Deanship of Graduate Studies
Al-Quds University

The Influence of the Interaction and Allelic Location of the C957T and SNP19 Polymorphisms of the Dopamine Receptor-2 Gene on Feedback-Based Learning

Anfal Ahmad Saleh Abu-Hilal

M.Sc. Thesis
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Thesis Approval

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Jerusalem – Palestine

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Dedication

Science is only real when shared. I dedicated this work to future scientists entering the field of research. May you find knowledge and inspiration.

Declaration

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and that this study has not been submitted for a higher degree to any other university or institution.

Signed: ...Anfal Abu-Hilal.....

Anfal Ahmad Saleh Abu-Hilal

Date: 10/6/2020

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This project would not have been possible without the support of many people. I feel honored to have been in the position to work with such leaders in the field who took the time to teach and guide me through this work. These are some, but not all of the people who have made this work possible. I would like to thank my supervisors, Dr. Mohammad M. Herzallah and Dr. Zaidoun Salah for their guidance, teaching, and insightful comments on my projects as well as advice on my future career path. I would also like to thank the members of my supervisory committee, my friends, and my colleagues. A special thanks to my parents, who have always believed in me and have supported me unconditionally through it all.

Abbreviations

SNP: Single Nucleotide Polymorphism

DA: Dopamine

SNpc: Substania Nigra pars compacta

VTA: Ventral Tegmental Area

DRD2: Dopamine Receptor D2

PCR: Polymerase Chain Reaction

µl: Microliter

°C: Caulis

Bp: Basepair

RFLP: Restriction Fragment Length Polymorphism

ARMS: Allele Refectory Mutation System

LD: Linkage Disequilibrium

D2S: Dopamine D2 short isoform

D2L: Dopamine D2 Long isoform

RT: Response Time

-25: Negative Feedback

-0: No Feedback Punishment

+0: No Feedback Reward

+25: Positive Feedback

Abstract

People vary in their cognitive performance. Multiple factors can perturb learning and forming associations. It has been shown that the neurotransmitter dopamine is an essential factor in modulating feedback-based learning. Dopamine exerts its effects via two families of postsynaptic receptors, type 1 and type 2. The release of dopamine is regulated by presynaptic dopamine type 2 receptors (DRD2). In this project, we pursued a multidisciplinary approach to examine the molecular and cognitive aspects of naturally-occurring variations in the dopamine system. We investigated feedback-based learning under the influence of two naturally occurring polymorphisms in the DRD2 gene, the C957T, which regulates binding affinity in postsynaptic DRD2 receptors; and SNP19, which modulates the number of presynaptic DRD2 autoreceptors. After using polymerase chain reaction (PCR), we utilized restriction fragment length polymorphism (RFLP) to identify the polymorphism variants. Further, we developed a PCR based technique to test whether the two polymorphisms are on the same allele or not. Our aim was two-fold: (1) to study the interaction between the C957T and SNP19 in modulating cognitive function, and (2) to investigate the cognitive effects of allelic location of C957T and SNP19 on cognitive performance. All heterozygotes for the two polymorphisms underwent amplification refractory mutation system (ARMS) PCR followed by ARMS to examine the allelic location of the two polymorphisms. We recruited a sample of 476 healthy undergraduate students at Al-Quds University, Palestine. All subjects completed a probabilistic categorical feedback-based learning task that differentiates learning from positive and negative feedback. Our results showed a gene dose effect, in which the T allele of C957T that is related to low expression of D2 postsynaptically is linked to the G allele of SNP19 that is related to high expression presynaptically. Also, we found that subjects with the lowest DRD2 postsynaptic affinity (CC-homozygotes for C957T) alongside the highest concentration of presynaptic DRD2 autoreceptors (GG- homozygotes for SNP-19) learned significantly better after receiving positive feedback or being in a potentially-positive state. These results are in line with previous findings that highlight the role of DRD2 in modulating tonic dopamine signaling. To our knowledge, this is the first project to examine the cognitive effects of the interaction of the C957T and SNP19 polymorphisms and their allelic locations. These results will further our understanding of the dopaminergic system and its regulation of cognition and involvement in a myriad of neurological and psychiatric disorders.

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Definitions

Single Nucleotide Polymorphism (SNP): is the variation at only one nucleotide location in the DNA sequence among individuals.

Haplotype: is a set of SNP alleles that are inherited together from a single parent, a haplotype is a group of SNPs in a region of a chromosome.

Chapter 1

Introduction and Literature Review

Individuals differ in their learning from positive or negative feedback. It has been shown that the neurotransmitter dopamine is an important factor in modulating feedback-based learning. The basal ganglia and dopamine levels facilitate feedback-based learning; an increase in dopamine mediates positive feedback learning, whereas a decrease in dopamine mediates negative feedback. By this framework, the dopaminergic system gives rise to a feedback system, allowing an organism to base its decision from past experiences to enhance its survival. Dopamine exerts its effects via two families of receptors that are located both pre and postsynaptically: dopamine type 1 receptors (DRD1) and dopamine type 2 receptors (DRD2). Presynaptic DRD2 functions as an autoreceptor and regulates the transmission of dopamine, while postsynaptic DRD2 transmits dopaminergic signals (Lindgren et al. 2003).

Prior studies have shown that DRD2 polymorphisms correlated with striatal D2 receptor affinity (Hirvonen, Laakso, et al., 2009). Other polymorphisms had differential effects on prefrontal and striatal D2 receptor expression. These points signify the need to study the regulatory role of D2 receptors on cognition and learning. Understanding these variations in the dopaminergic function can shed on its contribution to individual differences in cognitive functions. In this study, we pursued a multidisciplinary approach to examine the genetic, molecular, and cognitive effects of naturally-occurring polymorphisms on feedback-based learning. There is a plethora of research associating dopamine levels with addictive and impulsive behaviors, as well as neuropsychiatric disorders (Noble et al., 1993; Parsons et al., 2007). Our study utilizes the recently discovered differential expression of D2 receptors via the C957T and SNP19 polymorphism in the DRD2 gene (Duan et al., 2003; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009; Zhang et al., 2007). This study can provide us with an inlet to further understand the dopaminergic system and its regulation in healthy states and neuropsychiatric diseases.

The Dopaminergic System

Dopamine

Dopamine is a monoamine neurotransmitter produced in several areas in the brain, including the substantia nigra (SNc) and the ventral tegmental area (VTA) (Cragg, Rice, & Greenfield, 1997). Dopamine has many important influences on behavioral activation, locomotion, reward processing, and goal-directed behavior (Noble, 2003). Dysfunctions in the dopaminergic system have been implicated in a range of neurological and psychiatric disorders, most notably Parkinson's disease and schizophrenia.

Dopamine Receptors

Dopamine functions are mediated via five subtypes of G protein-coupled receptors. These receptors were differentiated based on their biochemical, physiological, pharmacological properties, and their anatomical distribution (Missale, Nash, Robinson, Jaber, & Caron, 1998). The main classification of dopamine receptors was based on their biochemical ability to stimulate/inhibit adenylyl cyclase (Kebabian & Calne, 1979). This classification allowed for two main families: D1-like and D2-like, to be coined (Jaber, Robinson, Missale, & Caron, 1996). D1-like receptors include D1 and D5 subtypes, and D2-like receptors include D2, D3, and D4 subtypes. The former has a stimulatory effect on adenylyl cyclase, while the latter has an inhibitory effect (Kebabian & Calne, 1979). Dopamine receptors are distributed in different brain areas at different densities such as the prefrontal cortex, thalamus, hippocampus, midbrain, and striatum (Meador-Woodruff et al., 1989).

The Basal Ganglia

The basal ganglia are a group of interconnected subcortical structures involved in the control of movement, learning, and cognition. The basal ganglia process information through cortical-striatal loops (Haber, 2016). Dopamine modulates basal ganglia function via two main pathways: the direct and indirect pathways (**Figure 1**). The direct pathway plays a role in enabling movement and goal-directed behaviors, while the indirect pathway inhibits behaviors that may result in a poor outcome (Macpherson, Morita, & Hikida, 2014). Increases in dopamine release from the substantia nigra pars compacta or ventral tegmental area (midbrain dopamine nuclei) favor activation of the direct pathway, along with inhibition of the indirect pathway via activation of postsynaptic D1 and D2 receptors on striatal medium spiny neurons, respectively (Macpherson et al., 2014).

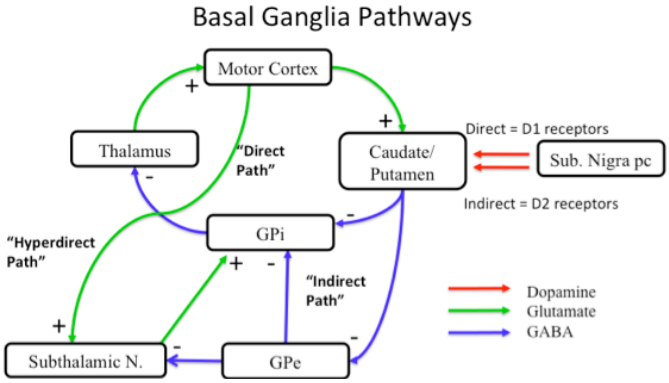

Figure 1. Basal Ganglia Pathways. D1-expressing MSNs predominantly send inhibitory GABAergic projections directly to the output nucleus of the basal ganglia: the internal globus pallidus (GPi) & substantia nigra pars reticulata. This is referred to as the direct pathway. D2-expressing MSNs predominantly send inhibitory GABAergic projections first to the external globus pallidus (GPe). The GPe, in turn, sends inhibitory GABAergic projections to the subthalamic nucleus (STN). The STN then sends excitatory glutamatergic projections to output nuclei of the basal ganglia. This is referred to as the indirect pathway (Haley Carpenter, 2012).

Dopamine Release

Dopamine release can take on two different modes of firing: tonic firing, which is a form of sustained spontaneous release regulated by prefrontal cortical afferents, or phasic burst firing, which is a transient peak of dopamine release caused by an increase in the firing of dopaminergic neurons (Grace & Bunney, 1985). Phasic bursts of midbrain dopamine encode "reward prediction errors" in terms of whether outcomes are better or worse than expected (Schultz, Dayan, & Montague, 1997). An increase in the binding of low-affinity D1-receptors by dopamine phasic bursts activates the medium spiny neurons of the direct pathway, leading to a selection of reward-associated actions. In contrast, simultaneous binding of high-affinity D2-receptors (DRD2) produces an inhibition of medium spiny neurons of the indirect pathway to further enhance action selection (Frank, Moustafa, Haughey, Curran, & Hutchison, 2007). When the outcome is worse than expected, a phasic dip of dopamine results in reduced D1-receptor binding, inactivating the direct pathway, while simultaneously reducing DRD2 binding, disinhibiting the indirect pathway (Frank, Woroch, & Curran, 2005; Hollerman, Tremblay, & Schultz, 1998; Schultz, Apicella, & Ljungberg, 1993). On the other hand, tonic dopamine release is proposed to cap the effect of phasic dopamine firing through its effect on extracellular dopamine levels. The tonic release sets the background level of dopamine receptor stimulation in both the midbrain dopamine neurons and striatal medium spiny neurons (Marcott, Mamaligas, & Ford, 2014).

Dopamine D2 Receptor

The dopamine D2 receptor (DRD2) is a G-protein-coupled receptor that is highly expressed in the striatum. It mediates an array of fundamental brain functions, including reward behavior, regulation of movement, and attention. The DRD2 is also important clinically as a target of pharmacotherapy for psychosis and Parkinson's disease. DRD2 auto-receptors serve as part of a negative feedback loop of dopamine release. These presynaptic auto-receptors can be located either somatodendritically, on the neuron body, or axonally, on nerve terminals. Somatodendritic autoreceptors mainly decrease neuronal firing rate (Bunney, Aghajanian, & Roth, 1973), while axonal autoreceptors inhibit dopamine synthesis by regulating tyrosine hydroxylase, reducing dopamine exocytosis, and increasing reuptake through the dopamine transporter activation (Jones et al., 1999; Lindgren et al., 2003; Schmitz, Schmauss, & Sulzer, 2002). Furthermore, it was shown that neurons in the ventral tegmental area show different firing patterns once stimulated. They were subdivided according to the pattern of firing and presence or absence of an autoreceptor into low-firing conventional dopaminergic neurons, fast-firing unconventional dopaminergic neurons with auto-receptors, and fast-firing unconventional dopaminergic neurons without auto-receptors (Lammel et al., 2008).

Dopamine D2 Receptor Isoforms

DRD2 has two isoforms named D2-long (D2L) and D2-short (D2S). They differ by 29-amino acids located in the third intracellular loop of the long isoform, a product of alternative splicing. Studies have shown that both isoforms differ in their affinity to different G-proteins (Iaccarino et al., 2002). The DRD2 is abundantly expressed in the basal ganglia and prefrontal cortex (Jackson & Westlind-Danielsson, 1994). D2L is widely expressed in the striatum and midbrain dopaminergic neurons compared to D2S, which is mainly expressed presynaptically on the somatodendritic and axonal portions of dopaminergic neurons (Wang et al., 2000).

Functionally, the roles of D2L and D2S are not interchangeable. The D2L is a predominant player postsynaptically, while the D2S functions presynaptically (Centonze et al., 2002). Lindgren et al. demonstrated that D2S receptors found on dopaminergic nigrostriatal presynaptic terminals regulate tyrosine hydroxylase phosphorylation and activity while the D2L receptors in medium spiny neurons are responsible for the regulation of dopamine- and cAMP-regulated phosphoprotein 32kDA (DARPP-32) dephosphorylation (Lindgren et al. 2003). A *DRD2* knockout mice (DRD2 -/-) study done by Schmitz et al. demonstrated that DRD2 is the auto-receptor that regulates dopamine synthesis and release (Schmitz et al., 2002). Another study of D2L knockout mice showed

that the D2S acts as an auto-receptor that reduces D1 receptor activation (Usiello et al., 2000). These studies motivated further studies of the molecular influences of the *DRD2* gene. One of these molecular aspects was the study of single nucleotide polymorphisms (Duan et al., 2003; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009; Zhang et al., 2007).

Dopamine D2 Receptor Gene

The DRD2 is encoded by the *DRD2* gene, which is located on chromosome 11 (Eubanks et al. 1992). *DRD2* mRNA is processed through alternative splicing, which results in two DRD2 isoforms, DRD2 long and DRD2 short (Zhang et al., 2007). The DRD2 short isoform is located presynaptically, whereas the DRD2 long is found mainly postsynaptically (Centonze et al., 2002).

The DRD2 plays an important role in reinforcement learning and has been implicated in the pathophysiology of various neurological and psychiatric disorders such as Parkinson's, schizophrenia, and drug addiction. (Noble et al., 1993; Parsons et al., 2007). Several genetic variants are linked to the variability of DRD2 expression as well as individual differences in reinforcement learning pharmacological responses and risk for psychiatric disorders (Duan et al., 2003; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009; Zhang et al., 2007). Human genetic variations are known to modulate DRD2 density and affinity in the striatum. Although polymorphisms in the *DRD2* have been linked to psychiatric disorders, such as schizophrenia (Vijayan et al., 2007; Lawford et al., 2005), other evidence found no such associations (Glatt, Trksak, Cohen, Simeone, & Jackson, 2004; Iwata et al., 2003). Studying *DRD2* polymorphism is a particular interest to warrant out grasp and advance our understanding of different cognitive functions. The most commonly described single nucleotide polymorphisms are the C957T and SNP19.

Single Nucleotide Polymorphisms in the DRD2 Gene

The C957T (C>T) is a functional synonymous single nucleotide polymorphism located in exon 7 of the *DRD2* gene (Seeman et al., 1993). Studies have shown that variations of the C957T polymorphism influence the expression of the DRD2 at the molecular level with a change from a C allele (common variant) to a T allele (polymorphism variant). Duan et al. demonstrated that cells transfected with the human *DRD2* cDNA, carrying the C957T polymorphism, exhibited differences in *DRD2* mRNA stability with no change in baseline membrane protein expression of DRD2 (Duan et al., 2003). However, when cells were pre-

treated with dopamine, there was no up-regulation of DRD2 membrane protein expression in cells transfected with the 957T variant of the *DRD2* compared to the wild type cells. Besides, Hirvonen et al. showed that the C957T polymorphism influenced the DRD2 binding potential where the common variant (C) was associated with lower binding potential (Hirvonen et al., 2005). This influence varied between striatal and extrastriatal (cortex and thalamus) locations. T-Homozygotes had the highest striatal binding potential, which reflects receptor number, of the DRD2 to [^{11}C]raclopride while simultaneously having the lowest cortical DRD2 binding potential compared to C-Carriers and C-Homozygotes (Hirvonen, Lumme, et al., 2009). In 2009, Hirvonen and colleagues moved a step further in identifying whether the effect of C957T on binding potential was due to a change in striatal DRD2 density or affinity (Hirvonen, Laakso, et al., 2009). Their results showed that the effect of C957T was due to a change in affinity with T-Homozygotes having the highest affinity of DRD2 in the striatum. Hirvonen related this increase in affinity to a decrease in dopamine tones, facilitating the binding of the competitive radioligand [^{11}C]raclopride in C-Carriers and C-Homozygotes (Hirvonen, Laakso, et al., 2009). However, studying the C957T polymorphism did not directly address the autoreceptor influence of DRD2 on dopamine regulation. Researchers found a polymorphism that could modulate the function of the DRD2 autoreceptor; SNP19 (Zhang et al., 2007).

SNP19 (rs1076560) (G>A) is an intronic single nucleotide polymorphism located in intron 6 of the *DRD2* gene (Zhang et al., 2007), it has been associated with the relative expression of the DRD2 two isoforms via mRNA splicing. It affects the balance of *DRD2* mRNA splicing, for which the A allele (polymorphism variant) has been associated with decreased expression of the D2S (expressed mainly presynaptically) to D2L (expressed postsynaptically) compared to the G allele (common variant). Zhang et al. adopted the allele expression imbalance approach on minigene constructs of the *DRD2* gene. It showed that cells transfected with the minigene containing the A construct had the least expression of D2S mRNA. The A allele is associated with lower expression of the DRD2 relative to the G allele. Thus, a higher expression of D2S will be expected with the GG relative to the GA genotype, which corresponds to lower synaptic levels of dopamine (Zhang et al., 2007).

Variations of the *DRD2* gene in the form of single nucleotide polymorphisms have been shown to impact behavior and cognition (Clarke et al., 2014; Frank & Hutchison, 2009; Jacobsen, Pugh, Mencl, & Gelernter, 2006; Rodriguez-Jimenez et al., 2006; Xu et al., 2007). C957T has been associated with working memory, executive functioning and avoidance-based learning, with the CC-Homozygotes showing poorer performance in these cognitive domains compared to T-Carriers (Frank & Hutchison, 2009; Frank et al., 2007; Jacobsen et al., 2006; Rodriguez-Jimenez et al., 2006; Xu et al. 2007). On the other hand, SNP19 A-Carriers exhibit better performance in positive feedback and worse performance in negative feedback compared to CC-Homozygotes. Moreover, CC-Homozygotes showed

a balanced performance in both positive and negative feedback, while A-Carriers displayed a bias toward learning from positive feedback. Besides, Frank and colleagues demonstrated that the reduction in learning from negative feedback in C-Carriers of the C957T polymorphism was not present in subjects who were also CC-Homozygotes in SNP19 (Frank & Hutchison, 2009).

The C957T and SNP19 are common genetic polymorphisms of the *DRD2* gene known to affect the DRD2 expression. These genetic polymorphisms (such as C957T and SNP19) can be appreciated in the context of basal ganglia network where neuronal activity in the striatum can modulate the activity of dopaminergic neurons. As mentioned, the DRD2 is inhibitory in the indirect pathway, both pre and postsynaptically. Higher postsynaptic DRD2 affinity increases the basal ganglia output but reduces dopamine release from the SNpc. Presynaptically, a higher DRD2 affinity reduces dopamine release in the SNpc. Although the underlying molecular mechanisms are yet not fully understood, the C957T C-homozygous (low affinity) could be associated with higher dopamine release. Hirvonen et al. found that the C957T genotype changes in DRD2 availability by altering its affinity and striatal dopamine levels (Hirvonen, Laakso, et al., 2009).

Allele frequencies could vary when there is heterozygosity for two or more polymorphisms at any location (Eberle, Rieder, Kruglyak, & Nickerson, 2006). In this case, a heterozygous haplotype includes two different alleles with two variable components of each polymorphism on each allele, one inherited from the mother and one from the father. These two copies of alleles are expressed under allelic balance (Tucci, Isles, Kelsey, & Ferguson-Smith, 2019). However, when the expression ratio is unequal between the two chromosomes, this is known as allelic imbalance (Wagner et al., 2010). In heterozygotes for C957T and SNP19, and given their proximity, we predict that the allelic location of these polymorphisms could be variable and produce different allelic genotypes. Studying the locations of polymorphism in the genome is a critical component of genetic analysis, especially when considering the position of polymorphism at the allelic level. Many studies reported that a trait is expressed when the polymorphisms that are responsible for its expression are in a linkage on the same allele (Eberle et al., 2006). For example, this is evident in studies that focus on haplotypes in the *DRD2* gene in alcohol dependence (Jasiewicz et al., 2014; McAllister, 2009; Preuss, Zill, Koller, Bondy, & Soyka, 2007) Identification of this association can help further our understanding of the functional contributions of naturally-occurring genetic polymorphisms.

Feedback-Based Learning

The dynamics of the dopaminergic system influence behavior and feedback-based learning, either directly or through other neuromodulators (Frank & Hutchison, 2009; Rogers, 2011; Schultz, 1998). Based on this, it is essential to focus on feedback-based learning in general. In an instrumental conditioning paradigm, an individual makes a decision that can result in either a poor or a good outcome. Accordingly, the individual will construct internal associations that will either increase or decrease the likelihood of choosing that particular choice for a specific stimulus depending on the obtained outcomes. If the choice results in a positive outcome (reward), the stimulus-outcome association is strengthened; this is referred to as positive reinforcement. On the other hand, if the choice results in a negative outcome (punishment), the stimulus-outcome association is weakened; this is referred to as negative reinforcement. The instrumental paradigm is represented anatomically and physiologically, in most part, by the dopaminergic system and the direct/indirect pathways in the basal ganglia. Particularly, Schultz and colleagues discovered how phasic dopamine firing encodes the reward prediction error signal for unexpected rewards, which reflects the difference between the probability of the actual stimulus-outcome association and that of the expected stimulus-outcome association (Schultz et al., 1997). Frank and colleagues explained how the balance between the direct (DRD1) and indirect (DRD2) pathways in the basal ganglia could modulate learning from positive and negative feedback (Frank, Seeberger, & O'Reilly, 2004).

Significance of The Study

To our knowledge, this is the first study to examine the cognitive correlates of haplotypes and their allelic location in dopamine genes. Understanding the genetic control of DRD2 function as a result of natural-occurring variations can significantly further our understanding of the role of dopamine in cognitive function. Many studies have related DRD2 density to neurological and psychiatric disorders, such as major depressive disorder, schizophrenia, attention-deficit hyperactivity disorder, and Parkinson's disease. Successful completion of the current project can advance our understanding of the pathophysiology of these disorders and warrant a better grasp of the underlying mechanisms that regulate learning from positive and negative feedback.

Study Questions:

- How does the balance between presynaptic and postsynaptic DRD2 receptors, as inferred from C957T and SNP19 modulate learning from positive and negative feedback?
- Can variations in the allelic location of C957T and SNP19 *DRD2* polymorphisms affect individual differences in learning from positive and negative feedback?

Study Goals:

- We will investigate the effects of two of naturally-occurring polymorphisms in the *DRD2* gene, namely, the C957T and the SNP19 and their interaction on modulating learning from negative feedback.
- We will investigate the effect of allelic locations of C957T and the SNP19 on individual differences in learning from positive and negative feedback.

Predictions

- Subjects who are TT homozygous for the C957T and CC-homozygous for the SNP19 will learn better from negative feedback.
- Heterozygous subjects with the T nucleotide of C957T and the G nucleotide of SNP19 on one allele (and the C nucleotide of C957T with the T nucleotide of SNP 19 on the other allele) will exhibit balanced learning from both positive and negative feedback.

Study Justification

Our study utilized the differential expression of the DRD2 via the C957T and SNP19 polymorphism in the *DRD2* gene to provide an inlet for further understanding of the dopaminergic system, its regulation of cognition, and involvement in related neurological and psychiatric disorders.

Chapter 2

Materials and Methods

Participants and Testing Sessions

We tested 476 healthy undergraduate students (age range 18-24 years), who are originally from Hebron, enrolled in faculties of the healthcare complex at Al-Quds University. This was to ensure the homogeneity of the ethnicity and educational achievement of our sample. All subjects are required to sign a consent form according to a research protocol that was approved by the Al-Quds University Research Ethics Committee. Subjects underwent a pre-enrollment screening to rule-out neurological and psychiatric disorders at the personal and family levels. The duration of the testing session was 60-90 minutes.

Blood Samples and Genotyping

Blood sampling and DNA extraction:

Blood samples of 5-9mL for genotyping were withdrawn from subjects. Each blood sample was placed in an EDTA tube and then centrifuged for 15 minutes at 1800rpm. After centrifugation, the buffy coat was taken, and then DNA was extracted using the MasterPure DNA Purification Kit for Blood Version II ©. All DNA samples were verified for quality and quantity using whole-genome gel electrophoresis (Settings: 1% agarose, 120V, 30 minutes).

Polymerase Chain Reaction:

PCR was used to amplify the polymorphism containing fragments of the *DRD2* gene. The polymorphisms of interest were C957T (rs6277) and SNP19 (rs1076560). The primers sequences and the protocol we used was obtained from a published study done by (Hirvonen, Lumme, et al., 2009; Koehler et al., 2011). The PCR protocol for each polymorphism was as follows:

C957T (rs6277): The primers used were: F1 (5'-ACC ACG GTC TCC ACA GCA CTC T-3'), F2 (5'-ACC ATG GTC TCC ACA GCA CTC T-3'), and R (5'-ATG GCG AGC ATC TGA GTG GCT-3'), which amplified a PCR product with a fragment length of 196bp. The PCR conditions were: initial denaturation for 2 minutes at 95°C, one cycle; denaturation for 30s at 95°C, annealing for 30s at 62°C, and extension for 30s at 72°C for 40 cycles; final extension for 5 minutes at 72°C.
SNP19 (rs1076560): The primers used were: F1(5'-CTC GAC CTC TAC CTC TAC GA-'3), and R (5'-CCT GAA CAG AGA GTA CCG GA-3'), which amplified a PCR product with a fragment length of 323bp. The PCR conditions were: initial denaturation for 2 minutes at 95°C, one cycle; denaturation for 30s at 95°C, annealing for 30s at 58°C, and extension for 30s at 72°C for 30 cycles; final extension for 5 minutes at 72°C.

Enzyme Digestion and Gel electrophoresis:

PCR products were treated with the appropriate restriction enzyme to identify the exact version of the two polymorphisms for each subject. This produces a restriction fragment length polymorphism (RFLP) that can be identified by using gel electrophoresis.
The enzyme digestion and gel electrophoresis protocol for each polymorphism were as follows:

C957T (rs6277): 5 µl of PCR product was digested for 1 hour at 65°C with Taq-alpha1 restriction enzyme. Fragment sizes: T allele, 196bp; C allele, 174bp, and 22 bp. Fragments were visualized using 3% agarose gel on gel electrophoresis (120V for 1.5 hours).
SNP19 (rs1076560): 5 µl PCR product was digested for 3 hours at 37°C with the Hph1 restriction enzyme. Fragment sizes: C allele, 175bp, and 148bp; A allele, 175bp, 114bp, and 34bp. Fragments were visualized using 4% agarose gel on gel electrophoresis (120V for 1.5 hours).

Allele-Specific PCR or Amplification Refectory Mutation System (ARMS)

After performing PCR-RFLP for all our samples, all heterozygote samples underwent ARMS for genotyping. The ARMS should detect any mutation or variation that involves a single base pair change or deletions. This technique is based on the use of allele-specific PCR primers that allow amplification when the target allele is contained within the sample. Following an ARMS reaction, the presence or absence of a PCR product is diagnostic for the presence or absence of the target allele. Our ARMS PCR was followed by second ARMS to test whether the two variations are on the same allele (on a chromosome) or not.

Primer Sets:

ARMS primers were designed using Prime 3.0 software for both the C957T and SNP19 polymorphisms. The way we designed the primers revealed to us if the two polymorphisms are located on the same allele (on a chromosome), we performed ARMS followed by ARMS. Given the proximity of the two polymorphisms to each other (229 bp), the SNP19 PCR was performed first, followed by the PCR for C957T. For SNP19 and C957T, we had to design a primer with a mismatch to increase the allele specificity of ARMS-PCR. Primer sets are shown in Table-1.

SNP19 Polymorphism:

Table1. SNP19 ARMS primer set.

Alleles	**Primer**	**Primer Location**
SNP19- ARMS(G):	**5'-ttgcaggagtcttcagagggg-3'**	**DRD2 Gene Ch11:113412580-113412986**
SNP19-ARMS(T):	**5'-ttgcaggagtcttcagagagt-3'**	**DRD2 Gene Ch11:113412580-113412986**
SNP19-ARMS(com)-Rev:	**5'-tggctttcttctccttctgc-3'**	**DRD2 Gene Ch11:113412580:113412756**

*Blue color refers to the mismatch.

*Red highlight refers to the target variation.

C957T Polymorphism:

Table2. C957T ARMS primer set.

Alleles	**Primer**	**Primer Location**
C957T-ARMS(C):	**5'-atggtctccacagcactacc-3'**	**DRD2 Gene Ch11:113412609:113412756**
C957T-ARMS(T):	**5'-atggtctccacagcactacc-3'**	**DRD2 Gene Ch11:113412609:113412756**
C957T-ARMS (com)-Rev:	**5'-ttcctacggctcatggtctt-3'**	**DRD2 Gene Ch11:113412580:113412756**

*Blue color refers to the mismatch.

*Red highlight refers to the target variation.

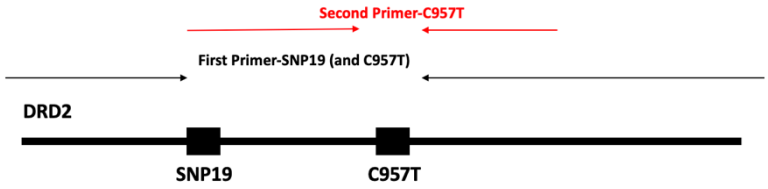

Figure 2. Schematic review of the primer-amplified region in the DRD2 gene.

ARMS-PCR genotyping was performed with specific primers, SNP19 reactions contained 1.0 µL of genomic DNA, 0.5 µL (pmol/µL) of a specific G primer, 0.5 µL (pmol/µL) of T primer, and 0.5 µL (pmol/µL) of the common reverse primer. The PCR cycling program included an initial denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s, which was followed by last extension step extension at 72°C for 5 min. ARMS-PCR products were resolved electrophoretically on 2% agarose gels.

The mixture of second-round PCR comprised of 1.0 µL of diluted SNP19 PCR, a 1:750 dilution of the first-round PCR mixture. The C957T reactions, 0.5 µL (pmol/µL) of a specific C primer, 0.5 µL (pmol/µL) of T primer, and 0.5 µL (pmol/µL) of the common reverse primer. The PCR cycling for the second round was performed with an initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturation at 95°C for 30 s, annealing at 64°C for 30 s, and extension at 72°C for 30 s, which was followed by last extension step extension at 72°C for 5 min.

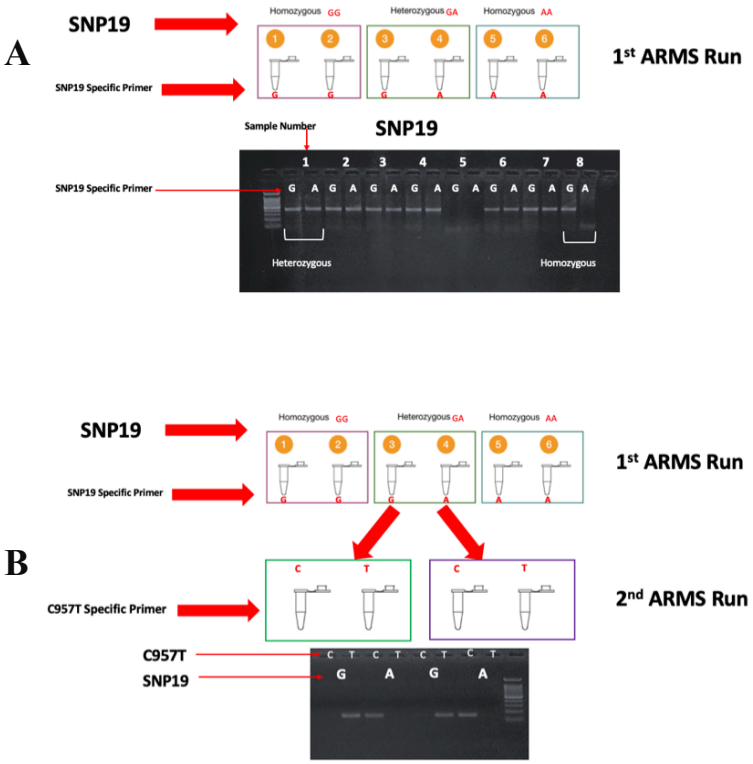

Figure 3. Schematic representation of the ARMS on ARMS technique.

The Computer-Based Cognitive Task

As a measure of basal ganglia functions in feedback-based associative learning, we used a probabilistic category-learning task that dissociates learning from positive and negative feedback (**Figure 4;** Herzallah et al., 2017). On each trial, the subject sees one of several stimuli, each of which is a different abstract geometric design. The subject is asked whether the stimulus predicts rainy weather (Rain) or sunny weather (Sun). The critical manipulation that differentiates this task from previous studies of probabilistic category learning is that half the stimuli are trained using only positive feedback for correct answers (and no feedback for incorrect answers) while the other half are trained using only negative feedback for incorrect answers (but no feedback for correct answers). Thus, across all stimuli, the no-feedback trials are ambiguous, with half occurring following correct responses and half occurring following incorrect responses. This makes it difficult for subjects to infer the implicit meaning of the no-feedback trials and encourages them to focus, instead, on learning from the positive and negative feedback trials. Across four blocks of 40 trials (160 total), subjects learn to categorize the stimuli into the two outcome

categories, Rain and Sun. This experimental design allowed us to measure and compare individuals' sensitivity to learning from positive feedback versus negative feedback.

Figure 4. The feedback-based probabilistic classification task. (a) On each trial, the participant saw one of four stimuli and was asked whether this stimulus predicts rain or sun. (b) No feedback is given for incorrect answers in rewarding stimuli or correct answers in punishing stimuli. (c) For rewarding stimuli, correct responses get rewarded with visual feedback and 25 points winnings. (d) For punishing stimuli, incorrect responses get punished with visual feedback and the loss of 25 points (Herzallah et al., 2017).

Statistical Analysis

We used SPSS version 20 to conduct the analyses. To examine the association between feedback based-learning and the two polymorphisms of the *DRD2* gene, the C957T, and SNP19 polymorphisms, we used repeated-measures ANOVA with accuracy and response time for trial type, prior trial, prior feedback as dependent variables, and the genotypes as between-subject variables.

Linkage Disequilibrium Analysis

The non-random association of alleles at two genetic loci is referred to as linkage disequilibrium (LD). We used LD analysis to measure the degree of linkage between the

C957T and SNP19 polymorphisms. First, we calculated the observed allele frequency for each polymorphism by coding the genotypes at each locus as 0, 1, and 2, by counting the number of C and T alleles for the C957T, and G and A alleles for the SNP19. Next, we defined the allele frequency at the two loci. Then alleles were defined based on the expected haplotype.

Table 3. Two locus haplotype frequencies Calculations

Locus 1	**Locus 2**		
		Allele 1	**Allele 2**
	Allele1	**p1**	**q1**
	Allele2	**p2**	**q2**

The strength and association of LD for two polymorphisms are expressed by LD parameters D' and r^2 (Devlin & Risch, 1995), providing values ranging from zero to 1, where 1 indicates full LD, and close to 1 means very strong LD. D' with a value of one indicates that two alleles exist on a shared haplotype and were co-inherited in all meiosis (Wall & Pritchard, 2003). The r^2 is the square of the statistical correlation coefficient between two loci, meaning that alleles with different allele frequencies can never reach an r^2 of one (VanLiere & Rosenberg, 2008).

Chapter 3

Results

Experiment 1: Genetic Results

PCR-RFLP:

We used the PCR-RFLP technique to amplify the polymorphism containing fragments of the two polymorphisms in the *DRD2* gene. The results of PCR amplification are shown in **Figure 5**. Digested PCR products are shown in **Figure 6**.

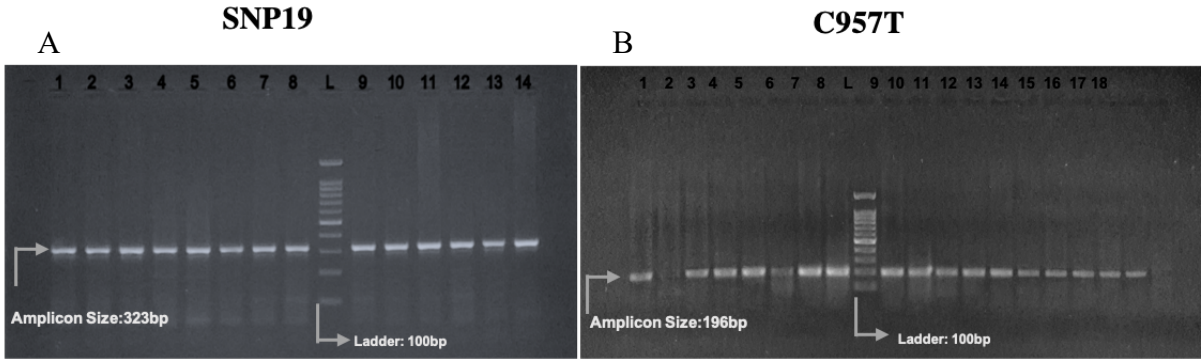

Figure 5. Gel electrophoresis of PCR product for SNP19 (A) and C957T (B). Band lengths are 323bp for SNP19, and 196bp for C957T.

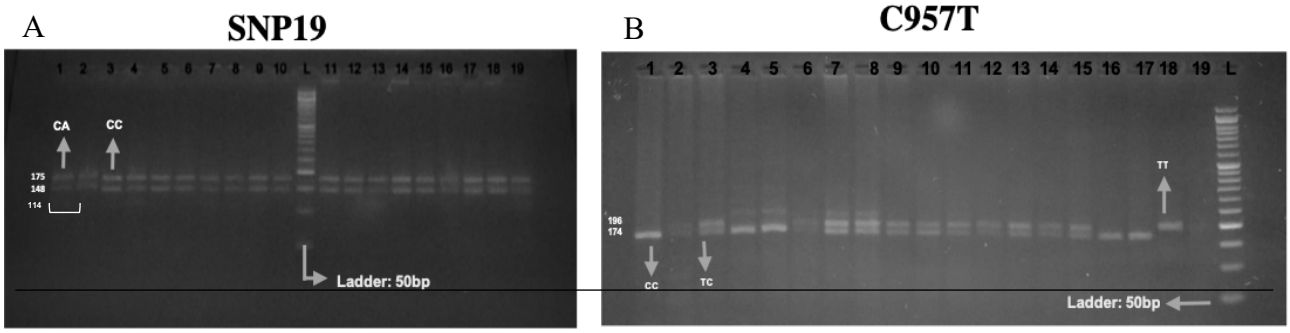

Figure 6. Enzyme digestion product for SNP19 (A) and C957T (B). **A.** Products of enzyme digestion for the G-variant of SNP19 are 175+148bp, and for A-variant of SNP19 175+114+34, the A-variant band was finite and not shown in the figure. **B.** For C957T, the digestion product for the C-variant allele is 174+22bp, and for the T- variant is 196bp.

ARMS Result:

To ensure that our designed primers are working and to ensure the appropriate PCR conditions, we performed an ARMS-PCR reaction. Two separate reactions were carried out in two different PCR tubes, one primer (common primer) is common to both tubes. However, the other primer that overlaps the polymorphism site differs between the two tubes. ARMS PCR is allele-specific; hence the sample that has the allele for the specific primer set will be amplified. The results of the ARMS-PCR analysis showing the SNP19 and C957T genotypes are displayed in (**Figure 7**). Our experimental results confirmed the results reported in the PCR-RFLP step. A comparison of the two methods showed that the ARMS-PCR was significantly more accurate than PCR-RFLP.

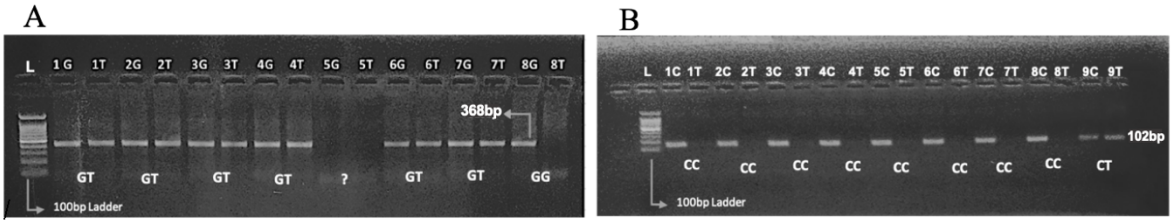

Figure 7. Gel electrophoresis of ARMS PCR product for SNP19 (A) and C957T (B). Band lengths are 368bp for SNP19, and 102 for C957T.

ARMS on ARMS Results:

Using the ARMS PCR technique, we aimed to test whether the two SNPs are located on the same allele or not. All heterozygote samples underwent ARMS for SNP19 **Figure 8-A**. This was followed by ARMS for the C957T polymorphism. The second ARMS PCR involved two sets of primers to detect the C957T, and the two PCR products from SNP19. Results of ARMS on ARMS are shown in **Figure 8-B.** Our ARMS technique showed an allelic balance, were G variant of SNP19 that is related to high gene expression is located on the same allele with the T allele of the C957T that is associated with low gene expression, and vice versa.

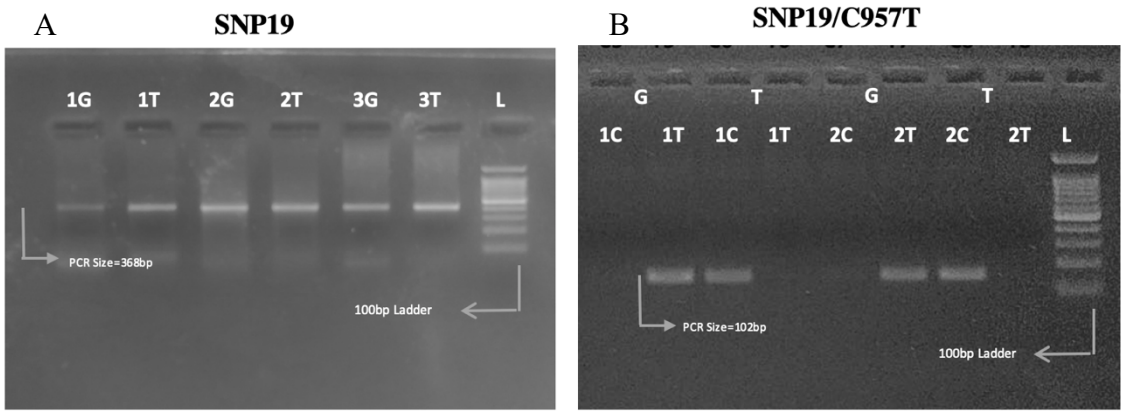

Figure 8. Gel electrophoresis of ARMS PCR product for SNP19 heterozygotes samples (A) the second ARMS on ARMS run to test whether the allelic location of the C957T variations (B).

Genotype Frequency:

The figure below shows the frequencies of the C957T and SNP19 polymorphisms haplotype in the *DRD2* gene. Our polymorphism frequencies corresponded with that reported in previous studies. In Frank & Hutchison 2009, the frequency of the A allele (polymorphism variant) of SNP19 was 16.9%, and the frequency of the C allele (common variant) of C957T was 18%. In our study, the frequency of the A allele (polymorphism variant) of SNP19 was 18%, and the frequency of the C allele (common variant) of C957T was 22%.

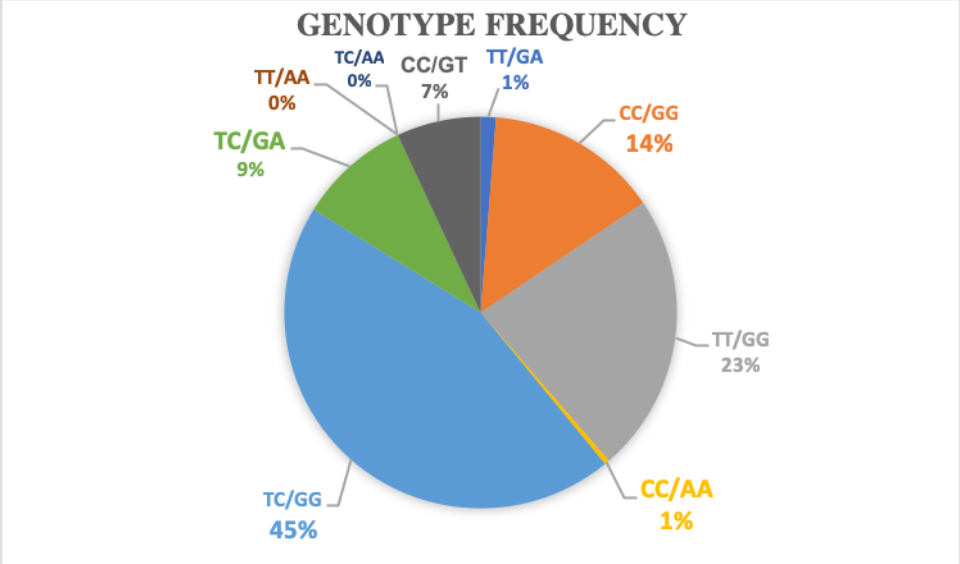

Figure 9. Pie chart representing the *DRD2* haplotype frequency of the C957T (first genotype) and SNP19 (second genotype) in our healthy population (n=476).

LD Analysis:

We found a significant correlation between alleles at two loci (SNP19 and C957T) in the *DRD2* gene (r^2=0.087). There was a significant linkage disequilibrium between the C957T and SNP19 polymorphisms (X^2 = 81.79, p<0.001).

Allele Frequency:

Table 4. Allele Frequency of C957T and SNP19

	p1	p2	q1	q2
	SNP19-G	**SNP10-A**	**C957T-C**	**C957T-T**
n	846	94	472	468
Frequency	0.9	0.1	0.50212766	0.4978723

Haplotype Frequency (Observed):

Table 5. Genotypes and Genotype Frequency of C957T and SNP19

	p11	p12	q21	q22
	GG	**GA**	**TC**	**TT**
n	384	460	90	6
Frequency	0.4519149	0.4893617	0.09574468	0.006383

Haplotype Frequency (Expected):

Table 6. Haplotype and Haplotype Frequency of C957T and SNP19

	p1q1	p1q2	p2q1	p2q2
	GC	**GT**	**AC**	**AT**
n	0.45191489	0.448085106	0.050212766	0.04978723

D=(p1q1*p2q2)-(p1q2*p2q1)

D= -0.04424627

D<0, then we use the Dmin

*Dmin is the larger between –p1q1 and –p2q2

D' = D/p2q2

-0.04424627/0.04978723

$$r^2 = D^2/p1*p2*q1*q2$$

-0.04424627/0.02249959

=0.087011886

$$X^2 = r^2 / n^2$$
$$= 0.087011886/940$$
$$= 81.79117313$$

p-value<0.00001

- D= Coefficient of linkage disequilibrium, and it's the measure of allelic association.
- D' = Normalized coefficient D
- r^2= Coefficient of correlation
- X^2=Chi square

Experiment2: Behavioral Results

Performance in the feedback-based learning task:

First, we grouped the sample into homozygotes and carriers for both C957T (C-Homozygotes, T-Carriers), and SNP19 (A-Carriers, G-Homozygotes) to investigate their influence on learning from positive and negative feedback (**Figures 10-12**). We used mixed-model ANOVA, with feedback type (negative and positive) as the within-subjects variables, genotype (C957T and SNP19) as the between-subjects variable, and accuracy of learning from positive and negative feedback as the dependent variables. Our analysis revealed a significant effect of feedback type ($F(1,472)=24.316$, $p<0.001$), a significant interaction between feedback type and SNP19 ($F(1,472)=3.918$, $p=0.048$). However, there was no significant effect of feedback type and C957T interaction ($F(1,472)=0.634$, $p=0.634$), nor an interaction between feedback type, C957T and SNP19 ($F(1,472)=0.482$, $p=0.488$). Further, there was no effect of C957T ($F(1,472)=1.575$, $p=0.210$) SNP19($F(1,472)=0.085$, $p=0.771$), or C957T and SNP19 interaction ($F(1,472)=1.249$, $p=0.264$).

Figure 10. The effect of the C957T genotype on performance on the feedback-based learning Task. There was no significant effect on learning from positive or negative feedback.

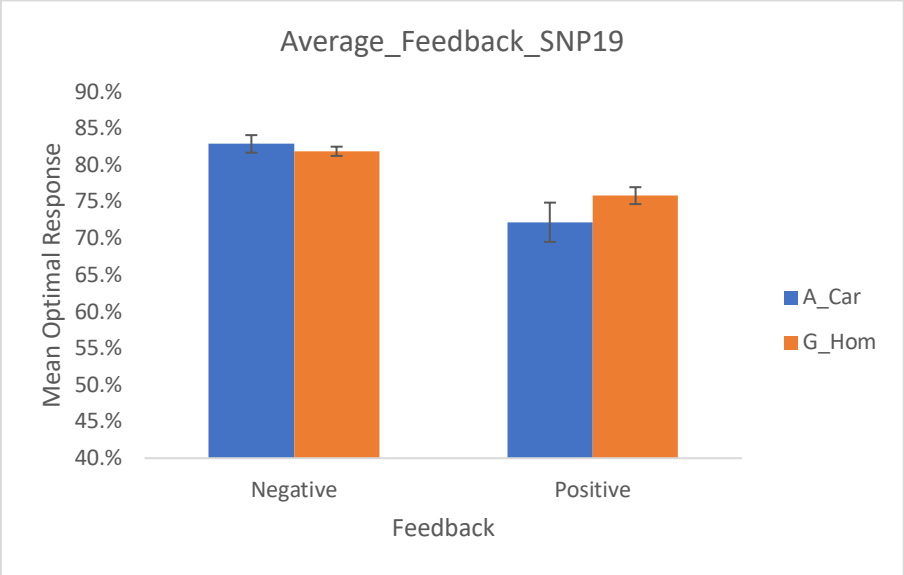

Figure 11. The effect of the SNP19 genotype on performance in the feedback-based learning Task. There was a significant effect of feedback type with A-Carriers learning better from negative feedback.

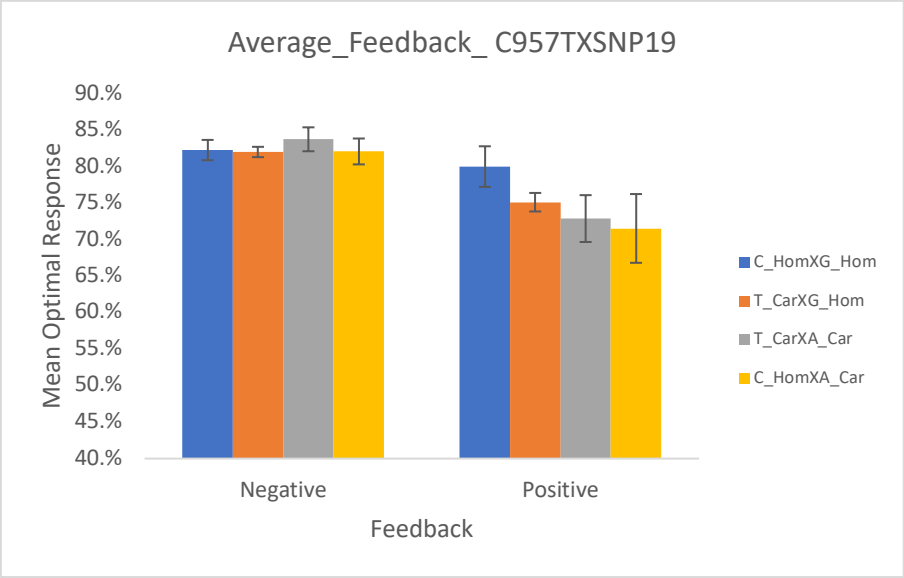

Figure 12. The genotype effect of the C957TXSNP19 interaction on performance in the feedback-based learning Task. C957TXSNP19 showed no significant effect on learning from positive or negative feedback.

To explore the balance between learning from positive and negative feedback, we examined relative avoidance; the difference between optimal responses in negative and positive feedback trials (**Figures 13-15**). We conducted a between-subject univariate analysis which revealed an effect of SNP19 ($F(1,472)=3.918$, $p=0.048$), but no effect of C957T ($F(1,472)=0.634$, $p=0.426$). Further, there was no significant effect of C957TXSNP19 ($F(1,472)=0.482$, $p=0.488$).

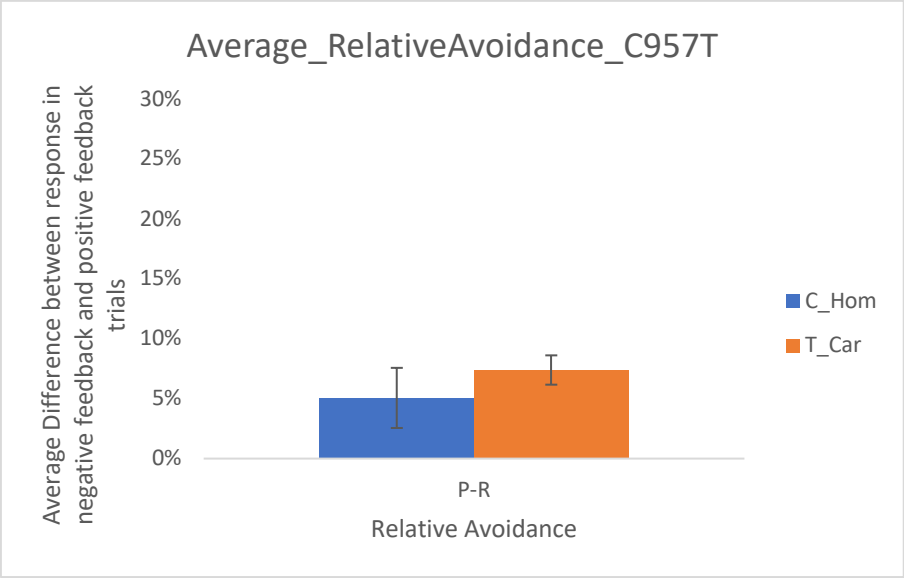

Figure 13. Illustration of the C957T genotype effect on relative avoidance. The C957T genotype did not show any significant effect.

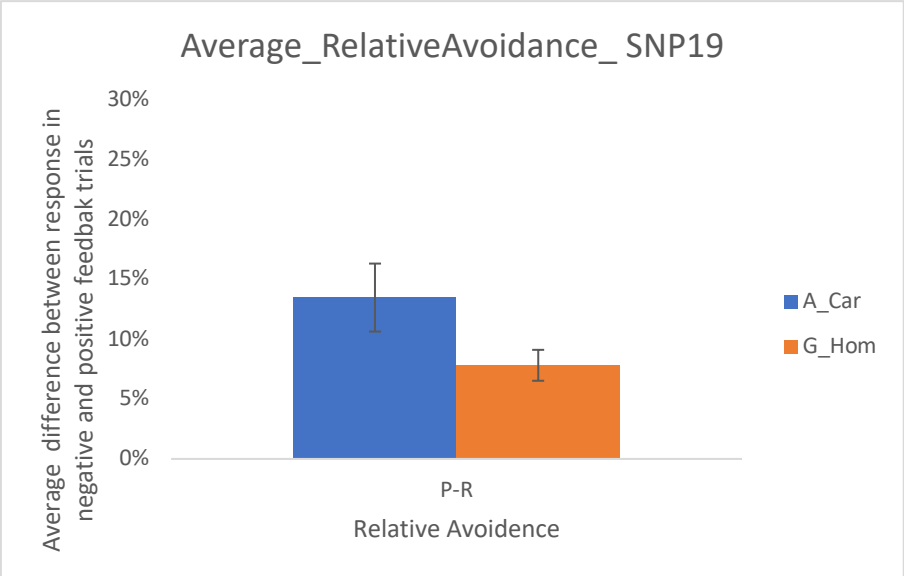

Figure 14. Illustration of the SNP19 genotype effect on relative avoidance. SNP19 A-Carriers had a higher bias toward negative feedback compared to G-Homozygotes.

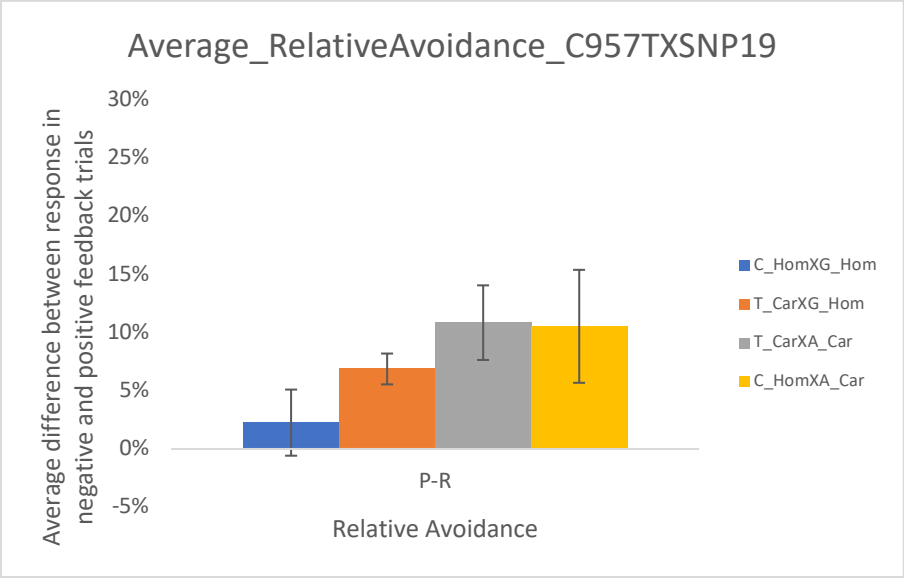

Figure 15. Illustration of the C957TXSNP19 interaction effect on relative avoidance. C957TXSNP19 showed no significant effect.

Performance-based on the prior outcome value (n-1):

Prior Trial Outcome

To investigate which dynamic aspects of prior experience would be incorporated by the dopamine signal on DRD2, we analyzed the effect of prior experience on the performance in the feedback-based learning task. We calculated the performance based on the current trial feedback and categorized the trials based on the previous trial outcome or valence. We built this approach on the value of prior distributions "negative feedback (-25)," "no feedback in negative feedback (-0)," "no feedback in positive feedback (+0)," or "positive feedback (+25),". We examined these categories in the context of *DRD2* haplotypes based on C957T and SNP19. Previous studies showed that past behavior influences later behavioral decisions (Lee, Seo, & Jung, 2012).

We explored the effect of prior valence and prior trial feedback using two mixed-model ANOVA (**Figure16-16**). One ANOVA was conducted with the current performance as the dependent variable, and the prior trial valence (positive and negative) as the within-subject variable, while genotype (C957T and SNP19) was the between-subject variable. Our ANOVA revealed an approaching-significance interaction between valence, C957T, and SNP19 ($F(1,472)=2.964$, $p=0.086$). There was no significant effect of valence ($F(1,472)=1.461$, $p=0.227$), no significant effect of valence type and C957T interaction

(F(1,472)=0.552, p=0.458), and valence type and SNP19 interaction (F(1,472)=0.662, p=0.431), genotype with SNP19 (F(1,472)=1.679, p=0.196), C957T (F(1,472)=0.317, p=0.574), and C957TXSNP19 (F(3,472)=0.998, p=0.394).

The second mixed-model ANOVA was conducted with prior trial feedback (positive (+25, -0), and negative (-25, +0)) as the within-subject variable, while genotype (C957T and SNP19) was the between- subject variable (**Figure17-21)**. There was a significant effect of feedback type (F(1,472)=3.703, p=0.05), but no effect of feedback and C957T interaction (F(1,472)=0.427, p=0.514), and feedback and SNP19 interaction (F(1,472)=0.534, p=0.465), no significant effect of C957T, SNP19 and feedback interaction (F(1,472)=0.118, p=0.731), and no significant effect of genotype with SNP19 (F(1,472)=1.679, p=0.196), C957T (F(1,472)=0.317, p=0.574), and C957TXSNP19 (F(3,472)=0.998, p=0.394).

In both mixed-model ANOVAs, we noticed that post-hoc power was significantly low, ranging between 20-40%. This indicated that we needed a larger sample to confirm the significance of the observed effects.

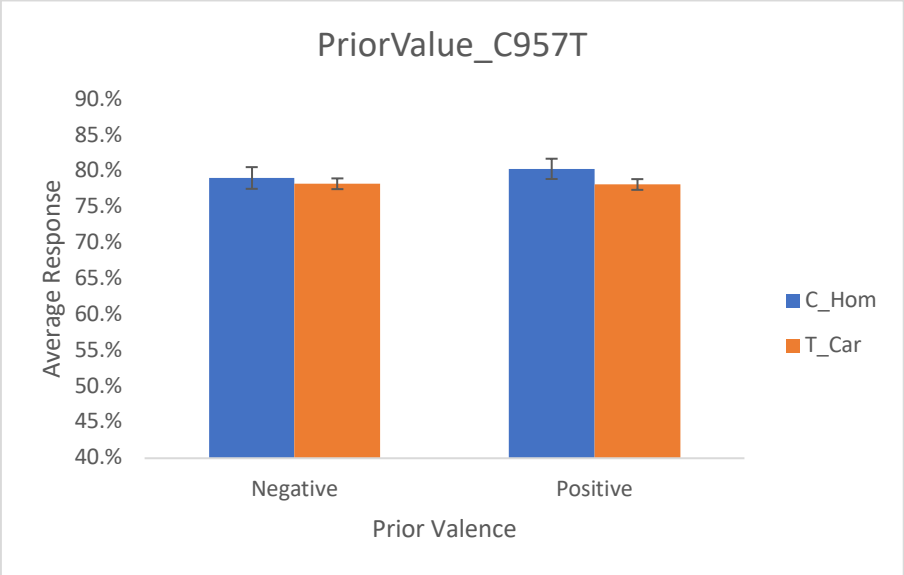

Figure 16. C957T effect on trials categorized according to the response of the subject in the prior trial divided by valence type.

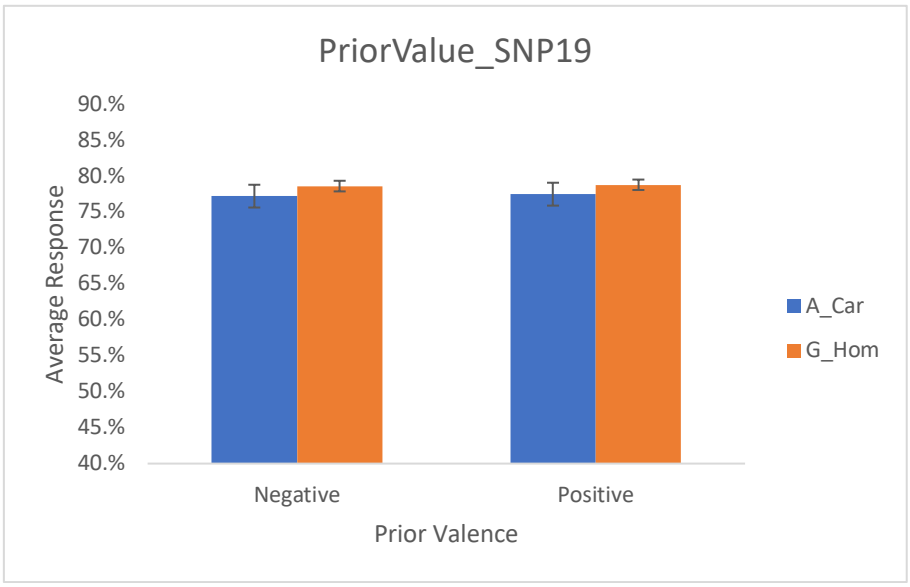

Figure 17. SNP19 effect on trials categorized according to the response of the subject in the prior trial divided by valence type.

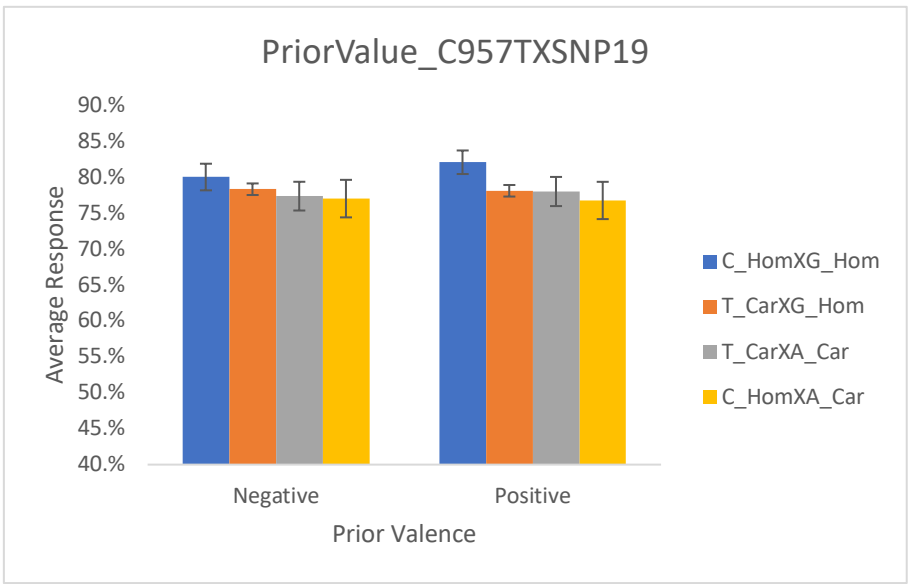

Figure 18. C957TXSNP19 effect on trials categorized according to the response of the subject in the prior trial divided by valence type.

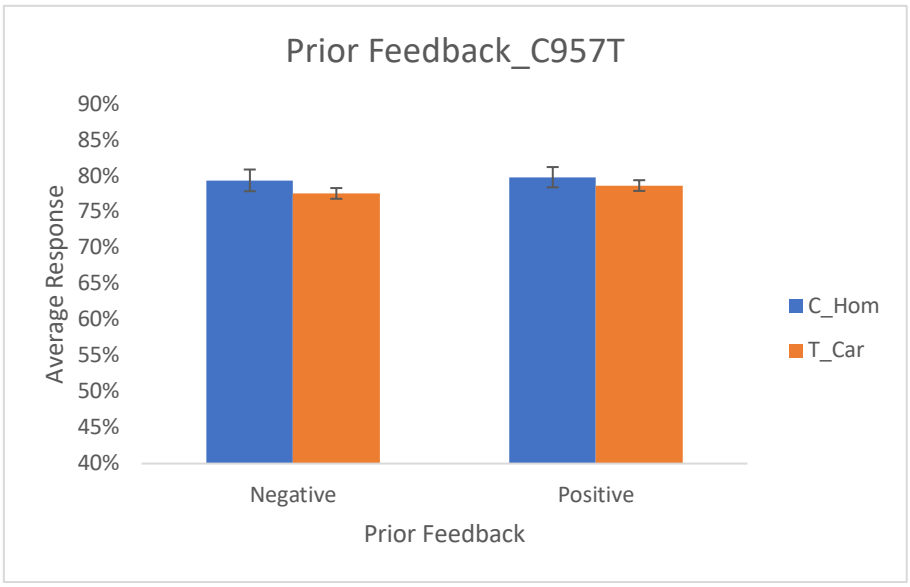

Figure 19. C957T effect on trials categorized according to the response of the subject in the prior trial divided by feedback type.

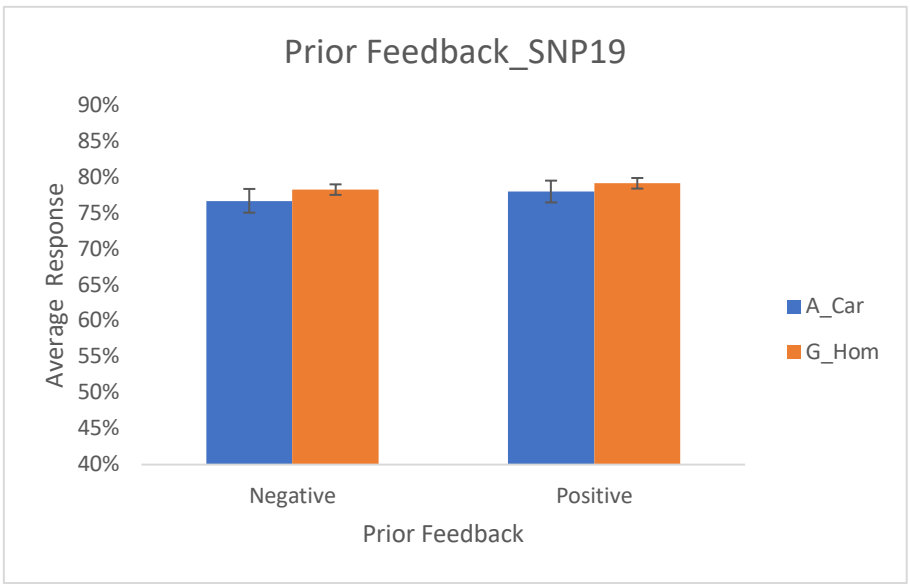

Figure 20. SNP19 effect on trials categorized according to the response of the subject in the prior trial divided by feedback type.

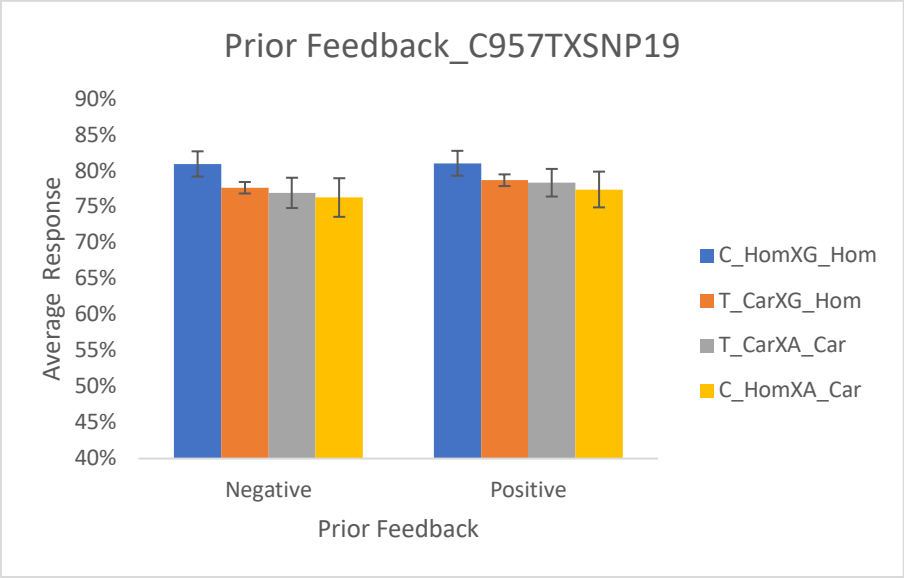

Figure 21. C957TXSNP19 effect on trials categorized according to the response of the subject in the prior trial divided by feedback type.

Chapter 4

Discussion

The goal of our study is to investigate the effects of two polymorphisms in the *DRD2* gene, namely, the C957T and the SNP19 and their interaction on modulating feedback-based learning. Our results showed a significant effect of SNP19 on relative avoidance with A-Carriers showing higher bias toward negative feedback. However, we did not find an effect of the C957T or SNP19 genotypes nor interaction between the genotypes on positive feedback learning. Prior outcome analysis revealed a significant effect of the prior trial valance with C-Homozygotes|G-Homozygotes performing better on current trials when the prior trial had a positive valence, regardless of the outcome.

Also, we investigated the effect of allelic locations of C957T and the SNP19 on individual differences in learning from positive and negative feedback. We found that there was a fixed distribution, with genotypes related to high allelic expression in the SNP19 is linked to that related to low allelic expression in C957T, thus creating a gene-dose allelic balance in the *DRD2* haplotype.

The influence of C957T and SNP19 on feedback-based learning

We investigated the cognitive effects of C957T and SNP19 polymorphisms in the *DRD2* gene. We based our genotype prediction of dopamine levels on previous findings (Frank & Hutchison; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009; Zhang et al., 2007). We could not find a significant difference among subjects with different variations in the C957T polymorphism, contrary to Hirvonen et al., who showed an increase in learning from negative feedback in TT-Homozygotes of the C957T polymorphism, but no difference in learning from positive feedback (Hirvonen, Laakso, et al., 2009). Regarding SNP19, there was a significant effect showing better performance in learning from negative feedback among A-Carriers compared to CC-Homozygotes. Still, we did not find a trend in learning from positive feedback. Besides, we found a significant effect in relative avoidance showing higher avoidance among A-Carriers compared to CC-Homozygotes. Frank and Hutchison revealed that SNP19 A-Carriers showed enhanced learning from positive feedback and poor learning from negative feedback, resulting in a positive learning bias (Frank & Hutchison, 2009). Contrary to their hypothesis, there was a significant difference in relative avoidance in SNP19, showing higher relative avoidance among A-Carriers. Frank and Hutchison studied SNP19 with SNP17, as both SNPs are in complete linkage disequilibrium. In our study, we examined SNP19 alongside C957T given their

proposed effect on pre- vs. post-synaptic DRD2 function. This provides a novel angle to the current study that was not examined heretofore. Further, the cognitive task that we used in our study is principally different from that used by Frank and colleagues.

SNP19 A-Carriers exhibited high levels of relative avoidance. A-Carriers are expected to have a lower density of DRD2 autoreceptors, and therefore higher levels of dopamine signaling and better learning from positive feedback, according to Frank and Hutchison (Frank & Hutchison, 2009). In our sample, subjects exhibited better learning from negative feedback compared to positive feedback, which is contrary to that reported by Frank and Hutchison (Frank & Hutchison, 2009). However, this discrepancy in our results may be due to the young age of our sample (18-23 years of age as opposed to 18-36 in the Frank & Hutchison, 2009 sample). Further, there is a huge difference in the subject pool we utilized in our study (N=476) as opposed to the used in Frank's study (N=69). Accordingly, the smaller sample size in Frank's study could have reflected an extreme value specific to the sample he studied rather than representing the effects DRD2 polymorphisms on cognitive function. It has been shown that the effects of single nucleotide polymorphisms are not as apparent compared to that in older individuals due to the complex, multifaceted "compensatory" mechanisms in neural circuits (Colzato, Zmigrod, & Hommel, 2013). Besides, it is difficult to pinpoint the effect of the SNP19 polymorphism on the exact dynamics of the dopaminergic system as a whole, due to its multitude of regulatory actions on tyrosine hydroxylase activity, dopamine transporter membrane expression, vesicular monoamine transporter-2 activation and dopamine firing frequency (Anzalone et al., 2012; Lindgren et al., 2003; Patel, Mooslehner, Chan, Emson, & Stamford, 2003). Furthermore, the effect of a single polymorphism or even two interacting polymorphisms in cognitive-behavioral studies is relatively small to be accurately measured. Other polymorphisms in the *DRD2* gene have been associated with feedback-based learning, such as SNP17 and TaqAI polymorphisms (Hirvonen et al., 2009; Zhang et al., 2007). These polymorphisms are also in linkage disequilibrium with SNP19 and C957T polymorphisms (Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009; Zhang et al., 2007). The relation of these polymorphisms that are most likely to be inherited as a single unit could be antagonistic, synergistic, or additive; one polymorphism could also have no effect and be a marker for another polymorphism which exerts an effect. Such a distinction will be ambiguous in the presence of linkage disequilibrium with the marker polymorphism.

The effect of C957T and SNP19 genetic variations can be appreciated in the context of the basal ganglia network, where neuronal activity in the striatum can modulate the activity of dopaminergic neurons. Positive feedback increases the phasic firing of dopaminergic neurons, whereas negative feedback learning leads to phasic dip/decease in dopaminergic neuronal firing (Schultz et al., 1997). *DRD2* polymorphisms may alter the

balance between tonic and phasic neurotransmission. The DRD2 is inhibitory in the indirect pathway, both pre- and postsynaptically. Higher postsynaptic DRD2 affinity increases the basal ganglia output but reduces dopamine release from the SNpc. Presynaptically, a higher DRD2 affinity reduces dopamine release in the SNpc. The G-Homozygotes of SNP19 is associated with increased presynaptic synthesis and high autoreceptor density (Zhang et al., 2007). The C-Homozygotes of the C957T is associated with a reduced striatal binding potential of the DRD2 but also increased presynaptic synthesis. SNP19 and C957T genotype variation changes DRD2 synthesis and availability, which may alter the balance between tonic and phasic dopamine signals.

In the case where the value of positive feedback is larger than expected, and in our case, when the feedback value equals +25, dopaminergic neurons will increase their phasic firing upon the arrival of the feedback. In the context of lower tonic dopamine, this will shape the dopamine signal to better predict outcomes in future trials. On the other hand, if the value of the positive feedback following the predictive cue is negative, in the case of -25, dopaminergic neurons will decrease firing, and thereby the synaptic tonic baseline dopamine-levels will drop (Tsai et al., 2009).

The C957T C-Homozygous genotype is associated with reduced binding of dopamine on postsynaptic DRD2 and subsequently increases disinhibition of the external globus pallidus, which further inhibits the subthalamic nucleus (Mamad, Delaville, Benjelloun, & Benazzouz, 2015). The subthalamic nucleus' glutamatergic input to the SNpc will be inhibited, thus leading to decreased dopamine release. This can limit the concentration of tonic dopamine that will linger in the synapses in medium spiny neurons. In turn, the G-Homozygous genotype of SNP19 is associated with a higher density of presynaptic autoreceptors that could truncate tonic dopamine levels even further (Grace, 1995). Therefore, we anticipate that the effect of C957T and SNP19 will be more pronounced when we examine performance based on preceding rather than current outcomes.

Influence of the prior outcome on performance:

Classical approaches that determine trial-based responses are not necessarily instructive. Dopamine activity may be modulated by predicted value during trials or events prior to the outcome (Lee et al., 2012). Schultz showed how decisions are informed by past rewards and modeled this process using reinforcement learning algorithms (Schultz, 2015). The dynamics of decision-making showed that feedback type (positive or negative) influences our future choices and our processing of decision outcomes (Gonzalez, 2014). Trial-by-trial variability of feedback is associated with the prediction error, which inspired the development of new analytical approaches to infer the influence of *DRD2* polymorphisms

on cognitive function in light of performance on prior trials. We created a trial-by-trial value history, which consisted of the value of the stimuli that the participant received in previous trials (n-1). The prediction error theory of dopamine states that the phasic activity of dopamine signals the difference between the predicated and the obtained feedback of a particular trail or event (Schultz et al., 1997). Given the structure of the DRD2 system with both pre- and postsynaptic elements, the effects of the C957T (postsynaptic) and SNP19 (presynaptic) will be more pronounced on performance based on preceding trials.

Our results showed a significant effect of the prior trial valance. C-Homozygotes|G-Homozygotes performed better on current trials when the prior trial had a positive valence, regardless of the outcome. This effect could reflect a more effective phasic dopamine signal due to better clearance of tonic dopamine in the prior trial. This is in line with previous experimental and computational modeling results suggesting that higher tonic dopamine could hinder the positive prediction error signal encoded by dopamine (Cools, Altamirano, & D'Esposito, 2006; Moustafa, Herzallah, & Gluck, 2013; Shohamy, Myers, Geghman, Sage, & Gluck, 2006). However, the lower affinity and lower tonic dopamine should also promote negative prediction error processing as well.

We found that subjects with the C-Homozygous|G-Homozygous genotype performed significantly better on current trials when they received negative feedback (negative prediction error) on the previous trial. Frank et al. showed that the T-Homozygotes in C957T of the *DRD2* gene were better at avoiding negative outcomes (Frank et al., 2007). Based on previous findings that the C957T T-Homozygotes have a higher affinity to dopamine, we conjecture that they will exhibit lower levels of negative prediction error and therefore learn less from negative feedback. In our results, we show that this effect is evident in subsequent rather than current trials.

Midbrain dopaminergic neurons evaluate the value of specific stimuli compared to the current internal state in the organism (Bromberg-Martin, Matsumoto, & Hikosaka, 2010). The dopamine projections from the presynaptic terminal to the postsynaptic play a role in the learning process and determine how we react to cues. The striatum maps states and creates different response options according to encountered values and signals (Daw, Gershman, Seymour, Dayan, & Dolan, 2011; Klein, Ullsperger, & Jocham, 2017). The striatum receives projections from the VTA, where it has two types of DA population, one population encoded the valence of the stimulus (either positive or negative). In contrast, the second population encoded the value of the stimulus (large or small) (Matsumoto & Hikosaka). Schultz showed that dopamine in the VTA encodes the outcome value and correlate it with future choices (Schultz, 2015). Our results show that the DRD2 system is a critical component in this chain of events to control the encoding of prior experience in cognitive performance during current encounters.

Influence of Allelic Location on DRD2 Gene Function

Humans have two copies of their genetic material, one maternal and one paternal. Variations exist between these two copies. Alleles can be regulated and expressed unequally, and this is termed allele-specific expression. This expression may lead to phenotypic variation. In our study, we quantified the extent of allele-specific expression to examine any potential cognitive effects.

Previous studies showed that naturally-occurring polymorphism are detected using a restriction enzyme specific to a polymorphism that is present in one allele and absent in the other. Subsequently, alleles are distinguished by their different lengths (Mamotte, 2006). However, using ARMS-PCR provided us with the locations of each allele variant in the *DRD2* polymorphisms we studied. However, ARMS-PCR did not distinguish whether the studied allele is from the maternal or paternal source. In our case, we aimed to measure the differential expression levels of the gene depending on different locations of C957T and SNP19 alleles. For this purpose, we selected heterozygous carriers of two polymorphisms of the *DRD2* gene. Surprisingly, our results showed a fixed distribution of alleles, where the "C" in the C597T is linked to that related to the "G" in SNP19, thus creating a gene-dose allelic balance in the *DRD2* haplotype. Genes-dose effects can occur due to the copy number of alleles. Alteration in gene dosages appears to be linked with genetic disease. For example, the duplication and triplication of the SNCA gene appear to be a rare cause of Parkinson disease (Konno, Ross, Puschmann, Dickson, & Wszolek, 2016).

Conclusions and Limitations

Our study revealed a significant cognitive effect of the *DRD2* haplotype composed on C957T and SNP19 polymorphisms. In particular, the interaction of the C-Homozygous in C957T and G-Homozygous in SNP19 showed the most efficient cognitive performance according to prior trials. In particular, when the trial was positively valenced, subjects C-Homozygous in C957T and G-Homozygous in SNP19 learned significantly better. They also outperformed other genotypes when they received negative feedback in prior trials. We conjectured that these effects are due to optimal dopamine signaling that facilitates subsequent learning.

Future studies ought to include a larger sample size, which would increase the power of the study for further examination of the full stratification of the different allelic combinations. Post-hoc power was significantly low, ranging between 20-40%. This indicated that we needed a larger sample to confirm the significance of the observed effects. Other molecular and imaging-based can help develop a more refined understanding of the underlying neural circuitry involved in these cognitive processes. Finally, future studies

should focus on further identifying the separate roles of the presynaptic and postsynaptic DRD2.

الملخص:

يتفاوت الناس في أدائهم الإدراكي، فهناك عدة عوامل تعيق التعلم وإنشاء الروابط. لقد تبين أن الناقل العصبي "الدوبامين" يلعب دورا مهما في التحكم في التعلم عن طريق التغذية الراجعة. يظهر تأثير الناقل العصبي دوبامين من خلال عائلتين من مستقبلات ما بعد الشق العصبي: مستقبلات الدوبامين النوع الأول D1 ومسقبلات الدوبامين النوع الثاني D2. يعمل المستقبل لدوبامين ما قبل الشق العصبي من عائلة D2 على التحكم في افراز الدوبامين ويعمل على تنظيم إطلاقه. في هذه البحث اتبعنا نهجًا متعدد الجوانب لفحص العوامل الجزيئية والادراكية للتغيرات الجينية التي تحدث بشكل طبيعي في نظام الدوبامين. ركزنا على دراسة تأثير اثنتين من الطفرات الجينية التي تحدث بشكل طبيعي في جين DRD2. C957T الذي ينظم الترابط و التشابك الملزم في مستقبلات الدوبامين DRD2 ما بعد الشق العصبي، وSNP19 الذي يعمل على تعديل عدد مستقبلات DRD2 ما قبل الشق العصبي.

في هذه الدراسة، استخدمنا تقنية ال PCR و RFLP لتحديد التغيرات في الطفرات الجينية. بالإضافة الى ذلك ،قمنا بتطوير نظام مبني على ال PCR لاختبار ما إذا كان تعدد الأشكال على نفس الأليل أم لا .وقد كان هدفنا ذو شقين:

1 - دراسة التفاعل بين C957T وSNP19 في تعديل الوظيفة الادراكية.

2 - التحقيق في التأثيرات الإدراكية للموقع الأليلي ل C957T وSNP19 في الأداء الادراكي. لذلك، خضعت جميع الزيجوتات المتغايرة لاثنين من الأشكال المتعددة ل ARMS PCR متبوعاً ب ARMS لفحص الموقع الأليلي لتعدد الأشكال.

فحصنا عينة مكونة من 429 طالبًا جامعيًا أصحاء (لا يعانون من أي مشاكل نفسية) في جامعة القدس. هؤلاء الطلاب أنهوا بنجاح لعبة محوسبة تهدف إلى دراسة مقدار تعلم الشخص من التغذية الراجعة الإيجابية والسلبية. أظهرت نتائج هذه الدراسة تأثير جرعة الأليل، حيث أن أليل "T" في C957T (انخفاض في مستقبلات الدوبامين ما بعد الشق العصبي) يرتبط دائماً بأليل "G" في SNP19 (زيادة في مستقبلات الدوبامين ما قبل الشق العصبي). أيضا، أظهرت نتائج دراستنا أن الأشخاص الذين لديهم نسب منخفضة لارتباط الدوبامين بمستقبلات DRD2 ما بعد الشق العصبي (CC-C957T)، بجانب أعلى تركيز لمستقبلات DRD2 ما قبل الشق العصبي (GG-SNP19) يتعلمون بشكل أفضل من ردود الأفعال الإيجابية . تتوافق نتائج دراستنا مع النتائج السابقة التي تسلط الضوء على دور DRD2 في تعديل إشارات الدوبامين اللحظية. يمكننا القول بأن دراستنا هذه هي الدراسة الأولى من نوعها التي تهدف لفهم الآثار الادراكية للتفاعل بين الطفرات الجينية C957T وSNP19 ومواقع الأليلات. نتائج هذه الدراسة ستلعب دورا مهما في تفسير نظام الدوبامين وكيفية تحكمه في المهام الوظيفية المختلفة، وبالتالي هذا سيساعدنا في دراسة العديد من الأمراض العصبية والنفسية.

Chapter 5

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