ELUCIDATING THE EFFECT OF KRATOM ON STRIATAL DOPAMINE TRANSPORTER LEVEL USING ^{99m}Tc TRODAT-1 SPECT-CT IN KRATOM USERS

BY

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DECLARATION

I hereby declare that this research was sent to Universiti Sains Malaysia or the Degree of Master of Medicine (Nuclear Medicine) and has not been sent to other universities. With that, this research can be used for consultation and will be photocopied for reference.

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DISCLAIMER

I hereby declare that this dissertation records the results of the study performed by me and that it is of my own composition. I declare that I have no financial interest in the instruments or materials used in this study.

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OUD	Opioid use disorder
DAT	Dopamine transporter
7-OH	7-hydroxymitragynine
MOR	μ-opioid receptor
KOR	κ-opioid receptor
DOR	δ-opioid receptor
ORL-1	Opioid receptor like-1
BRET	Bioluminescence resonance energy transfer
HEK	Human embryonic kidney
GC-MS	Gas chromatograph with mass spectrometry
SUD	Substance use disorders
D_1R	Dopamine D_1 receptor
D_2R	Dopamine D_2 receptor
D_3R	Dopamine D_2 receptor
DAT	Dopamine transporter
CRF	Corticotropin-releasing factor
cAMP	Cyclic adenosine monophosphate
NAcb	Nucleus accumbens
VTA	Ventral tegmental area
CN	Caudate nucleus
Put	Putamen
StN	Subthalamic nucleus
GPe	Globus pallidus externa
GPi	Globus pallidus interna
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
VMAT2	Vesicular monoamine transporter 2
MSNs	Medium-sized spiny neurons
GPCR	G-protein coupled receptor

NMDA	N-methyl-D-aspartate
MAO-A	Monoamine oxidase A
MAO-B	Monoamine oxidase B
COMT	Catechol-o-methyltransferase
SPECT	Single photon emission computed tomography
CT	Computed topography
MRI	Magnetic resonance imaging
VOI	Volume of interest
SUR	Striatal specific uptake ratio
SUV	Standard uptake value
SUVr	Standard uptake value ratio

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ABSTRAK

Objektif: Untuk memperjelas kesan ketum kepada kepadatan pengangkut dopamin di striatum pengguna ketum dan kontrol sihat dengan menggunakan skan ^{99m}Tc-TRODAT-1 *single photon emission computed tomography with computed tomography* (SPECT-CT) dan MRI otak.

Metodologi: Satu kajian tinjauan rentas telah dijalankan kepada 12 orang pengguna ketum dan 13 orang kontrol sihat di Unit Perubatan Nuklear, Institut Perubatan dan Perubatan Termaju (IPPT) USM. Semua subjek adalah lelaki berumur di antara 18 sehingga 55 tahun dan tiada penyakit neurologi mahupun psikologi. Pengguna ketum yang dikaji mestilah menggunakan ketum untuk tempoh lebih daripada setahun dan tiada ketagihan dadah terlarang. Mereka juga disaring menggunakan kriteria DSM-V untuk ketagihan opioid di samping kuesioner HAM-A, PHQ-9 dan MMSE. Semua subjek kemudian menjalani skan ^{99m}Tc-TRODAT-1 SPECT-CT dan MRI otak. *Standard uptake value* (SUV) untuk setiap bahagian kaudat, putamen and striatum serta oksipital dilukis berpandukan struktur otak pada imej MRI yang telah digabung bersama imej SPECT-CT yang telah diproses.

Keputusan: Ujian kualitatif, kedua-dua penilai bersetuju terdapat penurunan kepadatan DAT pada putamen kanan (κ=0.824, p=0.004), kaudat kiri (κ=0.824, p=0.004) dan striatum kiri (κ=1.0, p<0.001). Ujian semi kuantitatif menunjukkan kepadatan DAT pada kaudat kanan lebih tinggi dengan SUVr 2.06±0.67 dalam pengguna ketum dan SUVr 1.51±0.31 dalam kontrol normal (p=0.019). Turutan itu, striatum kanan juga menunjukkan keapdatan DAT lebih tinggi dengan SUVr 1.98±0.50 dalam pengguna ketum dan SUVr 1.62±0.37 dalam kontrol normal (p=0.055). Ratio kaudat kepada putamen kanan juga lebih tinggi dalam penagih ketum pada 1.09±0.25 berbanding dengan to 0.89±0.14 (p=0.026). Peratus

asimetri kaudat juga tinggi pada 32.97% dalam penagih ketum berbanding 14.37% dalam kontrol normal (p=0.04). Parameter kajiselidik tidak menunjukkan perbesaan yang signifikan.

Kesimpulan:

Kajian ini menunjukkan SUVr kaudat kanan lebih tinggi pada penguna ketum kronik, menyerupai efek stimulan seperti kokain dan bukan seperti pengguna heroin kronik. Walaupun efek merokok tidak dapat dibuang, ^{99m}Tc-TRODAT-1 SPECT-CT-MRI mungkin masih boleh digunakan sebagai modaliti untuk meengesan kesan penagihan ketum kronik dengan kajian tambahan di masa hadapan.

ABSTRACT

Objective: To evaluate and compare striatal dopamine transporter (DAT) level in kratom users and healthy controls utilizing ^{99m}Tc-TRODAT-1 single photon emission computed tomography with computed tomography (SPECT-CT) and brain MRI.

Methods: A cross-sectional study involving 12 kratom users and 13 healthy controls was done at Unit Perubatan Nuklear, Institut Perubatan dan Perubatan Termaju (IPPT) USM. All subjects were male, aged between 18 to 55 years old without neurological or psychological diseases. kratom users were dependent on kratom for more than a year period and were free from illicit drugs abuse. Urine toxicology was done to exclude illicit drug abuse. They were also screened using DSM-V criteria for opioid dependence along with HAM-A, PHQ-9 and MMSE questionnaires. All subjects underwent ^{99m}Tc-TRODAT-1 SPECT-CT and brain MRI. The standard uptake value corrected with body weight (SUVbw) of bilateral caudate, putamen and striatum as well as occipital were drawn guided structurally by brain MRI images which were co-registered with processed SPECT-CT images. SUVr was calculated by dividing the target SUV with the occipital SUV.

Results: Qualitative evaluation by two nuclear medicine physician showed almost perfect agreement that there were reduced tracer uptake in the right putamen (κ=0.824, p=0.004) and left caudate (κ=0.824, p=0.004), consequently abnormal finding in the overall image (κ=1.0, p<0.001). Semiquantitative evaluation revealed significantly higher right caudate SUVr of 2.06±0.67 in kratom users compared to 1.51±0.31 in healthy controls (p=0.019), accordingly higher right striatum SUVr of 1.98±0.50 in kratom user compared to 1.62±0.37 in healthy controls (p=0.055). The right caudate to putaminal ratio was also

significantly higher at 1.09±0.25 compared to 0.89±0.14 (p=0.026) accordingly. The caudate AI% was high at 32.97% in kratom users versus 14.37% in healthy controls (p=0.04). The questionnaires parameter showed no significant difference between group.

Conclusions:

In conclusion, chronic kratom usage causes significantly higher right caudate SUVr, mimicking a pattern more like a cocaine-stimulant effect rather than the chronic heroin-opioid effect. Although the effect of smoking in kratom users on striatal DAT binding is inevitable in this study, 99mTc-TRODAT-1 SPECT-CT-MRI may still be used as a potential imaging modality to assess the effect of chronic kratom use with further investigations in the future.

CHAPTER 1

INTRODUCTION

Kratom or air biak, ketum or krypton is a traditional medicinal plant indigenous to Southeast Asia including Malaysia, Thailand and Philippines. It was traditionally used by farmers for its analgesic, anti-anxiety, anti-depression and energy boosting properties (Z. Hassan et al., 2013). It exerts stimulating effect at low dose and opioid-like effects at high dose making it a favourite substitute for opioid addiction and to overcome withdrawal symptoms (Andrew C Kruegel & Grundmann, 2018). Due to its dose-dependent morphine-like effect, it is now used as a recreational drug substitute and is marketed globally. It became popular as it is cheaper and widely available. On top of that, there were rare reporting of overdose kratom toxicity.

Regardless, the chronic use of kratom is perilous as it may become an entry drug for users before introduction to other illicit drugs. The Star on 25 September 2017 has reported that kratom abuse has risen with over 14,000 people arrested over the past two years since 2015. This makes kratom a national concern as addressed by the Health Ministry of Malaysia in 2019. Currently, kratom abuse is under the Poison Act 1952 and interests have been voiced to include it under the Dangerous Drug Act 1952 to ensure proper control can be taken (Tan, Carvalho, Sivanandam, & Rahim, 2019).

Interest has also risen towards kratom as they were recently investigated for treatment of opioid withdrawal as it has been found to attenuate withdrawal opioid symptoms (R. Hassan et al., 2020). This led to other researchers studying the effect of kratom abuse. In 2018, researcher found that regular kratom use did not alter brain structure (Singh et al., 2018). This is conflicting as kratom abusers do experience

withdrawal symptom. Addiction involves the reward or dopaminergic mesolimbic pathway. Thus, it is of our interest to explore the effect of kratom abuse physiologically, particularly to the dopamine transporter (DAT) utilizing ^{99m}Tc-TRODAT-1 SPECT-CT.

We aimed to elucidate the effect of kratom on the striatal DAT density in kratom abuser subjects. If there is any effect, we would proceed to assess the severity of the reduction of DAT binding level when the subjects are dependent on kratom. We would also like to determine the baseline dopamine transporter level in normal subjects.

The effect of kratom dependence on DAT binding level may change the local regulations. Currently in Malaysia, kratom is listed under the Poison Act 1952 instead of the drug act. This study will also provide a model data of normal striatal DAT level in healthy men in Malaysia. Up to now, there is no data collection on the normal striatal DAT level in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Kratom

M. speciosa Kroth from Rubiaceae family is a tropical broad-leaved tree. It can grow to over 15 metres in height. It has erected and branching stem with dark green leaves. The leaves are ovate and has tapered ends. The size of the leaves can reach over 18 cm long and 10 cm wide. It produces clusters of yellow flowers. The leaf and smaller stems are used for consumption (Z. Hassan et al., 2013). The leaves are dried and can be consumed either by chewing fresh or smoked or made into extract. Most commonly, it will be brewed with hot water and drunk as tea. To mask the bitter taste sugar or honey or carbonated drinks were added. In Southern Thailand, a cocktail known as '4x100' or 'sii koon roi' is made up of kratom leaves, soft drink containing caffeine and codeine- or diphenhydramine-containing cough syrup giving added antidepressant, anxiolytic and analgesic effects (Tanguay, 2011). Fatalities have been reported after consumption of aforementioned cocktail due to its multidrug action (Tungtananuwat & Lawanprasert, 2010). They are also marketed commercially in many preparations including resin, dried leaf or in powder form.

To date, there have been more than 50 alkaloids identified within Kratom as listed by Manwill et al. in 2022 (Figure 1) (Manwill et al., 2022). Mitragynine (chemically 9-methoxy-corynantheidine) is the most abundant alkaloid (66%). It has antinociceptive effect. Another alkaloid, 7-Hydroxymitragynine, though has low and varied concentration up to 2%, is also a potent analgesic like mitragynine and morphine (Trakulsrichai et al., 2015). These alkaloids are responsible for the analgesic, antitussive and antidiarrheal effect (Z. Hassan et al., 2013). Mitragynine is metabolized in the liver into other

metabolites (Philipp et al., 2009; Trakulsrichai et al., 2015). Urine analysis using liquid chromatography-tandem mass spectrometry showed very low concentration of unchanged mitragynine at 0.14%, suggesting it is not a significant route of excretion. Adverse effect or acute toxicities reported in human studies include xerostomia, nausea, vomiting, abnormal urination, constipation, anorexia, nystagmus, weight loss. Chronic abusers have reported hyperpigmentation, anorexia, tremor and psychosis (K. M. Babu, C. R. McCurdy, & E. W. Boyer, 2008). Listed in Table 1 is the alkaloid profile of *Mitragyna speciosa* Korth which was adopted from (Z. Hassan et al., 2013).

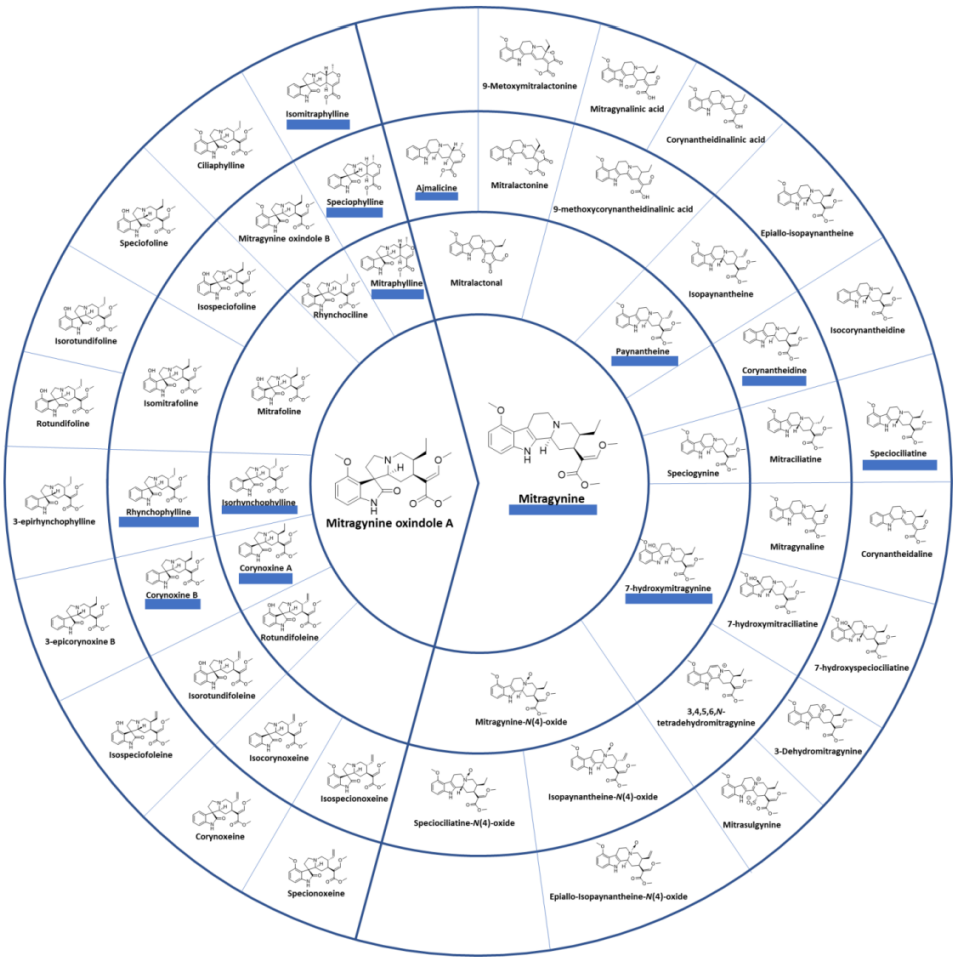

Figure 1 Schematic representation of the 54 compounds that have been reported from Mitragyna speciosa, showing the interrelatedness of the structures.

Compounds to the right are indole alkaloids, while those to the left are oxindole alkaloids. Compounds underlined in blue are commercially available (as of 2019); however, we strongly recommend verifying both the purity and identity of any purchased standards. Adopted from (Manwill et al., 2022)

Kratom is also known to exert an opioid-like activity (Thongpradichote et al., 1998; Yamamoto et al., 1999) with stimulant effects at lower doses and sedative-like effects at higher doses (Kavita M Babu, Christopher R McCurdy, & Edward W Boyer, 2008). Mitragynine, the primary indole alkaloid constituent, displays high affinity to opioid receptors which mediate respiratory depression, analgesia and euphoria. The antinociceptive activity was shown to be mediated mostly by the μ- and δ-opioid receptors (A. C. Kruegel et al., 2016; Meireles et al., 2019; Yamamoto et al., 1999). 7-hydroxymitragynine (7-OH), an oxidized derivative, though a minor alkaloid in kratom has been reported to induce analgesic effect through opioid-receptors exceeding the effect obtained from morphine (Matsumoto et al., 2004; Takayama et al., 2002). A study evaluating kratom compounds effect in human embryonic kidney (HEK) cells using bioluminescence resonance energy transfer (BRET) functional essays, found that Mitragynine act as partial agonist at human μ-opioid receptor (hMOR), competitive antagonist at human κ-opioid receptor (hKOR) and low potency antagonist at human δ-opioid receptor (hDOR) (A. C. Kruegel et al., 2016). They also found that 7-OH acts as a potent partial agonist at hMOR, competitive antagonist at hKOR and hDOR (A. C. Kruegel et al., 2016). Displaying a biased signalling at MOR has opened a possibility for kratom to be explored as an opioid analgesic with reduced side effects compared to current treatment options.

Alkaloid profile of *Mitragyna speciosa* Korth. The percentage is the estimated content in the alkaloid extracts.

Alkaloid	Percentage	Effect	Reference
Mitragynine	66%	Analgesic, antitussive, antidiarrheal, adrenergic, antimalarial	Hooper (1907); Field (1921); Lee et al. (1967); Ponglux et al. (1994)
Paynantheine	9%	Smooth muscle relaxer	Ponglux et al. (1994)
Speciogynine	7%	Smooth muscle relaxer	Lee et al. (1967); Shellard, 1974; Shellard et al. (1978b); Ponglux et al. (1994)
7-Hydroxymitragynine	2%	Analgesic, antitussive, antidiarrheal	Ponglux et al. (1994)
Speciociliatine	1%	Weak opioid agonist	Lee et al. (1967); Ponglux et al. (1994)
Mitraphylline	<1%	Vasodilator, antihypertensive, muscle relaxer, diuretic, antiamnesic, immunostimulant, anti-leukemic	Seaton et al. (1958); Shellard, 1974; Shellard et al. (1978b); Ponglux et al. (1994)
Isomitraphylline	<1%	Immunostimulant, anti-leukemic	Seaton et al. (1960); Shellard and Philipson (1966); Ponglux et al. (1994)
Speciophylline	<1%	Anti-leukemic	Shellard and Philipson (1966); Beckett et al. (1966)
Rhynchophylline	<1%	Vasodilator, antihypertensive, calcium channel blocker, antiaggregant, anti-inflammatory, antipyretic, anti-arrhythmic, antithelmintic	Seaton et al. (1960); Shellard, 1974; Shellard et al. (1978b)
Isorhynchophylline	<1%	Immunostimulant	Seaton et al. (1958); Seaton et al. (1960); Shellard, 1974; Shellard et al. (1978b)
Ajmalicine	<1%	Cerebrocirculant, antiaggregant, anti-adrenergic, sedative, anticonvulsant, smooth muscle relaxer	Beckett et al. (1966)
Corynantheidine	<1%	Opioid agonist	Takayama et al. (2002)
Corynoxine A	<1%	Calcium channel blocker, anti-locomotive	Shellard et al. (1978a)
Corynoxine B	<1%	Anti-locomotive	Shellard et al. (1978a)
Mitrafoline	<1%		Hemmingway et al. (1975); Shellard et al. (1978a)
Isomitrafoline	<1%		Hemmingway et al. (1975); Shellard et al. (1978a)
Oxindale A	<1%		Shellard et al. (1978a)
Oxindole B	<1%		Shellard et al. (1978a)
Speciofoline	<1%	Analgesic, antitussive	Hemmingway et al. (1975)
Isospeciofoline	<1%		Hemmingway et al. (1975); Shellard et al. (1978a)
Ciliaphylline	<1%	Analgesic, antitussive	Trager et al. (1968)
Mitraciliatine	<1%		Lee et al. (1967)
Mitragynaline	<1%		Houghton et al. (1991)
Mitragynalinic acid	<1%		Houghton et al. (1991)
Corynantheidalinic acid	<1%		Houghton et al. (1991)

Table 1 Alkaloid profile of *Mitragyna speciosa* Korth. The percentage is the estimated content in the alkaloid extracts. Adopted from Hasan et al. 2013.

2.2 Kratom Abuse in Malaysia

In Malaysia, kratom is commonly ingested as a brewed solution. The solution is either prepared at home or bought from familiar local sellers. Despite being banned, it is still widely available and most areas have no stigma towards kratom consumption since it is a traditional folk remedy which has been long used. Analysis using gas chromatograph with mass spectrometry (GC-MS) equipped with 7637B autosampler and electron capture showed that there was 24.06 to 28.93 mg of mitragynine in a 0.25 litre glass of kratom samples taken from Penang area. This sums up to a daily consumption of 72.18 to 86.79 mg of mitragynine for three medium-sized glass per day (Z. Hassan et al., 2013; Singh et al., 2018). This dose was widely held to ease opiate withdrawal symptoms.

Mean age of user was 38.7 years with majority had lower secondary education and had irregular employment (Vicknasingam, Narayanan, Beng, & Mansor, 2010). Vicknasingam and friends also found that around 90% of kratom users reported that they started using kratom to alleviate addiction to other drugs while around 84% others use it to minimize withdrawal symptoms of opioid addiction. Almost 78% of users reported failure of withdrawal from kratom dependence. Side effects reported include chronic fatigue, intense craving, insomnia, watery eyes and nose, sudden neuralgia, frequent sweating, and intermittent discomfort at lumbar region.

So far, there is no diagnostic nor withdrawal criteria specific for Kratom use either in DSM-V or ICD-10. In clinical setting, diagnosis of kratom abuse is generally and usually made utilizing Opioid dependence by DSM-V criteria (Association, 2013) with HAMA-R rating the anxiety level during withdrawal (Hamilton, 1959), as per opioid addiction management. Hence, diagnostic modality to investigate kratom abuse is gaining relevance. Laboratories can now detect kratom alkaloids in blood and urine. For imaging, brain MRI scans of 7 male kratom users for more than a year showed no significant differences in brain structure or volumes, diffusion tensor imaging (DTI) metrics, fractional anisotropy (FA) and mean diffusivity (MD), in comparison to normal controls (Singh et al., 2018). Nevertheless, the small sample size might limit the power to detect a significant difference.

Yet, no molecular imaging study has been reported for investigation of kratom use specifically. In line with having opioid-like effect, we followed through a study which showed there were reduced striatal DAT in subjects who were heroin and METH dependence and were abstinent during the study (Yuan et al., 2017).

2.3 Opioid Addiction and Dopamine System

Besides the notorious illicit heroin, synthetic opioids like fentanyl, morphine, oxycodone and codeine are widely used as analgesia. It also helps with cough, diarrhoea and cancer-related pain disorder. These opioids activate MORs in the reward centre to exert its effect. In addition to having analgesic effect, the MOR system also plays a role in enhancing mood and modulate euphoria by activating the dopamine reward pathways.

The roles of opioid and dopamine systems in substance use disorders (SUD) are well recognized (Koob & Volkow, 2016; Roy A. Wise & Robble, 2020). Dopamine transmission dysregulation appears the same across different addictions, but the correlation between neurochemistry and drug seeking behaviours appears to vary with the particular drug of abuse (Martinez et al., 2012). To date, four different opioid receptor systems mu (μ), delta (δ), kappa (κ), opioid receptor like-1 (ORL-1) and their genes has been characterized at the cellular, molecular and pharmacological levels.

During addiction, changes in opioid and dopamine signalling in the basal ganglia causes drug rewards and incentive salience (Koob & Volkow, 2016). Reward is defined as any event that increases the probability of a response with a positive hedonistic component. Drug reward happens when the drugs release dopamine and opioid peptides into ventral striatum. Consequently, the fast and high steep dopamine release activates low affinity dopamine D_1 receptors (D_1R), giving the rewarding sensation of the so-called 'high' (Caine et al., 2007; R. A. Wise, 2009). On the other hand, dopamine stimulation of high affinity dopamine D_2 receptors (D_2R) is inadequate for drug rewards. Thus, only drugs that trigger phasic dopamine firing is associated with rewarding stimuli. Key neurotransmitters involved in this neuroadaptation include dopamine, enkephalins, γ-aminobutyric acid, glutamate, dynorphin, norepinephrine, corticotropin-releasing factor (CRF), endocannabinoids and neuropeptide Y.

Incentive salience is defined as motivation for rewards derived from one's physiological state and previously learned associations about a reward cue that is mediated by the mesocorticolimbic dopamine system (Robbins, 1976). Conditioned reinforcement is the association of previously natural stimuli with drug use effect. Both these conditions promotes compulsive seeking of drug, self-administration behaviours and the transformation to habit-like compulsive drug seeking behaviour (Koob & Volkow, 2016). D_1R stimulates cyclic adenosine monophosphate (cAMP) signalling in acute drug reward and conditioning while D_2R which inhibits cAMP signalling, are not necessary for drug reward as they are stimulated by both phasic and tonic dopamine release (Koob & Volkow, 2016). High affinity dopamine D_3 receptors (D_3R) co-localise in the nucleus accumbens (NAcb) with D_1R, are also associated with drug-seeking behaviour.

Chronic irritability, malaise, emotional pain, stress, alexithymia, dysphoria and loss of motivation are experiences reported during withdrawal. Across all major drug abuse studied in laboratory animals, this stage is characterized by elevations in reward threshold. Chronic drug exposure-induced neurochemical changes in acute drug reward changes the brain in which it decreases dopaminergic and serotonergic transmission in the NAcb during drug withdrawal, increased in MOR responsivity during opioid withdrawal, decreased GABAergic and increased NMDA glutamatergic transmission in the NAcb (Koob & Volkow, 2016). These changes in reward system function may persist.

2.4 Mesolimbic Dopamine Pathway

The Striatum

The mesolimbic system exerts a central role in motivated behaviours, various type of rewards and cognitive processes. It is a central nervous system circuit which begins in the