ASSESSMENT OF DOPAMINE RECEPTOR DRD4 AND DRD5 mRNA EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTES OF OPIOID & AMPHETAMINE TYPE STIMULANT DEPENDENT MALAY MEN UNDERGOING METHADONE MAINTENANCE THERAPY

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by

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LIST OF SYMBOLS AND UNITS

%	Percentage
<	Less than
>	More than
α	Alpha
β	Beta
δ	Delta
Δ	Delta
°C	Celsius
µ	Micro
µL	Microlitre
Bp	Base pair
CT	Cycle threshold
dL	Decilitre
g	Gram
h	Hour
mg	Microgram
min	Minute
mL	Millilitre
mM	Millimolar
ng	Nanogram
nm	Nanometre
pg	Picogram
rpm	Rotation per minute

s	Seconds

LIST OF ABBREVIATIONS

ATS	Amphetamine type stimulant
MMT	Methadone Maintenance Therapy
DNA	Deoxyribonucleic acid
PCR	Polymerase Chain Reaction
RT PCR	Reverse transcription polymerase chain reaction
TBE	Tris Borate EDTA
CNS	Central nervous system
VTA	Ventral tegmental area
NAc	Nucleus accumbens
UV	Ultraviolet
SD	Standard deviation
IQR	Interquartile range
BMI	Body mass index
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
NADA	National Drug Agency
WHO	World Health Organization
OD	Optical density
PBL	Peripheral blood lymphocytes
RNA	Ribonucleic acid
cDNA	complementary DNA
MOH	Ministry of Health
ADHD	Attention Deficit Hyperactivity Disorder
UNODC	United Nations Office on Drugs and Crime
VNTR	Variable Number Tandem Repeat

DRD4	Dopamine D4 receptor
DRD5	Dopamine D5 receptor
DAT	Dopamine transporter
SEA	South East Asia
MDMA	Methylenedioxy-metamphetamine
TAAR	Trace amine-associated receptor
DSM	Diagnostic and statistical manual of mental disorder
APA	American Psychiatric Association
HIV	Human immunodeficiency virus
NSEP	Needle and syringe exchange program
OT	Olfactory tubercle
APUD	Amine precursor uptake and decarboxylation cells
PET	Positron emission tomography

PENILAIAN EKSPRESI mRNA RESEPTOR DOPAMIN DRD4 DAN DRD5 DI DALAM SEL LIMFOSIT PERIFERI DI KALANGAN SUBJEK LELAKI MELAYU DENGAN PERGANTUNGAN CAMPURAN JENIS OPIAT DAN PERANSANG AMFETAMIN YANG SEDANG MENJALANI TERAPI GANTIAN METHADONE

ABSTRAK

Di Malaysia dan lain-lain negara Asia, taburan pergantungan kepada campuran opiat dan peransang jenis amfetamin adalah tinggi dan telah menjadi masalah besar kesihatan. Opiat dan peransang jenis amfetamin menghasilkan tindakbalas untuk mencapai tujuan utama iaitu meningkatkan aras dopamin yang tinggi di luar sel di dalam otak dan akhirnya akan memberi kesan ganjaran dadah. Tindakbalas dopamin berlaku melalui perantaraan reseptor yang khusus yang berpasangan dengan protin G yang terdiri daripada 2 jenis yang berbeza; jenis D1 reseptor (D1 dan D5) dan jenis D2 reseptor (D2, D3 dan D4). Bukti dari kajian-kajian lepas telah mencadangkan bahawa sistem dopamin periferi mencerminkan aktiviti dan patologi sistem dopamin pusat, terutama dalam penyakit neuropsiakiatri. Kajian-kajian lepas telah banyak dilakukan ke atas sistem dopamin pusat sedangkan siasatan ke atas sistem dopamin dalam kalangan organ periferi masih terhad. Dalam kajian ini, kami mengkaji perbezaan di dalam ekspresi mRNA bagi reseptor dopamin DRD4 dan DRD5 di dalam sel limfosit dalam darah periferi dalam kalangan subjek lelaki Melayu dengan pergantungan campuran jenis opiat dan peransang jenis amfetamin yang sedang menjalani terapi gantian methadone berbanding lelaki Melayu yang sihat bertindak sebagai kawalan. Keseluruhan jumlah peserta adalah 72 orang; 36 subjek yang bergantung kepada opiate

dan ransangan jenis amfetamin dan 36 subjek kawalan telah direkrut daripada pelbagai tempat di Kuala Terengganu, Terengganu berdasarkan kriteria kelayakan dan pengecualian. Data demografi termasuk umur, tinggi, berat, indeks jisim badan (BMI) dan tekanan darah untuk semua peserta direkodkan. Kebenaran secara pemberitahuan dan bertulis diperolehi. Sampel darah diperolehi daripada setiap subjek. RNA telah diekstrak daripada sel limfosit. Ekspresi mRNA untuk reseptor DRD4 dan DRD5 di dalam sel darah limfosit periferi ditentukan menggunakan teknik "real-time PCR". Data demografi untuk subjek-subjek dikira menggunakan ujian Mann Whitney. Nilai $p<0.05$ digunakan untuk perbezaan bererti. Terdapat perbezaan yang signifikan di antara subjek yang bergantung kepada dadah dan subjek kawalan untuk parameter umur dan tekanan darah sistolik sementara parameter-parameter lain tidak menunjukkan statistik yang signifikan. Aras ekspresi mRNA untuk reseptor DRD4 dalam sel limfosit adalah menurun dengan signifikan di dalam subjek yang bergantung dadah berbanding dengan subjek kawalan ($p=0.039$). Walaubagaimanapun, aras ekspresi mRNA untuk reseptor DRD5 dalam sel limfosit subjek yang bergantung dadah kepada opiat dan peransang jenis amfetamin tidak menunjukkan statistik yang signifikan ($p=0.0251$). Sebagai kesimpulan, subjek yang bergantung dadah campuran opioid dan peransang jenis amfetamin yand sedang menjalani terapi gantian methadone mungkin mempamerkan corak yang berbeza pada ekspresi dopamin reseptor DRD4 dan DRD5 di dalam sel limfosit periferi. Kajian lanjut disarankan untuk menyokong penemuan dalam kajian ini.

ASSESSMENT OF DOPAMINE RECEPTOR DRD4 AND DRD5 MRNA EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTES OF OPIOID & AMPHETAMINE TYPE STIMULANT DEPENDENT MALAY MEN UNDERGOING METHADONE MAINTAINENCE THERAPY

ABSTRACT

In Malaysia and throughout the Asian region the mixed opioid and amphetamine type stimulants dependence is highly prevalent which has become a major health problem. Opioids and amphetamine-type stimulants (ATS) exert their effect by altering natural dopamine neurotransmission in the brain to achieve the ultimate goal of an extracellular hyper-dopamine state hence resulting drug reward effect. Dopamine actions are mediated by specific G proteins coupled receptors of two distinct families; D1-like receptor subtypes (D1 and D5) and the D2-like receptor subtypes (D2, D3, and D4). Evidence from previous studies has suggested that peripheral dopamine systems reflect the central dopamine system's activity and pathology, especially in neuropsychiatric diseases. Studies have been carried out widely on central dopamine systems, while investigation of dopamine systems in various peripheral organs is still limited. It has been reported that peripheral blood lymphocytes express dopamine in peripheral systems. In this study, we investigated the difference in the mRNA expression of the dopamine receptors DRD4 and DRD5 in peripheral blood lymphocytes of ATS and opioid dependent Malay male subjects undergoing methadone maintenance treatment and healthy Malay male serving as control subjects. A total of 72 participants with 36 drugs dependent subjects and 36 control subjects were recruited from various parts of Kuala Terengganu, Terengganu according to inclusion and exclusion criteria. Demographic data including age, height,

weight, body mass index and blood pressure of all participants were recorded. A questionnaire form was given to the drug dependent subjects to assess their drug addiction status. Informed and written consent was obtained, and blood samples were collected. RNA was extracted from lymphocytes. The mRNA expression of the dopamine receptors DRD4 and DRD5 in peripheral blood lymphocytes was assessed by real-time PCR method. The demographic data of the subjects were calculated using Mann Whitney Test. The p-value 0.05 was used for the statistical significance. There was a significant difference between drug dependent subjects and control subjects for age and systolic blood pressure parameters while other parameters were not statistically significant. The DRD4 mRNA expression level is significantly reduced in lymphocytes of drug dependent subjects compared to control subjects ($p=0.039$). However, DRD5 mRNA expression level in lymphocytes of drug dependent subjects was not statistically significant ($p=0.251$). In conclusion, drug dependent subjects on mixed opioid and ATS dependence undergoing methadone maintenance treatment may exhibit different patterns of dopamine receptors DRD4 and DRD5 mRNA expressions in the peripheral lymphocytes. Further studies are recommended to support the findings of the present study.

CHAPTER 1

INTRODUCTION

1.1 Drug Addiction

Drug addiction is a major public health threat and social issue worldwide which includes addiction of opioids (both natural and synthetic opioid), methamphetamine, amphetamine type stimulant (ATS), cocaine, cannabis and other psychoactive substances (Low *et al*., 2016). According to World Drug Report 2019 (UNODC, 2019) cannabis is the most widely used drug worldwide while opioids and untreated hepatitis C maintain to be the most harmful, that account for two-thirds of the deaths due to drug use disorders. These drugs are being administered into the body through various routes including smoking, injection, tablets/pills, snorting/intra nasal and transdermal route. Injectors subjects were reported to have higher level of drug dependence and more likely to overdose. They also have higher risk of the serious blood-borne infection including HIV, Hepatitis B and Hepatitis C compared to those non-injector subjects (Mehta *et al*., 2011; Keen *et al*., 2014).

Drug addiction is a chronic, relapsing and neurobiological brain disease involving multi factors including genetic, neurodevelopmental, and sociocultural factors that are manifested by compulsive use of drugs despite harmful consequence (Volkow & Morales, 2015). The fifth edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5) classify drug addiction under section substance-related and addictive disorders which defined as recurrent use of alcohol or other drugs that causes

clinically and functionally significant impairment, such as health problems, disability, and failure to meet major responsibilities at work, school, or home (Volkow *et al*., 2016). This disorder is further classified into mild, moderate, or severe depending on severity level. While for addiction, DSM-5 referred addiction term as synonymous to the severe substance-use disorder class where there is substantial loss of self-control evident by compulsive drug taking instead of the desire to stop drug taking (Volkow *et al*., 2016).

Brain reward system which predominantly located in the limbic structures of the brain is a system that control and regulate cognitive function, learning ability, emotion and memory (Drozak & Bryła, 2005). The activation of the reward system will increase dopamine level which in turn causes the person to feel pleasure and motivated. Thus, this system is the major target of most drugs. Both natural and chemical stimuli that give pleasure effect activate the same site which is the reward system. However only natural activities are controlled by feedback mechanisms that activate aversive centers, whereas in the artificial stimuli there is no such mechanism occur. Therefore chronic intake of drugs can cause adaptive changes, sensitization or tolerance in this system hence affecting the cognitive function which include decision making, learning as well as memory and behavior (Vetulani, 2001).

1.1.1 Prevalence of drug addiction in Malaysia

Malaysia has been facing a serious public health problem regarding drug addiction. It was initiated by the hippy culture in the 1970s and the Vietnam War which introduced cannabis and heroin to Malaysia (Singh *et al*., 2013). A cumulative total of 512,767 drug users were detected as reported by National Anti-Drugs Agency (NADA)

between 1988 and 2017 representing about 1.6% of total Malaysian population (National Anti-Drug Agency (NADA), 2017). The real total of drug users may exceed more than half a million as the national database only reported individuals who have been arrested and convicted for illicit of drug use and sent for mandatory institutional rehabilitation (Vicknasingam *et al.,* 2009).The annual number of reported drug addicts by the National Anti-Drugs Agency (NADA) in 2010 to 2017 is approximately 15,000 to 30,000 per annum with a peak of 30,844 in 2016 and the least cases detected was in 2012 of 15,101 cases.

Heroin maintains the first rank of the most frequently drug used every year since 2010 to 2013 with a peak of 75.07% of total abused drugs in 2013. The percentage of heroin addiction per year shows a steady decline from 2014 to 2017 in which it reduced by half in 2017 which accounts for 39.1%. (National Anti-Drug Agency (NADA), 2017).

The scenario is evidence by the growing problem of usage of amphetamine-type stimulants (ATSs) including crystal methamphetamine and various other methamphetamine and/or amphetamine-containing substances/pills in Malaysia and the surrounding countries (Chawarski *et al*., 2012). The rise of methamphetamine use in South East Asia has attracted major attention as it emerges as the world's fastest-growing methamphetamine market and the report that these drug~~s~~ is the main drug of concern in South East Asia (SEA) countries (UNODC, 2019). According to Chawarski *et al.,* (2012), ATS use in Malaysia has been insignificant before 1987. Over the next several years, the total ATS use increased slowly before spiking up after 1997. By 2018, the total ATS use was 75% of the participants. Vicknasingam *et al.,* (2010) found

that approximately 60% of opioid injection drug users report lifetime use of ATS with 29% also reporting lifetime injection of ATS in many regions in Malaysia. Between 2010 to 2017, it was observed that ATSs addiction ranging around 30-40% of total drug abused per year. The percentage started to rise steadily from 2014 and became more than half of total drug used in 2017 which exceeded heroin usage by 20.88% (National Anti-Drug Agency (NADA), 2017). The rising of ATSs usage also being evidenced by the increasing number of admissions in recent years for amphetamines use. In 2015, the total number for admission related with drug treatment was 6,032 which opiates accounts for 71% (4,287) while the amphetamines use related admission was 1,571 accounting for 26% of the total in 2015 and about a 47% increase compared to 2014 (839 admissions). Of this total, methamphetamine (crystalline form) represented 77% (1,213 admissions) of the amphetamines-related treatment admissions in 2015 (Global SMART Programme & UNODC, 2017).

Male continue to represent the majority (96.4%) of cumulative drug addict cases in Malaysia with a ratio of 1 female for every 26 males reported. About 41.7% of reported cases are amongst young people between the ages of 13-29 years old. In term of ethnicity, 80.6% of Malays, 7.5% of Chinese, and 6.8% of Indians were drug addict with other ethnics contributing 5.1% (National Anti-Drug Agency (NADA), 2017).

1.1.2 Overview of opioid and opioid addiction

Opioids include synthetic or naturally occurring alkaloid (benzylisoquinoline alkaloids) found in opium poppy plant (Manglik *et al*., 2012). Figure 1.1 and 1.2 showed the chemical structure of heroin and morphine molecules. They are substances

that stimulate brain's opioid receptors, a group of receptors that give pain and euphoria perceptions which also involved in the breathing regulation (Waldhoer *et al.*, 2004). Of all, morphine and heroin are the most known and used opioids while others include methadone, buprenorphine, codeine, tramadol, oxycodone and hydrocodone (UNODC, 2019). They are prescribed clinically for its analgesic properties and in treating opioid dependence. Excessive opioid used or in absence of proper medical supervision, can lead to fatal respiratory depression (UNODC, 2013). Opioids are being administered through injection, oral tablets and snorting. It also available in liquid, solid and powder form (Butler *et al.,* 2011). Apart from its powerful analgesic effects, opioid intake also may induce relaxation and 'high' feeling as well as other side effects that include physical dependence, tolerance, respiratory depression, sedation, constipation, nausea, and death (Bruehl *et al.,* 2013).

Opioid acts by directly binding and activating opioid receptors chiefly μ-opioid receptor which mediates its most potent analgesic and addictive effects. μ-opioid receptor activation leads to two different signaling pathways. First through the heterotrimeric G-protein Gi, resulting in sedative and analgesic properties including physical dependence and euphoria. Secondly is through arrestin signal pathway which is linked to opioid side effects such as tolerance, constipation and respiratory suppression (Huang *et al.,* 2015). The rewarding properties of opioid is achieved by indirectly elevating of post synaptic dopamine level in ventral tegmental area (VTA) which is considered as rewards system center. Activation of μ-opioid receptor results in inhibition of γ-aminobutyric acid (GABA)ergic normal inhibitory tone, leading to increase in dopaminergic neurons firing rate hence increasing dopamine level in synaptic cleft (Gysling, 2005).

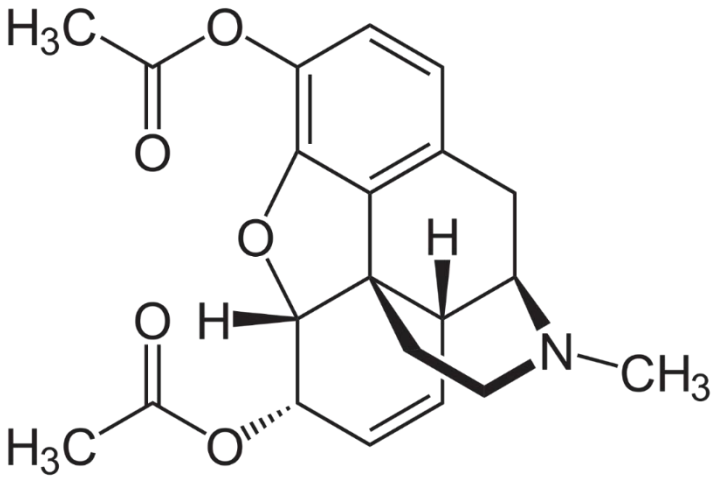

Figure 1.1 Chemical structure of heroin molecule

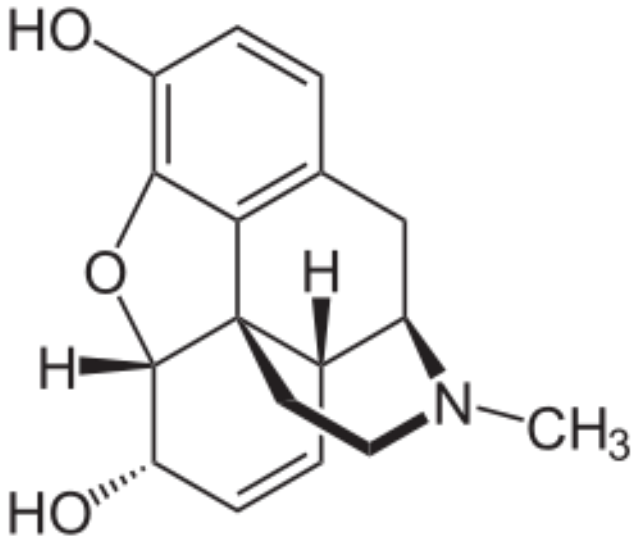

Figure 1.2 Chemical structure of morphine molecule

Most drugs of abuse including ATS and opioid exert trigger various mechanisms of actions in CNS system (including direct, indirect and multiple actions), hijacks the normal brain-reward circuitry in which natural dopamine neurotransmission is altered, to achieve the ultimate goal of extracellular hyperdopamine state (Daberkow *et al.*, 2013). Dopamine is an extremely important neurotransmitter that mediates the reinforcing and rewarding effects in our body. Repeated exposure and administration of illicit drugs produces persistent adaptive changes in structural dopaminergic neuron and neuroadaptations in reward and reinforcement circuits, all of which involving in augmentation of addiction process (Volkow *et al.*, 2003).

1.1.3 Overview of amphetamine type stimulant (ATS) and ATS addiction

Amphetamine type stimulants (ATS) are manufactured composite that consist of two main sub-type amphetamine-type and ecstasy-type substances. Figure 1.3 shows, the chemical structure of amphetamine ($C_9H_{13}N$). Amphetamine group substances include amphetamine, methamphetamine and their derivatives, such as methcathinone, fenetylline, and methyl-phenidate (UNODC, 2015). Methamphetamine (street name frequently known as “crystal”, “glass”, “speed,” “ice,”) is being manufactured simply in illicit laboratories from readily accessible, cheap elements. Its chemical properties consist of ten carbon molecules, fifteen hydrogen molecules and one nitrogen molecules ($C_{10}H_{15}N$) (Figure 1.4). Ephedrine or pseudoephedrine are the most commonly used precursors for methamphetamine synthesis (Colfax *et al.*, 2010). Amphetamine group substances are being prescribed for a number of clinical conditions under strict rules and regulation (Schifano & Naidoo, 2010).

Esctasy group substances are synthesized from amphetamine derivatives, including methylenedioxy-methamphetamine (MDMA) and MDMA-like drugs. They are classified as ‘entactogens’ with no therapeutic use has been recognized so far.

Unlike opioid which commonly being administered by injection route, amphetamine-type stimulants are available in various form including tablets, crystalline and liquid which can be smoked, snorted, injected, or used per rectal (Chooi *et al*., 2017). ATS administration, particularly the amphetamine-group substances render the users being euphoric, experience heightened confidence level and lead to increase alertness, arousal, libido, energy levels and physical strength. Besides, ATS also raise heart rate, respiratory rate, blood pressure (Barr *et al.*, 2006). Meanwhile, methamphetamine use

disorder may lead to serious complication including neurological damage to brain, acute renal failure and toxic effect to cardiovascular system. (Albertson *et al.*, 1999; Barr *et al.*, 2006)

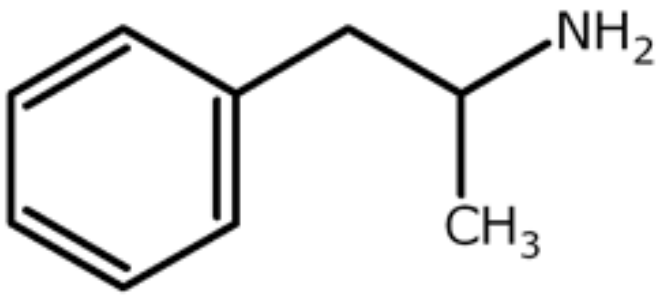

Figure 1.3 Chemical structure of amphetamine molecule

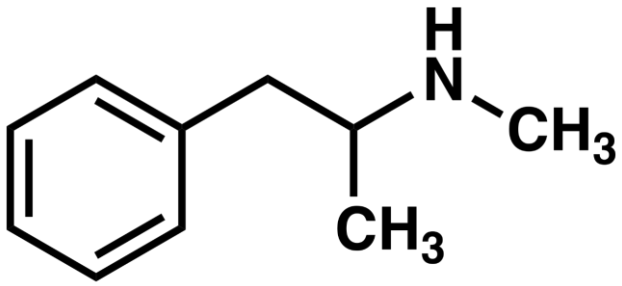

Figure 1.4 Chemical structure of methamphetamine molecule

ATS is a potent direct CNS stimulant that produces its rewarding effects by inducing a state of hyper-dopamine level extracellularly through three major mechanisms (Calipari & Ferris, 2013). First it inhibits dopamine uptake competitively by being dopamine transporter (DAT) substrate; second, it promotes dopamine release into the cytoplasm and out of vesicle; and third, independently to action potential induction to dopamine vesicular release, it promotes DAT to reversely transport dopamine into the synaptic cleft release (Goodwin *et al.*, 2009). It has been observed that ATS acts as powerful agonist for trace amine-associated receptor 1 (TAAR1) TAAR, where binding of amphetamine to this receptor lead to brain monoamines regulation including dopaminergic activity modulation (G. M. Miller, 2011).

1.1.4 Diagnostic and Statistical Manual of Mental Disorders (DSM-5)

Diagnostic and statistical manual of mental disorders (DSM) is the standard and manual used by clinicians and researchers for clinical diagnosis, research, policy, and reimbursement purpose which published by American Psychiatric Association (APA). APA is a national medical specialty society participated by more than 37,000 physician members who specialize in the diagnosis, treatment, prevention and research of mental illnesses, including substance use disorders. DSM has been reviewed five times since the first edition of DSM which was released in 1952. The most recent edition of the DSM (5^{th} edition) was published on May 18, 2013 at the 166^{th} Annual Meeting of the APA at San Francisco by APA President, Dr. Dilip Jeste (Vahia, 2013).

DSM-5 classify drug disorder under chapter of "Substance-Related and Addictive Disorders" that which merges substance abuse and substance dependence categories in DSM-IV into one disorder. Substance use disorder is diagnosed according to the presence of more than two symptoms from a list of 11 which include drug craving symptoms. Other symptoms include hazardous use of the drugs, social/interpersonal problems related to use, neglected major roles to use, withdrawal, tolerance, use larger amounts/longer, repeated attempts to quit/control use, much time spent using, physical / psychological problems related to use and activities given up to use (Brien *et al*., 2013).

1.1.5 Methadone maintenance therapy

Methadone maintenance was first discovered by Dole and Nyswander in 1960s in USA which later became model for opioid dependence substitutional therapy worldwide (Courtwright, 1997). Consistent evidence through extensive research had revealed the effectiveness and efficacy of methadone maintenance treatment in reduction of drug use, mortality rates (Gibson *et al*., 2008) , transmission of Hepatitis C (Alavian *et al*., 2013), HIV risk behaviours (Wong *et al*., 2003) and drug and property- related criminal behaviours (Russolillo *et al*., 2018). Besides, apart from being cost-effective, it helps to retain patients in treatment and significantly improving quality of life in the physical, social ,psychological,and environmental aspects (Ali *et al*., 2018).

In Malaysia, national harm reduction program was launched in 2004 to arrange and implement the harm reduction initiatives which include public education of drug use and HIV infection, introduction of needle & syringe exchange program (NSEP) and condom distribution (Reid *et al*., 2007). Initially, substitution therapy as part of harm

reduction programmes were rejected by Malaysia government as contradicting the aim of becoming a drug-free nation. However, extensive discussions and combined pressure from non-governmental organisations, medical expert and the public led to the approval of methadone maintenance therapy (MMT) as a pilot programme in early 2005 and became officially started started with ten MMT centers in October 2005 with two of them operated in private sector (Ng *et al*., 2009; MyTOS Report, 2018). By December 2013 there were 811 MMT centres (446 government facilities and 365 private setting) in this country that provided treatment for total of 65,259 opioid dependence patients (33444 in government facilities and 31, 805 in private settings) with 18 600 active patients (MyTOS Report, MOH, 2018).

Methadone is a synthetic opioid with high bioavailability and readily absorbed from the intestine (Dale *et al*., 2004). It has long half-life which will result in longer duration of action and reaches stable-state plasma levels rapidly after recurrent administration. It is a direct μ, δ and κ receptors agonist which indirectly will increase dopamine level extracellularly (Garrido & Trocóniz, 2000). Besides, it is a single dose on daily basis, taken orally and is effective to reduce cravings and withdrawal symptoms. This will help the patients to do their daily activities efficiently and get job hence improving their social functioning and stability (Baharom *et al*., 2012). At appropriate dose and right usage, methadone is non-toxic, non-sedating, with fewer side-effects mainly excessive sweating and constipation which slowly diminished over time (Ng *et al*., 2009). Apart from the physical benefits, as it is being taken orally, the risk of transmission of bloodborne diseases will subsequently reduce. Taken together, the pharmacokinetics and pharmacodynamic properties of methadone make it an ideal drug of choice for opioid dependence substitutional therapy.

1.2 Dopamine system and drug addiction

1.2.1 Dopamine system

Dopamine refers to 3,4-dihydroxy-L-phenylalanine (L-DOPA) derivative (Rub *et al.*, 2010). It is an important neurotransmitter which belongs to catecholamine group as it poses catechol structure (a benzene ring with two hydroxyl side groups) with one amine group attached to an ethyl chain (Figure 1.5). Dopamine presents in various systems in our body both central and peripherally.

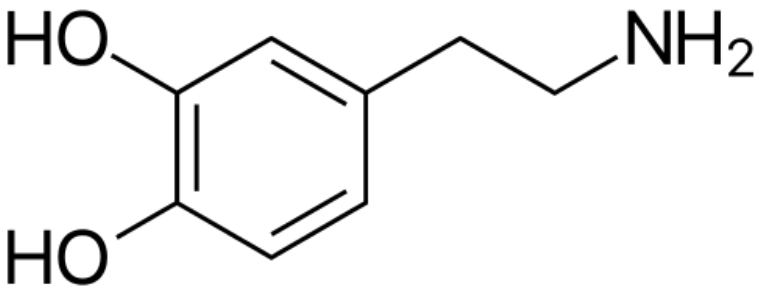

Figure 1.5 Structure of dopamine molecule

1.2.1 (a) Central dopamine systems

Dopamine is predominantly the most essential neurotransmitter in the brain. It forms approximately 80% of all brain catecholamine (Vallone *et al*., 2000). Apart of its well-known cardinal role in brain reward system, dopamine also plays important function which determines by dopamine's site release. Ventral midbrain (ventral tegmental area and substantia nigra) is the major brain dopamine's location from which projects to four axonal pathways: (1) nigro-striatal; (2) mesolimbic; (3) mesocortical; and (4) tuberoinfundibular.

The nigro-striatal pathway arises from the substantia nigra compacta (SNC) in the midbrain and projects to the dorsal striatum (caudate-putamen) (Qiu *et al*., 2019). This pathway regulates movement control and its degeneration results in Parkinson's disease, observed as tremors, dyskinesia and rigidity. Two pathways which begins from the ventral tegmental area (VTA) project out to innervate different regions of the frontal cortex forming meso-cortical pathway. The other one innervates parts of limbic system, the ventral striatum (nucleus accumbens), and the olfactory tubercle (OT) forming mesolimbic pathway. The meso-cortical pathway is responsible for memory and learning center whereas the mesolimbic pathway is important for behavior and motivation regulation. The last pathway which is the tuberoinfundibular pathway arises from hypothalamus (arcuate nuclei) and perventricular cells, projects to median eminence of the hypothalamus, whereby dopamine is released into the perivascular spaces of the capillary plexus of the hypothalamic–hypophyseal portal system. Then dopamine is transported to the anterior pituitary and inhibit prolactin release by its

action on lactotroph (Vallone *et al.*, 2000; Ikemoto *et al.*, 2010). Figure 1.6 summarizes the dopaminergic pathway and its function.

1.2.1 (b) Peripheral Dopamine System

Apart from its prominent role in central nervous system, dopamine is an important catecholamine peripherally. As dopamine does not pass through blood brain barrier, the peripheral dopamine system has different signaling pathway of brain dopamine system which is closely linked to peripheral sympathetic nervous system. Synthesis of peripheral dopamine is both related and not related to neuronal properties as it is originated at least from three sources mainly from chromaffin cells in adrenal medulla, sympathetic neuronal fibers and additional source from amine precursor uptake and decarboxylation cells (APUD) cells (Rubí & Maechler, 2014). Plasma dopamine level is determined by sympathetic neurons activation and chromaffin cells of adrenal medulla which synthesize dopamine from L-3,4-dihydroxyphenylalanine (L-DOPA) as they express L-DOPA decarboxylase enzyme (Goldstein & Holmes, 2008). Meanwhile APUD cells are found in kidney pancreatic exocrine and endocrine cells, retinal cells, and peripheral leukocytes in which dopamine's action varies according to the release site. In general, peripheral dopamine involves in regulation of sodium excretion and urine output in kidney as well as regulating blood pressure, respiration, gastrointestinal motility, and circadian rhythms in retina (Rubí & Maechler, 2014)

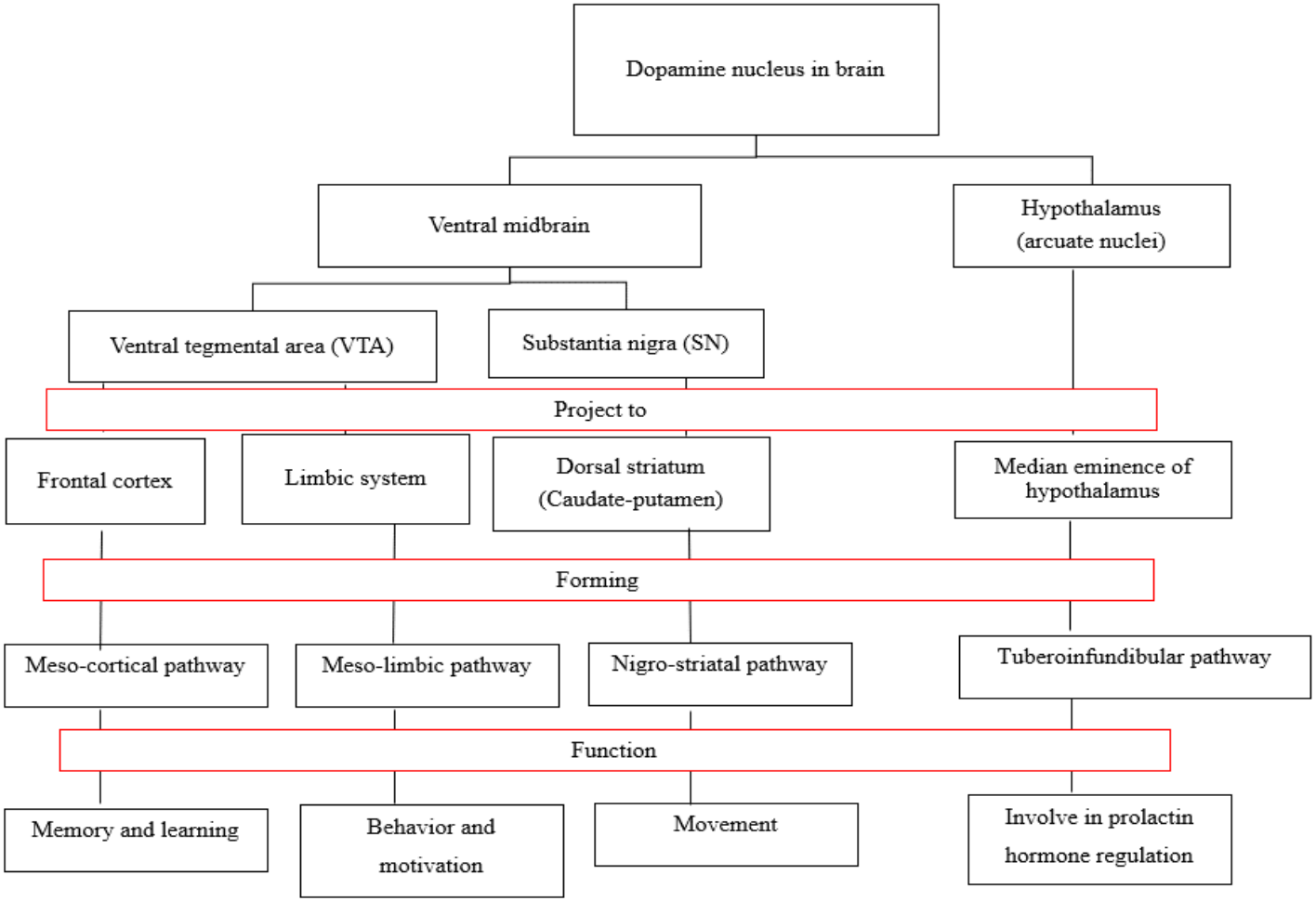

Figure 1.6 The doparminergic pathway and its function (Vallone *et al.*, 2000; Ikemoto *et al.*, 2010)

1.2.2 Dopamine Receptors

Dopamine plays its critical role in central nervous system and peripherally through actions mediated by specific receptors of multiple subtypes which include five distinct G protein-coupled receptor subtypes. Two D1-like receptor subtypes (D1 and D5) couple to the G protein Gs and activate adenylyl cyclase. The other receptor subtypes belong to the D2-like subfamily (D2, D3, and D4). They are prototypic of G protein-coupled receptors that inhibit adenylyl cyclase and activate K+ channels (Missale *et al.*, 1998).

Dopamine receptors belong to the D1-like and D2-like receptor subtypes that are believed to be situated post-synaptic and pre-synaptic respectively. Central D2 dopamine receptor activation regulate prolactin synthesis and release from anterior pituitary while D3 and D4 receptors are found in CNS area mediating cognitive function (Jaber *et al.*, 1996).

In cerebrovascular and renal system, D1-like receptors stimulation causes direct vasodilatation and reduce vascular resistance whereas D2-like receptors stimulation lead to indirect vasodilatation, by sympathetic vasoconstrictor tone inhibition. Besides, dopamine acts as potent positive inotropic agent towards cardiac muscle as well as induces diuresis and natriuresis. Dopamine receptor protein immunohistochemistry confirmed the localization of dopamine D1 and D5 receptors presence in the tunica media of systemic arteries and of presynaptic dopamine D2-D4 receptors closely

associated with sympathetic neuroeffector junctions. Arrighi *et al., (*2009) reported that renal system expresses all the five distinct dopamine receptors subtypes.

Human peripheral blood lymphocytes also express all dopamine receptors subtypes as well as vesicular dopamine transporters which is important in modulation the immune responses under physiological and pathological conditions (Buttarelli *et al.,* 2011). Peripheral dopaminergic system D1-like and D2-like receptors are detected in pulmonary nerve trunk which are found on pulmonary afferents of immunoreactive fibers (Amenta *et al.,* 2002). A concomitant stimulation of D1 and D2 receptors is required, a phenomenon known as the "requisite" D1/D2 synergism (Hasbi *et al*., 2011). Study by Capper-Loup *et al.,* (2002) also provide evidence that evocation of neural and behavioural sensitization to cocaine in rats only occurred in participation of both D1 and D2 receptors activation. On the other hand, the ability of D2 agonists to induce changes in basal ganglia single unit activity and spontaneous motor activity is dependent upon the presence of endogenous dopamine to stimulate D1 receptors (Walters *et al.,* 1987). These observations revealed that synergism effect is only being generated in the presence of both D1 and D2 receptors (Hasbi *et al.,* 2011). In addition, D1-like receptors stimulation may reduce the seek reward motivation whereas the D2-like receptors facilitate the addictive drugs rewarding effects (Li *et al.,* 2006).

1.2.2 (a) Dopamine receptor in peripheral blood lymphocytes

Human peripheral blood lymphocytes (PBL) has been reported to synthesize endogenous dopamine including other related catecholamine; norepinephrine and epinephrine through tyrosine hydroxylase dependent pathway as well as expressing dopamine receptors and transporters. As dopamine also exert its effects peripherally,

the dopamine receptors in the brain may be parallel to their homologous receptors in peripheral blood lymphocytes (PBLs) (Vousooghi *et al.,* 2015). Interestingly, previous studies reported that central neurological disorders characterized by dysfunctional central dopaminergic neurotransmission also concomitantly caused PBL dopaminergic pathway dysfunction (Ostadali *et al.,* 2004).

Studies in schizophrenia disorder had revealed a consistent evidence of the changes in PBL dopamine receptors expression in which D3 receptors up regulation were observed in most cases and closely related to the disease severity. Study by Kwak *et al.,* (2001) that compared between dopamine receptors in PBL from treated and untreated schizophrenic patients and healthy subjects observed increased D3 receptor mRNA expression in PBL from unmedicated in comparison with medicated patients and healthy subjects. On the other hand, the decreased dopaminergic transmission in striatum that has been observed in Parkinson's disease, also showed reduction in dopamine receptor expression in PBL which is consistent with the disease severity clinically (Nagai *et al.,* 1996).

In 2004, Czermak *et al.,* (2004) observed reduced expression of D4 mRNA in PBL in long-term abstinent alcohol and heroin addicts which proposed dopamine imbalance is persistent in abstinent addicts. It has been detected that D3 receptor mRNA expression in PBL, is increased in heroin-addicted and methadone-maintained subjects while D4 mRNA expression in PBL declined in heroin-abstinent and heroin-addicted subjects. In heroin-abstinent subjects solely showed reduction of D5 mRNA expression level (Goodarzi *et al.,* 2009).

Being relatively easily accessible and less invasive procedure to get blood cells instead of the technically difficulties in CNS in vivo study, these findings suggest that PBL dopamine system might represent an important promising tool to investigate the central dopamine pathologies as well as monitoring the efficiency of pharmacological and therapeutic intervention (Buttarelli *et al.,* 2011). In addition, to date there is no available dedicated biological marker designed to objectively measure the drug addiction severity (Ersche *et al.*, 2011).

1.2.3 Dopamine and drug addiction

Drugs of abuse is known to promote persistent neurobiological changes characterized by the compulsiveness of taking drug repeatedly hence causing craving and relapse. Being the brain reward centre, which acts as the common ultimate aim of majority of abused drugs, dopamine system (mesolimbic pathway) represents the most affected neurobiological systems of all (Czermak *et al.,* 2004). Administration of drugs of abuse particularly stimulant drugs (cocaine, amphetamine, methamphetamine, etc) either directly or indirectly, causing elevation of dopamine level in post synaptic region which in turn mediate the reinforcement, euphoria and experience of pleasure (rewarding effects) (Daberkow *et al.,* 2013).

Researches on dopamine's potential involvement in addiction has been dramatically expanded since 1970's pioneered by Olds *et al.,*(1997) discovery that observed the positive reinforcement in rats characterized by self-stimulation repetition on certain brain region with electricity. Imperato (1988) then developed the brain micro-dialysis technique in rats and found that dopamine level in nucleus accumbens was increased