPH revealed a trend in lever choices shifting from the LL-lever toward the SS-lever in a dose dependent manner [$F(3,33) = 3.782$, $p = 0.051$, Fig. 10]. At the 50% and 33% probability levels no effect on lever choices was detected [$F(3,33) = 2.519$, $p = 0.10$, $F(3,33) = 0.09276$, $p = 0.92$, respectively]. At the 25% probability level, AMPH caused a dose-dependent shift from the SS-lever toward the LL-lever [$F(3,33) = 6.022$, $p < 0.05$]. Post hoc analysis revealed significant differences in LL-lever choices between vehicle and 1.0 mg/kg AMPH ($p < 0.05$). At the 20% probability level, AMPH caused a dose-dependent shift from the SS-lever toward the LL-lever [$F(3,33) = 12.01$, $p < 0.001$]. Post hoc analysis revealed significant differences in LL-lever choices between vehicle and 0.3 mg/kg AMPH ($p < 0.01$) and vehicle and 1.0 mg/kg AMPH ($p < 0.01$).

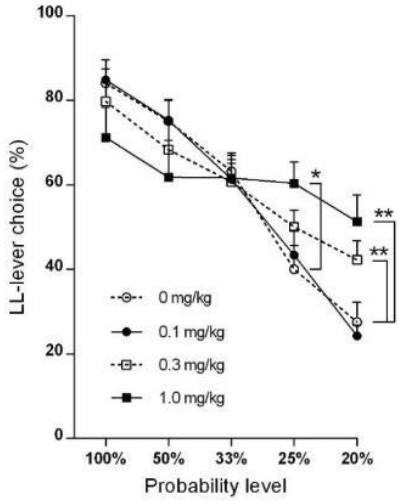

Figure 10. Effect of AMPH on the LL-lever choices of AA rats at different LL-lever probability levels. [LL-lever choice (%) = percentage of LL-lever choices out of the total lever responses, ±SEM, n = 12].

5.2.1 RIGHT-LEFT LEVER BIAS

We observed differences in AMPH effects during the data analysis based on the LL-lever disposition (left or right). Lever bias is observed when rats are divided based on the LL-lever (right-lever Fig. 11., left-lever Fig. 12.). The bias is observed during the LL-lever probability of 100%) where AMPH has no effect on lever choices of rats with LL-lever right [$F(3,15) = 0.65$, $p = 0.59$] but

has statistically significant effects on the rats with LL-lever left [$F(1.34,6.87) = 6.66$, $p < 0.05$]. However, no significant differences were found with the Bonferroni post hoc test. At the LL-lever probability level of 20%, a significant increase in LL-lever choices are observed in AA rats with LL-lever right after AMPH dose of 1.0 mg/kg [$F(1.21,6.03) = 12.76$, $p < 0.05$]. The effects were significant after Bonferroni correction between AMPH dose of 0.1mg/kg and 1.0mg/kg and 0.3mg/kg and 1.0mg/kg. In AA rats with LL-lever left no significant effects on LL-lever choices are observed [$F(1.49,7.43) = 4.82$, $p = 0.052$].

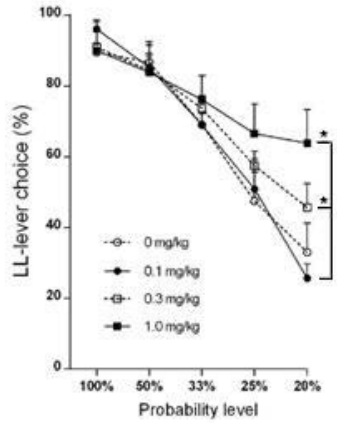

Figure 11. Effect of AMPH on the LL-lever choices of AA rats with LL-lever right at different LL-lever probability levels. [LL-lever choice (%) = percentage of LL-lever choices out of the total lever responses, ±SEM, n = 6].

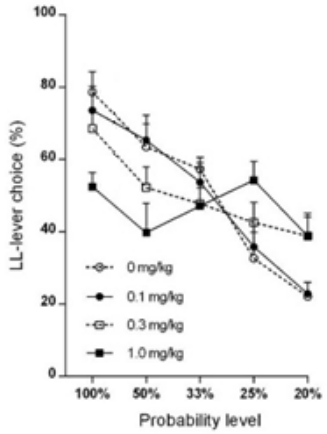

Figure 12. Effect of AMPH on the LL-lever choices of AA rats with LL-lever left at different LL-lever probability levels. [LL-lever choice (%) = percentage of LL-lever choices out of the total lever responses, ±SEM, n = 6].

From study II onwards, levers (SS or LL) were counterbalanced so that for each rat, the LL-lever was designated to be the side that the rat did not spontaneously prefer during training phase C.

For the full results and discussion of Study I see Oinio et al. 2017.

5.3 COMPARISON OF DOPAMINERGIC MODULATION IN AA AND WISTAR RATS (II)

In AA rats, a statistically significant increase in LL-lever choices was observed after administration of a single dose of AMPH at a dosage of 1.0 mg/kg compared to the vehicle [$F(2, 22) = 9.148$, $p < 0.01$, post hoc $p < 0.01$ between vehicle and dose 1.0 mg/kg)] and an AMPH dose of 0.3 mg/kg [$F(2, 22) = 9.148$, $p < 0.01$, no significance between vehicle and dose 0.3 mg/kg was detected, Fig. 13]. In Wistar rats, no statistically significant main effect on LL-lever choices was observed after administration of a single dose of AMPH at a dose of 0.3 or 1.0 mg/kg compared to the vehicle [$F(2, 22) = 2.107$, $p = 0.145$ (one rat missing due to zero responses after AMPH dose of 1.0 mg/kg), Fig. 13].

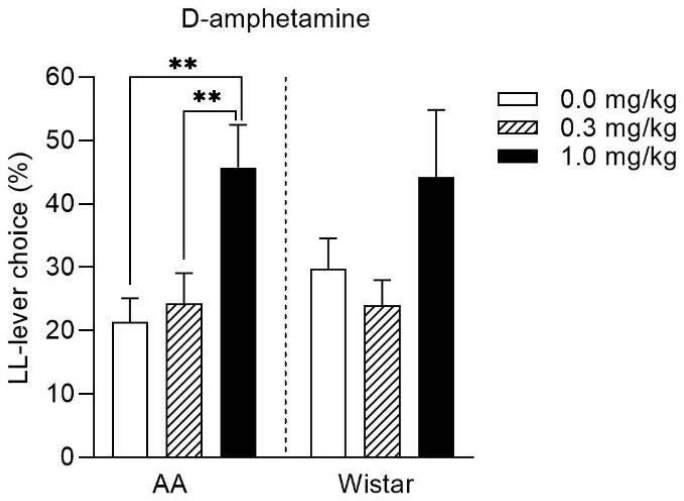

Figure 13. Single dose of AMPH increased LL-lever choices of AA rats at the dose of 1.0 mg/kg when compared to vehicle or AMPH dose of 0.3 mg/kg (n = 12, +SEM). A similar increase in LL-lever was not observed in Wistar rats (n = 12 +SEM).

Administration of AMPH promoted unprofitable decision-making of AA rats more robustly when compared to Wistar rats (Fig. 14). The comparison revealed high consistency in the LL-lever increasing effect of AMPH among the AA rats but considerable inconsistency among the Wistar rats resulting in relatively high distribution.

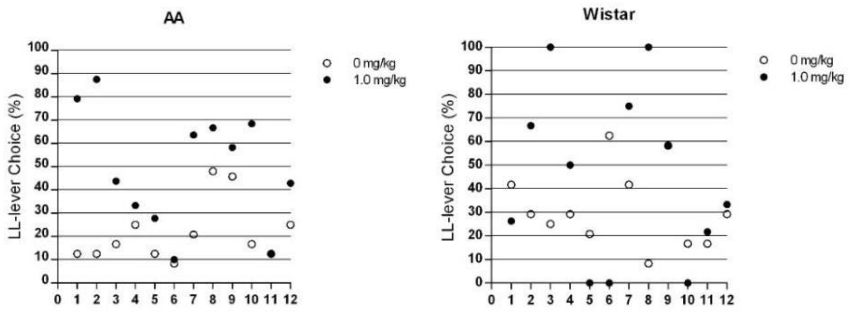

Figure 14. LL-lever choice of each rat after the AMPH dose of 1.0 mg/kg (white dot = LL-lever choice after vehicle, black dot = LL-lever choice after AMPH administration).

AMPH reduced lever-pressing responses of AA and Wistar rats (Table 3). Reduced lever pressing was found significantly greater in Wistar rats than in AA rats. AMPH reduced sucrose eating of AA and Wistar at doses of 0.3 mg/kg and 1.0 mg/kg compared to vehicle.

In satiety control, dramatic effects of D-amphetamine on the sucrose eating of AA and Wistar rats were observed (Table 3). Repeated measures ANOVA revealed significant main effects in both groups {[AA: $F(2, 22) = 63.920$, $p < 0.001$ followed by Bonferroni post hoc test results for vehicle and AMPH 0.3 mg/kg $p < 0.001$, and vehicle and AMPH 1.0 mg/ kg $p < 0.001$)], [Wistar: $F(2, 24) = 34.569$, $p < 0.001$, followed by Bonferroni post hoc test results for vehicle and AMPH 0.3 mg/kg $p < 0.01$, and vehicle and AMPH 1.0 mg/kg $p < 0.001$)]}.

Table 3. Effect of AMPH on lever responses and sucrose eating

Dose (mg/kg)	AA responses	Wistar responses
Vehicle	24.00	24.00
0.3	24.00	24.00
1.0	19.67 (± 1.71)*	11.38 (± 3.13)*

*p < 0.05 between AA and Wistar

	Sucrose (g/kg)	Sucrose (g/kg)
Vehicle	7.55 (± 0.81)	9.56 (± 0.91)
0.3	2.35 (± 0.63)***	5.38 (± 0.71)*
1.0	0.43 (± 0.17)***	1.34 (± 0.61)***

n = 12/12, ± SEM

*p < 0.05 versus vehicle; ***p < 0.001 versus vehicle

For the full results and discussion of Study II see Oinio et al. 2018.

5.4 COMPARISON EFFECTS OF OPIOIDERGIC MODULATION ON PROBABILITY-BASED RISKY DECISION-MAKING OF AA AND WISTAR RATS (III)

Morphine showed no statistically significant main effect on drug challenge [$F(2,40) = 1.06$, p = ns] or strain × drug challenge interaction [$F(2,40) = 2.44$, p = ns] was detected between AA and Wistar rats. However, in individual group analysis, we found a significant main effect when comparing morphine to the vehicle [$F(2,22) = 5.793$, $p < 0.01$] Fig. 15.) and post hoc test with Bonferroni revealed a significant difference between vehicle and morphine dose of 1.0 mg/kg (Fig. 15.).

Repeated measures ANOVA within the strain showed that the naltrexone significantly increased LL-responding in the Wistar rats [$F(2,20) = 11.05$, $p < 0.01$] and post hoc test with Bonferroni revealed a significant effect between vehicle and naltrexone dose of 0.3 mg/kg ($p < 0.01$) and vehicle and naltrexone dose of 1.0 mg/kg ($p < 0.01$). No significant effects in AA rats were detected [$F(2,18) = 0.93$, p= ns].

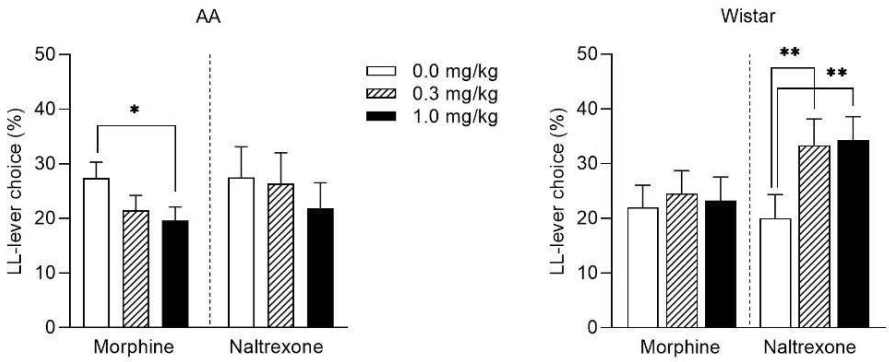

Figure 15. In AA rats, morphine dose of 1.0 mg/kg decreased LL-lever choices compared to vehicle. In Wistar rats, NTRX doses of 0.3 mg/kg and 1.0 mg/kg increased LL-lever choices compared to vehicle.

For the full results and discussion of Study III see Oinio et al. 2021.

5.5 PREDICTIVE VALIDITY OF SYSTEMIC NALTREXONE ON THE "GAMBLING-LIKE" BEHAVIOR OF AA RATS (IV, unpublished)

AMPH administered at Nacc increased the LL-lever choices of AA rats [$F(2,14) = 12.747$, $p < 0.001$] and Bonferroni post hoc analysis revealed a significant difference between the vehicle and AMPH dose of 20 µg /side ($p < 0.001$, Fig. 16).

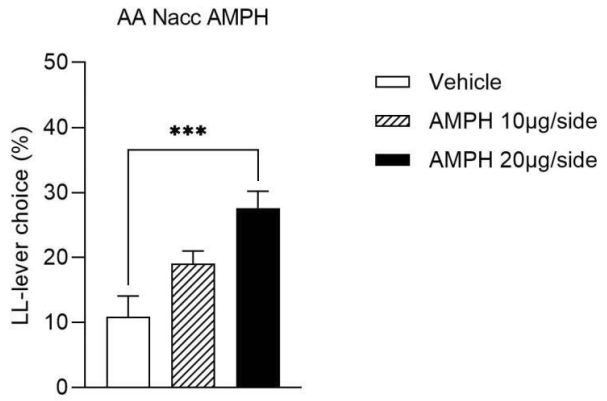

Figure 16. AMPH dose of 20 µg /side administered in Nacc increased LL-lever choices of AA rats (SEM, n8).

Pre-administered NTRX (1.0 mg/kg) failed to produce any changes in Nacc AMPH-promoted LL-lever choice increase in AA rats. Paired t-test [F(7) = -0.108 p=0.92] (Fig. 17).

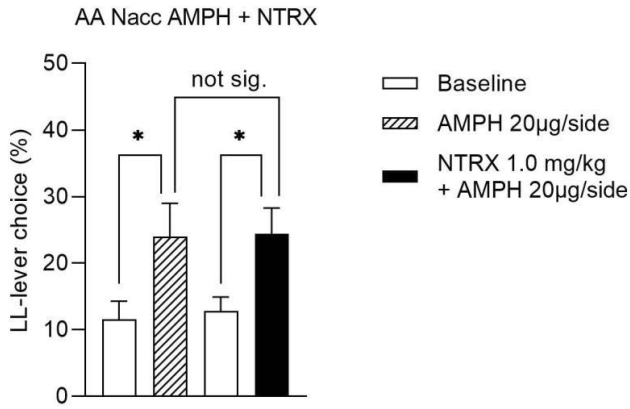

Figure 17. Pre-administered NTRX failed to produce any changes in Nacc AMPH-promoted LL-lever choice increase in AA rats (SEM, n8).

5.6. SUMMARY OF RESULTS

The main findings from all studies are summarized below.

Table 4. Summary of results (I,II, III, IV)

Study	Drugs	Strain	Ad. Route	LL-lever choices
I	AMPH	AA (n12)	Systemic	increase
	Quinpirole	AA (n7)	Systemic	increase (u-curve)
	SKF-81297	AA (n8)	Systemic	no effect
II	AMPH	AA (n12)	Systemic	increase
	AMPH	Wistar (n13)	Systemic	no effect
	AMPH (food restrict.)	Wistar (n8)	Systemic	increase
III	MOR	AA (n12)	Systemic	decrease
	MOR	Wistar (n10)	Systemic	no effect
	NTRX	AA (n10)	Systemic	no effect
	NTRX	Wistar (n11)	Systemic	increase
IV	AMPH	AA (8)	Nacc	increase
	AMPH + NTRX	AA (8)	Nacc	no effect with NTRX

6 DISCUSSION

The literature review outlines the fundamentals of gambling behaviors that are essential to adopt in order to understand the aims and results of this research. As shown, GD has numerous behavioral and neurobiological factors that must be considered when executing preclinical studies, making it challenging to perform a study where the face, construct, and predictive validities are at sufficient levels. This research aimed to validate a preclinical model for studying probability-based risky decision-making and investigate differences in this behavior between AA and Wistar rats. Therefore, in addition to the results, the fundamentals of the model are also discussed.

6.1 AA AND WISTAR RATS

Studies have produced a lot of data on neurobiological differences between AA and ANA (Sommer et al. 2006). Early studies also included Wistar rats but specialized quickly to comparative studies only between AA and ANA. Although ANA rats first appeared to be the perfect counterpart in search of a neurobiological factor in familial alcoholism-related GD, it was quickly shown ANA would not consume sucrose pellets enough to make them usable in this study.

Studies have shown that AA rats voluntarily consume more sweet agents than ANA (Sinclair et al. 1992b), but the preference for sweet taste is similar in AA and Wistar. The similarity in sweet preference between AA rats and Wistar rats, but the difference in the alcohol consumption, made this rat pair a valid choice in the study that aims to find differences in the decision-making behavior that are based on the genetic alcohol preference according to the fact that sucrose is used as a reinforcer to guide the decision-making in our research.

6.2 *AD LIBITUM* FEEDING

Ad libitum feeding is one of the most distinctive features of the model compared to widely used models in decision-making research with rodents. This approach was chosen because the aim was to capture the essence of pure probability-based risky decision-making, where only the excessive reward from the sucrose would guide the decisions. It is reliably shown that restricted feeding is a major motivational factor for rats to accomplish operant self-administration tasks (Sevak et al. 2008). However, a possible pitfall comes with the food restriction as it is shown to affect the dopaminergic functions in the brain by enhancing reinforcing effects (increased conditioned place preference and self-administration) of dopaminergic drugs AMPH and cocaine (Carroll et al. 1981, Stuber et al. 2002, Bell et al. 1997). Because of this, when trying to find differences in decision-making which is modulated via a

dopaminergic mechanism between rat strains, there is a possibility that food restriction affects the behavioral effects of dopaminergic drugs and therefore masks some differences that could be observed only with *ad libitum* fed animals.

Although *ad libitum* feeding provides a good tool for operant studies using palatable rewards, it has a few deficiencies. The most important is that the dosing of a drug must be carefully adjusted so that it does not disturb the appetitive behaviors of animals too much. During the pilot studies, we quickly realized that dopaminergic drugs diminished the appetitive motivation of *ad libitum* fed rats at similar doses used effectively in other behavior studies using restricted feeding methods (St Onge and Floresco 2009). In the case of the highest dose of AMPH, we also observed that some rats did not make all 24 lever presses during the 30 minutes, which was not observed in pilot studies conducted with rats under 85% food restriction.

An interesting finding was found in study IV, where AMPH was administered via microinjections to Nacc of AA rats. In this study, LL-lever choosing was increased and all rats accomplished all 24 lever presses. This finding suggests that a microinjection protocol would be a good way to study decision-making behavior using the *ad libitum* feeding approach from a methodological perspective.

6.3 CHOICE BEHAVIOR AT BASELINE

As the study involved rats from selected strains (AA and Wistar), one interesting question was the baseline decision-making behavior differences between strains. When we compared all animals used in the published studies, we did not discover a difference between the strains during the rational choice or probability discounting sessions. It must be noted that this data was gathered only from animals that fulfilled the RCC and RAC. However, preliminary data suggest a difference in baseline decision-making behavior between AA and Wistar rats when disqualified rats are involved. To clarify this, conducting a study where baseline decision-making of AA and Wistar are studied at all probability levels without disqualifying rats that do not fulfill RCC and RAC could be beneficial to obtain more knowledge of possible differences in the innate decision-making tendencies of these rats as a population.

The model used in this research captures the essence of 'normal' decision-making by showing the similar decision-making behavior in AA and Wistar rats, seen similarly in humans considering the theory published by Kahneman and Tversky in 1979. Both rat groups displayed a rational decision-making pattern in the first 15 sessions. Still, right after the LL-lever probability changed to 50%, both groups immediately started to shift the lever choosing toward the SS lever, although it would be beneficial to select the LL-lever. Similar behavior has been reported by Adriani and Laviola (2006) and indicates that instead of rational behavior, rats act as risk aversive in situations

where uncertainty is involved. Similar risk aversive behavior is seen in humans, and it has been proposed that risk aversion is the natural way healthy humans act in decision-making situations where uncertainty is involved (Kahneman and Tversky 1979). In this manner, the behavior of rats in this model resembles human behavior in similar situations.

At the probability level of 33%, lever choosing of rats is indifferent as both groups show approximately 50% preference towards LL-lever. At this level, choices make no difference in sucrose gain, which is shown in the preference of the levers. After moving to the probability level of 25%, choices shift toward the SS-lever, indicating that rats can make choices that optimize reward gain. For the nature of the model used in this study, we must note that the rats continually learn to choose the better option. Because of this, the LL-lever choices at the probability level of 25% are slowly decreasing throughout the study. Although it is a definite con of the study, it is also a major pro. Instead of fixating on specific choice behavior, rats are continually weighing the costs vs. benefits of the lever choices. This slowly changing baseline level was controlled using the Latin square design and also by comparing the drug effects to the 3-day baseline preceding the drug challenges.

6.4 VALIDATING THE MODEL (I)

AMPH and quinpirole promoted irrational and unprofitable decision-making in AA rats. Effects that were observed, however, are not unambiguous. When AMPH is administered in systemic blood circulations, it enters the whole brain, affecting numerous dopaminergic nerve terminals, making it impossible to point out a specific neurobiological correlate for behavioral effects. In the Nacc, AMPH is known to cause phasic DA increases in the synapse as it releases DA from postsynaptic nerve endings of dopaminergic neurons and blocks DA intake (Miller 2013). As a result, DA binds to postsynaptic D_1 and D_2 type receptors, which are shown to modulate probability-based risky decisions (St Onge and Floresco 2009). Based on our findings, the effect of AMPH is likely due to the activation of DA $D_{2/3}$ receptors because DA D_1 receptor activation failed to produce any behavioral changes. This is in line with other studies where it has been shown that AMPH and the DA $D_{2/3}$ receptor agonist quinpirole facilitate the modulation of reward expectancy during a rat slot machine task, prompting rats to make erroneous decisions (Winstanley et al. 2011). In addition, systemic treatment with the D_2 agonist bromocriptine has been shown to increase the frequency of choosing large but uncertain rewards in a probabilistic discounting task (St Onge and Floresco 2009). In the case of SKF-81297, it was challenging to interpret results because lever responses were decreased to a level that made it difficult to make reliable conclusions about the role of D_1 receptors. SKF-81297 is found to increase trial omission (Floresco et al. 2009) and this trial omission might be pronounced in our study due the *ad libitum* feeding.

When considering AMPH, we must acknowledge that it has a high potential to produce stereotypical behavioral patterns, and animals could become fixed to a specific behavioral protocol (Miller 2013). In light of this research, it is implausible that this would be a reason for the effects of AMPH on the lever choosing behavior because, during the long periods of sessions, rats are more prone to the SS-lever than LL-lever. Therefore, if the fixation occurs, it should be towards SS-lever. Another possible reason for changes in lever choice behavior after AMPH is that the drug might disturb the learned behavior of rats and provoke more impulsive decisions towards either one of the levers. As this might be the case with AMPH, it must be noted that GD is considered partly as an impulsive disorder and disturbances in the dopaminergic neurotransmission could provoke individuals to make an irrational and impulsive choice during gambling (e.g., betting too much and/or favor high risk-reward options) and also initiate gambling action more frequently.

6.4.1 DOPAMINE RECEPTOR ANTAGONISTS

At the beginning stage of the research, we decided not to study the impact of DA receptor antagonists on rats' decision-making. This was done for two reasons: First, the initial aim of the research was not to conduct a formal decision-making study but a study where the objective was to produce gambling-like behaviors in rats. The second reason was more practical because in our pilot studies, all DA receptor antagonists that were used had a major decreasing effect on the appetitive motivation of rats (most likely because of the *ad libitum* approach), and rats were not motivated to press levers in operant tasks in a manner that was needed to get reliable results.

6.4.2 RIGHT-LEFT LEVER BIAS

Based on the results of lever bias, the rat's innate lever preference was taken to account in studies II, III, and IV. Based on these findings and the finding of (Haluk and Floresco 2009), we modified our model so that the levers were not randomized anymore but balanced. The LL-lever was chosen to be the opposite lever that rats spontaneously chose. This was conducted to ensure that learning of optimizing rewards during the Rational Choice Task was genuinely based on the choice behavior of rats rather than initial fixation to a certain lever.

Lever bias results from study I create an interesting question concerning the role of increased dopaminergic neurotransmission in learning, which unfortunately cannot be theorized in this thesis's frame. However, it must be mentioned that this finding might reveal something important for the role of DA neurotransmission as activating behavioral patterns that are strongly consolidated at the neuronal level as inherent properties of an individual or, in case of addiction, developed during years of neuronal adaptation by neuronal plasticity.

6.5 DIFFERENCES IN PROBABILITY-BASED RISKY DECISION-MAKING BETWEEN AA AND WISTAR RATS (II, III)

After validating the model with AMPH, two latter studies showed promising results of strain-specific differences in risky decision-making after dopaminergic and opioidergic modulation. These results suggest that the gambling behavior of gamblers with a genetic vulnerability to high alcohol drinking should be studied more profoundly to find neurobiological correlations responsible for differences compared to the normal population.

Study II indicated that AMPH's risky decision-increasing effect was more robust in AA than in Wistar rats. Although the AMPH dose of 1.0 mg/kg did show a similar impact on group comparison, it was not significant in Wistar rats. After examining individual data analysis with each individual rat (Fig. 14), we found differences in the high distribution of effects of AMPH in Wistar rats. As the heterogenic Wistar rats represent the "normal" population, this finding might partly explain the clinical findings indicating a high discrepancy in gambling behaviors and etiology in human subjects (Blaszczynski and Nower 2002, Nower et al. 2013, Devos et al. 2020).

Results in study II indicated that AMPH is a more potent inducer for risky decisions in AA than in Wistar based on two findings:

1. An increase in LL-lever choices after AMPH is more consistent with the AA population, indicating that a genetic preference for high alcohol drinking increases the likelihood of DA-derived reinforcement in gambling behavior.

2. Despite the similar sucrose preference, AMPH reduces total lever responses significantly more in Wistar than in AA rats, which indicates that AMPH induced the "wanting" more in AA than in Wistar rats.

As Berridge and colleagues have proposed, the "wanting" of rewards can be separated from the "liking" of rewards resulting in behavior where the motivation towards the addictive action occurs without the positive feelings of the reward itself (Berridge et al. 2008). In this study, the AMPH showed decreased "liking" in both groups, which is observed as a similar decrease in sucrose eating (Table 3). Despite the reduced "liking" of sucrose, AA rats were more motivated to press levers than Wistar rats indicating that "wanting" occurs more strongly in AA rats, although the effects of AMPH to "liking" is similar in AA rats as in Wistar. This finding underlines the importance of the *ad libitum* approach in this research because the differences between AA and Wistar rats could not have been detected if rats were kept under restricted feeding due to the higher motivation to pursue sucrose rewards (Table 3). Altogether our results indicate that this neurobiological separation could be more prominent in AA than in Wistar rats.

In addition to the dopaminergic mechanism, study III indicated that brain functions that mediate probability-based risky decisions respond differently to opioidergic drugs morphine and NTRX in AA and Wistar rats. The clear and expected finding was that NTRX decreased the motivation to pursue rewards in AA and Wistar. This was hypothesized to occur because the opioidergic system has a significant role in the control of appetitive factors as the 'liking' and 'wanting' of sucrose is modulated by the opioidergic mechanisms (Berridge 1996, Berridge and Kringelbach 2015, Eikemo et al. 2016, Majuri et al. 2017, Nummenmaa et al. 2018). We also find that NTRX increased session time in both groups, and in AA rats it also reduced the number of lever presses rats made during the sessions, indicating reduced motivation towards pursuing rewards. These results were in line with other studies where NTRX decreases lever responding in operant conditions (Sanchez-Roige et al., 2015) and reduces alcohol drinking or the urge to gamble (Grant et al. 2006, Grant et al. 2008, Potenza 2008).

On the other hand, the effects on decision-making were surprising and not as hypothesized. The initial hypothesis was that morphine would increase the LL-lever choices, and this increase would be pronounced in AA rats because morphine modulates dopaminergic neurotransmission via blocking GABAergic inhibition in medium spiny neurons and should theoretically cause similar effects as DA release in Nacc (Spanagel et al. 1992). NTRX, on the other hand, should block the effects of endogenous opioids and thus decrease the DA neurotransmission in mesolimbic areas. According to this, it was unexpected that NTRX increased the LL-lever choices of Wistar rats and therefore biased the lever choices toward riskier and unbeneficial options. In AA rats, morphine reduced LL-lever presses, thus, promoting rats to make more beneficial choices by choosing the SS-lever more.

The lack of scientific support from preclinical studies made results challenging to interpret. However, some clinical evidence shows that small to moderate doses of morphine may enhance cognitive performance in humans (van Steenberg et al. 2019), which may also be true in our study, but only in AA rats. Morphine is also shown to enhance the performance of cognitive functions by improving accuracy on the choice reaction time task (Hanks et al. 1995, O'Neill et al. 2000) and to shift choices toward high-value rewards in a two-choice paradigm conducted with human participants (Eikemo et al. 2017), so there is a possibility that the dose range of 0.3 to 1.0 mg/kg of morphine acted in a similar way in AA rats. More studies with higher doses of morphine are needed in future studies to fully understand how opioid agonists may interact with decision-making mechanisms.

NTRX has been shown to reduce the craving to gamble, gambling-related thoughts, and gambling frequency (Victorri-Vigneau et al. 2018). However, the mechanism of action of NTRX on the control of decision-making during gambling is unclear and preclinical studies are few. NTRX is shown to improve decision-making in rGT with a subset of animals that favored the disadvantageous choice at the baseline (Di Ciano and Le Foll 2016). In a

clinical setting with recreational gamblers, NTRX modulated the responsiveness to wins, but in the opposite direction that was predicted (Porchet et al. 2013), making test subjects more confident in the decision-making and showing heightened responsiveness to wins. Our results may occur due to similar behavioral responses, but only in Wistar rats. According to our results, the overall effect of NTRX seen on AA rats seems to act as a demotivating drug, causing decreasing motivation to pursue rewards overall.

6.6 MICROINJECTION STUDY, LEAP TOWARDS DRUG SCREENING (IV)

The fourth and final study was conducted to address two critical subjects. First, we wanted to strengthen the theory of Nacc-derived dopaminergic function as a driver of risky decision-making. Another aim was to study the possible impact of opioid antagonist NTRX on this gambling-induced behavior and its predictive validity on the DA-based theory of GD. Our hypothesis was that NTRX would block the behavioral excitation towards the riskier choice and thus decrease the "gambling-like" behavior promoted by the hyperdopaminergic activity of the Nacc.

Execution and results from study IV were encouraging. AMPH dose-dependently increased the LL-lever choices of AA rats, indicating that phasic DA activity at Nacc increases the risky choices. Compared to systemic administration, AMPH did not affect motivational factors like time to accomplish sessions or amount of lever pressing. However, based on study IV, NTRX lacked any effects on the LL lever choices in AA rats, indicating that opioid receptor blockade does not affect AMPH-induced probability-based decision-making in the AA rats at the dose range used in the study. However, NTRX increased the time to accomplish the sessions, indicating the lack of overall motivation towards "wanting" the rewards, which supports the clinical use of opioid antagonists to reduce gambling urges, but probably not to decrease the risk-taking tendencies in GD patients. However, this conclusion should be verified with a study using a wider range of NTRX and/or naloxone doses.

As the microinjection protocol was shown to be a very robust method to induce "gambling-like" behavior in rats, it should be considered a valid approach for possible drug-screening studies in the future. One thing to consider is to conduct stereotaxic surgery at the very beginning of the study to avoid any breaks because of the recovery time of rats in the middle of training sessions.

6.7 FACE, CONSTRUCT, AND PREDICTIVE VALIDITY

When interpreting the data from animal models, we must always consider how the modeled behavior appears in terms of *face, construct,* and *predictive validity*. Face validity refers to how the model captures the studied behavior

(Willner 1984). The construct validity answers the question, is the modeled behavior result what we suppose it is (Willner 1984). The third, predictive validity, answers how the obtained results predict human behavior (Willner 1984).

When considering *face validity*, we can confidently acknowledge that the model used in this research is validly capturing the behavior aimed to study. The main reason for this is *ad libitum* feeding, which ensures that rats pursue sucrose based on reward-related factors more than a need for food-seeking based on nutritional needs. Rats also react rapidly to changes in probabilities, indicating that lever choices are made based on probabilistic estimations of available rewards.

As *the construct validity* of the model, we can interpret that the baseline decision-making behavior is replicating validly similar decision-making behavior that is observed in humans. This "human-kind" decision-making is disturbed pharmacologically by increasing the DA levels by systemic administration or locally at the Nacc regions, which increase the favoring of bigger but riskier rewards, indicating that we can replicate key factors of gambling behavior.

However*, the predictive validity* is more problematic to ensure based on just this model. The are several reasons for this which the most prominent is that we cannot use *de facto* money as a reward, which is one of the most significant differences when comparing animal studies to studies conducted with humans. Although GD is considered an addiction, there are no external chemicals responsible for the rewarding effects of gambling. As money is the primary outcome of a successful gamble, it is not the agent producing the reward in the brain, like in substance-based addictions. We can promote "gambling-like" behavior in rats, but the final question that needs to be answered is how well this correlates to human behavior in GD? To answer this question, more preclinical studies with a variety of approaches and methods should be conducted in the future.

6.8 LIMITATIONS OF THE RESEARCH

Firstly, all but in one study, drugs were administered to systemic blood circulation resulting in effects in the 'whole brain,' which makes interpreting exact neurobiological mechanisms impossible. The findings emphasize the need for more specific preclinical studies focusing on specific areas of the brain, which was done in study IV. Excluding study IV, drugs were examined at the baseline decision-making of the rats and only in rats that fulfilled the criteria for 'normal' decision-making. The outcome could be different in a setting where the dopaminergic activity is already disturbed (as in study IV) as it is supposed to be in individuals with GD. One possibility would be using a larger cohort of rats without selection based on RCC and RAC. This could give us more precise information on population-based effects.

Some concerns were also present, considering the model itself. Although the fact that rats' LL-lever choosing is not fully stabilized during the task is at the same time a major pro and con of the model, it creates challenges during the drug manipulations because the time window should be kept as short as possible to avoid wrong interpretations. This was addressed by using individual baseline comparisons before the drug manipulations. When this is done, we can obtain a time window that can be considered short enough that no significant learning can occur at this time (statistic analyses controlled learning).

One con, when considering gambling, is the probabilities used in the study. From a gambling point of view, the optimal probability would be 50% or so that the expected value would be similar to slot machine gambling. This is one factor that can be easily conducted in future studies by adjusting the EV of SS and LL levers. At this research, the "gambling level" was LL 25% vs. SS 100%, which leaves the EV of LL lever 0,75 pellets/choice. However, the main problem is not the EV, but the LL lever percentage, which might cause rats to express aversive behavior towards choosing the LL lever.

One con is that this model does not allow us to use DA antagonists very effectively due to the effects on the feeding. This does not allow us to conduct a basic pharmacological agonist/antagonist comparison. Although, from the standpoint of the model's aims, this is not a significant flaw as it first seems, as the model aims to produce "normal" decision-making behavior that could be modified to "gambling-like" behavior.

Only male rats were used in this research. This was done mainly for practical reasons but must be mentioned as a limitation because preclinical studies have shown some differences in the decision-making based on the sex of the rats (van den Bos et al. 2012, van den Bos et al. 2013). On the other hand, gambling problems are shown to be more prevalent among men than women (Calado and Grifths 2016).

6.9 SUMMARY AND FUTURE DIRECTIONS

In this research, we completed the aim to validate an animal model for studying the probability-based risky decision-making behavior in AA rats. This allowed us to study the impact of genetic alcohol-preference to "gambling-like behavior" by comparing the behavior of AA rats to heterogenic laboratory Wistar rats.

We showed results that strongly indicate that dopaminergic modulation of risky decisions is pronounced in AA rats and validated that this behavior is, at least partly, modulated by the dopaminergic actions of the Nacc. Results also indicate that the opioidergic mechanisms differently modulate the decision-making behavior of AA and Wistar rats. These studies create a platform for future studies aiming to point out the specific neurobiological mechanisms that control the behavior of the gambler subgroup with a genetic vulnerability to alcohol use disorder.

As the work of this thesis only goes as far as showing behavioral differences between AA and Wistar, it would be mandatory in future studies to precisely map neurobiological differences between AA and Wistar, as this would be beneficial to truly address the question what are neurobiological correlates that are responsible for the behavioral differences between AA and Wistar. Also, other alcohol-preferring ratlines should be studied. Microinjection studies also have endless possibilities to variate different drug combinations to different brain areas. At least AMPH mixed with different DA antagonists and morphine or NTRX injections to Nacc should be investigated in future studies.

7 CONCLUSIONS

This research included three main goals, which were all achieved.

1. We validated a preclinical model which reliably models similar decision-making behavior in probabilistic decision-making that is observed in humans and validated the model by using AMPH as a risky decision-making promoter.

2. We showed differences in the probability-based risky decision-making based on the genetic alcohol preference by showing that DA- and opioidergic drug manipulations modulated the decision-making of AA and Wistar rats differently. AMPH acted as a more potent inducer for risky decisions in AA rats than in Wistar rats, indicating heightened reinforcing properties of dopaminergic functions in AA rats' "gambling-like" behavior. Although the difference in the decision-making was observed with opioidergic drugs between AA and Wistar rats, exact predictions of the relevance of opioids could not be made.

3. We showed that microinjections to Nacc are a valid approach for studying DA-induced risky decision-making and strengthened the role of NTRX in the treatment of GD to affect the motivational aspect of gambling.

Altogether results of this research create a good platform for future studies aiming to find individual differences in the neurobiology of GD in individuals with a genetic risk factor for alcohol use disorder.

ACKNOWLEDGMENTS

The work described in this thesis was carried out at the Division of Pharmacology and Pharmacotherapy at the University of Helsinki Faculty of Pharmacy and at the Finnish Institute for Health and Welfare from 2013 to 2022. I am grateful to both institutions for this exciting opportunity to accomplish my doctoral studies in the field of addiction behavior.

The Finnish Foundation for Alcohol Studies supported my research and doctoral training for more than four years, allowing me to accomplish most of this research, for which I am extremely grateful. I want to thank also other supporters, Orion Research Foundation and the Finnish Pharmaceutical Society. All these grants also allowed me to present my research at four international conferences and meet colleagues from other countries. I am very grateful for this.

I would like to thank my pre-examiners, Professor Catharine Winstanley and Associate Professor Juho Joutsa, for reviewing this thesis. I was very flattered that I could present my work to these two experts in gambling disorder research. Winstanley's work in this field has inspired me from the beginning of this research in 2013.

I would like to thank Professor Emeritus Hannu Alho for agreeing to act as my opponent. It is exciting that one of the pioneers of clinical research in gambling disorder in Finland will be my opponent.

I want to thank Professor Raimo Tuominen, the Head of the Division of Pharmacology and Pharmacotherapy, at the time of this research. Raimo has the incredible skill of creating a supportive atmosphere where everybody can feel their work being appreciated.

My three supervisors Docent Petteri Piepponen, Professor Emeritus Atso Raasmaja, and Professor Emeritus Kalervo Kiianmaa, deserve my greatest gratitude. I also want to thank Petteri for agreeing to act as custos. I want to thank you all for giving me freedom and responsibility that I would not have expected. In times I was insecure about the direction of the research, you always managed to get me back on track. Without Petteri's support with the statistical analysis, I would have been lost for endless time trying to figure it out myself. My first touch with addiction research was when I started my Master's Thesis work in the research group of Kalervo in 2009. Since then, I have always been fascinated by addiction research, especially the preclinical side of it. Thank you Kalervo, for being incredibly supportive throughout these years and sharing the knowledge and expertise in the writing process of research articles. I also want to thank all of you three for being my support till the end of this research despite my progress during the last few years has been slow due to combining thesis, business, and family.

I would like to express gratitude to my colleagues Dr. Pia Bäckström and Dr. Johanna Uhari-Väänänen for all the help and support you gave me. Pia's expertise in animal handling and planning research protocols was essential for

this research to be accomplished. There were no times I couldn't ask for your advice, for which I am very grateful. Johanna's attitude and devotion to work have always amazed me, and despite having much of her own work, she was always there to help and support me.

I also want to thank M.Sc. Mikko Sundström, who conducted his Master's thesis under my supervision and put a lot of effort into the laboratory work of our published studies. Numerous hours in the dark lab with the rats would have been impossible without his high work ethic and dedication to the research.

I want to thank Leena Tanner-Väisänen and Marjo Vaha for helping me with the tasks where my laboratory skills would not have been good enough. You both teach me many things that only real professionals can accomplish. Since we first met in 2009, I have always admired Leena's animal handling expertise, especially in stereotaxic operations. Thank you Leena, for teaching all those things many years ago.

I also want to thank Antti "Apteekkari Antti" Hyytiäinen for encouraging me over the last few years to accomplish this thesis. I greatly appreciate the opportunity to combine work in pharmacy and thesis writing during my time in Pori.

I want to thank Dr. Katrina Albert for proofreading this thesis.
Despite personally knowing, I want to thank Joan Bolker, who wrote the book, "Writing Your Dissertation in Fifteen Minutes a Day." Without reading this book, I think this thesis would still be in the drawer because combining family and a career as an entrepreneur and this thesis felt more than often overwhelming during the last few years.

I want to thank my mom and dad for their constant support and belief in me. Also, in those times, this work has been proceeding slowly. Despite having a research background, they have always been interested in how my work is going.

Last but not least, I want to express my deepest gratitude to my family, my son Sisu, my daughters Jade and Perla, and especially my wife Älis. Family as the bedrock of my life allows me to take risks without fear of losing all if something does not go as planned. Throughout these years, support from Älis has been crucial for completing this thesis. Despite her hands full of work (an insane amount of it!), she has always arranged for me time to write by taking care of our children and house. Älis, I love you!

Helsinki, August 2022

Ville

REFERENCES

Abler B, Walter H, Erk S, Kammerer H, Spitzer M (2006) Prediction error as a linear function of reward probability is coded in human nucleus accumbens. Neuroimage 31:790–795.

Adriani W, Laviola G (2006) Delay aversion but preference for large and rare rewards in two choice tasks: Implications for the measurement of self-control parameters. BMC Neuroscience 7:1–11.

Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM (1984) Endogenous opioids: biology and function. Annu Rev Neurosci 7: 223-255.

Akil H, Owens C, Gutstein H, Taylor L, Curran E, Watson S (1998) Endogenous opioids: overview and current issues. Drug Alcohol Depend 51: 127-140.

APA (1994) Diagnostic and statistical manual of mental disorders (DSM-IV). American Psychiatric Association.

APA (2013) Diagnostic and statistical manual of mental disorders (DSM-5). American Psychiatric Association.

Baik JH (2013) Dopamine signaling in reward-related behaviors. Front Neural Circuits 11:7:152.

Barrus MM, Winstanley CA (2016) Dopamine D3 receptors modulate the ability of win-paired cues to increase risky choice in a rat gambling task. Journal of Neuroscience 36:3:785–794.

Beaulieu JM, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63:1:182-217.

Bechara A, Damasio AR, Damasio H, Anderson SW (1994) Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 50:7–15.

Bell SM, Stewart RB, Thompson SC, Meisch RA (1997) Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. Psychopharmacology 131:1:1-8.

Berridge KC (1996) Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev 20:1–25.

Berridge KC (2003) Pleasures of the brain. Brain and Cognition 52:1:106–128.

Berridge KC (2007) The debate over dopamine's role in reward: The case for incentive salience. Psychopharmacology 191:3:391–431.

Berridge KC, Robinson TE, Aldridge JW (2008) Dissecting components of reward: 'liking', 'wanting', and learning. Current opinion in pharmacology 9:1:65-73.

Berridge KC, Kringelbach ML (2015) Pleasure Systems in the Brain. Neuron 86:3:646–664.

Bischof A, Meyer C, Bischof C, Kastirke N, John U, Rumpf H-J (2013) Comorbid Axis I-disorders among subjects with pathological, problem, or at-risk gambling recruited from the general population in Germany: Results of the PAGE study. Psychiatry Research 210:3:1065–1070.

Blanco C, Hanania J, Petry NM, Wall MM, Wang S, Jin CJ, Kendler KS (2015) Towards a comprehensive developmental model of pathological gambling. Addiction 110:8:1340–1351.

Blaszczynski A, Nower L (2002) A pathways model of problem and pathological gambling. Addiction 97:5:487–499.
Blaszczynski A, Walker M, Sharpe L, Nower L (2008) Withdrawal and tolerance phenomenon in problem gambling. Int. Gambl. Stud 8:179–192.
Blum K, Braverman ER, Holder JM, Lubar JF, Monastra VJ, Miller D, Lubar JO, Chen TJ, Comings DE (2000) Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviours. Journal of Psychoactive Drugs 32:1–112.
Boileau P, Payer D, Chugani B, Lobo DSS, Houle S, Wilson AA, Warsh JK, Zack M (2014) In vivo evidence for greater amphetamine-induced dopamine release in pathological gambling: a positron emission tomography study with [11C]-(+)-. Molecular psychiatry 19:12:1305-1313.
Bonci A, Williams JT (1997) Increased probability of GABA release during withdrawal from morphine. J Neurosci 17: 796-803.
Bozarth MA (1987) Neuroanatomical boundaries of the reward-relevant opiate-receptor field in the ventral tegmental area as mapped by the conditioned place preference method in rats. Brain Res 414: 77-84.
Brevers D, Bechara A, Cleeremans A, Noël X (2013) Iowa Gambling Task (IGT): twenty years after – gambling disorder and IGT Front Psychol 4: 665.
Brevers D, Bechara A, Cleeremans A, Kornreich C, Verbanck P, Noël X (2014) Impaired decision-making under risk in individuals with alcohol dependence. Alcohol Clin Exp Res 38(7):1924–1931.
Bullock SA, Potenza MN (2012) Pathological gambling: neuropsychopharmacology and treatment. Curr Psychopharmacol 1:67-85.
Calado F, Grifths MD (2016). Problem gambling worldwide: An update and systematic review of empirical research (2000–2015). Journal of Behavioral Addictions 5:4:592–613.
Cardinal RN, Howes NJ (2005) Effects of lesions of the nucleus accumbens core on choice between small certain rewards and large uncertain rewards in rats. BMC Neuroscience 6:1–19.
Carroll ME, France CP, Meisch RA (1981) Intravenous self-administration of etonitazene, cocaine and phencyclidine in rats during food deprivation and satiation. The Journal of pharmacology and experimental therapeutics 217:2:241-247.
Castro DC, Berridge KC (2014) Advances in the neurobiological bases for food 'liking' versus 'wanting'. Physiology & behavior 136:22-30.
Chesselet MF (1984) Presynaptic regulation of neurotransmitter release in the brain: facts and hypothesis. Neuroscience 12: 347-375.
Clark L (2010) Decision-making during gambling : an integration of cognitive and psychobiological approaches 365:1538:319–330.
Clark L (2014) Disordered gambling: the evolving concept of behavioral addiction. Annals of the New York Academy of Sciences 1327:1:46-61.
Cocker PJ, Winstanley CA (2015) Irrational beliefs, biases and gambling: Exploring the role of animal models in elucidating vulnerabilities for the development of pathological gambling. Behavioural Brain Research 279:259–273.

Corbett AD, Paterson SJ, McKnight AT, Magnan J, Kosterlitz HW (1982) Dynorphin and dynorphin are ligands for the kappa-subtype of opiate receptor. Nature 299:79-81.
Cowlishaw S, Merkouris S, Chapman A, Radermacher H (2014) Pathological and problem gambling in substance use treatment: A systematic review and meta-analysis. Journal of Substance Abuse Treatment 46:2:98–105.
Croson R, Sundali J (2005) The Gambler's Fallacy and the Hot Hand: Empirical Data from Casinos. Journal of risk and uncertainty 30:3:195-209.
Cunningham-Williams RM, Cottler LB, Compton WM, Spitznagel EL (1998) Taking chances: problem gamblers and mental health disorders–results from the St. Louis Epidemiologic Catchment Area Study. Am. J. Public Health 88:1093–1096.
Davey B, Cummins R (2018) Testing an incentive-sensitisation approach to understanding problem slot-machine gambling using an online slot-machine simulation. Journal of Gambling Studies 34:3:773–784.
de Castro V, Fong T, Rosenthal RJ, Tavares H (2007) A comparison of craving and emotional states between pathological gamblers and alcoholics. Addict. Behav 32: 1555–1564.
Devos MG Clark L, Bowden-Jones H, Grall-Bronnec M, Challet-Bouju G, Khazaal Y, Maurage P, Billieux J (2020) The joint role of impulsivity and distorted cognitions in recreational and problem gambling: A cluster analytic approach. Journal of Affective Disorders 260:473–482.
Di Ciano P, Le Foll B (2016). Evaluating the impact of naltrexone on the rat gambling task to test its predictive validity for gambling disorder. PloS One 11:5:e0155604.
Djamshidian A, Jha A, O'Sullivan SS, Silveira-Moriyama L, Jacobson C, Brown P, Lees A, Averbeck BB (2010) Risk and learning in impulsive and non impulsive patients with Parkinson's disease. Mov Disord 25:2203–2210.
Djamshidian A, Cardoso F, Grosset D, Bowden-Jones H, Lees AJ (2011) Pathological gambling in Parkinson's disease-a review of the literature. Movement disorders 26:11:1976-1984.
Driver-Dunckley E, Samanta J, Stacy M (2003) Pathological gambling associated with dopamine agonist therapy in Parkinson's disease. Neurology 61:3:422-423.
Eikemo M, Løseth GE, Johnstone T, Gjerstad J, Willoch F, Leknes S (2016). Sweet taste pleasantness is modulated by morphine and naltrexone. Psychopharmacology 233:3711–3723.
Eikemo M, Biele G, Willoch F, Thomsen L, Leknes S (2017). Opioid modulation of value-based decision making in healthy humans. Neuropsychopharmacology 42:1833–1840.
Eriksson K (1698) Genetic Selection for Voluntary Alcohol Consumption in the Albino Rat Science 159:3816:739-741.
Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, Robbins TW (2008) Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. Philos Trans R Soc Lond B Biol Sci 363:1507:3125-35.
Fiorillo CD, Tobler PN, Schultz W (2003) Discrete Coding of Reward Dopamine Neurons. Science 299:1898–1902.

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6, 968-973.
Floresco SB, Block AE, Tse MT (2008) Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behav Brain Res 190:1:85-96.
Floresco SB (2016) Dopamine neurons, input integration, and reward prediction errors: E pluribus Unum. Neuron 91:6:1192–1194.
Flórez G, Saiz PA, Santamaría EM, Álvarez S, Nogueiras L, Arrojo M (2016) Impulsivity, implicit attitudes and explicit cognitions, and alcohol dependence as predictors of pathological gambling. Psychiatry Research 245:392–397.
French MT, Maclean JC, Ettner SL (2008) Drinkers and bettors: Investigating the complementarity of alcohol consumption and problem gambling. Drug and Alcohol Dependence 96(1–2):155–164.
Fugariu V, Zack MH, Nobrega JN, Fletcher PJ, Zeeb FD (2020) Effects of exposure to chronic uncertainty and a sensitizing regimen of amphetamine injections on locomotion, decision-making, and dopamine receptors in rats. Neuropsychopharmacology 45(5):811–822.
Gainsbury SM, Suhonen N, Saastamoinen J (2014) Chasing losses in online poker and casino games: Characteristics and game play of Internet gamblers at risk of disordered gambling. Psychiatry research 217:3: 220-225.
Gianoulakis C (2001) Influence of the endogenous opioid system on high alcohol consumption and genetic predisposition to alcoholism. J Psychiatry Neurosci 26:4:304-18.
Gilpin NW, Koob GF (2008) Neurobiology of Alcohol Dependence: Focus on Motivational Mechanisms. Alcohol Res Health 31: 185-195.
Granero R, Jiménez-Murcia S, Del Pino-Gutiérrez A, Mena-Moreno T, Mestre-Bach G, Gómez-Peña M, Moragas L, Aymamí N, Giroux I, Grall-Bronnec M, Sauvaget A, Codina E, Vintró-Alcaraz C, Lozano-Madrid M, Camozzi M, Agüera Z, Martín-Romera V, Sánchez-González J, Casalé G, Sánchez I, López-González H, Munguía L, Valenciano-Mendoza E, Mora B, Baenas-Soto I, Menchón JM, Fernández-Aranda F (2020) Gambling Phenotypes in Older Adults. Journal of Gambling Studies 36:3:809–828.
Grant JE, Potenza MN, Hollander E, Cunningham-Williams R, Nurminen T, Smits G, Kallio A (2006). Multicenter investigation of the opioid antagonist nalmefene in the treatment of pathological gambling. Am J Psychiatry 163:303–312.
Grant JE, Kim SW, Hollander E, Potenza MN (2008). Predicting response to opiate antagonists and placebo in the treatment of pathological gambling. Psychopharmacology 200:521–527.
Grant JE, Chamberlain SR (2015) Gambling disorder and its relationship with substance use disorders: implications for nosological revisions and treatment. Review Am J Addict 2:126-131.
Grant JE, Odlaug BL, Chamberlain SR (2016) Neural and psychological underpinnings of gambling disorder: A review Progress in neuro-psychopharmacology & biological psychiatry 65:188-193.

Griffiths M (1991) Psychobiology of the near-miss in fruit machine gambling. J. Psychol 125:347–357.
Griffiths M (1995) The role of subjective mood states in the maintenance of fruit machine gambling behaviour. J Gambl Stud 11:123–135.
Haluk DM, Floresco SB (2009). Ventral striatal dopamine modulation of different forms of behavioral flexibility. Neuropsychopharmacology 34:2041 2052.
Hanks GW, O'Neill M, Simpson P, Wesnes K (1995). The cognitive and psychomotor effects of opioid analgesics I1. A randomized controlled trial of single doses of morphine, lorazepam and placebo in healthy subjects. Eur J Clin Pharmacol 48:455–460.
Hansen C, Spuhler K (1984) Development of the National Institutes of Health genetically heterogeneous rat stock. Alcohol Clin Exp Res 8: 477-479.
Hirose N, Murakawa K, Takada K, Oi Y, Suzuki T, Nagase H, Cools AR, Koshikawa N (2005) Interactions among mu- and delta-opioid receptors, especially putative delta1- and delta2-opioid receptors, promote dopamine release in the nucleus accumbens. Neuroscience 135: 213-225.
Hodgins DC, Stea JN, Grant JE (2011) Gambling disorders. Lancet 378:9806:1874–1884.
Honkanen A, Mikkola J, Korpi ER, Hyytiä P, Seppälä T, Ahtee L (1999) Enhanced morphine- and cocaine-induced behavioral sensitization in alcohol-preferring AA rats. Psychopharmacology (Berl) 142:3:244-52.
Huang YF, Soon CS, Mullette-Gillman OA, Hsieh PJ (2014) Pre-existing brain states predict risky choices. NeuroImage 101:466–472.
Huettel SA, Stowe CJ, Gordon EM, Warner BT, Platt ML (2006) Neural signatures of economic preferences for risk and ambiguity. Neuron 49:5:765–775.
Hyytiä P, Sinclair JD (1990) Differential Reinforcement and Diurnal Rhythms of Lever Pressing for Ethanol in AA and Wistar Rats. Alcoholism: clinical and experimental search 14:3: 375-9.
Hyytiä P, Sinclair JD (1991) Stimulus-Controlled Responding for Ethanol in AA and Wistar Rats. Alcohol 3:229-34.
Hyytiä P, Sinclair JD (1993) Oral etonitazene and cocaine consumption by AA, ANA and Wistar rats. Psychopharmacology (Berl) 111:4:409-14.
Jiang ZG, North RA (1992) Pre- and postsynaptic inhibition by opioids in rat striatum. J Neurosci 12:356-361.
Johnson SW, North RA (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 12:483-488.
Kahneman D, Tversky A (1979) Prospect Theory: An Analysis of Decision under Risk. Econometrica 47:2:263–292.
Kampov-Polevoy AB, Garbutt JC, Khalitov E (2003) Family History of Alcoholism and Response to Sweets. Alcoholism: Clinical and Experimental Research 27:11:1743–1749.
Katner SN, Weiss F (2001) Neurochemical characteristics associated with ethanol preference in selected alcohol-preferring and -nonpreferring rats: A quantitative microdialysis study. Alcoholism: Clinical and Experimental Research 25:2:198–205.

Khanbhai Y, Smith D, Battersby M (2016) Gender by Preferred Gambling Activity in Treatment Seeking Problem Gamblers: A Comparison of Subgroup Characteristics and Treatment Outcomes Journal of gambling studies 33:1:99-113.
Kim SW, Grant JE, Adson D, Shin YC (2001) Double-blind naltrexone and placebo comparison study in the treatment of pathological gambling. Biol Psychiatry 49:914–921.
Koistinen M, Tuomainen P, Hyytiä P, Kiianmaa K (2001) Naltrexone suppresses ethanol intake in 6-hydroxydopamine-treated rats. Alcohol Clin Exp Res 11:1605-12.
Kovács I, Richman MJ, Janka Z, Maraz A, Andó B (2017) Decision making measured by the Iowa Gambling Task in alcohol use disorder and gambling disorder: a systematic review and meta-analysis. Drug and alcohol dependence 181:152-161.
Koob GF (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends in pharmacological sciences 13:177-184.
Koob GF, Le Moal M (1997) Drug Abuse: Hedonic Homeostatic Dysregulation Science 278:5335:52-58.
Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytiä P, Merlo-Pich E, Weiss F (1998) Neurocircuitry Targets in Ethanol Reward and Dependence Alcoholism, clinical and experimental research 22:1:3-9.
Koob GF, Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. Lancet Psychiatry 8:760-773.
Kovic M, Kristiansen S (2019) The gambler's fallacy fallacy (fallacy). Journal of risk research 22:3:291-302.
Kraus SW, Etuk R, Potenza MN (2020) Current pharmacotherapy for gambling disorder: a systematic review. Expert Opin Pharmacother 3:287-296.
Law PY, Wong YH, Loh HH (2000) Molecular mechanisms and regulation of opioid receptor signaling. Annu Rev Pharmacol Toxicol 40:389-430.
Ledgerwood DM, Petry NM (2006) What do we know about relapse in pathological gambling? Clin. Psychol. Rev 26:216–228.
Leeman RF, PotenzaMN (2013) A targeted review of the neurobiology and genetics of behavioural addictions: an emerging area of research. Can. J. Psychiatr 58:5:260–273.
Li TK, Lumeng L, Doolittle DP, Carr LG (1991) Molecular associations of alcohol-seeking behavior in rat lines selectively bred for high and low voluntary ethanol drinking. Alcohol Alcohol Suppl 1:121-124.
Linnet J, Frøslev M, Ramsgaard S, Gebauer L, Mouridsen K, Wohlert V (2012) Impaired Probability Estimation and Decision-Making in Pathological Gambling Poker Players. Journal of Gambling Studies 28:1:113–122.
Linnet J (2013) The Iowa Gambling Task and the three fallacies of dopamine in gambling disorder. Front Psychol 4:709:1–11.
Linnet J (2014) Neurobiological underpinnings of reward anticipation and outcome evaluation in gambling disorder. Front Behav Neurosci 8:100.
Lorains FK, Cowlishaw S, Thomas SA (2011) Prevalence of comorbid disorders in problem and pathological gambling: systematic review and meta-analysis of population surveys. Addiction 106:490–498.

Mai B, Sommer S, Wolfgang H (2015) Dopamine D1/D2 Receptor Activity in the Nucleus Accumbens Core But Not in the Nucleus Accumbens Shell and Orbitofrontal Cortex Modulates Risk-Based Decision Making. Int J Neuropsychopharmacol 18:10:pyv043.

Majuri J, Joutsa J, Johansson J, Voon V, Alakurtti K, Parkkola R, Lahti T, Alho H, Hirvonen J, Arponen E, Forsback S, Kaasinen V (2017) Dopamine and Opioid Neurotransmission in Behavioral Addictions: A Comparative PET Study in Pathological Gambling and Binge Eating. Neuropsychopharmacology 42:5:1169–1177.

Mann K, Lemenager T, Zois E, Hoffmann S, Nakovics H, Beutel M, Vogelgesang M, Wölfling K, Kiefer F, Fauth-Bühler M (2017) Comorbidity, family history and personality traits in pathological gamblers compared with healthy controls. European Psychiatry 42:120–128.

Mansour A, Khachaturian H, Lewis ME, Akil H, Watson SJ (1987) Autoradiographic differentiation of mu, delta, and kappa opioid receptors in the rat forebrain and midbrain. J Neurosci 7:2445-2464.

Merims D, Giladi N (2017) Dopamine dysregulation syndrome, addiction and behavioral changes in Parkinson's disease. Parkinsonism & related disorders 14:4:273-280.

Mick I, Myers J, Stokes PRA, Erritzoe D, Colasanti A, Bowden-Jones H, Clark L, Gunn RN, Rabiner EA, Searle GE, Waldman AD, Parkin MC, Brailsford AD, Nutt DJ, Lingford-Hughes AR (2014) Amphetamine induced endogenous opioid release in the human brain detected with [^{11}C]carfentanil PET: replication in an independent cohort. Int J Neuropsychopharmacol 12:2069-74.

Mick I, Myers J, Ramos AC, Stokes PRA, Erritzoe D, Colasanti A, Gunn RN, Rabiner EA, Searle GE, Waldman AD, Parkin MC, Brailsford AD, Galduróz JCF, Bowden-Jones H, Clark L, Nutt DJ, Lingford-Hughes AR (2016) Blunted Endogenous Opioid Release Following an Oral Amphetamine Challenge in Pathological Gamblers. Neuropsychopharmacology 41:7:1742–1750.

Miller PM (2013) Neuropharmacology of Cocaine and Amphetamine Biological Research on Addiction 2:573-577.

Murakawa K, Hirose N, Takada K, Suzuki T, Nagase H, Cools AR, Koshikawa N (2004) Deltorphin II enhances extracellular levels of dopamine in the nucleus accumbens via opioid receptor-independent mechanisms. Eur J Pharmacol 491:31-36.

Nautiyal KM, Okuda M, Hen R, Blanco C (2017) Gambling disorder: an integrative review of animal and human studies. Annals of the New York Academy of Sciences 1394:1:106–127.

Nestler EJ (2005) Is there a common molecular pathway for addiction? Nature neuroscience 8:11:1445-1449.

Nower L, Martins SS, Lin K, Blanco C (2013) Subtypes of disordered gamblers: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Addiction 108:4:789-798.

Nummenmaa L, Saanijoki T, Tuominen L, Hirvonen J, Tuulari JJ, Nuutila P, Kalliokoski K (2018). μ-opioid receptor system mediates reward processing in humans. Nat Commun 9:1500.

Ojala KE, Janssen LK, Hashemi MM, Timmer MHM, Geurts DEM, Ter Huurne NP, Cools R, Sescousse G (2018) Dopaminergic drug effects on probability weighting during risky decision making. eNeuro 5(2).
Okutsu H, Watanabe S, Takahashi I, Aono Y, Saigusa T, Koshikawa N, Cools AR (2006) Endomorphin-2 and endomorphin-1 promote the extracellular amount of accumbal dopamine via nonopioid and mu-opioid receptors, respectively. Neuropsychopharmacology 31: 375-383.
Olive MF, Anton B, Micevych P, Evans CJ, Maidment NT (1997) Presynaptic versus postsynaptic localization of mu and delta opioid receptors in dorsal and ventral striatopallidal pathways. J Neurosci 17:7471-7479.
O'Neill WM, Hanks GW, Simpson P, Fallon MT, Jenkins E, Wesnes K (2000) The cognitive and psychomotor effects of morphine in healthy subjects: a randomized controlled trial of repeated (four) oral doses of dextropropoxyphene, morphine, lorazepam and placebo. Pain 85:209–2.
Orsini CA, Hernandez CM, Singhal S, Kelly KB, Frazier CJ, Bizon JL, Setlow B (2017) Optogenetic Inhibition Reveals Distinct Roles for Basolateral Amygdala Activity at Discrete Time Points during Risky Decision Making The Journal of neuroscience 37 :48:11537-11548.
Paxinos P, Watson C (1998) The rat brain in stereotaxic coordinates. Academic Press, San Diego.
Peciña S (2008) Opioid reward 'liking' and 'wanting' in the nucleus accumbens. Physiol Behav 945:675–680.
Petrovic P, Pleger B, Seymour B, Klöppel S, De Martino B, Critchley H, Dolan RJ (2008) Blocking central opiate function modulates hedonic impact and anterior cingulate response to rewards and losses. Journal of Neuroscience 28:42:10509–10516.
Pettorruso M, Zoratto F, Miuli A, De Risio L, Santorelli M, Pierotti A, Martinotti G, Adriani W, di Giannantonio M (2020) Exploring dopaminergic transmission in gambling addiction: A systematic translational review. Neuroscience and Biobehavioral Reviews 119:481–511.
Petry NM, Stinson FS, Grant BF (2005) Comorbidity of DSM-IV pathological gambling and other psychiatric disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. J. Clin. Psychiat 66:564–574.
Porchet RI, Boekhoudt L, Studer B, Gandamaneni PK, Rani N, Binnamangala S, Müller U, Clark L (2013) Opioidergic and dopaminergic manipulation of gambling tendencies: a preliminary study in male recreational gamblers. Front Behav Neurosci 7:138.
Potenza MN (2008) The neurobiology of pathological gambling and drug addiction: an overview and new findings. Philos Trans R Soc Lond B Biol Sci 363:1507:3181-9.
Potenza MN (2013a) How central is dopamine to pathological gambling or gambling disorder? Frontiers in behavioral neuroscience 7:206.
Potenza MN (2013b) Neurobiology of gambling behaviors. Current Opinion in Neurobiology 23:4:660–667.

Prescott CA, Kendler KS (1999) Genetic and environmental contributions to alcohol abuse and dependence in a population-based sample of male twins. American Journal of Psychiatry 156:1:34–40.
Prescott CA, Aggen SH, Kendler KS (1999) Sex differences in the sources of genetic liability to alcohol abuse and dependence in a population-based sample of U.S. twins. Alcoholism: Clinical and Experimental Research 23:7:1136–1144.
Preuschoff K, Bossaerts P, Quartz SR (2006) Neural differentiation of expected reward and risk in human subcortical structures. Neuron 51:381-390.
Ribeiro EO, Afonso NH, Morgado P (2021) Non-pharmacological treatment of gambling disorder: a systematic review of randomized controlled trials. BMC psychiatry 21:1:105-105.
Robinson TE, Berridge KC (1993) The neural basis of drug craving: An incentive-sensitization theory of addiction. Brain Research Reviews 18:3:247–291.
Robinson TE, Berridge KC (2003) Addiction. Annual Review of Psychology 54:25–53.
Salonen A, Hagfors H, Lind K, Kontto J (2020) Rahapelaaminen ja peliongelmat: Suomalaisten rahapelaaminen 2019: Rahapelien pelaaminen riskitasolla on vähentynyt. https://urn.fi/URN:NBN:fi-fe2020041618876.
Sanchez-Roige S, Ripley TL, Stephens DN (2015). Alleviating waiting impulsivity and perseverative responding by μ-opioid receptor antagonism in two inbred mouse strains. Psychopharmacology 232:1483–1492.
Sarne Y, Fields A, Keren O, Gafni M (1996) Stimulatory effects of opioids on transmitter release and possible cellular mechanisms: overview and original results. Neurochem Res 21:1353-1361.
Schultz W (2016) Dopamine reward prediction error coding. Dialogues Clin Neurosci 1:23-32.
Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:5306:1593–1599.
Schultz W (1998) Predictive reward signal of dopamine neurons. J. Neurophys 80:1–27.
Sevak RJ, Koek W, Owens WA, Galli A, Daws LC, France CP (2008) Feeding conditions differentially affect the neurochemical and behavioral effects of dopaminergic drugs in male rats. Eur. J. Pharmacol 592:1–3: 109–115.
Shaffer HJ, Hall MN, Vander BJ (1999) Estimating the prevalence of disordered gambling behavior in the United States and Canada: a research synthesis. Am. J. PublicHealth 89:9:1369–1376.
Sharif NA, Hughes J (1989) Discrete mapping of brain Mu and delta opioid receptors using selective peptides: quantitative autoradiography, species differences and comparison with kappa receptors. Peptides 10:499-522.
Sharman S, Aitken MRF, Clark L (2015) Dual effects of 'losses disguised as wins' and near-misses in a slot machine game. International gambling studies 15:2:212-223.

Sinclair JD, Lê AD, Kiianmaa K (1989) The AA and ANA rat lines, selected for differences in voluntary alcohol consumption. Experientia 45:9:798–805.
Sinclair JD, Hyytiä P, Nurmi M (1992a) The limited access paradigm: Description of one method. Alcohol 9:5:441–444.
Sinclair JD, Kampov-Polevoy A, Stewart R, Li TK (1992b) Taste preferences in rat lines selected for low and high alcohol consumption. Alcohol 9:2:155–60.
Slutske WS, Eisen S, True WR, Lyons MJ, Goldberg J, Tsuanget M (2000) Common genetic vulnerability for pathological gambling and alcohol dependence in men. Archives of General Psychiatry 57:7:666–673.
Slutske WS, Ellingson JM, Richmond-Rakerd LS, Zhu G, Martin NG (2013) Shared genetic vulnerability for disordered gambling and alcohol use disorder in men and women: Evidence from a national community-based ustralian twin study. Twin Research and Human Genetics 16:2:525–534.
Smith KS, Berridge KC (2005) The ventral pallidum and hedonic reward: Neurochemical maps of sucrose 'liking' and food intake. Journal of Neuroscience 25:38:8637–8649.
Sommer W, Hyytia P, Kiianmaa K (2006) The alcohol-preferring AA and alcohol-avoiding ANA rats: neurobiology of the regulation of alcohol drinking Addiction biology 11:3:289-309.
Sommer M, Hauber W (2015) Dopamine D1/D2 receptor activity in the nucleus accumbens core but not in the nucleus accumbens shell and orbitofrontal cortex modulates risk-based decision making. International Journal of Neuropsychopharmacology 18(10): p.1–9.
Spanagel R, Herz A, Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci U S A 89: 2046-2050.
St Onge JR, Floresco SB (2009) Dopaminergic modulation of risk-based decision making. Neuropsychopharmacology 34:3:681–697.
St Onge JR, Chiu YC, Floresco SB (2010) Differential effects of dopaminergic manipulations on risky choice. Psychopharmacology 211: 209–221.
St Onge JR, Ahn S, Phillips AG, Floresco SB (2012) Dynamic fluctuations in dopamine efflux in the prefrontal cortex and nucleus accumbens during risk-based decision making. J Neurosci 32:47:16880-91.
Stewart SH, Zack M, Collins P, Klein RM (2008) Subtyping pathological gamblers on the basis of affective motivations for gambling: relations to gambling problems, drinking problems, and affective motivations for drinking. Psychol Addict Behav 22:257–268.
Stopper CM, Khayambashi S, Floresco SB (2013) Receptor-specific modulation of risk-based decision making by nucleus accumbens dopamine. Neuropsychopharmacology 38:5:715-28.
Stuber GD, Evans SB, Higgins MS, Pu Y, Figlewicz DP (2002) Food restriction modulates amphetamine-conditioned place preference and nucleus accumbens dopamine release in the rat. Synapse 46:2:83-90.
Suri R, Schultz W (1998) Learning of sequential movements by neural network model with dopamine-like reinforcement signal. Exp Brain Res 121:350–354.

Tackett J, Krieger H, Neighbors C, Rinker D, Rodriguez L, Gottheil E (2016) Comorbidity of Alcohol and Gambling Problems in Emerging Adults: A Bifactor Model Conceptualization. Journal of gambling studies 33:1:131-147.

Tedford SE, Holtz NA, Persons AL, Napier TC (2014) A new approach to assess gambling-like behavior in laboratory rats: using intracranial self-stimulation as a positive reinforcer Frontiers in behavioral neuroscience 8:215-215.

Toneatto T, Blitz-Miller T, Calderwood K, Dragonetti R, Tsanos A (1997) Cognitive Distortions in Heavy Gambling Journal of gambling studies 13:3:253-266.

Toneatto T, Brands B, Selby P (2009) A randomized, double-blind, placebo-controlled trial of naltrexone in the treatment of concurrent alcohol use disorder and pathological gambling. Randomized Controlled Trial Am J Addict 18:3:219-25.

Trigo JM, Martin-Garcia E, Berrendero F, Robledo P, Maldonado R (2009) The endogenous opioid system: A common substrate in drug addiction. Drug Alcohol Depend 108: 183-94.

van den Bos R, Jolles J, van der Knaap L, Baars A, de Visser L (2012) Male and female Wistar rats differ in decision-making performance in a rodent version of the Iowa Gambling Task. Behavioural brain research 234:2:375-379.

van den Bos R, Homberg J, de Visser L (2013) A critical review of sex differences in decision-making tasks: focus on the Iowa Gambling Task. Behav Brain Res 238:95-108.

van den Bos R, Koot S, de Visser L (2014) A rodent version of the Iowa Gambling Task : 7 years of progress Frontiers in psychology 5:203-203.

van Steenbergen H, Eikemo M, Leknes S (2019) The role of the opioid system in decision making and cognitive control: A review. Cognitive, Affective and Behavioral Neuroscience 19:3:435–458.

Victorri-Vigneau C, Spiers A, Caillet P, Bruneau M, Challet-Bouju G, Grall-Bronnec M (2018) Opioid Antagonists for Pharmacological Treatment of Gambling Disorder: Are they Relevant? Current neuropharmacology 16:10:1418-1432.

Voon V, Napier TC, Frank MJ, Sgambato-Faure V, Grace AA, Rodriguez-Oroz M, Obeso J, Bezard E, Fernagut PO (2011) Dopamine agonists and risk: Impulse control disorders in Parkinson's Disease. Brain 134:5: 1438–1446.

Willner P (1984) The validity of animal models of depression Psychopharmacology 83:1:1-16.

Winstanley CA, Cocker PJ, Rogers RD (2011) Dopamine Modulates Reward Expectancy During Performance of a Slot Machine Task in Rats: Evidence for a 'Near-miss' Effect Neuropsychopharmacology 36:913–925.

Winstanley CA, Clark L. Translational Neuropsychopharmacology p. 93. Editors : Trevor W. Robbins and Barbara J. Sahakian. Published by Springer Nature 2016.

Winstanley CA, Floresco SB (2016) Deciphering Decision Making: Variation in Animal Models of Effort- and Uncertainty-Based Choice Reveals Distinct Neural Circuitries Underlying Core Cognitive Processes. J Neurosci 36:48:12069-12079.
Wise RA (1998) Drug-activation of brain reward pathways Drug and alcohol dependence 51:1:13-22.
Wise RA (2002) Brain reward circuitry: insights from unsensed incentives. Neuron 36:229-240.
Yau YHC, Potenza MN (2015) Gambling disorder and other behavioral addictions: recognition and treatment. Harvard review of psychiatry 23:2:134-146.
Yip SW, Potenza MN (2014) Treatment of Gambling Disorders Current treatment options in psychiatry 1:2:189-203.
Yoshida Y, Koide S, Hirose N, Takada K, Tomiyama K, Koshikawa N, Cools AR (1999) Fentanyl increases dopamine release in rat nucleus accumbens: involvement of mesolimbic mu- and delta-2-opioid receptors. Neuroscience 92:1357-1365.
Zack M, Featherstone RE, Mathewson S, Fletcher PJ (2014) Chronic exposure to a gambling-like schedule of reward predictive stimuli can promote sensitization to amphetamine in rats. Frontiers in Behavioral Neuroscience 8:1–15.
Zack M, St George R, Clark L (2020) Dopaminergic signaling of uncertainty and the aetiology of gambling addiction. Progress in Neuro-Psychopharmacology and Biological Psychiatry 99:109853.
Zeeb FD, Robbins TW, Winstanley CA (2009) Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task. Neuropsychopharmacology 34:10:2329–2343.
Zeeb FD, Li Z, Fisher DC, Zack MH, Fletcher PJ (2017) Uncertainty exposure causes behavioural sensitization and increases risky decision-making in male rats: Toward modelling gambling disorder. Journal of Psychiatry and Neuroscience 42:6:404–413.
Zois E, Kortlang N, Vollstädt-Klein S, Lemenager T, Beutel M, Mann K, Fauth-Bühler M (2014) Decision-making deficits in patients diagnosed with disordered gambling using the Cambridge Gambling task: the effects of substance use disorder comorbidity. Brain and behavior 4:484-494.

Figures 1, 2, 3, 6 and 8 were created with BioRender.com.