THE BEHAVIOURAL CHARACTERIZATION OF DOPAMINE D2 RECEPTOR-RELATED PROTEIN-PROTEIN INTERACTIONS

by

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Graduate Department of Physiology
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Abstract

Dopamine is one of the prominent catecholamine neurotransmitters in mammalian central nervous system. Dopamine is critically involved in a wide range of physiological functions including movement, motivation, reward, learning and memory, etc. The dopaminergic system exerts or modulates these physiological actions through four major signaling pathways. Clinically, the dysfunctions of the dopaminergic system are implicated in the patho-physiology of disorders such as Parkinson’s disease (PD), schizophrenia, attention deficit/hyperactivity disorder (ADHD), etc. At the molecular level, dopamine exerts its actions via its binding to dopaminergic receptors such as dopamine D2 receptors (D2R). In this study, two D2R-related protein-protein interactions were examined \textit{in vivo} regarding their behavioural effects. The disruption of D2R-DAT interaction was found to elevate voluntary movement in normal animals as well as in animals modeling acute dopamine depletion. On the other hand, the interference in the D2R-DISC1 protein complex exhibited anti-psychotic actions in two animal models of schizophrenia.
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List of Abbreviations

ADHD = Attention Deficit/Hyperactivity Disorder
AMPT = α-methyl-DL-tyrosine
cAMP = Cyclic adenosine monophosphate
CNS = Central Nervous System
D2R = Dopamine D2 Receptor
DISC-1 = Disrupted-in-Schizophrenia-1
GABA = γ-Aminobutyric acid
GDP = Guanine Diphosphate
GPCRs = G-protein coupled receptors
GRK = G-protein-coupled receptor kinase
GTP = Guanine Triphosphate
i.c.v. = intracerebroventricular
i.p. = Intra-peritoneal
L-DOPA = L-3, 4-dihydroxyphenylalanine
MSN = Medium Spiny Neuron
PD = Parkinson’s disease
S1 = the treatment peptide that disrupts the D2R-DAT interaction
S2 = the control peptide for the D2R-DAT experiment
s.c. = Subcutaneous
SNc = Substantia Nigra Pars Compacta
VTA = Ventral Tegmental Area
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1. Introduction

Neurons often communicate with one another through the means of chemical signaling, which a presynaptic neuron will release a large quantity of neurotransmitters into the synaptic cleft \[^1\]. These neurotransmitters will bind to different classes of receptors or ion channels sitting on the membrane of a post-synaptic neuron, influencing the neural activities of the post-synaptic neurons by activating different downstream signaling pathways. According to Purves et al, there are more than 100 neurotransmitters which are divided into two broad categories simply based on size: i) neuropeptides and ii) small-molecule neurotransmitters \[^1\]. Neuropeptides are neurotransmitters composed of 3 to 36 amino acids. Meanwhile, small-molecule neurotransmitters include catecholamines, acetylcholine, serotonin, histamine, and single amino acids such as glutamate, γ-aminobutyric acid (GABA), glycine, etc. The main focus of this thesis is on an important member of the catecholamine neurotransmitter family: **DOPAMINE**.

1.1. The Structure of Dopamine

As mentioned in the previous section, dopamine is a member of the catecholamine neurotransmitter family which also includes members such as norepinephrine and epinephrine \[^2-3\]. Being classified as a small molecule neurotransmitter, dopamine only weighs approximately 190 Daltons \[^2\]. Being a member of the catecholamine family, dopamine must share certain structural backbone: a nucleus of catehol (a benzene ring with two adjacent hydroxyl groups) and an ethylamine side chain (illustrated in Figure 1). Aside from the similarities in chemical structure, it is worth noting that dopamine is the predominant catecholamine in mammalian brain as it constitutes about 80% of the catecholamine content in the central nervous system. The biosynthesis, actions, reuptake and degradation of dopamine will be discussed in the following sections.
1.2 The Synthesis of Dopamine

The biosynthesis of dopamine usually takes place in the cytoplasm of presynaptic terminals of dopaminergic neurons\[^1\]. The precursor of dopamine is tyrosine, an aromatic amino acid. The transformation from tyrosine to dopamine mainly involves two chemical reactions: i) hydroxylation and ii) decarboxylation. As the synthesis begins, tyrosine hydroxylase adds an hydroxyl group to the benzene ring of tyrosine, converting it into L-3,4-dihydroxyphenylalanine (L-DOPA) \[^1-2\]. This step is considered the rate-limiting reaction of the synthesis \[^1,3\]. Following hydroxylation, DOPA decarboxylase will remove the carboxylic group from L-DOPA, yielding dopamine as the final product. Dopamine can undergo further chemical reactions and transform into other catecholamines (i.e. norepinephrine and epinephrine) \[^1-3\]. The metabolic pathways of dopamine biosynthesis and degradation are summarized in Figure 2. After the biosynthesis of dopamine, dopamine will be packaged into vesicles via vesicular monoamine transporter as preparation for future releases into the synaptic clefts.
Figure 2 – The Biosynthesis and Degradation of Dopamine. This figure aims to summarize the major metabolic processes for the synthesis and degradation of dopamine. The synthesis begins with a hydroxylation reaction, which tyrosine hydroxylase converts tyrosine into L-DOPA by adding a hydroxyl group to tyrosine. Please note that this is the rate-limiting reaction of the whole synthetic process. Subsequently, the carboxylic group of L-DOPA will be removed by DOPA decarboxylase, yielding the neurotransmitter of dopamine. Eventually, dopamine will be degraded by catechol o-methyltransferase into homovanillic acid. This figure was modified from *Neuroscience 8th Edition* [1]
1.3 Dopaminergic Receptors

Upon being released from the presynaptic neuron, dopamine will travel through the synaptic cleft and bind to different dopaminergic receptors localizing on the postsynaptic membrane. The physiological actions of dopamine will then be carried out by these post-synaptic dopaminergic receptors. Up to date, five distinct but related dopaminergic receptors have been discovered and studied. On the basis of structural, biochemical, genetics and pharmacological properties, these five dopaminergic receptors were often classified as either D1-like (D1 and D5 receptors) or D2-like dopamine receptors (D2, D3 and D4 receptors) [3-5].

1.3.1 The General Structure of Dopaminergic Receptors

All dopaminergic receptors are G-protein coupled receptors (GPCRs) that are composed of seven transmembrane domains, three extracellular loops, three intracellular loops, an extracellular N-terminal tail and an intracellular C-terminal tail (Figure 3) [1,3-5]. The N-terminus possesses a similar number of amino acids among all five members and contains several consensus N-glycosylation sites, even though the amount of these sites varies across different members [5]. For example, members of the D1-like family have two such sites, one in the N-terminal tail and one in the second extracellular loop [5-7]. On the other hand, the D2 receptor has four potential glycosylation sites while the D3 receptor has three. However, the D4 receptor possesses only one N-glycosylation site.

The C-terminal tail is approximately seven times longer in the D1-like receptors than in the D2-like receptors. This C-terminus is rich in serine and threonine residues and contains a cysteine residue that is conserved in all G-protein coupled receptors [5-7]. In spite of the similarities in amino acid composition, there are still differences in where these residues are located within the C-terminus. In particular, the cysteine residue is located near the beginning of
the C-terminus in D1-like receptors while it is located near the end of the C-terminus in D2-like receptors \cite{5-7}. Regardless of all the differences found in the C-terminal tail, the N-terminus possesses a similar amount of amino acid residues among all five dopaminergic receptors as aforementioned \cite{4-5}.

Similar to all other GPCRs, all five dopaminergic receptors have two cysteine residues encoded in their extracellular loops 2 and 3 \cite{5-7}. These cysteine residues are believed to interact with each other and form a disulfide bridge, which serves to stabilize the structure of a dopaminergic receptor \cite{8-9}.

O’Dowd suggested that the binding pocket for dopamine in all dopaminergic receptors is composed of several aspartic residues found in transmembrane domains 2 and 3, several serine residues found in transmembrane domain 5, and cysteine residues-mediated disulfide bridge between transmembrane domains 2 and 3 \cite{7}.
Figure 3 – The Structure of Dopaminergic Receptor.

This is a simplistic representation of a dopaminergic receptor. It aims to illustrate the general features shared by all five dopaminergic receptors. All dopaminergic receptors are seven-transmembrane GPCRs. Therefore, each receptor possesses seven transmembrane domains, depicted by seven cylinders in this diagram. In addition, each dopaminergic receptor contains an extracellular N-terminal tail (labeled as NH₂) and an intracellular C-terminal tail (labeled as COOH). This diagram was modified from Vallone et al.\textsuperscript{[3]}. The lipid bilayer portion was obtained online from http://www.biologyguide.net/biol1/3b_exchange.htm on November 12th, 2012. Please note that the length and size of each protein component was not drawn proportionally. IL = intracellular loop; EL = extracellular loop
1.3.2 The G-protein Interaction

Like any other GPCRS, dopaminergic receptors use their third intracellular loop to interact with the coupled G-protein (Figure 3). However, each member, depending on its corresponding family (i.e. D1-like or D2-like), couples with a different set of G-proteins due to their structural difference in the third intracellular loop. The D1-like receptors (e.g. D1 and D5) possess a short third intracellular loop which allows them to couple to G\(\alpha_{s/olf}\)\[^{3-5}\]. In contrast, having a longer third intracellular loop, the D2-like receptors interact with G\(\alpha_{i/o}\)\[^{3-5}\].

In general, a G-protein is composed of three subunits: \(\alpha, \beta\) and \(\gamma\). Piece et al suggested that the classification of a G-protein is based on the properties of the \(\alpha\)-subunit it associates with\[^{10}\]. When the receptor is not activated in the absence of agonist binding, the \(\alpha\)-subunit is attached to guanosine diphosphate (GDP) and tightly associated with the \(\beta\gamma\)-complex to form an inactive trimeric G-protein complex. Upon agonist-mediated activation of dopaminergic receptor, it will result in GDP release and guanosine triphosphate (GTP) binding to the \(\alpha\)-subunit. Such GTP binding will free the \(\alpha\)-subunit from the \(\beta\gamma\)-complex. The \(\alpha\)-subunit can then exert the actions of the receptors by influencing its downstream signaling pathways (Figure 5). For instance, the activation of D1-like receptors will release G\(\alpha_{s/olf}\), which will then stimulate adenylate cyclase, elevating the production of cyclic adenosine monophosphate (cAMP)\[^{1-5}\]. On the other hand, the activation of D2-like receptors will allow G\(\alpha_{i/o}\) to inhibit adenylate cyclase, repressing cAMP level. After the hydrolysis of GTP, the \(\alpha\)-subunit will become GDP-bound and reunite with the \(\beta\gamma\)-complex to be an inactive G-protein complex again\[^{10}\].

In spite of this generalized distinction in G-protein coupling between D1-like and D2-like receptors, there is an exception as George et al reported that D1 and D2 can function as a dimer and interact with G\(\alpha_{q/11}\) instead of G\(\alpha_{s/olf}\) and G\(\alpha_{i/o}\), subsequently activating the PLC/IP\(_3\)
downstream pathway \textsuperscript{[11-12]}. This PLC/IP3 pathway has no effects on modulating the effects of cAMP levels. At last, it is also worth noting that dopaminergic receptors can also link to downstream signalling pathways other than the two general pathways listed above (e.g. via $G_{\beta\gamma}$ complex) but the details of those pathways would be beyond the scope of this research report\textsuperscript{[4,10]}.

1.3.3 The Genetic Variants

Aside from differences in their structural properties and downstream signalling cascades, D1-like and D2-like receptors also possess unique genetic characteristics from one and other. First of all, receptors from the same family share a high level of homology in their transmembrane domains \textsuperscript{[3-5]}. For example, the D1-like receptors share 80% homology in their transmembrane domains. Similarly, the D2 receptor shares 75% homology with D3 receptor and 53% with D4 receptor in their transmembrane domains.

Other than the level of homology in transmembrane domain among family members, the presence of introns is another genetic feature to distinguish between D1-like and D2-like receptors. Both D1 (5q35.1) and D5 (4p16.1) receptor genes do not contain any introns in their respective coding sequence \textsuperscript{[3-5]}. In contrast, the gene (11q23.1) encoding for D2 receptors contains six introns and eight exons. The alternative splicing of an 87-bp exon between introns 4 and 5 gives rise to two important splice variants of D2 receptors: D2-Long (D2L) and D2-Short (D2S) \textsuperscript{[13-16]}. Due to the alternative splicing, D2L has an additional 29 amino acids within the third intracellular loop, while such amino acid sequence is absent in D2S (Figure 4) \textsuperscript{[3-5,16]}. These two isoforms of D2 receptors actually possess different anatomical, physiological and pharmacological properties. For example, D2S as an auto-receptor is predominantly expressed on the cell body membrane and along the axonal projection of dopaminergic neurons originating from the midbrain and hypothalamus \textsuperscript{[17]}. Meanwhile, D2L is the post-synaptic counterpart that is
expressed by the neurons in striatum and nucleus accumbens to mediate the physiological actions of dopamine \[^{17}\]. In addition, D2S is generally activated at a lower concentration of dopamine agonists than the long isoform of D2 receptors \[^{4}\].

Similar to D2 receptors, the gene (3q13.3), encoding D3 receptor, contains five introns \[^{3-5}\]. Several splice variants of D3 receptors have been discovered. For example, one variant is derived from a deletion of 54bp between its fifth and sixth transmembrane domain. Another D3 isoform is found to have a 113-bp deletion in its third transmembrane domain and a frame-shift resulting in a stop codon, encoding a 100-amino-acid long truncated form of D3 receptor. It is worth noting that these splice variants of D3 receptors have been considered non-functional \[^{18}\].

The gene (11p15.5) encoding the last member of the D2-like family also has several splice variants. These D4 splice variants only differ in the number of 16-amino-acid repeat sequence in their third intracellular loop \[^{4-5}\]. It has been reported that a splice variant can contain up to 11 repeat sequences \[^{19}\]. In human, the predominant isoform is the one with four repeat sequences in its third intracellular loop, constituting up to 60% of D4 expression \[^{5}\]. There is a summary of the molecular and genetic properties of each dopaminergic receptor listed in Table 1.
Figure 4 – The Schematic Comparison between D2 Receptor Isoforms.
The top panel is a schematic representation of D2L, whereas the bottom panel is a representation of D2S. The dotted line in the top panel depicts the 29-amino-acid sequence which is absent in all D2S isoforms. The D2L isoform encodes 443 amino acids while the D2S isoform only contains 414 amino acids. This diagram was modified from Vallone et al.\cite{3}. The lipid bilayer portion was obtained online from \url{http://www.biologyguide.net/biol1/3b_exchange.htm} on November 12th, 2012. Please note that the length and size of each protein component was not drawn proportionally. IL = intracellular loop; EL = extracellular loop
1.3.4. Pharmacological Profiles

D1-like and D2-like receptors also possess different pharmacological profiles. In general, dopaminergic ligands can easily distinguish between D1-like and D2-like receptors. For instance, SKF-38393 (a D1-like dopaminergic agonist) has a $K_i$ of 1-150nM and 0.5 – 100nM for D1 and D5 respectively, whereas its $K_i$ value for D2-like receptors ranges from 150nM – 956nM [3]. Meanwhile, SCH-23390 (a D1-like dopaminergic antagonist) displays a $K_i$ value of 0.11 – 0.54 for D1-like receptors but a $K_i$ value of 270nM – 3560nM for D2-like receptors. On the other hand, apomorphine (a D2-like agonist) has a $K_i$ value of 0.5nM – 5nM for D2-like receptors but a $K_i$ value of 50nM – 5000nM for D1-family members [5]. In addition, the $K_i$ value of haloperidol (a D2-like antagonist) for D2-like receptors is 0.6 – 7.8nM, whereas it is 27-203nM for D1-like receptors [3]. In spite of the drastic binding affinity difference between D1-like and D2-like subfamily, it is generally difficult to discriminate among members with in the same sub-family based on the binding affinity of one ligand alone.

1.3.5. The Distribution of Dopamine Receptors in CNS

The D1 and D2 receptors are the most widespread and predominantly expressed dopaminergic receptors within human central nervous system (CNS). For example, D1 receptors are found in striatum, nucleus accumbens, olfactory tubercle, cerebral cortex, amygdala, the subthalamic nucleus and substantia nigra pars reticulata [3,20]. Meanwhile, D2 receptors are mainly expressed in striatum, olfactory tubercle, nucleus accumbens, substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) [3,20].

Unlike the widespread distribution of both D1 and D2 receptors, D3 receptors are only expressed in SNc, hypothalamus and several regions of thalamus and cerebellum [3,20]. D4 receptors are found in mesencephalon, hypothalamus, hippocampus, amygdala, the olfactory
bulb and the frontal cortex \cite{3,20}. At last, D5 receptors are localized in the hippocampus, lateral mammillary nucleus and in the parafascicular nucleus of the thalamus \cite{3,20}.

1.3.6. Receptor Desensitization and Internalization

During agonist-mediated activation, the dopaminergic receptors are also subjected to regulation processes such as desensitization and internalization (Figure 5) \cite{10,21-22}. As the dopaminergic receptors are activated by the agonist binding and mediate their G-protein-dependent signaling pathways, it also leads to the rapid phosphorylation of the receptors by G-protein-coupled receptor kinases (GRKs)\cite{10,21,23-24}. These GRKs phosphorylate specific residues on the third intracellular loop and the C-terminus of the dopaminergic receptors. The phosphorylation of the dopaminergic receptors will recruit β-arrestins (scaffolding proteins). This binding will uncouple and prevent the dopaminergic receptors from subsequent G-protein activation even in the presence of agonist-bindings, leading to the desensitization of receptors \cite{4,21,25-27}. In addition to desensitization, the protein complexes of receptors and β-arrestins will also bind to clathrin adaptor protein AP2 complex and clathrin. Such clathrin-coated protein complex will signal for clathrin-mediated internalization of the receptors. There are two destinies for the internalized receptors: 1) they will subsequently be re-sensitized and recycled back to the cell surface or 2) they will be subjected to degradation \cite{4,26-29}.
Table 1 – Genetics and Molecular Characteristics of Dopamine Receptors

<table>
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<th>Gene Symbol</th>
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This table attempts to summarize the general information regarding the genetics and structural properties of each dopaminergic receptor. All the information presented in this table was compiled from several scientific reviews \cite{3-5}. * indicated that the number of amino acids depends on the number of repeat sequences found in the third intracellular loop.
Figure 5 – The Signaling Cascades of Dopaminergic Receptors.
The figure is showing the three possible signaling cascades which any dopaminergic receptor can undergo upon activation: i) the classical G-protein dependent signaling pathway, ii) the novel G-protein independent pathway and iii) desensitization and internalization. In the classical pathway, the receptor activation can result in upregulation or downregulation of adenylate cyclase, depending on the type of the coupling G-protein. The G-protein independent pathway is a newly discovered cascade, which is believed to work alongside the classical pathway to carry out the physiological functions of dopamine. The desensitization and internalization pathway marks the termination of the dopaminergic neurotransmission. The figure was obtained and slightly modified from Beaulieu et al.\textsuperscript{[26]}. 
1.4. Dopaminergic Pathways in the CNS

The dopaminergic system is an essential nervous system in the mammalian brain as it modulates or controls a wide variety of physiological functions such as voluntary movement, motivation, reward, learning, working memory, neuro-endocrine system, etc [2-3,5]. All the aforementioned functions are considerably mediated through four major dopaminergic pathways in the brain: i) tuberoinfundibular, ii) mesocortical, iii) mesolimbic and iv) nigrostriatal (Figure 6) [1-3,5].

1.4.1. Tuberoinfundibular Pathway

This pathway originates from cells of the periventricular and arcuate nuclei of the hypothalamus (Figure 6) [2-3]. These cells send their axonal projection to median eminence of the hypothalamus and release dopamine into the perivascular spaces of the capillary plexus of the hypothalamic. As median eminence is a part of the hypophyseal portal system, it serves as a bridge between hypothalamus and the anterior pituitary gland. Therefore, dopamine released in this area will then be transported to the anterior pituitary gland and these neurotransmitters will subsequently act on the lactotrophs to inhibit the release of prolactin, thereby dampening milk production from the mammary gland.
Figure 6 – An Overview of the Major Dopaminergic Pathways.
The left panel is a simplistic illustration of the four major dopaminergic pathways, while the right panel provides an anatomical representation of the pathways in mammalian CNS. The purple line represents the tuberoinfundibular tract. It is noteworthy that dopamine is released into medium eminence and subsequently diffused into the anterior pituitary gland through the hypophyseal portal system [2,3,30-31]. The blue line represents both the mesolimbic and mesocortical pathways, which both originate from VTA but project to the limbic system and the frontal cortex respectively. At last, the green line represents the nigrostriatal pathway which arises from SNc and has its axonal projection to the dorsal striatum. For both diagrams, solid circles depict the origins of the dopaminergic pathways while the arrows or “Y”-shape arrows represent their corresponding axonal projections. The left panel was modified from Principles of Medical Pharmacology 7th Edition [2]. The right panel was obtained from http://neuropolitics.org/defaultdec09.asp on the date of November 14th, 2012. Abbreviations: SNc, substantia nigra pars compacta; VTA, ventral tegmental area; ME, medium eminence; Ant. Pit., anterior pituitary gland; Hypo, hypothalamus; Amyg, amygdala; NAc, nucleus accumbens and OT, olfactory tubercle.
1.4.2. Mesocortical and Mesolimbic Pathways

The mesocortical pathway arises from the dopaminergic neurons in the VTA (Figure 6). The axonal projection of these dopaminergic neurons innervates several regions of the frontal cortex \[^{[2-3]}\]. This pathway is considered to be critically involved in some aspects of learning and memory \[^{[3,31]}\]. Similarly, the mesolimbic pathway also originates from the dopaminergic neurons in the VTA of the midbrain. Instead of innervating the frontal cortex, these dopaminergic neurons projects to limbic areas such as nucleus accumbens, olfactory tubercle and amygdala \[^{[2-3,31]}\]. Functionally, the mesolimbic pathway is considered the neural mechanisms of motivation and reward behaviours. In addition, this pathway is generally implicated in the patho-physiology of drug addiction.

1.4.3. Nigrostriatal Pathway

The nigrostriatal pathway originates from the A9 dopaminergic neurons of SNc in the midbrain (Figure 6) \[^{[2-3,30]}\]. These dopaminergic neurons send their axonal projections to innervate the dorsal striatum (i.e. caudate-putamen complex). This pathway is critically involved in the coordination of voluntary movement. In addition, the degeneration of the nigrostriatal pathway is heavily implicated in the pathogenesis of Parkinson’s disease, characterized by tremors, rigidity and akinesia \[^{[1-5]}\].

How is the nigrostriatal pathway involved in voluntary movement? Let’s take a closer look at the motor circuit in the basal ganglia (Figure 7). The majority of neurons in the dorsal striatum are medium spiny neurons (MSNs) \[^{[4,32]}\]. These MSNs are classified into two distinct classes of neural pathways: i) direct and ii) indirect \[^{[32-34]}\]. The direct pathway is so named because it has a “direct” monosynaptic projection to the output regions of basal ganglia: the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulate (SNr) \[^{[32-35]}\].
In contrast, the indirect pathway must go through the external segment of globus pallidus (GPe) and the subthalamic nucleus (STN) before reaching the output station of basal ganglia \[^{32-35}\]. In general, the striatum serves as an input station for basal ganglia, receiving glutamatergic inputs from the cortex. Subsequently, this glutamatergic signaling will excite both the direct and indirect pathways. When the direct pathway is activated, the MSNs will send their GABAergic signals to both GPi and SNr, inhibiting these regions. The inhibition of both GPi and SNr will lead to the disinhibition of thalamic neurons, which will in turn send excitatory glutamatergic signals to the cortex, facilitating voluntary movements \[^{32-33}\]. On the other hand, when the indirect pathway is activated, its MSNs will release inhibitory (GABAergic) signals to GPe, which normally serves to hinder the activities of STN. The MSN-mediated inhibition of GPe will result in the disinhibition of STN, which in turn will excite the output station of basal ganglia (i.e. GPi and SNr). The activation of GPi and SNr by STN will lead to a decrease in the excitability of thalamus and therefore a reduction/termination in voluntary movements \[^{32-33}\].

There are two models explaining how the direct and indirect pathways cooperate to modulate voluntary movements: i) scaling and ii) focusing \[^{33}\]. In the “scaling” model, the direct pathway must first get activated to initiate and facilitate movements. After the duration of delay, the indirect pathway will get activated to terminate the ongoing/pre-existing movements. In the focusing model, the direct pathway serves to facilitate intended movements while the indirect pathway aims to suppress any unintended movements and therefore complements the direct pathway to better mediate the desired movement.

The MSNs in the direct pathway only express D1 receptors while the MSNs in the indirect pathway selectively express D2 receptors \[^{32,34,36}\]. Therefore, as the stimulated nigrostriatal projection releases dopamine onto striatum, dopamine will activate D1 receptors in
the direct pathway and lead to the stimulation of adenylate cyclase, therefore enhancing the Cyclic adenosine monophosphate (cAMP) production. The increased cAMP level modulates several intracellular pathways and eventually leads to an increase in the excitability of D1-expressing MSNs, promoting the direct pathways [32-33]. Simultaneously, the dopamine released from the nigrostriatal projection will also bind to the D2 receptors expressed on the membrane of MSNs in the indirect pathways. Since D2 receptors are coupled to Gαi/o as previously mentioned, the activation of D2 receptors will result in a decrease in cAMP level. This reduction is believed to reduce the excitability of these D2-expressing MSNs in the indirect pathways and subsequently inhibit the indirect pathways [1,3-5,32-33]. All in all, the overall effects of nigrostriatal dopamine release are to relieve the inhibitory output from GPi/SNr to the thalamus, which in turn results in increased activities of the thalamus-cortex projection to enhance the facilitation of voluntary movements (Figure 7).
Figure 7 – A Schematic Diagram of the Direct-Indirect Circuitry in the Basal Ganglia

In the direct pathway, the MSNs project their GABAergic axons to inhibit the activities of both GPi and SNr. The inhibition of GPi and SNr will lead to the disinhibition of thalamus, which will be able to send excitatory signals to the cortex to facilitate voluntary movements. In contrast, the activation of the indirect pathway will inhibit the output of GPe, disinhibiting STN. STN will in turn stimulate GPi and SNr to impose a stronger inhibition on thalamus and therefore prevent the initiations of any voluntary movements. This schematic diagram also shows how dopamine modulates both direct and indirect pathways to promote voluntary movements. When dopamine is released into the striatum, due to the expression difference of dopamine receptor between the direct and indirect pathway, dopamine will stimulate the direct pathway while inhibit the indirect pathway. In general, dopamine will lead to a stronger output from the thalamus to cortex and subsequently result in an increased level of movement facilitation. This schematic diagram is modified from Wichman and Delong [33].
1.5. The Reuptake of Dopamine

The physiological functions of dopamine are terminated by dopamine transporters (DATs), which transport dopamine from the synaptic cleft back into the presynaptic neuronal terminals \[^{37-40}\]. These transporters are localized in the plasma membrane of dopaminergic neurons derived from the aforementioned dopaminergic pathways \(^{1-3,37-39}\). It is noteworthy that not only does DAT terminate dopaminergic neurotransmission via its reuptake action, but it is also responsible for restoring the dopamine content within the presynaptic dopaminergic neurons for subsequent releases \(^{37}\).

The dopamine transporters belong to the SLC6 (solute carrier 6) family of Na\(^+\)/Cl\(^-\)-dependent transporters \(^{37-39}\). Other family members include transporters for neurotransmitters norepinephrine (i.e. NET), serotonin (i.e. SERT), GABA (i.e. GAT) and glycine (i.e. GLYT) \(^{37,41-42}\). All these SLC6 transporters share the same molecular structure, which is composed of 12 transmembrane domains and intracellular N- and C-termini (depicted in Figure 8) \(^{37-38,42-43}\). Such structure was later confirmed by Yamashita et al. using the crystallography of a bacterial homologue (i.e. LeuT) of the SLC6 transporter \(^{44}\). As its name suggested, DAT utilizes the Na\(^+\) gradient created by the plasma membrane Na\(^+\)/K\(^+\) ATPase as the driving force to transport dopamine and to co-transport Cl\(^-\) ions across the cellular membrane \(^{45-46}\). Interestingly, there is a considerable homology in transmembrane domains among the SLC6 members, which may imply a common mechanism of substrate translocation shared by all the SLC6 transporters \(^{38}\). However, the extracellular and intracellular components of these transporters are more divergent in terms of sequence, which may be responsible for their corresponding substrate specificity and affinity.
Figure 8 – A Structural Overview of the Dopamine Transporter (DAT).

The dopamine transporter is composed of 12 transmembrane domains as depicted by the cylinders. Both the N-terminal and C-terminal tails of DAT are situated in the intracellular side. In addition, it is also composed for four extracellular loops and four intracellular loops connecting all twelve transmembrane domains. The arrows denote all the serine residues along DAT and these serine residues are considered to be involved in the PKC-mediated phosphorylation of DAT \[47\]. Most of these serine residues are concentrated in the N-terminus, implicating the important role of N-terminus in DAT phosphorylation. It is also noteworthy that the second extracellular loop is the primary site for N-glycosylation, which is essential to the membrane localization of DAT \[37,39,47\]. Please note there is a considerable homology in transmembrane domain sequence among all SLC6 transporters \[38\]. This diagram was obtained from Foster et al. \[47\].
1.6. The Regulation of DAT

The reuptake activity of DAT is greatly dependent on its membrane expression level and its surface expression is constantly under dynamic regulation. The membrane expression of DAT is tightly regulated and maintained through constant internalization via endocytosis as well as constant recycling from the endosomal pool back to the surface via exocytosis \[^{38}\]. Despite the exact molecular mechanisms underlying these cellular events still remain elusive, there are three plausible mechanisms considerably responsible for these dynamic regulations of DATs: i) phosphorylation, ii) ubiquitination and iii) protein-protein interaction.

1.6.1. Phosphorylation

Several studies have revealed the presence of potential phosphorylation sites within the amino acid sequence of DAT: 15 serine, 9 threonine and 5 tyrosine intracellular residues \[^{48-49}\]. Many of these residues are positioned within consensus sequences for protein kinase C (PKC), cAMP-dependent protein kinase, cGMP-dependent protein kinase and calcium calmodulin-dependent protein kinase \[^{38,50}\]. The existence of cellular phosphorylation of DAT was discovered, when Huff \textit{et al}. and Vaughan \textit{et al}. respectively reported a basal level of DAT phosphorylation in the absence of exogenous treatments \[^{51-52}\]. Furthermore, they showed that the activation of PKC increased the level of DAT phosphorylation in a concentration-dependent manner. Interestingly, Vaughan \textit{et al}. showed that the inhibition of protein phosphatases also led to an increase in DAT phosphorylation, indirectly confirming the presence of constitutive dephosphorylation on DATs \[^{52}\]. Moreover, Foster \textit{et al}. revealed that the PKC-mediated phosphorylation primarily happens to the serine residues within the N-terminus of DAT (Figure 8) \[^{47}\]. Functionally, the activation of PKC has reportedly reduced the reuptake actions and membrane expression of DAT \[^{52-56}\]. It has been proposed that such down-regulation is facilitated through internalization \[^{50}\]. However, the effects of PKC on DAT internalization may not be
directly related to the phosphorylation of DAT N-terminus because the truncation or mutation of the N-terminus did not abolish the PKC-mediated down-regulation of DAT \textsuperscript{[57]}. Therefore, future studies are required to further determine the exact role of PKC-enhanced phosphorylation in the down-regulation of DAT.

1.6.2. Ubiquitination

It is widely accepted that ubiquitination is a cellular mechanism for regulating protein membrane expression level \textsuperscript{[58-61]}. The ubiquitin tags on any transmembrane protein serves as cellular signal for endocytosis-mediated internalization, while such tags will lead to lysosomal degradation of proteins if present in endosomes. Miranda et al. reported that DATs undergo constitutive ubiquitination under normal cellular/physiological condition \textsuperscript{[62]}. In addition, they also showed that the level of DAT ubiquitination enhanced in response to the activation of PKC \textsuperscript{[62]}. Miranda et al. later discovered that the ubiquitination of DAT was dependent on the presence of three lysine residues (i.e. Lys\textsubscript{19}, Lys\textsubscript{27}, Lys\textsubscript{35}) within the N-terminus of DAT (Figure 8) \textsuperscript{[63]}. More interestingly, mutation of these lysine residues resulted in a complete abolishment of both DAT-ubiquitination and PKC-mediated DAT down-regulation \textsuperscript{[63]}. This experimental finding prompted a potential role of ubiquitination in regulating DAT membrane expression and especially for the PKC-mediated internalization of DAT. \textsuperscript{[63]}

1.6.3. Protein-Protein Interaction

The last means of regulating DAT membrane expression is through protein-protein interactions. Until now, many proteins have been reported to modulate the activities of DAT by interacting with either its N-terminal tail or its C-terminal tail. For instance, \(\alpha\)-synuclein, a protein implicated in the pathogenesis of Parkinson’s disease, was showed to interact with the C-
terminus of DAT through its non-Aβ amyloid component\textsuperscript{[64]}. Through this interaction, α-synuclein recruited intracellular DAT to the cell surface and therefore up-regulate the reuptake of dopamine. Unlike α-synuclein, Hic-5 (a scaffolding protein) also interacted with the C-terminal of DAT but it dampened the expression of DAT at the cell membrane, leading to a reduction in the activity of DAT\textsuperscript{[65]}. On the other hand, synaptogyrin-3 (a synaptic vesicle protein) formed a protein complex with DAT through their respective N-termini\textsuperscript{[66]}. Through this interaction, synaptogyrin-3 elevated the DAT-mediated reuptake of dopamine. At last, it was reported that D2S also modulated the activity of DAT via a direct protein-protein interaction which will be discussed in later sections\textsuperscript{[67]}.

1.7. The Dopamine Theory of Schizophrenia

Given its physiological significance, a dysfunctional dopaminergic system will often result in drastic medical consequences such as schizophrenia. The dopamine theory of schizophrenia was first derived from \textit{in vivo} studies in 1960s where neuroleptics drugs were reported to either impair dopamine signaling or block dopamine receptors\textsuperscript{[68-70]}. The theory was further conceptualized in 1970s when several reports showed that the clinical efficacy of neuroleptics drugs or anti-psychotic drugs was directly dependent on their binding affinities for dopamine receptors\textsuperscript{[71-73]}. Since then, related research viewed a global excessive dopaminergic neurotransmission as the core patho-physiology of schizophrenia\textsuperscript{[74-76]}. Due to the advancement in experimental technologies, it was later found that dopamine content was not universally elevated in the cerebrospinal fluid or serum of patients with schizophrenia, implying a regional specificity of hyperdopaminergia\textsuperscript{[77]}. In addition, the lesioning of frontal dopaminergic neurons was reported to elevate dopamine and its metabolites level as well as D2R density in the striatum\textsuperscript{[78]}. Furthermore, the administration of dopaminergic agonists into the prefrontal areas lowered
the striatal content of dopamine metabolites, suggesting a causational relationship between frontal hypodopaminergia and striatal hyperdopaminergia \(^{79}\). Based on these findings, Davis et al in 1991 refined the dopamine theory by proposing that the striatal hyperdopaminergia was a consequence of the hypodopaminergic activity in the frontal cortex \(^{80}\). However, due to the lack of clear evidence of frontal hypodopaminergia in schizophrenia, the “hypofrontality” was soon fallen out of favour \(^{77}\).

In the past decade, the emphasis of the dopamine theory began to shift to presynaptic dopamine abnormalities based on new research findings. First of all, several studies have reported elevated presynaptic striatal dopamine synthesis capacity in patients with schizophrenia \(^{81-84}\). Aside from synthesis capacity, schizophrenic patients also exhibited a greater level of dopamine release in striatum upon challenge compared to control subjects \(^{85-87}\). Based on its abnormalities in synthesis and release, it would not be surprising to observe greater baseline D2R occupancy in schizophrenia patients \(^{88}\). Taken together, it seems clear that the presynaptic dopamine dysregulation leads to the striatal dopamine overactivity, which is then translated into psychotic behaviours \(^{77}\). The last but not least, scientists have begun to incorporate environmental factors into the theory as studies revealed that social isolation and subordination rendered hyperdopaminergic activity in animals \(89-91\).

### 1.8. Disrupted-in-schizophrenia-1 in Schizophrenia

Aside from the dopamine theory, the appearance of schizophrenia is also believed to be a consequence of genetic susceptibility. In the past decade, a number of genetic risk factors have been identified by genetic analyses and disrupted-in-schizophrenia-1 (DISC1) appears to be one of the most promising susceptibility genes. Disrupted-in-schizophrenia-1 (DISC1) was first identified in a Scottish family whose members inherited a balanced translocation between
chromosome 1 and chromosome 11, resulting in a loss of 257 amino acid residues in its C-terminal [92-95]. More importantly, members within this Scottish pedigree showed a remarkably high incidence of mental disorders including schizophrenia, depression, autism, etc. With the use of statistical analysis, Blackwood et al. showed that the inheritance of this genetic translocation increases the risk for developing one of these mental disorders by approximately 50 folds compared to the general population [96]. Other than the mutation of DISC1 found in the Scottish pedigree, two common single-nucleotide polymorphisms, Leu607Phe in exon 9 and Ser704Cys in exon 11, were also reported in patients with schizophrenia [97]. In addition, a 4-base-pair deletion in exon 12 of the DISC1 gene was also discovered in an American family with schizophrenia [98]. This mutation renders a frame-shift in the DISC1 gene and results in a truncated protein with nine abnormal amino acids in its C-terminus [95,99]. Aside from schizophrenia, several other studies also reported “a modest but significant” association with mental disorders such as autism and major depression, prompting its pathological implications in these disorders [98,100-102]. Taken together, these association studies reinforced the notion that DISC1 is a strong genetic risk factor for mental illnesses, especially for schizophrenia.

This DISC1 gene contains 13 exons and encodes a protein, which is composed of 854 amino acid residues and weighs 100 – 105 kDa [95,103-104]. Several studies reported that the expression of DISC1 is highly localized in the forebrain regions including the hippocampus, cerebral cortex and olfactory bulbs [105-108]. Structurally, the DISC1 protein contains several coiled-coil domains but none of them provides any clear indications of its potential functions [99]. Therefore, researchers have been working to identify its potential interacting partners, which would hopefully help deduce its cellular and physiological functions. With the utilization of the yeast two-hybrid screening studies, Camargo et al. derived a diverse sets of cellular proteins,
which DISC1 could potentially interact with \[^{109}\]. Subsequently, some of these potential DISC1-related protein-interactions were further examined and validated by other research groups \[^{110-112}\]. For example, several studies have reported that DISC1 interacts with either or both nuclear distribution element-like-1 (NDEL1) and elongation protein zeta-1 (FEZ-1) to facilitate neurite outgrowth \[^{105-106}\]. In addition, an interactome study reported that DISC1 associates with a number of proteins which are critical in regulating synaptic structure (e.g. post-synaptic density) and plasticity \[^{109}\]. During neurodevelopment, DISC1 is involved in radial neuronal migration possibly through its interaction with the dynein motor complex (e.g. LIS1, NDEL1 and BBS) \[^{113}\]. Moreover, Mao et al. reported that DISC1 is essential for the proliferation of neural progenitor cells and therefore neurogenesis through its involvement in the Wnt signalling cascade \[^{114}\]. Hippocampal neurogenesis is potentially implicated in the pathology of schizophrenia as a recent report revealed an impairment of adult hippocampal neurogenesis in individuals with schizophrenia \[^{115}\]. Taken together, DISC1 seems to be significantly involved in neurodevelopment, synaptic maturation and hippocampal neurogenesis. Although the exact role of DISC1 in the pathogenesis of schizophrenia and other mental illnesses still remains elusive, it is plausible that the mutations of DISC1 lead to abnormalities in neurodevelopment, rendering the individual to be more susceptible to environmental risk factors for the emergence of the disorders \[^{95}\].
2. Objectives

With the introduction presented above, it should become clear that the dopaminergic system takes on a significant role in modulating mammalian physiological functions. Moreover, the introduction also elucidated how dopamine elicits its actions through its binding to the dopaminergic receptors and how the dopaminergic signaling is terminated (i.e. via DAT). The sole objective of this study was to functionally characterize two D2R-related protein-protein interactions in rodents: a) D2R-DAT interaction and b) D2R-DISC1 interaction.

3. Rationale and Hypothesis

3.1. Part A: D2R-DAT Interaction

The interaction between D2R and DAT was first identified by Lee et al from our lab [67]. Lee et al. showed that the third intracellular loop of D2S interacted with the N-terminus of DAT, forming the D2R-DAT protein complex [67]. The interacting sequence was M1-P26 for DAT and I311-Q344 for D2S. The functional effects of this interaction were further examined in vitro. Through the interaction, D2S upregulated the membrane expression of DAT in an exocytosis-dependent manner. In addition, such up-regulation of DAT elevated the reuptake of dopamine. Based on the interacting region sequence of DAT (M1-V15), Lee et al. also developed an interfering peptide to disrupt the interaction between DAT and D2S [67]. The objective of this part of the study was to examine how this interaction can potentially affects the symptoms of Parkinson’s disease, however, what is Parkinson’s disease?

Parkinson’s disease (PD) is a progressive neurodegenerative movement disorder and the second most common chronic neurodegenerative disorder, affecting 1-2% of the population aged over 50 [30,116-117]. The predominant movement abnormalities observed in patients with PD include bradykinesia, muscular rigidity, postural instability and tremor at rest. According to the literature, the etiology of PD is primarily due to the progressive degeneration of dopaminergic
neurons originating from the SNc\textsuperscript{[2,30,116-117]}. This neuronal degeneration results in an impaired dopaminergic transmission within the nigrostriatal pathway, severely disrupting the motor circuitry. It is noteworthy that the motor abnormalities caused by PD will only appear after 70 – 80\% loss of dopaminergic signalling in the striatum\textsuperscript{[30]}. Due to the pathology of PD, any measures that can increase synaptic dopamine, especially in the nigrostriatal pathway, would in principle be able to normalize the motor defects of PD.

Since the D2R-DAT interaction results in upregulation of DAT at the plasma membrane, it is plausible to believe that the disruption of such interaction will lead to an elevation in synaptic dopamine content. Therefore, this part of the study was intended to validate the following hypothesis \textit{in vivo}. In hypothesis, as the interfering peptide dissembles the interaction, it will impair D2R-mediated upregulation of DAT and as a result the level of DAT at the cell membrane should decrease. Due to the decrease in DAT membrane expression, the uptake of dopamine should be reduced, therefore leaving a higher dopamine content in the synaptic cleft. This increase in synaptic dopamine level will translate into an elevation in voluntary movement level and more importantly it should alleviate motor abnormalities in animals modeling PD.

3.2. Part B – D2R-DISC1 Interaction

Other than the interaction between D2R and DAT on the presynaptic membrane, this study also examined a D2R-related protein-protein interaction on the post-synaptic membrane: D2R-DISC1 interaction. This interaction is essentially involved in the downstream signalling of postsynaptic D2R activations. As aforementioned, upon the binding of agonists, D2R will get activated and exert its physiological actions through the G-protein-dependent cellular signalling pathway\textsuperscript{[1-5]}. Recently, several evidences are prompting the existence of a G-protein-independent signalling pathway downstream of D2R activation. This signalling pathway indeed
is mediated through a cellular mechanism that is originally recognized to be involved only in the
desensitization and internalization of dopaminergic receptors: β-arrestins\(^{[4,26,118-120]}\).

For instance, the knock-out of β-arrestin 1 (one of the β-arrestin isoforms) abolished both
cocaine-induced and apomorphine-induced hyperactivity in mice compared to wild-type\(^{[21]}\). Under normal condition, however, β-arrestin 1 knock-out mice do not exhibit any physiological
or behavioural abnormalities\(^{[121]}\). All these findings together suggest that β-arrestin 1, other than
its role in the desensitization and internalization of the dopaminergic receptors, may also have a
role in signalling. In addition, knock-down of another isoform of β-arrestins (β-arrestin 2) also
resulted in mice that were less responsive to both amphetamine and apomorphine compared to
wild-type\(^{[21,118]}\). More interestingly, Beaulieu et al. confirmed that β-arrestin 2 is involved in the
regulation of the Akt/GSK-3 signaling pathway downstream of D2R activation\(^{[26,118]}\).

The details of this β-arrestin mediated G-protein-independent signalling pathway are as
followed: upon the binding of dopaminergic agonists, D2R will become activated while GRKs
phosphorylate it. The phosphorylation of D2R allows for the recruitment of β-arrestin 2 to the
receptor. The association of β-arrestin 2 will subsequently facilitate and form a protein complex
with PP2A (i.e. a serine/threonine protein phosphatase) and Akt (i.e. a serine/threonine protein
kinase)\(^{[26,118]}\). Through the formation of this multi-protein complex, PP2A will inactivate Akt by
dephosphorylating Akt at its Ser308 residue. Due to the PP2A-induced inactivation of Akt, GSK-
3 (glycogen synthase kinase -3) retains its constitutively active state and which will continue to
elicit dopamine-dependent behaviours.

Interestingly, this G-protein independent signalling pathway seems to be implicated in
Schizophrenia. Foremost, several studies have recently reported an association of Akt1 with
schizophrenia in several independent pools of schizophrenic patients \textsuperscript{[122-124]}. In addition, Emamian \textit{et al.} reported that the expression of Akt is reduced in individuals with schizophrenia compared to unaffected controls \textsuperscript{[125]}. Furthermore, both \textit{in vitro} and \textit{in vivo} studies showed that atypical anti-psychotics can either enhance or mimic Akt-mediated inhibition of GSK3 \textsuperscript{[126-127]}.

Recently, our research group discovered the involvement of the D2R-DISC1 interaction in this G-protein independent signaling pathway. For example, the activation of D2R reduces the phosphorylation of GSK-3α/β (i.e. less inhibition) in the presence of DISC1. Also, the stimulation-triggered internalization of D2R is significantly lowered in the presence of DISC1. Furthermore, agonist-stimulation promotes the formation of D2R/DISC1 protein complex. More importantly, the disruption of the D2R/DISC1 coupling by a TAT-carried interfering peptide (generated based on the D2R/DISC1 interacting region, namely TAT-DISC1 Pep) abolished D2R-mediated reduction of GSK-3alpha/beta phosphorylation.

Despite the controversy of the pathogenesis of schizophrenia, it is generally accepted that hyper-activation of the dopaminergic system is implicated in schizophrenia \textsuperscript{[77,128]}. Based on this concept and our recent discoveries, we therefore hypothesized that the disruption of D2R-DISC1 interaction will normalize the hyperactivation of dopaminergic system and especially its downstream β-arrestins-PP2A-Akt-GSK3 signaling pathway. As a result, the disruption of such protein complex will suffice to exert anti-psychotic actions \textit{in vivo} similar to classical anti-psychotics.
4. Method

4.1. Part A: D2R-DAT Interaction

4.1.1. The Locomotor Effects of D2R-DAT Disruption on Normal Animals

*Animals*

Twenty-one male Sprague-Dawley rats weighing 225-250g upon arrival were obtained from Charles River Laboratories (Montreal, Quebec, Canada). After their arrivals, the animals were double-housed in plastic cages with corn-cob bedding (The Andersons Lab Bedding) in a vivarium maintained on a 12:12 light-dark cycle and at 22-24°C room temperatures. Furthermore, all animals were given free access to food (5001 Rodent Diet from LabDiet) and water. In addition, the animals were given a week to acclimatize to the animal facility before being subject to any surgical and experimental procedures.

After the acclimatization period, each animal was subject to brain cannulation surgery, which a guide cannula was implanted into its third ventricle. On the day of surgery, each rat was weighed and recorded. Subsequently, the animal was placed into an induction chamber filled with 5% isofluorane (Halocarbon Products Corp., North Augusta, USA). After being sufficiently anaesthetized indicated by the loss of pedal reflex, the skull of the animal was shaved using a #40 clipper blade and loose hair was gently removed. The scalp area was then sterilized with betadine surgical scrub (Purdue Pharma, Pickering, Ontario, Canada). Following the anti-septic measures, the animal was placed on a stereotaxic frame with its head positioned on the incisor bar of the stereotaxic frame. After being placed on the stereotaxic frame, ear bars were carefully inserted into the animal’s ear canals and tightened to secure the animal without rupturing the animal’s tympanic membranes. Throughout the entire surgery, the animal was maintained in deep anaesthesia with a dose of 1-3% isofluorane continuously supplied by a vaporizer (Benson Medical Industries Inc. Markham, Ontario, Canada). It was also worth noting that lacrilube was
applied throughout the surgery to keep the animal’s eyes moist. Buprenorphine was injected subcutaneously around the incision site. A 2-cm midline incision was made through the scalp to expose bregma and lambda. Meanwhile, periosteum were scraped away and bulldog-clamps were applied to retract fasica. Subsequently, a guide cannula (8 mm in length under the pedestal; HRS Scientific, Montreal, Quebec, Canada) was accurately positioned to reach the designated coordinates to bregma (AP + 1.0 mm, LM + 1.4mm, DV – 3.6mm). After the cannula reaching its final dorsal/ventral coordinate, three stainless steel screws (HRS Scientific, Montreal, Quebec, Canada) were mounted around the cannula to help secure the subsequent head-cap. At last, both cannula and mounting screws were cemented in place with dental cement, forming the head-cap. It was worth noting that the surface of the head-cap was made smooth and free of rough edges to prevent any irritation. Once the cement hardened, bulldog clamps were removed from the animal and a dummy cannula was inserted into the guide cannula. In addition, nitrofurazone power was applied around the skin to further prevent infections. Sutures were also applied around the head-cap before the animal was removed from the stereotaxic frame.

After the surgery, the animal was transferred to a clean cage and placed under a heat lamp to maintain its body temperature. After re-gaining its full consciousness, the animal was returned to its home cage and singly housed for the rest of the study. Furthermore, a week of post-surgical recovery period was allowed and during which the animals were monitored and handled daily.

Drugs

Nitrofurazone was directly purchased from Canadian Compounding Pharmacy O/B Richard Stein Pharmacy Ltd. (Toronto, Ontario) and was ready to use upon arrival. Buprenorphine hydrochloride (Schering-Plough Ltd, Welwyn Garden City, Hertfordshire, UK)
was prepared to reach a final concentration of 0.05 mg/mL using sterilized saline. Both S1 and S2 peptides were prepared in powder form by GenScript USA Inc. (Piscataway, NJ, USA). The peptides were subsequently dissolved in filter-sterilized saline to reach a final concentration of 10 mM. It was worth noting that saline also served as a vehicle control in this experiment.

**Apparatus**

The dimensions of each open-field chamber (Med Associates Inc., St. Albans, VT, USA) were 43 cm long × 43 cm wide × 30 cm high. The walls of each open-field chamber were made of Plexiglas with a ventilated top-cover. In addition, each chamber was equipped with six 16-beam infrared arrays along its walls, allowing automated measurements of horizontal locomotor activity (Program Activity Monitor version 5.08; Med Associates Inc.).

**Peptide-Induced Locomotor Activity**

After the post-surgical recovery period, the animals were placed in an open-field chamber and allowed to explore freely for 30 minutes daily on three consecutive days, which constituted their baseline locomotor activities. At the end of each session, the chambers were cleaned with diluted clinicline solution (Vétoquinol Canada Inc., Lavaltrie, Quebec, Canada). Subsequently, based on their corresponding baseline activities, the animals were strategically randomized into 3 groups: Saline (vehicle control), TAT-S1 (positive treatment) and TAT-S2 (control peptide). The purpose of such strategic randomization was to ensure a baseline level without any statistically significant differences among all three groups.

On the experiment day, the animals received an intra-cranial injection of their corresponding treatments (saline, S1 or S2) 30 minutes before the open-field test. For the intra-cranial injection to proceed, an injector needle connected to a 5μL-Hamilton syringe via PE20 polyethylene tubing (Becton Dickinson and Company, Canada) was inserted into the guide
cannula on each rat’s head. Then, the injection was manually maintained at 2µL/min for two minutes followed by another minute to allow free diffusion from the injection site. Throughout the intra-cranial injection, the animals were placed in a plastic cage without any physical constraints. Thirty minutes after the injection, the rats were placed into the centre of the open field chambers and their locomotor activities were recorded for 30 minutes in 5 minute-intervals. At the end of the experiment, all animals were euthanized by the method of CO₂/O₂ gas mixture.

Statistical Analysis

All the data were presented as distance travelled (cm) ± standard error of the mean (cm). One-way ANOVA with Games-Howell post-hoc test was employed to analyze both the baseline locomotor activity and peptide-induced activity in this study. Two-way ANOVA with Games-Howell post-hoc analysis was used to examine the effects of peptide on locomotor behaviour across six different time-intervals. In addition, the effects of different treatment groups were further studied at each time point using one-way ANOVA with Games-Howell post-hoc test. All the statistical tests were performed by SPSS Version 16.0 (IBM Software). All the graphs were plotted using Microsoft Excel (Microsoft Software) and GraphPad Prism 5 (GraphPad Software).
4.1.2 The Locomotor Effects of D2R-DAT Disruption on Dopamine-Depleted Animals

4.1.2.1 Trial 1

Animal

Twenty-five male Sprague-Dawley rats weighing 225 – 250g were purchased from Charles River Laboratories (Montreal, Quebec, Canada). Upon arrival, animals were housed in the same facility previously described in Section 4.1.1. After a week of acclimatization to the facility, the animals were subjected to unilateral intracerebroventricular (i.c.v.) cannulation under deep isofluorane anaesthesia. The surgical procedures were detailed in Section 4.1.1. No experiments were conducted on the animals for a week, which they were singly housed and allowed to recover from the surgical procedures. Throughout the recovery period, the animals were handled daily since the degree of handling reduces the irritability of the animals. Following the recovery period, the animals were put in open-field chambers (Med Associates Inc., St. Albans, VT, USA) and given 30 minutes daily to explore on three consecutive days. The measurements were considered their baseline locomotor activities. Based on their baseline levels, the animals were strategically randomized into 3 groups: TAT (control peptide), S1 (treatment peptide) and S2 (control peptide).

Drug

Reserpine was dissolved with a drop of glacial acetic acid and further diluted with saline to reaching a final concentration of 3.5 mg/mL. α-methyl-DL-tyrosine ester hydrochloride (AMPT; Sigma Aldrich, USA) was dissolved in saline with a final concentration of 250 mg/mL. The S1, S2 and TAT peptides were synthesized and prepared in powder form by GenScript USA Inc. (Piscataway, NJ, USA). The peptides were subsequently dissolved in filter-sterilized saline with a final concentration of 1 mM.
Apparatus

Eight open-field chambers (Med Associates Inc., St. Albans, VT, USA) were used in this experiment and the details of the chambers were described in Section 4.1.1.

Locomotor Activity of Dopamine-Depleted Rats

Reserpine (3.5 mg/kg, s.c.) was injected into the animals 20 hours prior to testing. Two hours before the testing, an intra-peritoneal injection of AMPT (250 mg/kg) was given to the animals. Following the AMPT injection, the animals also received an intra-cranial injection of their corresponding treatments (TAT, S1 or S2) 30 minutes before the open-field test. The procedures of intra-cranial injection were as followed: an injector needle connected to a 5µL-Hamilton syringe via PE20 polyethylene tubing (Becton Dickinson and Company, Canada) was inserted into the guide cannula on the animals’ heads. Then, the injection was manually maintained at 2µL/min for two minutes followed by another minute to allow free diffusion from the injection site. Throughout the intra-cranial injection, the animals were placed in a plastic cage without any physical constraints. Half an hour after the injection, the rats were put into the centre of the open field chambers and their locomotor activities were recorded for 30 minutes. At the end of the experiment, all animals were euthanized by the method of CO₂/O₂ gas mixture.

Statistical Analysis

All the data were presented as distance travelled (cm) ± standard error of the mean (cm). One-way ANOVA with Games-Howell post-hoc test was employed to analyze both the baseline and post-treatment activities in this study. All the statistical tests were performed by SPSS Version 16.0 (IBM Software). All the graphs were plotted using Microsoft Excel (Microsoft Software).
4.1.2.2. Model Verification

Animals

A total of 11 male Sprague-Dawley rats with body weight of 225g – 250g were attained from Charles River Laboratories (Montreal, Quebec, Canada). Upon arrival, animals were housed in the same facility previously described in Section 4.1.1. The animals were allowed to acclimatize to the facility for a week without being subjected to any experimentation. During the time, the animals were handled daily since the degree of handling reduces the irritability of the animals. One week later, the animals were placed in an open-field chamber (Med Associates Inc., St. Albans, VT, USA) and allowed to explore freely for 30 minutes daily on three consecutive days, which constituted their baseline locomotor activities. Subsequently, based on their corresponding baseline activities, the animals were strategically randomized into 2 groups (5 – 6 animals per group): Sham or AMPT (dopamine depletion).

Drugs

α-methyl-DL-tyrosine ester hydrochloride (AMPT; Sigma Aldrich, USA) was dissolved in filter-sterilized saline with a final concentration of 5 mg/mL. The AMPT solution was made freshly for each experiment.

Apparatus

Eight open-field chambers (Med Associates Inc., St. Albans, VT, USA) were used in this experiment and the details of the chambers were described in Section 4.1.1.

Locomotor Activity of Dopamine-Depleted Animals

On the day of experiment, the animals from the AMPT group received two intra-peritoneal injections of AMPT (25 mg/kg each), separated by two hours. This protocol was adapted and modified from McDougall et al [131]. AMPT was dissolved in saline with a final
concentration of 5 mg/mL. On the other hand, a needle was inserted into the abdomens of the animals from the sham group as their “sham” injections. Immediately after the second intraperitoneal injection, the animals were transported to the testing room and given ten minutes to acclimatize the environment. After the acclimatization, the animals were placed into the open-field chambers to record their locomotor activities for 30 minutes. At the end of the experiment, all animals were euthanized by the method of CO$_2$/O$_2$ gas mixture.

**Statistical Analysis**

All the data were presented as distance travelled (cm) ± standard error of the mean (cm). Two-tailed t-test statistical analysis was used to compare both baseline and post-treatment locomotor activities between the sham group and the AMPT group. In addition, paired t-test (two-tailed) was also included to analyze the difference in locomotor behaviours between baseline and post-treatment within the same treatment group. Microsoft Excel (Microsoft Software) was employed to perform all statistical analyses as well as to plot all the graphs presented in this experiment of the thesis.
4.1.2.3. Trial 2

Animals

A sample size of 25 male Sprague-Dawley rats with body weight of 225-250g was purchased from Charles River Laboratories (Montreal, Quebec, Canada). Upon arrival, animals were housed in the same facility previously described in Section 4.1.1. After a week of acclimatization to the facility, the animals were subjected to unilateral intracerebroventricular (i.c.v.) cannulation under deep isofluorane anaesthesia. The surgical procedures were detailed in Section 4.1.1. Following the surgery, the animals were given a week to recover from the surgical procedures. During it, the animals were handled daily by the experimenter in an attempt to reduce the irritability of the animals.

Immediately after the recovery period, the animals were subjected to a daily 30-minute period of locomotor activity measurement for three consecutive days and such measurements were used to constitute their baseline levels. Based on their baseline levels, animals were subsequently strategically divided into three treatment groups (7 – 10 animals per group): saline (vehicle control), S1 peptide (treatment peptide) and S2 peptide (control peptide).

Drugs

α-methyl-DL-tyrosine ester hydrochloride (AMPT; Sigma Aldrich, USA) was dissolved in filter-sterilized saline with a final concentration of 5 mg/mL. The AMPT solution was made freshly for each experiment. The S1, S2 and TAT peptides were synthesized and prepared in powder form by GenScript USA Inc. (Piscataway, NJ, USA). The peptides were subsequently dissolved in filter-sterilized saline with a final concentration of 1 mM.
**Apparatus**

Eight open-field chambers (Med Associates Inc., St. Albans, VT, USA) were used in this experiment and the details of the chambers were described in Section 4.1.1.

**Locomotor Activity of Dopamine-Depleted Animals**

On the day of experiment, the animals from all three groups received two intra-peritoneal injections of AMPT, separated by two hours. Thirty minutes after the second intra-peritoneal injection, the animals were given an intra-cranial injection of saline or S1 or S2. The injection was manually maintained at 2µL/min for two minutes followed by another minute to allow free diffusion from the injection site. Throughout the intra-cranial injection, the animals were placed in a plastic cage without any physical constraints. After the intra-cranial injection, the animals were transported to the testing room and given ten minutes to acclimatize the environment. After the acclimatization, the animals were placed into the open-field chambers to record their locomotor activities for a period of 30 minutes. At the end of the experiment, all animals were euthanized by the method of CO$_2$/O$_2$ gas mixture.

**Statistical Analysis**

All the data were presented as distance travelled (cm) ± standard error of the mean (cm). One-way ANOVA with Games-Howell post-hoc test was employed to analyze both the baseline and post-treatment activities among three treatment groups. Paired t-test (two-tailed) was also included to analyze the difference in locomotor behaviours between baseline and post-treatment within the same treatment group. All the statistical tests were performed by SPSS Version 16.0 (IBM Software) and Microsoft Excel (Microsoft Software). In addition, Microsoft Excel was also used to plot the graph presented in this part of the thesis.
4.1.2.4. Trial 3 – AMPT-Induced Dopamine Depletion (40nmol peptide)

Animal

A total of 28 male Sprague-Dawley rats weighing 225-250g upon arrival were procured from Charles River Laboratories (Montreal, Quebec, Canada). Upon arrival, animals were housed in the same facility previously described in Section 4.1.1. After a week of acclimatization to the facility, the animals were subjected to unilateral intracerebroventricular (i.c.v.) cannulation under deep isofluorane anaesthesia. The surgical procedures were detailed in Section 4.1.1. Following the surgery, the animals were given a week to recover from the surgical procedures. Throughout the recovery period, the animals were handled daily since the degree of handling reduces the irritability of the animals. After the post-surgical recovery period, the animals were placed in an open-field chamber (Med Associates Inc., St. Albans, VT, USA) and allowed to explore freely for 30 minutes daily on three consecutive days, which constituted their baseline locomotor activities. Subsequently, based on their corresponding baseline activities, the animals were strategically randomized into 4 groups: Saline (vehicle control), TAT-S1 (positive treatment), TAT-S2 and TAT alone (control peptides). The purpose of such strategic randomization was to ensure a baseline level without any statistically significant differences among all four groups.

Drugs

Nitrofurazone was directly purchased from Canadian Compounding Pharmacy O/B Richard Stein Pharmacy Ltd. (Toronto, Ontario) and was ready to use upon arrival. Buprenorphine hydrochloride (Schering-Plough Ltd, Welwyn Garden City, Hertfordshire, UK) was diluted with filter-sterilized saline to reach a final concentration of 0.05 mg/mL. α-methyl-DL-tyrosine ester hydrochloride (AMPT; Sigma Aldrich, USA) was dissolved in filter-sterilized saline with a final concentration of 5 mg/mL. The AMPT solution was made freshly for each
experiment. On the other hand, the S1, S2 and TAT peptides were synthesized and prepared in powder form by GenScript USA Inc. (Piscataway, NJ, USA). The peptides were subsequently dissolved in filter-sterilized saline with a final concentration of 10 mM. It was worth noting that saline served as a vehicle control in this experiment.

**Apparatus**

Eight open-field chambers (Med Associates Inc., St. Albans, VT, USA) were used in this experiment and the details of the chambers were described in Section 4.1.1.

**Locomotor Activity of Dopamine-Depleted Animals**

On the day of experiment, the animals from all three groups received two intra-peritoneal injections of AMPT, separated by two hours. AMPT was dissolved in saline with a final concentration of 5 mg/mL. Immediately after the second intra-peritoneal injection, the animals were transported to the testing room and given ten minutes to acclimatize the environment. After the acclimatization, the animals were placed into the open-field chambers to measure their locomotor activities for a period of 15 minutes. After the fifteen-minute recording, the animals were removed from the open-field chambers and given an intra-cranial injection of saline or S1 or S2 or TAT. The injection was manually maintained at 2µL/min for two minutes followed by another minute to allow free diffusion from the injection site. Throughout the intra-cranial injection, the animals were placed in a plastic cage without any physical constraints. The animals were immediately returned to the open-field chambers after the i.c.v. injection and their locomotor activities were recorded for another hour. At the end of the experiment, all animals were euthanized by the method of CO₂/O₂ gas mixture.
Statistical Analysis

All the data were presented as distance travelled (cm) ± standard error of the mean (cm). One-way ANOVA with Games-Howell post-hoc test was employed to analyze baseline, AMPT-impaired and peptide-induced locomotor activities in this study. Two-way ANOVA with Games-Howell post-hoc analysis was used to examine the effects of peptide on locomotor behaviour across 15 five-minute intervals. In addition, the effects of different treatment groups were further studied at each time point using one-way ANOVA with Games-Howell post-hoc test. All the statistical tests were performed by SPSS Version 16.0 (IBM Software). All the graphs were plotted using GraphPad Prism 5 (GraphPad Software).
4.2 Part B: D2R-DISC1 Interaction

4.2.1 The Effects of D2R-DISC1 Disruption on Apomorphine-Induced PPI Deficits

**Animal**

A total of 80 male Wistar rats (Charles River Laboratories, Montreal, Quebec, Canada), weighing 250 – 275g upon arrival, were included in this experiment. Upon arrival, animals were housed in the same facility and condition as previously described in Section 4.1.1. After a week of acclimatization to the facility, the animals were subjected to unilateral intracerebroventricular (i.c.v.) cannulation under deep isofluorane anaesthesia. The surgical procedures were detailed in Section 4.1.1. Following the surgery, the animals were given a week to recover from the surgical procedures. Throughout the recovery period, the animals were handled daily since the degree of handling reduces the irritability of the animals.

**Drug**

(R)-(−)-Apomorphine hydrochloride (Tocris Bioscience) was dissolved in saline containing 0.1% L-Ascorbic Acid (Fisher Scientific) with a final concentration of 0.3 mg/mL. Haloperidol (Toronto Research Chemical Inc., North York, Ontario, Canada) was first dissolved in acetic acid and was further diluted 100 times with filter-sterilized saline to reach a final concentration of 0.1 mg/mL. It was worth noting that both apomorphine and haloperidol solutions were freshly prepared on the day of experiment. Both the D2R-DISC1 peptide and scramble peptides were synthesized and prepared in powder form GenScript USA Inc. (Piscataway, NJ, USA). The peptides were subsequently dissolved in filter-sterilized saline to reach a final concentration of 10 mM. It was worth noting that saline also served as a vehicle control in this experiment.
Apparatus

The startle chambers (SR Lab, San Diego Instruments, San Diego, CA) were used to measure the startle responses of the animals. Each chamber contained a ventilated Plexiglas cylinder (8.2 cm in diameter and 20 cm long) mounted on a platform. The platform was positioned upon a piezoelectric accelerometer unit, which detected and transduced the motion within the Plexiglas cylinder. The startle response was subsequently recorded by a computer interfaced with all the startle chambers. In each chamber, a speaker was mounted approximately 24 cm above the cylinder for providing the auditory stimuli. It was worth noting that the response sensitivities among all chambers were calibrated using the SR-Lab Startle Calibration System before the experiment.

Pre-pulse Inhibition (PPI)

After the post-surgical recovery period, the animals were randomly divided into eight groups with different combinations of treatments (i.e. haloperidol, saline, scramble peptide and D2R-DISC1 peptide) and disruptive treatment (i.e. saline and apomorphine). On the experiment day, animals were administered with one of the treatments: saline (i.c.v.), scramble peptide (i.c.v.), D2R-DISC1 peptide (i.c.v.) and haloperidol (0.1 mg/kg, s.c.). It is worth noting that all i.c.v. injections were delivered at a flow rate of 2μL/min for 2 minutes followed by another minute to allow free diffusion. Throughout the i.c.v. injection, the animals were placed in a plastic cage without any physical constraints. Twenty-five minutes after the treatment administration, all animals received a subcutaneous injection of either saline (1mL/kg) or apomorphine (0.3mg/kg). Immediately after the subcutaneous injections, the rats were placed into the startle apparatus for a 10-minute acclimatization period. Throughout the acclimatization period, a 65-dB background of white noise was present. After the acclimatization period, animals
were presented with a series of five startle pulse (110 db) alone trials. This series of stimuli was followed by 64 randomized trials consisting of no pulse (0 dB, no additional stimuli other than the background noise were presented), a startle pulse (110 db, 40 milliseconds) or three prepulse intensities (70, 75, and 80 db, 20 milliseconds) presented alone or 100 milliseconds preceding a startle pulse. Finally, another series of five startle pulse alone trials were presented. The time between trials ranged from 10 to 20 seconds. The startle response was measured every 1 ms for a 100-ms period from the onset of the startle stimulus. At the end of the experiment, all animals were euthanized by the method of CO₂/O₂ gas mixture.

Statistical Analysis

All the data were presented as prepulse inhibition on startle response (%) ± the standard error of mean (%). The percent prepulse inhibition (PPI) was calculated by the following formula: 

\[ \% \text{ PPI} = 100 - \frac{[\text{P} + \text{S}]}{\text{S}} \times 100 \]

where (P+S) is the mean response amplitude for prepulse plus startle pulse trials and S is the mean response amplitude for the startle pulse alone trials. The experimental data were analyzed by three-way ANOVA with repeated measures, followed by a post-hoc analysis of Turkey’s test. Subsequently, one-way ANOVA analyses were used to examine the differences in PPI at each prepulse level among specific treatment groups. All the statistical tests were performed by SPSS Version 16.0 (IBM Software) and the bar graph was plotted using Microsoft Excel (Microsoft Software).
4.2.2 The Effects of D2R-DISC1 Disruption on Amphetamine-Induced Hyperactivity

**Animals**

A total of 26 male Wistar rats (Charles River Laboratories, Montreal, Quebec, Canada), weighing 250 – 275g upon arrival, were tested in this experiment. After arriving at the animal facility, animals were housed in the same vivarium and condition as previously described in Section 4.1.1. After a week of acclimatization to the facility, the animals were subjected to unilateral intracerebroventricular (i.c.v.) cannulation under deep isofluorane anaesthesia. The surgical procedures were detailed in Section 4.1.1. Following the surgery, the animals were given a week to recover from the surgical procedures. Throughout the recovery period, the animals were handled daily to reduce the irritability of the animals. At the end of the post-surgical recovery period, the baseline locomotor activities of the animals were measured on three consecutive days. Throughout those three days, the animals were placed in open-field boxes and given 30 minutes daily to explore the chambers. Based on their baseline locomotor levels, they were divided into 4 groups (6 – 7 rats per group): saline (vehicle control), scramble peptide (control peptide), D2R-DISC1 peptide (treatment peptide) and haloperidol (positive control).

**Drug**

D-Amphetamine sulphate (a generous gift from Dr. P.J. Fletcher) was dissolved in saline with a final concentration of 3 mg/mL. Haloperidol (Toronto Research Chemical Inc., North York, Ontario, Canada) was first dissolved in acetic acid and was further diluted 100 times with filter-sterilized saline into a final concentration of 0.1 mg/mL. It was worth noting that both apomorphine and haloperidol solutions were freshly prepared on the day of experiment. Both the D2R-DISC1 peptide and scramble peptide were synthesized and prepared in powder form by GenScript USA Inc. (Piscataway, NJ, USA). Both peptides were then dissolved in filter-sterilized saline with a final concentration of 10 mM.
**Apparatus**

Eight open-field chambers (Med Associates Inc., St. Albans, VT, USA) were used in this experiment and the details of the chambers were outlined in Section 4.1.1.

**Amphetamine-Induced Hyperactivity**

On the experiment day, animals were injected with one of the treatments: saline (i.c.v), scramble peptide (i.c.v), D2R-DISC1 peptide (i.c.v) and haloperidol (s.c.). It is worth noting that all intra-cranial injections were delivered at a flow rate of 2μL/min for 2 minutes followed by another minute to allow free diffusion away from the injection site. Throughout the i.c.v. injection, the animals were placed in a plastic cage without any physical constraints. Twenty minutes after the treatment administration, all animals received a subcutaneous injection of amphetamine at the dose of 3 mg/kg. Ten minutes later, animals were transferred into open-field chambers and had their respective locomotor activity recorded for one hour in 5-minute intervals. At the end of the experiment, all animals were euthanized by the method of CO₂/O₂ gas mixture.

**Statistical Analysis**

All the data will be presented as distance travelled (cm) ± standard error of mean (cm). One-way ANOVA with Games-Howell post-hoc test was employed to compare the baseline locomotor activities of treatments and their corresponding effects on amphetamine-induced hyperactivity in the study. Two-way ANOVA with Games-Howell post-hoc analysis was used to examine the effects of treatments on locomotor behaviour across six different time-intervals. In addition, the effects of different treatment groups were further studied at each time point with one-way ANOVA with Games-Howell post-hoc test. All the statistical tests were performed by SPSS Version 16.0 (IBM Software). All the graphs were plotted using GraphPad Prism 5 (GraphPad Software).
5 Result

5.1 Part A: D2R-DAT Interaction

5.1.1 The Locomotor Effects of Disruption in Normal Animals

One-way ANOVA analysis indicated that there were no significant differences in baseline locomotor activities among the saline group, S1 group and S2 group (F_{2,18} = 0.282; p = 0.757; n = 6 – 8 for each group). The saline group exhibited a baseline locomotor level of 3410.01 cm ± 610.83 cm, while it was 3638.33 cm ± 545.10 cm and 3131.69 cm ± 255.23 cm for S1 and S2 group, respectively (Figure 9).

After the treatment administration, the S1 group on average travelled a total distance of 23200.8 cm ± 3320.5 cm. Meanwhile, the saline group and S2 group exhibited a total locomotor activity of 3921.62 cm ± 884.23 cm and 2646.0 ± 494.3 cm, respectively (Figure 9). One-way ANOVA analysis showed that there was a significant treatment effect on the total locomotor activity (F_{5,36} = 26.693, p < 0.001). In addition, Games-Howell post-hoc analysis illustrated that the S1 peptide significantly promoted locomotor behaviours in rats from its baseline level (p = 0.005). Furthermore, the effects of S1 peptide were significantly different from the effects of saline (p < 0.005) and S2 peptide (p = 0.005). Meanwhile, the effects of saline and S2 peptide on locomotor behaviours were statistically similar (p > 0.05).

The data were further broken down into interval locomotor activity at six different time point as shown in Figure 10. Two-way ANOVA with repeated measures analysis revealed a significant time (F_{5,96} = 7.291, p < 0.01) and treatment (F_{2,96} = 26.827, p < 0.001) effect on the interval locomotor activity. However, no significant effects of time×treatment interaction (F_{10,96} = 0.291, p = 0.891) effect were observed. Games-Howell post-hoc suggested that the S1 peptide had significant effects on interval locomotor activities among all three treatment groups (p <
Furthermore, one-way ANOVA analysis Games-Howell post-hoc analysis confirmed that the S1 peptide significantly elevated voluntary movement in rats at all six time points compared to both saline and the S2 peptide. \((p < 0.01; \text{Figure 10})\).

**Figure 9 - The Effects of D2R-DAT Protein Complex Disruption on Locomotor Behaviours in Rats.**

The experiment contained a sample size of 21 Sprague Dawley rats, 6 – 7 animals per group. This diagram displayed the baseline and treatment-induced locomotor activity for all three groups. The saline group exhibited a baseline locomotor level of 3410.01 cm ± 610.83 cm, while it was 3638.33 cm ± 545.10 cm and 3131.69 cm ± 255.23 cm for S1 and S2 groups, respectively. One-way ANOVA analysis reported no significant differences in baseline locomotor activities among these three groups. The administration of S1 peptide significantly triggered a hyperactivity of 23200.8 cm ± 3320.5 cm. Such stimulant effects were not observed in both saline and S2 groups, as they only displayed a locomotor activity of 3921.62 cm ± 884.23 cm and 2646.0 ± 494.3 cm, respectively. One-way ANOVA with Games-Howell post hoc analysis indicated that the effects of S1 peptide were statistically significant from both saline \((p < 0.005)\) and S2 peptide \((p = 0.005)\). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = \(p < 0.05\), ** = \(p < 0.01\) and *** = \(p < 0.001\)
Figure 10 – The Locomotor Effects of D2R-DAT Protein Complex Disruption at Each Time Interval
The experiment contained a sample size of 21 Sprague Dawley rats, 6 – 7 animals per group. This figure displayed the locomotor activity of each treatment group across six 5-minute intervals. Two-way ANOVA with repeated measures analysis revealed a significant time (F_{5, 96} = 7.291, p < 0.01) and treatment (F_{2, 96} = 26.827, p < 0.001) effect on the interval locomotor activity. However, no significant effects of time×treatment interaction (F_{10, 96} = 0.291, p = 0.891). Games-Howell post hoc test reported that the S1 peptide resulted in a significantly higher locomotor activity among all six time points compared to saline (p < 0.001). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = p < 0.05, ** = p < 0.01 and *** = p < 0.001
5.1.2. The Locomotor Effects of D2R-DAT in Dopamine-Depleted Animals

5.1.2.1. Trial 1

Before dopamine depletion, the TAT group exhibited a baseline locomotor level of 5631.32 cm ± 576.46 cm (Figure 11). Meanwhile, the S1 group and S2 group travelled 5799.15 cm ± 619.10 cm and 6441.37 cm ± 732.96 cm, respectively. One-way ANOVA indicated that there were no statistically significant differences in baseline movement among all three groups (F2,22 = 0.440, p = 0.65).

Both reserpine (3.5 mg/kg, s.c.) and AMPT (250 mg/kg, i.p.) were used to deplete dopamine in the animals. After the induction of dopamine-depletion and the administration of the corresponding treatments, the locomotor activity for the TAT group was reduced to 41.71 cm ± 21.76 cm (Figure 12). Meanwhile, the S1 and S2 groups exhibited a locomotor activity of 75.89 cm ± 53.94 cm and 27.72 cm ± 10.03 cm, respectively. One-way ANOVA reported no significant difference in post-treatment locomotor activity (F2,22 = 0.572, p = 0.572). Additionally, one-way ANOVA analysis Games-Howell post-hoc analysis confirmed that the effectiveness of the dopamine-depletion paradigm by comparing the locomotor activities between baseline and post-treatment (F5,44 = 49.331, p < 0.001).
Figure 11 – The Baseline Locomotor Activity among Three Treatment Groups over a Period of 30 Minutes.

The experiment contained a sample size of 25 Sprague Dawley rats, 8 – 9 animals per group. The baseline locomotor activities for all three groups were measured prior to any peptide injections. The TAT group displayed a baseline locomotor level of 5631.32 cm ± 576.46 cm, while it was 5799.15 cm ± 619.10 cm and 6441.37 cm ± 732.96 cm for S1 and S2 groups, respectively. One-way ANOVA analysis reported no significant differences in baseline locomotor activities among these three groups (F$_{2, 22}$ = 0.440, p = 0.65). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM).
The experiment contained a sample size of 25 Sprague Dawley rats, 8 – 9 animals per group. Upon the induction of dopamine depletion by both reserpine and AMPT, the corresponding peptide treatment was also delivered to each group. The right panel displayed the post-treatment locomotor activity of each group. For example, the TAT group exhibited a horizontal movement level of 41.71 cm ± 21.76 cm. In the meantime, the S1 and S2 groups exhibited a locomotor activity of 75.89 cm ± 53.94 cm and 27.72 cm ± 10.03 cm, respectively. One-way ANOVA did not detect any statistical significances in post-treatment locomotor activity ($F_{2,22} = 0.572, p = 0.572$). It is worth noting that one-way ANOVA analysis Games-Howell post-hoc analysis confirmed that the effectiveness of the dopamine-depletion paradigm by comparing the locomotor activities between baseline and post-treatment ($F_{5, 44} = 49.331, p < 0.001$). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM).
5.1.2.2. Model Verification

After the habituation period, the sham group showed a baseline locomotor activity of 5061.35 cm ± 340.80 cm (Figure 13). Meanwhile, the “designated” group for dopamine depletion travelled on average 5733.05 cm ± 382.94 cm over the three days. T-test did not detect any statistical differences in their baseline levels (two-tailed, \( p = 0.221 \)).

The purpose of this experiment was to verify whether AMPT at the dose of 25 mg/kg was sufficient to induce significant locomotor impairment in animals (Figure 13). It is worth noting that the sham group did not receive any AMPT injections and after the “sham” injection, the sham group displayed a movement level of 4822.62 cm ± 670.71 cm. Paired t-test revealed that the post-treatment locomotor activity of the sham group was statistically similar to its baseline (two-tailed, \( p = 0.636 \)). Meanwhile, the locomotor behaviours of the AMPT group were reduced to 2679.09 cm ± 460.32 cm and paired t-test reported a significant difference compared to its baseline (two-tailed, \( p < 0.001 \)). In addition, the post-treatment locomotor activity of the AMPT group was also significantly lower than the sham group’s (two tailed t-test, \( p < 0.05 \)).
Figure 13 – The Verification of Dopamine-Depletion Model Using AMPT. The study only contained 11 male Sprague-Dawley rats, 5–6 animals per group. The blue bars illustrated the baseline movement level for each group, which was 5061.35 cm ± 340.80 cm and 5733.05 cm ± 382.94 cm for the sham and AMPT group, respectively. As expected, t-test analysis did not detect any statistical significance in their baseline levels (two-tailed, p = 0.221). After the measurement of their baseline levels, the AMPT group was given two i.p. injections of AMPT (25 mg/kg each) while the sham group only received two sham injections. The post-treatment activity for each group was represented by the purple bars and which was 4822.62 cm ± 670.71 cm for the sham group and 2679.09 cm ± 460.32 cm for the AMPT group. Not only was the post-treatment activity in the AMPT group was significantly lower than the sham group’s (two tailed t-test, p < 0.05), but paired t-test also indicated a significant difference compared to its own baseline (two-tailed, p < 0.001). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.
5.1.2.3. Trial 2

Throughout the three days of habituation, all three groups exhibited a statistically similar level of locomotor behaviours (One-way ANOVA, $F_{2, 22} = 0.634, p = 0.540$). For example, the saline group on average travelled $5215.23 \text{ cm} \pm 344.39 \text{ cm}$. In the meantime, the horizontal activities for the S1 and S2 groups were $4865.35 \text{ cm} \pm 490.34 \text{ cm}$ and $5549.62 \text{ cm} \pm 418.05 \text{ cm}$, respectively. The baseline locomotor activities for all three groups were illustrated in Figure 14.

After the administration of AMPT, the animals were also given their designated treatments (i.e. saline, S1 or S2). After the delivery of AMPT and treatment, the average locomotor activity of animals in the saline group was $1864.42 \text{ cm} \pm 382.84 \text{ cm}$ (Figure 14). The post-treatment movement levels for the S1 and S2 groups were $2989.18 \text{ cm} \pm 603.39 \text{ cm}$ and $2152.44 \text{ cm} \pm 271.85 \text{ cm}$, respectively. Paired t-test indicated that the post-treatment locomotor activity for each group was significantly lower than their respective baseline level (two-tailed, $p < 0.001$). In addition, one-way ANOVA indicated that the S1 treatment peptide did not exert any significant effects ($F_{2, 22} = 1.525, p = 0.240$) on normalizing the motor deficits induced by AMPT compared to the control groups (i.e. saline and S2).
Figure 14 – The Effects of Peptide Treatments on Locomotor Activity in Dopamine-Depleted Rats. This study was composed of 25 male Sprague-Dawley rats, 7 – 10 animals per group. The blue bars represented the baseline movement level for each group, which was 5215.23 cm ± 344.39 cm, 2989.18 cm ± 603.39 cm and 5549.62 cm ± 418.05 cm for the saline, S1 and S2 groups, respectively. One-way ANOVA analysis reported no significant differences in baseline locomotor activities among these three groups (F\(_{2, 22}\) = 0.634, \(p = 0.540\)). After the collection of their baseline data, the animals were injected with AMPT (i.p.) to induce dopamine-depletion as well as their corresponding treatment (i.c.v.). The post-treatment locomotor activity of each group was subsequently recorded and illustrated by the purple bars. The saline group exhibited a post-treatment locomotor activity of 1864.42 cm ± 382.84 cm, while the post-treatment locomotor activities for the S1 and S2 groups were 2989.18 cm ± 603.39 cm and 2152.44 cm ± 271.85 cm, respectively. Paired t-test further verified the locomotor behaviours were significantly impaired for all three groups compared to their respective basal level (two-tailed, \(p < 0.001\)). One-way ANOVA reported no significant treatment effects (F\(_{2, 22}\) = 1.525, \(p = 0.240\)) on the locomotor behaviours in those dopamine-depleted animals. All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = \(p < 0.05\), ** = \(p < 0.01\) and *** = \(p < 0.001\)
5.1.2.4. Trial 3

One-way ANOVA showed that there were no significant differences in basal locomotor activity among all four groups ($F_{3,23} = 0.129; p = 0.942; n = 5 – 8$ per group). The baseline locomotor activity for the saline group, S1 group, S2 group and TAT group was $6055.61 \text{ cm} \pm 449.23 \text{ cm}$, $5545.35 \text{ cm} \pm 447.50 \text{ cm}$, $5905.49 \text{ cm} \pm 524.98 \text{ cm}$ and $5568.65 \text{ cm} \pm 632.70 \text{ cm}$, respectively (Figure 15).

After the administration of AMPT, the saline group on average travelled $2005.32 \text{ cm} \pm 295.81 \text{ cm}$ over a period of 15 minutes. Meanwhile, the S1 group, S2 group and TAT group travelled $2552.42 \text{ cm} \pm 433.99 \text{ cm}$, $2388.35 \text{ cm} \pm 245.68 \text{ cm}$ and $2347.44 \text{ cm} \pm 238.58 \text{ cm}$, respectively. One-way ANOVA indicated that there were no significant differences in the level of locomotor activity among all four groups after the administration of AMPT ($F_{3,23} = 0.596, p = 0.624$, Figure 16). In addition, two-way ANOVA with repeated measures was employed to examine whether the treatment groups had any significant effects at different time points across the fifteen-minute period. In fact, the statistical analysis reported no significant treatment effect ($F_{3,51} = 0.596, p = 0.624$) and timextreatment interaction effect ($F_{6,51} = 0.879, p = 0.505$) on locomotor activity at three different five-minute intervals (Figure 18). However, time had statistically significant effects on locomotor activity across those three time-points ($F_{2,51} = 150.39, p < 0.01$).

Following a fifteen-minute period of recording, the animals were subjected to their corresponding treatment and were subsequently placed in the open-field chamber to measure their locomotor behaviours for another hour. The saline group and TAT group exhibited a locomotor activity of $1891.96 \text{ cm} \pm 883.56 \text{ cm}$ and $1781.89 \text{ cm} \pm 221.77 \text{ cm}$, respectively (Figure 17). Meanwhile, the S1 group and S2 group travelled $24644.66 \text{ cm} \pm 3925.97 \text{ cm}$ and
13268.66 cm ± 3192.51 cm, respectively. One-way ANOVA revealed that there was a significant treatment effect on the locomotor activity of the dopamine-depleted rats ($F_{3, 23} = 15.471, p < 0.001$). In addition, Bonferroni post-hoc analysis showed that the S1 peptide significantly enhanced locomotor activity in the dopamine-depleted rats compared to the other three groups ($p < 0.001$ compared to saline and TAT; $p < 0.05$ compared to S2). Meanwhile, there was a trend that the effects of S2 peptide were different from TAT peptide ($p = 0.053$) and saline ($p = 0.065$), however, it did not reach statistical significance ($p > 0.05$). On the other hand, the effects of TAT peptide was indeed statistically identical to saline ($p = 1.0$).

The locomotor activity across twelve time intervals was also examined and illustrated in Figure 18. Two-way ANOVA with repeated measures reported significant time ($F_{11, 281} = 3.707, p < 0.05$), treatment ($F_{3, 281} = 15.831, p < 0.001$) and time×treatment interaction ($F_{33, 281} = 4.184, p < 0.001$) effects on locomotor activity across all twelve time-points. Games-Howell post-hoc analysis further showed that both S1 and S2 had significant effects on interval locomotor activity compared to saline. Meanwhile, the effects of TAT were found to be statistically insignificant. Additional one-way ANOVA reported that the effects of S1 peptide were significant from 25 minutes to 50 minutes ($p < 0.05$ or 0.01). Meanwhile, the effects of S2 peptide were different from saline at five different time point, which were 5-minute, 10-minute, 30-minute, 45-minute and 50-minute ($p < 0.05$ or 0.01; Figure 18). It was worth noting that the effects of TAT peptide were statistically similar to the effects of saline at all twelve time points ($p > 0.05$).
Figure 15 – The Baseline Horizontal Locomotor Activity of Normal Rats among Four Treatment Groups over a Period of 30 Minutes. The study included a sample size of 28 male Sprague Dawley rats, 5-8 animals per group. The baseline locomotor activity for the saline group, S1 group, S2 group and TAT group was 6055.61 cm ± 449.23 cm, 5545.35 cm ± 447.50 cm, 5905.49 cm ± 524.98 cm and 5568.65 cm ± 632.70 cm, respectively. One-way ANOVA reported no significant differences in basal locomotor activity among all four treatment groups (F\(_{3, 23}\) = 0.129; \(p = 0.942\)). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = \(p < 0.05\), ** = \(p < 0.01\) and *** = \(p < 0.001\).

Figure 16 – The Baseline Horizontal Locomotor Activity of AMPT-Treated (Dopamine-Depleted) Animals among Four Treatment Groups over a Period of 15 Minutes. After the administration of AMPT, the saline and TAT groups travelled on average 2005.32 cm ± 295.81 cm and 2347.44 cm ± 238.58 cm over a period of 15 minutes. On the other hand, the S1 and S2 groups exhibited a locomotor activity of 2552.42 cm ± 433.99 cm and 2388.35 cm ± 245.68 cm, respectively. One-way ANOVA indicated that there were no significant differences among all four groups (F\(_{3, 23}\) = 0.596, \(p = 0.624\)). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = \(p < 0.05\), ** = \(p < 0.01\) and *** = \(p < 0.001\).
Figure 17 – Treatment-Induced Locomotor Activity of AMPT-Treated (Dopamine-Depleted) Rats over a Period of 60 Minutes. After the impairment of locomotor behaviours by AMPT, the saline group and TAT group exhibited a locomotor activity of 1891.96 cm ± 883.56 cm and 1781.89 cm ± 221.77 cm, respectively. On the other hand, the S1 group and S2 group travelled 24644.66 cm ± 3925.97 cm and 13268.66 cm ± 3192.51 cm, respectively. One-way ANOVA revealed that there was a significant treatment effect on the locomotor activity of the dopamine-depleted rats (F₃, 2₃ = 15.471, p < 0.001). In addition, Bonferroni post-hoc analysis showed that the S1 peptide significantly enhanced locomotor activity in the dopamine-depleted rats compared to the other three groups (p < 0.001 compared to saline and TAT; p < 0.05 compared to S2). Meanwhile, the right panel indicated a trend that the effects of S2 peptide were different from TAT peptide (p = 0.053) and saline (p = 0.065), however, it did not reach statistical significance (p > 0.05). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = p < 0.05, ** = p < 0.01 and *** = p < 0.001.
**Figure 18 – The Locomotor Activity of Dopamine-Depleted Animals Before and After Treatments across 75 minutes.** The graph shown here was composed of experimental data combined from Figure 16 and Figure 17. As aforementioned and shown here, there were no significant differences among treatment groups in post-AMPT baseline level. After the intra-cranial delivery of corresponding treatment, two-way ANOVA with repeated measures reported significant time ($F_{11, 281} = 3.707, p < 0.05$), treatment ($F_{3, 281} = 15.831, p < 0.001$) and time×treatment interaction ($F_{33, 281} = 4.184, p < 0.001$) effects on locomotor activity across all twelve time-points. Games-Howell post-hoc analysis further showed that both S1 and S2 had significant effects on interval locomotor activity compared to saline. Meanwhile, the effects of TAT were found to be statistically insignificant. Furthermore, one-way ANOVA reported that the effects of S1 peptide were significant from 25 minutes to 50 minutes following the treatment administration ($p < 0.05$ or $0.01$). Meanwhile, the effects of S2 peptide were different from saline at five different time-points, which were 5-minute, 10-minute, 30-minute, 45-minute and 50-minute ($p < 0.05$ or $0.01$). It was worth noting that the effects of TAT peptide were statistically similar to the effects of saline at all twelve time points ($p > 0.05$). The dotted line represented the time of intra-cranial injection. All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.
5.2 Part B: D2R-DISC1 Interaction

5.2.1 The Effects of D2R-DISC1 Disruption on Apomorphine-Induced PPI Deficits

It was observed in all groups that a higher intensity of the prepulse stimuli resulted in a greater level of prepulse inhibition response (Figure 19). Three-way ANOVA with repeated measures reported that there were significant effects of pre-pulse stimuli on the startle responses \( (F = 100.08, p < 0.001) \). In addition, the three-way ANOVA also indicated that there were significant model treatment effects \( (F_{1,155} = 58.349, p < 0.001) \), treatment effect \( (F_{3,155} = 4.244, p < 0.01) \), treatment×model treatment interaction effects \( (F_{3,155} = 3.295, p < 0.05) \). It is also worth noting that the statistically analyses also reported the significances of prepulse×model treatment interaction effects \( (F_{2,155} = 10.011, p < 0.001) \) and prepulse×treatment×model treatment interaction effects \( (F_{6,155} = 2.511, p < 0.05) \).

In the saline group with normal prepulse inhibition responses, the 70dB prepulse led to a 41.2% ± 8.3% reduction in startle response, while the 75dB and 80dB prepulse resulted in 50.7% ± 8.3% and 68.4% ± 4.7 reduction in startle response, respectively (Figure 19). One-way ANOVA indicated that, compared to saline, all haloperidol, D2R-DISC1 peptide, and scramble peptide did not have any significant effects on prepulse inhibition in normal animals (i.e. animals that did not receive an injection of apomorphine) at all three prepulse: 70dB \( (F_{3,36} = 0.331, p = 0.803) \), 75dB \( (F_{3,36} = 0.284, p = 0.836) \) and 80dB \( (F_{3,36} = 0.140, p = 0.963) \).

The disruptive effects of apomorphine on PPI were observed in both saline group and the scramble peptide group (Figure 19). For instance, under apomorphine-induced impairment of prepulse inhibition, a prepulse stimulus of 70dB only triggered a 16.7% ± 1.0% reduction in startle response compared to a 41.2% ± 8.3% reduction in non-apomorphine-treated animals \( (F_{1,18} = 8.596, p < 0.01) \). At 75dB and 80dB prepulse stimuli,
prepulse inhibition were respectively reduced from 50.7% ± 8.3% to 18.9% ± 1.8% (F1, 18 = 14.111, p < 0.01) and from 68.4% ± 4.7% to 21.4% ± 1.9% (F1, 18 = 84.692, p < 0.001) under the influence of apomorphine. The disruptive effects of apomorphine on prepulse inhibition were also observed in the scramble peptide group: 70dB (F1, 18 = 14.997, p < 0.01), 75dB (F1, 18 = 19.604, p < 0.001) and 80dB (F1, 18 = 43.333, p < 0.001).

In contrast to saline and scramble peptide, the D2R-DISC1 interfering peptide significantly alleviated the disruptive effects of apomorphine on prepulse inhibition at all three prepulse levels: 29.3% ± 2.6% PPI at 70dB (F1, 18 = 20.065, p < 0.001), 38.5% ± 3.1% PPI at 75dB (F1, 18 = 29.669, p < 0.001) and 50.7% ± 3.3% PPI at 80dB (F1, 18 = 58.670, p < 0.001). Meanwhile, at the dose of 0.1mg/kg, haloperidol was only able to normalize the impairment of prepulse inhibition at 80dB (F1, 18 = 93.853, p < 0.001) but not at 70dB (F1, 18 = 2.823, p = 0.110) and 75dB (F1, 18 = 1.741, p = 0.204).
The study had a sample size of 80 male Wistar rats and 10 animals per group. Without the apomorphine-induced impairment, one-way ANOVA indicated no significant differences in prepulse inhibition at all three prepulse levels among the treatment groups: 70dB (F\(_{3,36} = 0.331, p = 0.803\)), 75dB(F\(_{3,36} = 0.284, p = 0.836\)) and 80dB(F\(_{3,36} = 0.140, p = 0.963\)). The subcutaneous injection of apomorphine (0.3 mg/kg) significantly impaired the prepulse inhibition in both saline and scramble peptide groups at all three prepulse levels (\(p < 0.01\) or 0.001; One-way ANOVA with Turkey HSD post-hoc). On the other hand, compared to saline, the D2R-DISC1 peptide was able to alleviate the disruptive effects of apomorphine on prepulse inhibition at all three prepulse levels (\(p < 0.001\)). Meanwhile, at the dose of 0.1 mg/kg, haloperidol only sufficed to alleviate apomorphine-induced prepulse inhibition deficits at the 80dB prepulse (\(p < 0.001\)). In addition, three-way ANOVA with repeated measures reported that there were significant effects of pre-pulse stimuli(F\(_{2,155} = 100.08, p < 0.001\)), apomorphine effect (F\(_{1,155} = 58.349, p < 0.001\)), treatment effect (F\(_{3,155} = 4.244, p < 0.01\)), treatment × apomorphine interaction effects (F\(_{3,155} = 3.295, p < 0.05\)), prepulse × apomorphine interaction effects (F\(_{2,155} = 10.011 p < 0.001\)) and prepulse × treatment × apomorphine interaction effects (F\(_{6,155} = 2.511, p < 0.05\)). ** = \(p < 0.01\), *** = \(p < 0.001\) compared to apomorphine-free saline group, while ### = \(p < 0.001\) compared to apomorphine-treated saline group.

Figure 19 – The Effects of Saline, D2R-DISC1 peptide, Scramble Peptide and Haloperidol on Normal Prepulse Inhibition and Apomorphine-Induced Prepulse Inhibition Deficits. The study had a sample size of 80 male Wistar rats and 10 animals per group. Without the apomorphine-induced impairment, one-way ANOVA indicated no significant differences in prepulse inhibition at all three prepulse levels among the treatment groups: 70dB (F\(_{3,36} = 0.331, p = 0.803\)), 75dB(F\(_{3,36} = 0.284, p = 0.836\)) and 80dB(F\(_{3,36} = 0.140, p = 0.963\)). The subcutaneous injection of apomorphine (0.3 mg/kg) significantly impaired the prepulse inhibition in both saline and scramble peptide groups at all three prepulse levels (\(p < 0.01\) or 0.001; One-way ANOVA with Turkey HSD post-hoc). On the other hand, compared to saline, the D2R-DISC1 peptide was able to alleviate the disruptive effects of apomorphine on prepulse inhibition at all three prepulse levels (\(p < 0.001\)). Meanwhile, at the dose of 0.1 mg/kg, haloperidol only sufficed to alleviate apomorphine-induced prepulse inhibition deficits at the 80dB prepulse (\(p < 0.001\)). In addition, three-way ANOVA with repeated measures reported that there were significant effects of pre-pulse stimuli(F\(_{2,155} = 100.08, p < 0.001\)), apomorphine effect (F\(_{1,155} = 58.349, p < 0.001\)), treatment effect (F\(_{3,155} = 4.244, p < 0.01\)), treatment × apomorphine interaction effects (F\(_{3,155} = 3.295, p < 0.05\)), prepulse × apomorphine interaction effects (F\(_{2,155} = 10.011 p < 0.001\)) and prepulse × treatment × apomorphine interaction effects (F\(_{6,155} = 2.511, p < 0.05\)). ** = \(p < 0.01\), *** = \(p < 0.001\) compared to apomorphine-free saline group, while ### = \(p < 0.001\) compared to apomorphine-treated saline group.
5.2.2 The Effects of D2R-DISC1 Disruption on Amphetamine-Induced Hyperactivity

The baseline locomotor activity was 6322.68 cm ± 970.17 cm, 5998.25 cm ± 731.36 cm, 5542.61 cm ± 936.24 cm and 5820.81 cm ± 748.19 cm for the saline group, haloperidol group, D2R-DISC1 peptide group and scramble peptide group, respectively (Figure 20). One-way ANOVA analysis indicated that there were no significant differences in baseline locomotor activity among all four groups (F<sub>3, 22</sub> = 0.145, p > 0.05)

After being treated with amphetamine (3 mg/kg), the saline group exhibited hyperactivity and travelled 23383.33 cm ± 3276.58 cm in 60 minutes of recording (Figure 21). Meanwhile, the scramble peptide group also showed a high level of locomotor activity at 23603.66 cm ± 5980.22 cm. On the other hand, the haloperidol group and the D2R-DISC1 peptide group only travelled 5998.25 cm ± 731.36 cm and 5542.61 cm ± 936.24 cm, respectively. One-way ANNOVA analysis reported significant treatment effects on amphetamine-induced hyperactivity (F<sub>3, 22</sub> = 0.9641, p < 0.01). Games-Howell post-hoc further revealed that both haloperidol and D2R-DISC1 peptide significantly alleviated the effects of amphetamine compared to saline (p < 0.01). It was worth noting that the effects of the scramble peptide on amphetamine-induced hyperactivity were statistically similar to the effects of saline (p > 0.05)

The data were dissected and presented as interval locomotor activity at twelve different time points as illustrated in Figure 22. Two-way ANOVA with repeated measures analysis reported significant time (F<sub>11, 254</sub> = 9.079, p < 0.01), treatment (F<sub>3, 254</sub> = 9.147, p < 0.01) and timex-treatment interaction effects (F<sub>33, 254</sub> = 2.476, p < 0.05) on amphetamine-induced hyperactivity across the twelve intervals being analyzed. Games-Howell post-hoc analysis suggested that the effects of both haloperidol and D2R-DISC1 peptide on amphetamine-induced hyperactivity were significantly different from saline (p < 0.01). Meanwhile, the effects of
scramble peptide were found statistically indifferent from the effects of saline ($p > 0.05$). Additional one-way ANOVA analyses indicated that haloperidol significantly alleviated the effects of amphetamine compared to saline at eight time points ($p < 0.05$ or 0.01, please refer to Figure 22 for details on specific time points), while the D2R-DISC1 peptide was effective six time points ($p < 0.05$).
The study included a sample size of 26 male Wistar rats, 6 – 7 animals per group. The baseline locomotor activity was 6322.68 cm ± 970.17 cm, 5998.25 cm ± 731.36 cm, 5542.61 cm ± 936.24 cm and 5820.81 cm ± 748.19 cm for the saline group, haloperidol group, D2R-DISC1 peptide group and scramble peptide group, respectively. One-way ANOVA analysis indicated that there were no significant differences in baseline locomotor activity among all four groups (F¬3, 22 = 0.145, p > 0.05). All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM). * = p < 0.05, ** = p < 0.01 and *** = p < 0.001
Figure 21 – The Effects of Saline, Haloperidol, D2R-DISC1 Peptide and Scramble Peptide on Amphetamine-Induced Hyperactivity.

A subcutaneous injection of 3 mg/kg amphetamine was used to trigger hyperactivity in 26 male Wistar rats. The saline and scramble peptide group demonstrated a total locomotor activity of 23383.3 cm ± 3276.6 cm and 23603.7 cm ± 5980.2 cm respectively. Meanwhile, the haloperidol (positive control) group and the D2R-DISC1 peptide group elicited an overall locomotor activity of 4858.7 cm ± 838.8 cm and 6571.0 cm ± 947.1 cm, respectively. One-way ANOVA analysis revealed a significant treatment effect (F(3, 21) = 9.641, p < 0.001) on the total locomotor level in the amphetamine-treated animals. In addition, Games-Howell post-hoc test indicated that both haloperidol and the DISC1 peptide significantly reduced the amphetamine-induced hyperactivity compared to saline (p < 0.01). Meanwhile, the scramble peptide group was statistically similar to the saline group in locomotor activity (p > 0.05). *, ** and *** = p < 0.05, 0.01 and 0.001 respectively compared to the saline group. All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM)
A subcutaneous injection of 3 mg/kg amphetamine was used to trigger hyperactivity in 26 male Wistar rats. Two-way ANOVA with repeated measures analysis reported significant time ($F_{11, 254} = 9.079, p < 0.01$), treatment ($F_{3, 254} = 9.147, p < 0.01$) and time x treatment interaction effects ($F_{33, 254} = 2.476, p < 0.05$) on amphetamine-induced hyperactivity across the twelve intervals being analyzed. Games-Howell post-hoc analysis suggested that the effects of both haloperidol and D2R-DISC1 peptide on amphetamine-induced hyperactivity were significantly different from saline ($p < 0.01$). The effects of scramble peptide on amphetamine-induced hyperactivity were statistically similar to the effects of saline ($p > 0.05$). Furthermore, one-way ANNOVA analyses indicated that haloperidol significantly alleviated the effects of amphetamine compared to saline at eight time points ($p < 0.05$ or 0.01) while the D2R-DISC1 peptide was effective six time points ($p < 0.05$). *, ** and *** = $p < 0.05$, 0.01 and 0.001 respectively compared to the saline group. All the data were presented as distance travelled (cm) ± standard error of mean (cm). The Y-error bar indicated “plus” the standard error of means (SEM).
6. Discussion

6.1. Part A: D2R-DAT Interaction

6.1.1. The Disruption of D2R-DAT complex in Locomotor Activity

As Lee et al. demonstrated *in vitro*, the third intracellular loop of D2R can interact with the N-terminus of DAT \(^{[67]}\). Through this interaction, D2R can enhance the expression of DAT as well as DAT-mediated reuptake of dopamine. On the other hand, the opposite will hold true for the disruption of such protein complex. Therefore, it is plausible to presume that a higher dopamine level in the synapse should equate behavioural alterations such as an elevated level of voluntary movements since dopamine is critically involved in movement initiation and facilitation through its actions on both direct and indirect pathways in the basal ganglia circuitry \(^{[32,34-35]}\). This is exactly what was observed in this study. After the intracranial delivery of the S1 interfering peptide, the animals exhibited a hyperactivity level of 23200.8 cm ± 3320.5 cm over a period of 30 minutes which was significantly higher than its baseline level (i.e. 3638.33 cm ± 545.10 cm) (Figure 9, \( p = 0.005 \)). In addition, one-way ANOVA with Games-Howell post-hoc analysis indicated that the effects of S1 peptide were statistically significant from the other two treatments (i.e. saline, \( p < 0.005 \); and S2 peptide, \( p = 0.005 \)). It is worth noting that the reason of using Games-Howell post-hoc analysis was due to unequal variance among the treatment groups indicated by Levine’s test (\( p < 0.05 \)). It is worth noting that the disruption of D2R-DAT interaction by the peptide resulted in a level of locomotor activity six times higher than its baseline level (\( p = 0.005 \)). Such dramatic enhancement in locomotor behaviours further confirmed the critical roles of DAT-mediated reuptake in the termination of dopaminergic neurotransmission. Similarly, hyperactivity due to an elevated dopamine content in the synapse has been reported in mice lacking DAT \(^{[40,132-133]}\) and rats with pharmacological blockade of DAT \(^{[134-135]}\). Furthermore, Jones et al. suggested that the lifetime of synaptic dopamine prolongs
by approximately 300 times and the synaptic dopamine content increases by 5 times in the absence of DAT\textsuperscript{[136]}. With that being said, we were interested in evaluating the disruption of D2R-DAT protein complex as a potential therapeutic measures for Parkinson’s disease.

Some may question that the locomotor enhancement observed in the S1 group was due to a greater level of novelty rather than an improved dopaminergic neurotransmission. As illustrated in Figure 10, however, the locomotor activities of animals from the S1 group were significantly elevated at all six time-points compared to both saline and S2 groups ($p < 0.01$). It prompted that the differences in locomotor behaviours were not due to novelty since the novelty-induced hyperactivity is usually observed within the first 5 – 10 minutes and dramatically normalize afterwards. Moreover, all the animals were habituated to the open-field boxes on three consecutive days prior to the testing, therefore eliminating novelty as a potential confounding factor (Please refer to the method section for more details).

6.1.2. The Disruption of D2R-DAT complex in Dopamine-Depleted Animals

6.1.2.1. Dopamine Depletion Model of Reserpine and AMPT

The effects of interfering D2R-DAT protein complex on reverting Parkinsonian abnormalities were first evaluated in a dopamine-depletion animal model using both reserpine and AMPT. Reserpine is a vesicular monoamine transporter inhibitor which blocks the reuptake of monoamine neurotransmitters such as dopamine, norepinephrine and serotonin\textsuperscript{[2]}. Meanwhile, AMPT exerts its inhibitory actions on tyrosine hydroxylase, which will lead to a severe impairment in the endogenous production of dopamine\textsuperscript{[2]}. This protocol is commonly used to induce an acute-parkinsonian state characterized by akinesia and rigidity that becomes stable 18 hours after the subcutaneous injection of reserpine\textsuperscript{[130]}. The administration of AMPT serves to further stabilize the impaired locomotor activities induced by resperine\textsuperscript{[137]}. 
After the administration of the S1 interfering peptide, the animals remained to be virtually immobile (e.g. 75.89 cm ± 53.94 cm). One-way ANOVA analysis showed that the therapeutic effects of the S1 peptide were statistically similar to both TAT and S2 peptides, indicating that the S1 peptide did not suffice to normalize the locomotor impairment ($F_{2,22} = 0.572$, $p = 0.572$). One of the plausible explanations for the ineffectiveness of the treatment peptide was due to the synergic effects of both reserpine and AMPT, resulting in a complete clearance of endogenous dopamine content. As aforementioned, the D2R-DAT interaction only regulates the membrane expression of DAT but does not have any effects on the biosynthesis of dopamine. Therefore, if the combination of reserpine and AMPT resulted in a complete depletion of dopamine (i.e. no dopamine was available for synaptic release), the disruption of D2R-DAT complex by the peptide would still be found ineffective in enhancing the synaptic dopamine level in spite of its action on DAT membrane expression. As demonstrated in Figure 11, the extremely low level of locomotor activities over a period of 30 minutes observed in all three groups implied the virtual absence of synaptic dopamine content after the administration of reserpine and AMPT (41.71cm ± 21.76cm for TAT group, 75.89cm ± 53.94cm for S1 group and 27.72cm ± 10.03cm for S2 group respectively). Consequently, an alternative model with moderate dopamine-depletion was needed to accurately examine the effects of the treatment peptide in vivo.

Another possible explanation was that, at the dose of 4 nmol, the S1 interfering peptide did not suffice to reverse the pharmacologically-induced akinesia. In other words, at the dose being tested in this experiment, the S1 peptide did not suppress the DAT-mediated reuptake of dopamine enough to result in a decent level of synaptic dopamine. Consequently, the animals still remained immobile even after the administration of the S1 peptide. Based on this
explanation, a higher dose of S1 peptide should instead be tested to determine its potential therapeutic effects for Parkinson’s disease.

6.1.2.2. Dopamine Depletion Model of AMPT

As discussed in the previous section, a model of moderate dopamine depletion was preferred to a severe dopamine depletion model. As a result, a dopamine depletion model using two intra-peritoneal injections of AMPT (25 mg/kg each) modified from McDougall et al. was evaluated. As illustrated in Figure 13, this depletion protocol reduced the locomotor behaviours approximately by half compared to the baseline level (two-tailed t-test, \( p < 0.001 \)). More importantly, after the administrations of AMPT, the animals were still able to display a decent level of horizontal movements (i.e. 2679.09 cm ± 460.32 cm), indicating that this protocol did not entirely deplete the endogenous dopamine content. Based on this result, this protocol of dopamine depletion was considered suitable for testing the potential therapeutic effects of disrupting the D2R-DAT interaction on moderate akinesia.

In the modified model of dopamine depletion, the S1 peptide still did not suffice to normalize the impaired motor behaviours at the dose of 4nmol (two-tailed paired t-test, \( p < 0.001 \); Figure 14). In contrast, at the dose of 40 nmol, the S1 peptide clearly showed to improve the motor behaviours in the dopamine-depleted animals compared to the other three treatment groups (\( p < 0.05 \), Figure 17). Taken together, the S1 peptide at 4nmol was below its effective dose and which accounted for its ineffectiveness in alleviating motor impairment. In other words, at 4nmol, the peptide could not suppress the membrane expression of DAT to a level where it would be beneficial to animals with compromised dopaminergic neurotransmission. Based on the data collected from this part of the study, it is plausible that the ineffectiveness of the S1 peptide observed in the reserpine-AMPT depletion experiment was due to its sub-effective dose. In order
to further validate the effectiveness of the S1 peptide as a potential therapy for PD, the reserpine-AMPT model should be revisited with a higher dose of S1 peptide (e.g. 40 nmol).

Aside from the effects of the S1 peptide in dopamine-depleted animals, it was also interesting to discuss the effects of the S2 peptide observed in this experiment. As summarized in Figure 9 and 10, the S2 peptide did not show to have any effects on the locomotor behaviours in normal animals at the dose of 40 nmol ($p > 0.05$ compared to saline). On the other hand, the S2 peptide seemed to improve the impaired motor behaviours in dopamine-depleted animals and it was very close to reaching statistical significance compared to TAT peptide ($p = 0.053$) and saline ($p = 0.065$, Figure 17). Furthermore, the effects of S2 peptide were significantly different from saline and TAT peptide at several time-points as illustrated in Figure 18. Even though the S2 peptide was synthesized based on the sequence of a segment near the interacting region of DAT, Lee et al. showed that this segment was not involved in facilitating the D2R-DAT interaction and more importantly, the S2 peptide did not disrupt the D2R-mediated up-regulation of DAT. The findings by Lee et al. completely aligned with the data observed in normal animals but did not support those observed in the dopamine depletion model. Unfortunately, we could not explain the results based on our current knowledge and further studies are necessary. For example, we can conduct an in vivo microdialysis experiment to identify whether the S2 peptide-mediated locomotor improvement was due to a rise in extracellular dopamine within the nigrostriatal pathway (e.g. striatum). If indeed the effects of the S2 peptide observed in the dopamine-depleted animals were due to a direct result of an increase in dopamine release, we should revisit the D2R-DAT interaction in vitro. Perhaps the structural properties of the D2R-DAT interaction changes when the dopamine level becomes scarce. In other words, when the endogenous dopamine content is at its normal physiological level, the first 15 amino acid
residues of the DAT N-terminus are sufficient to facilitate the D2R-DAT interaction as shown by Lee et al.\textsuperscript{[67]}. When the dopamine content is far below its normal physiological range, a bigger interacting region of DAT (including the next 10 amino acids A\textsubscript{16}-G\textsubscript{25}) would be required for a stable D2R-DAT interaction, securing DAT at the cell membrane and preventing it from being trafficked back into the cytoplasm \textsuperscript{[141-142]}. Regardless, further studies are definitely required to decipher a clear explanation for such interesting findings.
6.2. Part B: D2R-DISC1 Interaction

6.2.1. The Anti-Psychotics Actions of D2R-DISC1 complex disruption in Apomorphine-Induced Prepulse Inhibition Deficits

Pulse inhibition (PPI) was first described by Graham in 1974 as a phenomenon where an individual exhibits a diminished startle response to the startling auditory stimulus in the presence of a preceding weak prepulse stimulus \[143\]. PPI is not an acquired behaviour and does not habituate across trials. After being introduced to the field, it is widely accepted as a central information buffering system which filters out all the “extraneous or redundant environmental inputs” in order to selectively focus on processing important stimuli/information \[144\]. PPI deficits in patients with schizophrenia were first reported by Braff et al. in 1978 \[144-145\] and since then several studies showed that PPI impairments correlated with thought disorder and positive symptoms of schizophrenia \[146-148\]. More importantly, PPI has become a valuable experimental paradigm for schizophrenia-related research because its protocol for animals is very similar to the one used in humans \[149\]. In this experiment, we focused on examining the potential anti-psychotic effects of the interfering peptide on apomorphine-induced PPI disruption, one of the commonly used animal models of the hyperdopaminergic pathophysiology of schizophrenia \[150-151\].

As presented in Figure 19, apomorphine (0.3 mg/kg) severely impaired PPI in animals at all three prepulse levels \((p < 0.01\) or \(0.001\)). Both saline and scramble peptide did not show any corrective effects on these pharmacologically induced deficits. On the other hand, haloperidol was able to normalize these sensorimotor gating abnormalities at the 80dB prepulse \((p < 0.001\). Meanwhile, the interfering peptide alleviated these PPI deficits at all three prepulse levels \((p < 0.001\). As aforementioned, the interfering peptide does not possess any effects on the G-protein-dependent signalling pathway. If the \(\beta\)-arrestin/PP2A/Akt/GSK3 pathway as part of the D2R
downstream cascade was not responsible for the hyperdopaminergism-mediated PPI deficits, the interfering peptide would not show any beneficial effects since it is not involved in the G-protein dependent pathway. As the opposite was observed in this experiment, we can therefore conclude that this β-arrestin/PP2A/Akt/GSK3 signaling pathway is also essential to normal dopaminergic neurotransmission alongside with the G-protein-dependent pathway.

### 6.2.2. The Anti-Psychotics Actions of D2R-DISC1 complex disruption in Amphetamine-Induced Hyperactivity

After examining the potential anti-psychotic effects of the D2R-DISC1 disruption on pharmacologically disrupted PPI, we extended our study to another animal model of schizophrenia: amphetamine-induced hyperactivity. Pharmacologically, amphetamine acts on DAT to trigger an extraordinarily great level of dopamine release, which will then translate into hyperactivity at the behavioural level \[^{2,151}\]. This animal model of schizophrenia has construct validity because it mimics the hyper-dopaminergic pathogenesis of schizophrenia \[^{77,152-153}\]. Furthermore, it also provides certain level of predictive validity, especially for anti-psychotics that act on the dopaminergic system. However, this model does not possess any face validity as hyperactivity is not necessarily a characteristic of patients with schizophrenia[^128].

As presented in Figure 21, amphetamine-induced hyperactivity was observed in the saline group as well as the scramble peptide group. In contrast, both haloperidol and the interfering peptide successfully normalized the amphetamine-induced hyperactivity compared to saline \((p < 0.01)\). Moreover, statistical analyses also prompted that the “anti-psychotic” effects of both haloperidol and the DISC1 peptide persisted approximately throughout the first half of the experiment, but gradually faded out near the end of the study (Figure 22). It is worth noting that
the “fade-out” of their therapeutic effects at later time-points was possibly due to the declining stimulant effects of amphetamine as observed in both saline and scramble peptide groups.

In this model, the interfering peptide once again exhibited anti-psychotic actions similar to haloperidol (i.e. a classical anti-psychotic). Given that the peptide does not interfere the G-protein-dependent pathway, the data collected in this experiment further reinforced the essence of the β-arrestin/PP2A/Akt/GSK3 signaling pathway in dopaminergic neurotransmission. In the meantime, it also revealed the critical role of the D2R-DISC1 interaction as the mediator of this downstream signaling pathway. Based on the data from the PPI experiment as well as this part of the study, it has become very clear that the D2R-DISC1 protein complex and its downstream β-arrestin/PP2A/Akt/GSK3 signaling pathway indeed possess great potentials be a target site for the development of novel anti-psychotics.
7. Conclusion

7.1. Part A: D2R-DAT Interaction

D2R are localized on both pre-synaptic and post-synaptic membrane. Post-synaptic D2R serves to exert physiological effects of dopamine, while it acts as an auto-receptor on the pre-synaptic side \cite{3-5}. Its auto-receptor role is equally important as its post-synaptic counterpart because it modulates the biosynthesis of dopamine and therefore dopaminergic neurotransmission \cite{154-156}. Aside from D2R, DAT is another essential protein in the regulation of dopamine signalling. DAT generally sits on the axonal terminal of dopaminergic neurons and is primarily responsible for the removal of dopamine from the synapse back into the neuron terminal \cite{37-39}. Interestingly, our lab revealed that D2R and DAT can engage in a direct protein-protein interaction with each other and through this interaction D2R can up-regulate the membrane expression of DAT \cite{67}. Given the essence of DAT in the reuptake of dopamine, the disruption of the D2R-DAT protein complex \textit{in vitro} dampens the level of DAT at the cell membrane and therefore results in a decreased dopamine reuptake \cite{67}.

Based on the findings by Lee \textit{et al.}, this study focused on examining whether such alterations at the molecular level would result in significant effects on the behavioural level. First of all, the disruption of D2R-DAT protein complex via the interfering peptide led to hyperactivity in Sprague-Dawley rats. The explanation for such experimental observation was that the disruption of D2R-DAT complex led to a reduced membrane expression of DAT and the subsequent dopamine reuptake, hence, the excessive dopamine content in the synapse potentially enhanced the motor circuitry (both direct and indirect pathways) to facilitate a higher level of locomotor behaviours. In addition, the disruption of D2R-DAT complex sufficed to render hyperactivity in animals subjected to a moderate dopamine-depletion (i.e. Parkinson’s disease)
model, suggesting that this protein complex can be a potential target site for the PD therapeutic measures.

7.2. Part B: D2R-DISC1 Interaction

D2R is one of the major receptors to mediate the physiological functions of dopaminergic neurotransmission. The G-protein dependent pathway was originally perceived as the only D2R downstream cascade, which the activation of D2R led to the inhibition of adenylate cyclase via the action of \( G\alpha_i \)\(^{[3-5]} \). Recently, Caron’s research group corroborated that the \( \beta \)-arrestin/PP2A/Akt/GSK3 pathway is another essential signaling cascade along with the G-protein dependent pathway for the actions of D2R \(^{[26,118]} \).

D2R is not only important for carrying out the physiological actions of dopamine, but also implicated in both the patho-physiology of schizophrenia and its mainstay treatment. Traditionally, the underlying pathogenesis of schizophrenia was believed to be an overly active dopaminergic neurotransmission. The general perception of the pathology of schizophrenia is continually subjected to refinements over the years based on new findings. Recently, the focus has shifted to the presynaptic striatal dopaminergic dysregulation: a higher dopamine synthesis capacity and greater release upon cocaine challenge \(^{[81-84]} \). Furthermore, it was reported that schizophrenic patients exhibited higher baseline occupancy at D2R compared to normal individuals \(^{[88]} \). To further solidify the importance of D2R in schizophrenia, the typical anti-psychotics (e.g. haloperidol) have been designed to antagonize D2R and interestingly, their clinical efficacy is directly linked with their respective binding affinity for D2R \(^{[71-73]} \).

Recently, our lab revealed that disrupted-in-schizophrenia-1 (DISC1) interacts with D2R and \( \textit{in vitro} \) such protein interaction showed to be essential for mediating the G-protein
independent pathway (i.e. β-arrestin/PP2A/Akt/GSK3 signaling cascade) of D2R. Due to the relevant implication of D2R signaling in schizophrenia, we hypothesized that the disruption of D2R-DISC1 complex would yield anti-psychotic actions. In this study, an interfering peptide was developed to disrupt the D2R-DISC1 protein interaction. Subsequently, the anti-psychotic effects of the disruption were examined in two animal models of schizophrenia: i) apomorphine-induced prepulse inhibition (PPI) deficits and ii) amphetamine-induced hyperactivity. In the PPI model, the disruption of D2R-DISC1 interaction normalized the pharmacologically induced deficits at all three prepulse levels (i.e. 70dB, 75dB and 80dB). Meanwhile, the same disruption of D2R-DISC1 interaction restored normal locomotor behaviours in amphetamine-treated Wistar rats. As the anti-psychotic effects being demonstrated in both animal models, it is clear to suggest that the D2R-DISC1 interaction and its downstream β-arrestin/PP2A/Akt/GSK3 signaling pathway have great potentials to be a target site for future schizophrenia research and the development of novel anti-psychotics.
8. Future Directions

8.1. Part A: D2R-DAT Interaction

Throughout this study, the interaction between D2R and DAT has extensively examined as a potential treatment target for Parkinson’s disease. Given that the dopaminergic system is also implicated in the patho-physiology of attention deficit/hyperactivity disorder (ADHD), we are looking forward to studying the same protein interaction in the context of attention deficit/hyperactivity disorder (ADHD) in the near future.

ADHD are often characterized by abnormal behaviours such as hyperactivity, inattention and impulsivity\textsuperscript{[157]}. Clinically, the onset of ADHD generally starts in childhood and may even persist into adulthood. Although the abnormalities with the mesocortical and mesolimbic dopaminergic pathways are generally accepted as the core patho-genesis of ADHD, it still remains controversial whether this disorder is the consequence of hypo-dopaminergic or hyper-dopaminergic neurotransmission\textsuperscript{[158]}. Regardless of the controversy, several studies reported an elevated expression of DAT in patients with ADHD compared to controls\textsuperscript{[159-161]}. In addition, two of these studies demonstrated that chronic administration of a DAT inhibitor, methylphenidate, significantly suppressed the elevated expression of DAT found in individuals with ADHD\textsuperscript{[160-161]}. It is also worth noting that methylphenidate is one of the mainstay medications for ADHD patients. Based on these findings, it seems clear that the reduction of DAT activity can potentially normalize the abnormalities of ADHD. Since the disruption of D2R-DAT protein complex can dampen the expression of DAT at the cell surface, we strongly believe the disruption of such interaction will exert the same beneficial effects.

We are looking forward to examining our hypothesis using an animal of ADHD, for example, the spontaneously hypertensive rat (SHR). This SHR rat model shows behaviour
abnormalities similar to those observed in ADHD patients (e.g. hyperactivity, learning deficits, etc.)\textsuperscript{[162-163]}. In addition, these SHR rats display a dysfunctional dopaminergic neurotransmission in the prefrontal cortex and striatum \textsuperscript{[163]}. In order to evaluate the effects of the D2R-DAT interaction on ADHD, we must prove that the disruption of such interaction can correct the behavioural deficits observed in the SHR rats. For example, the disruption of D2R-DAT interaction should suffice to attenuate the hyperactivity in the SHR rats. In addition, the breakdown of the D2R-DAT interaction should also correct the cognitive deficits of the SHR rats in behavioural tests such as Y-maze, Morris water maze, etc. If its disruption normalizes these behavioural deficits as expected, the D2R-DAT interaction will possess great potentials to be a site for the development of new ADHD treatments.
8.2. Part B: D2R-DISC1 Interaction

In this study, the anti-psychotic properties of the D2R-DISC1 complex disruption were examined. First, the disruption of D2R-DISC1 interaction alleviated the PPI deficits in Wistar rats, which was caused by apomorphine. Also, the same disruption led to normal locomotor activities in amphetamine-treated rats. It is clear that the disruption of D2R-DISC1 interaction can exert anti-psychotic effects on animals modeling hyperdopaminergism-mediated schizophrenia. However, there is growing evidence that dysfunctional dopamine system in the CNS is not the only cause of schizophrenia. For instance, abnormalities within the glutamatergic system are also implicated in the patho-physiology of schizophrenia [164-166]. Studies showed that pharmacological antagonism of the NMDA receptor leads to psychotic symptoms such as delusions and hallucinations in healthy individuals [167-168]. Meanwhile, the blockade of NMDA receptor also caused negative symptoms such as social withdrawal and speech deficits similar to the ones observed in schizophrenic patients [169-170]. In order to accurately validate the anti-psychotics potentials, we must examine the effects of the D2R-DISC1 complex disruption in animal models of schizophrenia involving neurotransmitter systems (e.g. glutamatergic system) other than dopaminergic system.

One example of such animal models would be a pharmacological model of schizophrenia involving an antagonist for NMDA receptor: phencyclidine (PCP). This animal model exhibits a wide range of behavioural deficits that are akin to the symptoms observed in schizophrenia patients: hyperactivity, PPI deficits, social withdrawal and cognitive impairment [170-174]. One of the advantages this PCP model has over the dopaminergic model is its resemblance of the negative symptoms (i.e. cognitive deficits and social withdrawals) [170]. Therefore, it will provide a platform to evaluate whether the D2R-DISC1 interaction is implicated or involved in
mediating these cognitive and social deficits. In the future, we are looking forward to examine the antipsychotic effects of the D2R-DISC1 complex disruptions on all the behavioural abnormalities observed in the PCP rat models. If the disruption of D2R-DISC1 interaction can normalize abnormalities in this glutamate-based animal model of schizophrenia, it will strongly suggest that the interaction is implicated in the patho-physiology of schizophrenia involving different neurotransmitter systems and more importantly, it will brighten its prospect as a target site for the development of novel anti-psychotics.
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