CHARACTERIZATION OF THE REWARD STRUCTURAL CONNECTIVITY IN FEMALE MALAY ADOLESCENTS USING DIFFUSION MAGNETIC RESONANCE IMAGING

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by

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LIST OF SYMBOLS

B_0	Strong external magnetic field
b0	No diffusion image where b-value is zero
v_n	eigenvector
λ_n	eigenvalue
α	Alpha which is the probability of finding significance where there is none.
β	Beta which is the probability of not finding significance when it is there.

LIST OF ABBREVIATIONS

AADK	Agensi Antidadah Kebangsaan
ACC	anterior cingulate cortex
AD	axial diffusity
BAS	Behavioural Activation System
bedpostX	Bayesian Estimation of Diffusion Parameters Obtained using Sampling
BET	Brain Extraction Tool
BIS	Behavioural Inhibition System
DA	dopamine
dMRI	diffusion magnetic resonance imaging
dlPFC	dorsolateral prefrontal cortex
DTI	diffusion tensor imaging
DWI	diffusion-weighted imaging
FA	fractional anisotropy
FDT	FMRIB diffusion toolbox
fMRI	functional magnetic resonance imaging
FMRIB	Functional Magnetic Resonance Imaging of the Brain
FOV	field of view
FSL	FMRIB Software Library
FSLEyes	FMRIB Software Library image viewer
GABA	gamma aminobutyric acid
HD	heavy drinkers
HP	hippocampus
JEPeM	*Jawatankuasa Etika Penyelidikan Manusia*
LH	lateral hypothalamus
M	mean
MD	medial diffusivity
MID	monetary incentive delay
MRI	magnetic resonance imaging
mOFC	medial orbitofrontal cortex
mPFC	medial prefrontal cortex

MPRAGE	magnetized-prepared rapid gradient-echo
NAcc	nucleus accumbens
OFC	orbitofrontal cortex
PFC	prefrontal cortex
probtrackX	probabilistic tracking with crossing fibres
RD	radial diffusivity
RF	radiofrequency
ROI	region of interest
RRS	reward responsive scale
SN	substantia nigra
SN/VTA-HP	substantia nigra/ventral tegmental area-hippocampal loop
SN	substantia nigra pars compacta
T	tesla
TE	Echo time
TR	Repetition time
USM	Universiti Sains Malaysia
V	volume
vlPFC	ventrolateral prefrontal cortex
VS	ventral striatum
VTA	ventral tegmental area

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MENEROKA HUBUNGAN STRUKTUR BAGI GANJARAN DI DALAM REMAJA PEREMPUAN MELAYU MENGGUNAKAN PENGIMEJAN MAGNET RESONAN DIFUSI

ABSTRAK

Pengenalan: Rangkaian otak berkaitan dengan sistem ganjaran banyak dikaji selidik kerana ia berkaitan dengan ketagihan dan kepekaan ganjaran. Kaum belia telah dibuktikan di dalam kajian lepas mempunyai lebih kepekaan ganjaran jika dibandingkan dengan kumpulan umur yang lain. Kajian lepas juga telah menunjukkan bahawa litar jirim putih antara bahagian otak yang berkaitan dengan sistem ganjaran mempunyai kaitan dengan kepekaan ganjaran.

Objektif: Oleh sebab kekurangan kajian terhadap sistem rangkaian ganjaran remaja perempuan Melayu Malaysia, tujuan penyelidikan ini adalah untuk mengkaji struktur penyambungan jirim putih berkaitan ganjaran pada 15 belia Melayu Malaysia perempuan yang sihat dengan mengira kebarangkalian sambungan relatif diantara kawasan punca nukleus akumbens (NAcc) ke 6 kawasan sasaran iaitu amigdala, *anterior cingulate cortex* (ACC), *medial orbitofrontal cortex* (mOFC), hipokampus, *ventrolateral prefrontal cortex* (vlPFC) dan *dorsolateral prefrontal cortex* (dlPFC). Kajian ini juga telah menyelidik corak distribusi daripada operasi *parcellation* pada NAcc yang menunjukkan sambungan dengan 6 kawasan sasaran yang dikaji.

Kaedah: Pengimejan magnet resonan difusi (dMRI) digunakan untuk mengkaji struktur penyambungan jirim putih pada litar berkaitan ganjaran dengan menggunakan traktografi kebarangkalian untuk setiap peserta dengan mengira

bilangan aliran diantara kawasan punca dan setiap kawasan sasaran (amigdala, ACC, mOFC, hipokampus, vlPFC dan dlPFC).

Hasil kajian: Hasil kajian menunjukkan sampel peserta mempunyai respons ganjaran yang biasa pada peserta sihat. Mereka mempunyai kebarangkalian sambungan relatif yang paling tinggi antara NAcc dengan mOFC dan corak *parcellation* juga menunjukkan paling luas kawasan sambungan di NAcc dengan mOFC jika dibandingkan dengan 5 kawasan sasaran lain yang dikaji pada dua-dua belah otak.

Kesimpulan: Hasil kajian tersebut menunjukkan bahawa NAcc paling kuat sambungannya dengan mOFC berbanding dengan 5 kawasan sasaran yang lain. Oleh itu, ia memberi sokongan bahawa penyambungan NAcc sangat khusus kepada mOFC. Selain daripada itu, hasil penyelidikan ini juga boleh dikaitkan dengan bukti kajian lepas yang menunjukkan kematangan yang awal pada litar NAcc-mOFC.

CHARACTERIZATION OF THE REWARD STRUCTURAL CONNECTIVITY IN FEMALE MALAY ADOLESCENTS USING DIFFUSION MAGNETIC RESONANCE IMAGING

ABSTRACT

Introduction: The reward network is highly investigated as it is known to be involved in substance addiction and reward sensitivity. Adolescents have been shown to be more reward sensitive compared to other age groups. Previous studies have also shown that the white matter tracts between the frontostriatal reward-related brain regions was associated with reward sensitivity.

Objective: Since the reward network of female Malaysian Malay adolescents is understudied, the aim of this study was to characterize the white matter structural connectivity of the frontostriatal reward circuit of 15 healthy female Malaysian Malay adolescents by determining the relative connection probability of nucleus accumbens (NAcc) seed region to amygdala, anterior cingulate cortex (ACC), medial orbitofrontal cortex (mOFC), hippocampus, ventrolateral prefrontal cortex (vlPFC) and dorsolateral prefrontal cortex (dlPFC). This study also investigated the pattern of distribution from the parcellation of the NAcc corresponding to the connectivity of the 6 targets.

Methodolgy: Diffusion magnetic resonance imaging (dMRI) was used to study the reward structural connectivity via probabilistic tractography which was performed for each subject by calculating the number of streamlines between the seed (NAcc) and each target mask (amygdala, ACC, mOFC, hippocampus, vlPFC and dlPFC).

Results: The result showed that the sample with typical reward responsiveness for healthy participants had significantly the highest relative connection probability of NAcc to mOFC, while the NAcc parcellation showed the widest distribution of connection to mOFC compared to the other 5 targets in both sides of the brain.

Conclusion: Both of these findings support that NAcc and mOFC have the highest connection strength compared to the 5 targets. This supports previous study that shows NAcc is highly specific to the connection to mOFC. This finding can be explained by prior evidence showing early maturing of the NAcc-mOFC tract.

CHAPTER 1

INTRODUCTION

1.1 Background of study

The reward structural connectivity has previously been studied especially concerning reward sensitivity and addiction. Adolescents, which are defined as 10 to 25 years of age from previous brain studies, are known to be associated with higher risk-taking behaviour and impulsivity (Ikuta et al., 2018; Sawyer et al., 2018; van Duijvenvoorde et al., 2016). Adolescence is also often the time of onset for substance abuse (Arain and Johal, 2013; Jaworska and MacQueen, 2015; van Duijvenvoorde et al., 2016). This may be due to the constant development of brain regions during the adolescence period (Arain and Johal, 2013; Sawyer et al., 2018; Somerville, 2016). Adolescents have also been shown to have higher reward sensitivity compared to other age group (Karlsgodt et al., 2015; Schreuders et al., 2018; Steinberg et al., 2018; van den Bos et al., 2015). Hence, researchers have been studying the reward structural connectivity of this age group especially in relation to substance abuse in order to understand further the inner workings of their brain. Diffusion MRI is an excellent tool for investigating the white matter integrity connecting the reward regions of the adolescent's brain (Squeglia et al., 2015; van den Bos et al., 2015; Yuan et al., 2018).

1.2 Problem Statement & Study Rationale

This research was done to identify the white matter structural connectivity of the frontostriatal reward circuit of healthy Malaysian Malay female adolescents for future research comparisons. This enabled comparisons between those with disorders relating to the reward circuit especially addiction (Dubourg et al., 2017; Squeglia et al., 2016). The reward structural connection was tracked according to regions

identified from previous studies related to impulsivity, reward sensitivity and addiction (van den Bos et al., 2014, 2015; Yuan et al., 2018). The white matter was characterized in terms of strength of its connectivity specifically through relative connection probability between the NAcc and 6 reward-related regions which are the amygdala, anterior cingulate cortex (ACC), medial orbitofrontal cortex (mOFC), hippocampus, ventrolateral prefrontal cortex (vlPFC) and dorsolateral prefrontal cortex (dlPFC). To ensure participants had typical reward responsiveness, an online questionnaire was given to them which includes the Reward Responsiveness scale and the Behavioural Inhibition System and Behavioural Activation System (BIS/BAS) scale (Assari et al., 2020; Atkinson, 2018; Carver and White, 1994; Van den Berg et al., 2010). The data obtained enabled future studies to make comparisons based on the calculated white matter connectivity, which was indexed by streamlines that represent the probability of the structural reward connections.

1.3 Research Questions

1. What are the characteristics of the reward structural connectivity in terms of connection strength which are obtained from the number of streamline in Malaysian Malay female adolescents?
2. What are the characteristics of the reward structural connectivity in terms of parcellation of NAcc in Malaysian Malay female adolescents?

1.4 Study objectives

1.4.1 General objective

To identify the characteristic of the reward structural connectivity in Malaysian Malay female adolescents.

1.4.2 Specific objective

1. To determine the connection strength from the relative connection probability of NAcc to amygdala, ACC, mOFC, hippocampus, vlPFC and dlPFC.

2. To parcellate the NAcc according to its connectivity with amygdala, ACC, mOFC, hippocampus, vlPFC and dlPFC and to describe the pattern of distribution.

1.5 Research Hypothesis

1. Relative connection probability and parcellation of the NAcc to the 6 reward-related target regions would show the highest connection probability hence strongest connectivity to the mOFC (van den Bos et al., 2014, 2015; Yuan et al., 2018) compared to the other 5 target regions.
2. Parcellation of the NAcc would show more widely distributed pattern of connectivity to the mOFC compared to the other 5 target regions.

1.6 Operational definition

a) Diffusion magnetic resonance imaging

Diffusion magnetic resonance imaging (dMRI) is potentially capable of defining the reward network structural connectivity in a healthy or even lesioned brain. This imaging technique is MRI-based and can be used to obtain the orientation of the brain's white matter fibre tracts (Johansen-berg et al., 2007; Jung and Kim, 2020; Leuze et al., 2021; Morie et al., 2017). The three-dimensional shape and direction of water molecule diffusion through tissues in the white matter tracts will be measured voxel by voxel in the brain (Leuze et al., 2021; Meoded et al., 2020; Pajevic and Pierpaoli, 2000; Poretti et al., 2013). The directional dependence of diffusivity of the water

molecules is called anisotropy. During adolescence, an atypical reward network has been implicated to be an initiation of the substance use (Cservenka et al., 2014; Squeglia et al., 2015, 2017; Squeglia and Cservenka, 2017; Squeglia and Gray, 2016). Hence, the healthy typical reward network of adolescents should be studied as future studies may be able to make comparisons with those having atypical reward network or those with disorders related to the reward system.

b) Probabilistic tractography

Probabilistic tractography is a diffusion MRI analyses technique that can be used to investigate the brain structural connection in terms of microstructure indices and connection strength via the number of streamlines. This technique allows the reconstruction of fibre tracts within the brain based on the water diffusivity in dMRI (Caan, 2016; Heidi Johansen-Berg et al., 2010; Mollink et al., 2016). Particularly, this experiment focuses more on the strength of the white matter connectivity indexed by streamlines that putatively represent the probability of the structural connections.

c) Relative Connection Probability

The relative connection probability is the relative connection strength between the investigated seed region to the respective targets. Initially, the connection probability of the seed voxel to specific target regions is extracted which is represented by the number of fiber samples that reached a certain target area divided by the total number of samples propagated from a voxel (Samsir et al., 2018; Thanarajah et al., 2016). The connection probability value can be obtained through probabilistic tractography. The current study examined the relative connection probability between the NAcc region and the 6 target regions amygdala, ACC, mOFC, hippocampus, vlPFC and dlPFC.

d) NAcc segmentation

The NAcc segmentation is segregating the grey matter seed in accordance with the route of white matter projections to the cortical and subcortical target regions examined (Jbabdi and Johansen-berg, 2011; Sparks et al., 2020; van den Bos et al., 2015; Zhao et al., 2017). This can be done via the parcellation technique which was available in FSLeyes which is part of the FSL software package. Other than obtaining the relative connection probabilities between the target regions for comparison, parcellation allowed the researcher to visually determine the difference in relative connection strength between the seed and the targets which can help with the analysis.

e) Reward responsiveness

In order to determine that the sample of adolescents of the current study had a typical reward sensitivity and impulsivity of healthy adolescents, scales that tests reward responsiveness were recently given to the participant in the form of an online questionnaire. The Reward Responsive scale and the Behavioural Inhibition System and Behavioural Activation System (BIS/BAS) scale (Assari et al., 2020; Atkinson, 2018; Carver and White, 1994; Van den Berg et al., 2010) provided information on reward responsiveness and would be compared with the average scores of healthy adolescents in previous studies.

CHAPTER 2

LITERATURE REVIEW

2.1 The reward system

The reward system is an essential part of the brain for both humans and animal for survival. The system helps in assessing outcomes to guide behaviour (Andreou et al., 2017). It regulates pleasure and related emotions (Gibson, 2017). The system interacts with several brain components such as regions involved in cognitive and emotional processing. Dopamine is the main neurotransmitter that plays an important role in the brain reward system (Gibson, 2017). Dopamine is released by neurons and binds to dopamine receptors on other neurons. The dopaminergic reward system creates feelings of desire and operant conditioning such as positive reinforcement (Nutt et al., 2015). Dopamine encourages repeated consumption of substances or repeated activities that causes pleasure. Dopamine originates from the ventral tegmental area (VTA) and the mesolimbic dopamine pathways related to reward includes the ventral striatum, nucleus accumbens (NAcc), lateral hypothalamus, amygdala and hippocampus (Adcock et al., 2006; Gibson, 2017). Ventral striatum and hippocampus are a part of the substantia nigra/ventral tegmental area-hippocampal (SN/VTA-HP) loop (Adcock et al., 2006). The nigrostriatal pathway connecting the substantia nigra pars compacta (SNpc) to the dorsal striatum also influences reward (Luo and Huang, 2016). The mesocortical dopamine pathway involved in reward is the orbitofrontal cortex (OFC), prefrontal cortex (PFC) and the anterior cingulate cortex (ACC) (Elliott et al., 2020; Squeglia and Cservenka, 2017).

The hypothalamus is also considered to be one of the important regions for both homeostatic behaviours and rewards (Castro et al., 2015; Higgs et al., 2017; Morales and Berridge, 2020; Stuber and Wise, 2016). The NAcc, ventral pallidum and lateral

hypothalamus (LH) which are part of the hypothalamic-mesocorticolimbic circuitry play a role in managing the "wanting" and "liking" for food rewards (Castro et al., 2015; Higgs et al., 2017; Morales and Berridge, 2020). This is based on a related theory that showed evidence for a dissociable neural basis of "wanting" which represents motivation driven from incentives and "liking" which is the feeling of pleasure from consuming a rewarding stimulus (Higgs et al., 2017; Morales and Berridge, 2020). However, this pathway involves glutamate and GABA neurotransmitters aside from dopamine. There is D1 and D2 pattern of dopamine receptors. Both D1 and D2 receptors express the NAcc neurons projects indirectly to VTA via the VP and LH while D1 neurons that project directly to the VTA (Morales and Berridge, 2020; Soares-Cunha et al., 2020).

2.4.1 The reward network and addiction

Addiction to a substance such as drugs, tobacco and alcohol are examples of problems happening globally including here in Malaysia. According to a report by the *Agensi Antidadah Kebangsaan* (AADK), their report stated that majority of the drug addicts are youths and many of them started taking drugs when they were in secondary school ((AADK), 2010). A study even found a majority of drug addicts were youths or adolescents who were trapped with the habit which started from when they were in secondary schools ((AADK), 2010). The reward network has been extensively researched as it is also involved in addiction. Bjork and Pardini (2015) discussed that in the United States, the peak of impulsive behaviours such as binge drinking and risk-taking occurs in the ages of 19-23 even though the peak imbalance within the brain circuitry system was shown to occur between the ages of 14 and 16 years old (Bjork and Pardini, 2015; R. Li, 2017; Patrick et al., 2019; Steinberg et al., 2018). Hence, it is

crucial to study the characteristics of the reward network of adolescents' brain as it may be valuable for future studies on addiction or other disorders related to the network.

In their review article, Camara and colleagues highlighted that to study the reward system, the functional magnetic resonance imaging (fMRI) analysis should be complemented with VTA-VS system structural connection data (Camara et al., 2009). Diffusion MRI is one way to enable the characterisation of anatomical connectivity. Many recent studies have look more into the structural connection of the reward network using this neuroimaging technique (Damme et al., 2017; Dubourg et al., 2017; Galinowski et al., 2019; Squeglia and Cservenka, 2017; Yuan et al., 2018). Researchers classify reward regions into reward valuation, reward expectation and the role of reward in addiction (Haber, 2017; Umemoto and Holroyd, 2017; Volkow et al., 2019).

Reward valuation involved the fronto-subcortical-limbic network. This includes the ventral striatum, superior frontal cortex, cingulate cortex, inferior parietal lobe, insular cortex, hippocampus, thalamus, and caudate nuclei (Camara et al., 2009; Haber, 2017; Mosley et al., 2019). The NAcc, insular cortex and OFC, the amygdala, the hippocampus and the SN/VTA midbrain regions responded to reward and punishment valuation and are engaged in the HP-VTA learning circuit. Amygdala projections connecting with the hippocampus, OFC and ventral striatum were also involved (Camara et al., 2009; Haber, 2017; Mosley et al., 2019).

For reward expectation, the fronto-subcortical-limbic network was also involved which includes the ventral striatum, the PFC and insular cortex (Camara et al., 2009; Haber, 2017; Mosley et al., 2019). One connection called the accumbofrontal tract which connects the NAcc and the OFC were often studied in relation to reward including reward hypersensitivity in adolescents (Cha et al., 2016; Damme et al., 2017; Dubourg et al., 2017; Karlsgodt et al., 2015; Shott et al., 2015; Squeglia et al., 2015).

The NAcc was found to have a major role and it is more activated when it comes to cues that signal potential rewards compared to cues that signal no reward. During reward delivery, the insular cortex was found to interact with the ventral striatum (Camara et al., 2009; Boecker-Schlier et al., 2017; Mollick et al., 2021). The imbalance of the reward network may be the explanation to the adolescents having tendencies for risky behaviours and to make a suboptimal decision. Adolescents' prefrontal cortex continued to develop into early adulthood while having a mature limbic system (Arain and Johal, 2013; Sawyer et al., 2018; Somerville, 2016). Hence, the adolescents showed to be more biased toward reward evaluation by the limbic system compared to the prefrontal system.

For reward and addictive behaviour, it was to distinguish craving states (Camara et al., 2009; Squeglia and Cservenka, 2017; Squeglia and Gray, 2016; Volkow et al., 2019). Drug-seeking behaviour is a goal-directed behaviour whereby a person ingests the drug to obtain its rewarding effects. The connectivity of ventral and dorsal striatal regions are in different stages of addiction. So a defined large region of interest encompassing both, ventral and dorsal striatum, is set in addition to ROIs encompassing the SN/VTA, the mPFC and the OFC. The damage to the VTA–VS dopamine system has shown to suppress the free feeding and the willingness of the rats to press a lever for food rewards (Camara et al., 2009; Nicola, 2016; Verharen et al., 2018). Connectivity between NAcc, amygdala and the OFC is also involved.

2.4.2 Reward sensitivity and reward responsiveness test

Substance addiction is characterized by risk behaviour and impulsivity (Boecker-Schlier et al., 2017; Galinowski et al., 2019; Volkow et al., 2019). Regarding adolescents' risk behaviour, neurodevelopment theories of risk behaviour hypothesize

that this behaviour is due to low control of behaviour combined with high reward sensitivity (Demidenko et al., 2020; Fryt, 2017; Kim-Spoon et al., 2016; M. Li et al., 2019; Peeters et al., 2017). A longitudinal study that did not include brain imaging by Peeters and colleagues (2017) examined whether the risk taking behaviour in adolescents can be attributed to the imbalance between behavioural control and reward sensitivity. They analyzed data from 715 national samples of adolescents. They assessed behavioural control by self-report (effortful control) on the revised Early Adolescent Temperament Questionnaire (Putnam et al., 2002) and behavioural measures of cognitive control which includes working memory and response inhibition. They used the Bangor Gambling Task to assess reward sensitivity whereby responses to reward under arousing circumstances were assessed in which behavioural decisions lead to real gains and losses. Participants joined at the age of 11 and were followed through until they were at least 25 years old. They found that effortful control at the age of 11 significantly predicts risk-taking behaviour (cannabis and alcohol use) at the age of 16 especially among those who were more reward sensitive. Adolescents with weak effortful control that was present prior to the onset of their cannabis or alcohol use, developed a stronger use of cannabis and alcohol in comparison to those with relatively good behavioural control (Peeters et al., 2017).

A diffusion MRI study by Galinowsky and colleagues recently did a study on how the brainstem microstructure and reward sensitivity showed a difference within heavy drinking adolescents (Galinowski et al., 2019). The dorsal midbrain is associated with the reward-related regions. It had been shown that there is a structural connection between the upper dorsal pons and DA-containing areas of the VTA in humans (Sesack and Grace, 2010). There were three groups in this study which were heavy drinkers (HD) at the age of 14 (HD14), abstainers becoming HD at 16 years of age (HD16) and

abstainers. This study found that the upper dorsal pons of HD14 had both lower FA and higher RD values while HD16 had higher RD value compared to abstainers (Galinowski et al., 2019). Participants also did the monetary incentive delay (MID) task which was able to assess their performance in reward sensitivity in terms of reward expectation. HD14 was found to obtain higher success scores on the MID task compared to abstainers. All adolescents showed higher success score together with a lower number of tracts. Hence, the sensitivity for reward expectation was found to be associated significantly with lower white matter integrity in the upper dorsal pons in adolescents at the age of 14 years with the sensitivity increased significantly into current heavy drinkers which were HD16.

In many reward sensitivity and impulsivity studies, researchers include a self-report scale such as the Behavioural Inhibition System and Behavioural Activation System (BIS/BAS) scale to obtain behavioural data. The BIS/BAS scale is often used in order to obtain behavioural data on two basic brain mechanism which is the behaviour inhibition system that is responsive to punishment and behavioural activation system that can assess sensitivity to reward (Atkinson, 2018; Carver and White, 1994). The BIS/BAS scale has 20 items which are rated on a four-point likert scale with 1 indicating strong disagreement and 4 indicating strong agreement. An example of an item in the scale is “It would excite me to win a contest.”

An example of a study that uses the BIS/BAS scale is an fMRI study by Kim-Spoon and colleagues (2016) that investigated how inhibitory control interacts with reward and punishment sensitivity to predict substance use severity and the age of onset among early adolescents (Kim-Spoon et al., 2016). They had a total of 157 participants age 13 to 14 (52% male). They analyzed a survey given to the participants which included the BIS/BAS scale and substance use severity and onset. They also assessed

the adolescents' inhibitory control along with fMRI imaging based on adolescents behavioural performance on the Multiple Source Interference Task (MSIT). The MSIT task enables detection and response to conflict measures to be obtained and this was associated with both the flanker and spatial interference. They found that higher levels of BAS in adolescents, which did not include BAS Reward Responsive, together with low inhibitory control predicted an earlier start of substance use. During an interference control task, showing poor performance and increased prefrontal activity indicated the participants had low or weak inhibitory control and they had high reward sensitivity which makes them vulnerable to early onset of substance abuse. Their fMRI finding gave empirical evidence which emphasized the role of inhibitory control in the regulation of reward sensitivity in determining onset of substance use among early adolescents (Kim-Spoon et al., 2016).

Another longitudinal magnetic resonance imaging (MRI) study had also found evidence of a developmental increase in reward sensitivity which occurred from early adolescence into late adolescence which eventually decreased in early adulthood (Duell et al., 2016; Steinberg et al., 2018; Urošević et al., 2012a). They also measure reward sensitivity using the BIS/BAS scale. In addition, they found that adolescents had higher NAcc volume compared to adults whereby volume peaks at 13 to 17 years of age (Urošević et al., 2012). They found an association of the decline of reward sensitivity with the brain volume decrease of the left NAcc from late teens to early 20s. However, the decline of reward sensitivity may also be associated with the maturity of the adolescents' prefrontal cortex which is usually the last region to become mature (Arain and Johal, 2013; Demidenko et al., 2020; Fryt, 2017). Table 2.1 shows the BIS/BAS scores of the age group between 18 to 23 to compare score with participants in the current study. Table 2.2 is BIS/BAS score obtained from a different study with healthy

adolescents within the age range of 18 to 25 years of age. From both of these studies, the typical score of BIS, BAS reward responsive, BAS drive and BAS fun-seeking for healthy adolescents can be presented.

Table 2.1 BIS/BAS score of healthy adolescents age 18-23 (Urošević et al., 2012).

Variables	Mean (SD)
BIS score	20.15 (3.42)
BASRR score	17.64 (1.61)
BASD score	11.12 (1.58)
BASFS score	12.51 (2.00)

Table 2.2 BIS/BAS score of healthy adolescents age 18-25 (Atkinson, 2018).

Variables	Mean (SD)	Range
BIS score	20.60 (2.43)	10-28
BASRR score	17.50 (2.45)	9-20
BASD score	11.20 (2.44)	5-16
BASFS score	11.70 (2.45)	5-16

Another scale developed based on the BIS/BAS scale which attempts to focus only on testing reward sensitivity is the Reward Responsiveness scale (RRS) (Assari et al., 2020; Van den Berg et al., 2010). The RRS is an 8-item scale that is able to measure reward responsiveness which is rated on a four-point likert scale with 1 indicating strong disagreement and 4 indicating strong agreement. An example of an item in the scale is "I am someone who goes all-out." Since the participants' age when the RRS data was taken for the current study is around the young adult age (26-29 years of age), data from studies that include adolescents and young adults were included for comparison. Table 2.3 presents the RRS scores of healthy adolescents obtained from previous studies

(Ameral et al., 2017; Linke and Wessa, 2017; Oumeziane et al., 2019; Umemoto and Holroyd, 2017a).

Table 2.3 Reward Responsive score of healthy adolescents to young adult age group from previous studies

Study	Age range	Mean (SD)	Range
Oumeziane (2019)	19 (1.15)	26.83 (3.47)	18-32
Ameral (2017)	22.2	25.60 (2.69)	20-31
Linke (2017)	23.3(19-30)	24.3 (2.80)	-
Umemoto (2017)	17-26	26.4 (2.7)	20-32

2.4.3 Region of interest related to reward sensitivity

A notable feature in addiction is the preference for rewards that is attainable sooner even though the reward have relatively low overall value. This phenomenon has been taken into consideration in reward-related and addiction studies. Temporal discounting is a task developed by Kirby (2009) which is often used within these studies to measure impulsive choice (Bari et al., 2020; Hampton et al., 2017; Kirby, 2009; Urošević et al., 2016). This behavioural task is commonly used in many studies including fMRI studies which help determine the region related to reward anticipation and reward valuation. The task enables researchers to identify "the indifference points" when a person equally likely chooses an immediate smaller reward rather than a later higher value reward (Bari et al., 2020). An example is getting $100 now or $200 in 3 months.

A most recent study diffusion MRI study via probabilistic tractography by Bari and colleagues (2020) investigated smoking addiction in 197 healthy adolescents (age 22-25 years old) with 45 having a history of tobacco smoking. Their subjects were sampled from the Human Connectome database (Bari et al., 2020). Based on a previous study on brain regions related to smoking addiction, they used the amygdala as seed

and 7 regions were chosen as targets which were orbitofrontal cortex (OFC), rostral anterior cingulate cortex (rACC), dorsolateral prefrontal cortex (dlPFC), insular cortex, nucleus accumbens (NAcc), brainstem and hippocampus. The participants within the database also did the temporal discounting task. Their main findings included the parcellated amygdala connectivity to show the strongest connectivity was to the hippocampus, which was followed by OFC and brainstem. They also found that the connectivity of amygdala with the hippocampus was associated with preference for the delayed higher value rewards while connectivity with the OFC, rACC and insula was associated with preference for immediate lower value rewards (Bari et al., 2020).

Another tractography study on smoking examined whether a particular striatal tract strength with participants in the satiated condition was related to the percentage change of craving to smoke. In addition, they also verified whether specific striatal tract strength in the satiated condition can predict a smoking lapse induced by a 12-hour abstinence (Yuan et al., 2018). This study used the striatum as seed and 10 a priori target masks which were ACC, posterior cingulate cortex (PCC), dorsal ACC (dACC), mOFC, IFG, supplementary motor area (SMA), dlPFC, vlPFC, hippocampus and amygdala which was chosen according to previous studies which were consistent with the frontostriatal circuits including primates and other human diffusion MRI studies. (van den Bos et al., 2014, 2015). They had only male participants with 53 of them nicotine-dependent cigarette smokers age 20.98 (1.69) years and 58 age- and education-matched male non-smokers age 20.69 (1.50) years. They found weaker tract strengths of left striatal circuit with mOFC, vlPFC, IFG and PCC were detected in the young smokers relative to the non-smokers. They also found the tract strength of left striatum-vlPFC, left striatum-mOFC and left-striatum dlPFC have the potential to become

neuroimaging biomarkers for abstinence-induced craving and to predict lapsers in smoking.

A multimodal approach study by van den Bos and colleagues (2015) combined the measures of behaviour, structural connectivity and functional connectivity focusing on the reward connectivity and adolescents. This study used both fMRI and diffusion MRI imaging while also testing impulsive behaviour measures including via the temporal discounting task (van den Bos et al., 2015). The study specifically examined developmental changes in the structural and functional connectivity of different frontostriatal tracts (van den Bos et al., 2015). They had 50 adolescent participants (26 females) between the age of 18 and 25 years old. They reported that adolescents were more impatient on an intertemporal choice compared to young adults. In addition, they found a developmental increase in structural connectivity strength in the right dlPFC tract were related to increased negative functional coupling with the striatum and an age-related decrease in discount rates hence less impulsivity.

Their results implied that the reduction in impatience across adolescence was driven by mainly increased control, and the integration of future-oriented thought (van den Bos et al., 2015). Similar to Yuan and colleagues (2018), they used the striatum as seed and the 10 a priori target as target masks. Furthermore, they did segmentation of the striatum according to these target masks along with another study by van der Bos and colleagues (2014) and it clearly showed that the striatal subregions were connected in specific spatial patterns such that: NAcc with mOFC, caudate with dlPFC and putamen with vlPFC (van den Bos et al., 2014, 2015; Yuan et al., 2018).

Hence, from these three studies and many other studies related to reward network and a task that requires reward anticipation and reward valuation, the NAcc was chosen as the seed while the amygdala, ACC, mOFC, hippocampus, vlPFC and

dlPFC were chosen as targets. Since the distribution pattern of the ventral striatum (NAcc) was found from segmentation of striatum to show pattern spatially specific to mOFC from these past studies, it is hypothesized that the current study may find the highest relative connection probability between NAcc and mOFC compared to the other 5 target regions (van den Bos et al., 2014, 2015; Yuan et al., 2018).

a) NAcc

Many of the previous studies investigating reward and addiction had focused on the brain regions within the frontostraital network (Demidenko et al., 2020; van den Bos et al., 2014, 2015; Wilmer et al., 2019; Yuan et al., 2018) In these past studies involving diffusion MRI, the striatum or specifically ventral striatum was chosen as the seed mask. The ventral striatum, specifically the nucleus accumbens (NAcc), is the central hub for processing information regarding reward and pleasure (Coenen et al., 2011; Haber, 2017; Leong et al., 2016; Misaki et al., 2016; Soares-Cunha et al., 2020). So, the NAcc is very much an important component of the reward circuit in the brain (Misaki et al., 2016; Soares-Cunha et al., 2020).

The NAcc in particular integrates emotional and cognitive input to modulate goal-directed behaviour when it comes to reward processing (Floresco, 2015; Haber, 2017; Soares-Cunha et al., 2020) In other words, the NAcc receives input from both the cortical and subcortical regions of the brain to modulate the processing of incentive (Floresco, 2015; Haber, 2017; Yuan et al., 2018). Being the central hub and integration of input of reward processing, the NAcc is shown to be an optimal seed region for analyses in the current study.

The NAcc is also known to be divided into two components commonly known as the “shell” which is located at the medial and the lateral “core” (Haber, 2017; Soares-Cunha et al., 2020; Zhao et al., 2017). A study by Zhao and colleagues (2017) used

connectivity based parcellation of the NAcc into the shell and core portions in a study related to the investigation of temporal lobe epilepsy patients (Zhao et al., 2017). However, the current study will not use parcellation to segment the NAcc into core and shell.

b) Amygdala

The amygdala is a small almond-shaped group of nuclei near the hippocampus which is often associated with emotions. In relation to reward, the amygdala has shown that it has a role in processing positive stimuli which is stimulus-reward learning (Bari et al., 2020; Haber, 2017; Walker et al., 2017). This means that the amygdala is also involved in goal-directed behaviour (Bari et al., 2020; Damme et al., 2017; Haber, 2017; Walker et al., 2017). So, the amygdala was chosen as one of the targets in the current study. According to previous findings, the central nucleus of the amygdala is involved in reward outcome to guide the modulation of behaviours through the NAcc while the basolateral amygdala gives input to the NAcc on reward prediction related to reward learning (Janak and Tye, 2015; Kolada et al., 2017; Volkow et al., 2019). In past studies, connectivity between the amygdala and NAcc relates to reward sensitivity (Casey et al., 2016; Costumero et al., 2013; Damme et al., 2017). The diffusion MRI study on the reward-related NAcc-amygdala tract found that higher hypo/mania proneness is associated with stronger structural connectivity between the NAcc-amygdala tract and the NAcc-mOFC tract (Damme et al., 2017). Both these tracts were chosen to be investigated in the current study.

c) ACC

The ACC is one of the main components of evaluating reward value and outcome together with the orbitofrontal cortex (OFC) (Bari et al., 2020; Haber, 2017; Volkow et al., 2019; Wang et al., 2017) A previous study highlighted a dissociation between the

ACC and vmPFC (very close to the medial OFC location) which are both associated with reward prediction and outcome (Vassena et al., 2014; Wang et al., 2017). They found that the ACC codes for positive prediction errors while the vmPFC responds to outcome regardless of probability. This further support the role of ACC in intentional decision-making and taking value associated with the actions into account while vmPFC show more stimulus-based value processing (Arulpragasama et al., 2018; Rolls, 2019; Vassena et al., 2014). In a rat study investigating the functional interactions between ACC and NAcc found that crossed lesion of ACC and NAcc impaired effort-based decision making. However, both unilateral lesion of either ACC or NAcc and ipsilateral lesions of both structures did not impair effort-based decision making (Hauber and Sommer, 2009).The importance of ACC and NAcc regions in effort-based decision making was also shown in human studies (Bernacer et al., 2016; Ludwiczak et al., 2020). Thus, ACC is shown to be important for an intentional effort-based decision regarding reward and has been chosen as a target in the current study.

d) mOFC

The role of mOFC related to reward processing is its role in encoding reward value and accessing the probability of reward receipt (Fettes et al., 2017; Peters and D'Esposito, 2016; Y. Wang et al., 2017; Yan et al., 2016) A previous human study investigated patients with mOFC lesion or damage performance on an intertemporal choice task (Peters and D'Esposito, 2016). The mOFC lesions interfere with the choice-free valuation ratings and decrease self-control during the intertemporal choice task (Peters and D'Esposito, 2016). Similar to the temporal discounting task mentioned previously, the intertemporal choice task is where participants get to choose between lesser immediate rewards or larger postponed rewards.

As previously mentioned, the NAcc and the mOFC tract have been extensively studied in regards to reward and addiction (Damme et al., 2017; Ikuta et al., 2018; Karlsgodt et al., 2015; Squeglia and Cservenka, 2017). In addition, the role of excitatory white matter tracts from the mOFC to the NAcc in modulating reward valuation was documented in both human and non-human animal research (Bailey et al., 2016; Damme et al., 2017; Peters and D'Esposito, 2016; Z. Wang et al., 2019). A diffusion MRI study found that the tract's FA value significantly increases which peaked at 14.8 years of age followed by a decrease and levelled out. Hence, it showed that the tract matures around the mid-adolescence period. So the mOFC was easily chosen to become an ROI in the current study.

c) Hippocampus

Value-based learning is one of the major role of the hippocampus in association with reward. An fMRI study scanned healthy participants while learning value-based contingencies which is where the players have to try and win money within the game prepared in the context of a probabilistic learning task. The activation of the hippocampus was shown, other than the expected activation of the ventral striatal (NAcc) which is known to accompany this type of learning (Palombo et al., 2019). Furthermore, an fMRI reward system study of adolescents using probabilistic reinforcement learning task found that adolescents showed better reinforcement learning with a stronger link between reinforcement learning and episodic memory for rewarding outcomes (Davidow et al., 2016; Palombo et al., 2019). The brain imaging showed that there was an increased prediction error-related BOLD activity in the hippocampus and during the time of reinforcement, the hippocampus and the striatum showed stronger functional connectivity (Davidow et al., 2016). Thus, this study showed that the hippocampus has a crucial role in reinforcement learning in adolescents.

In addition, their findings suggest that reward sensitivity in adolescence is related to adaptive differences in how adolescents learn from their experiences (Davidow et al., 2016).

d) vlPFC

The cognitive control processes which is able to help in delving into relevant information is one of the roles of the vlPFC. This region is studied as it is shown to be associated with activities which includes goal-directed behaviour (Cho et al., 2016; Leong et al., 2018; Yuan et al., 2018). For directing attention, the vlPFC interacts with motor-related regions in the brain (Cho et al., 2016; Corbetta and Shulman, 2002; Leong et al., 2018). This phenomenon suggests that orienting attention to relevant stimuli in reward sensitive individuals may be associated with the increase in connectivity with vlPFC. Previous findings suggest that responses to choice might be different with different individuals due to individual traits such as reward sensitivity and this can be detected by the vlPFC (Cho et al., 2016). Hence, the vlPFC was chosen as a target region in the current study.

e) dlPFC

The dlPFC has an important role in integrating reward and goal information (Chung and Barch, 2016; Wilmer et al., 2019; Yuan et al., 2017; Yuan et al., 2018). This region encodes reward amount and becomes active when anticipated rewards signal future outcomes (Bartolo and Averbeck, 2020; Haber, 2017; Q. He et al., 2016). A prior study has found a decrease in impulsivity with the increase of age can be attributed to the development of the striatal connections with the lateral prefrontal cortex specifically the right dlPFC (van den Bos et al., 2015). Particularly what they found was that participants with greater medial striatum–right dlPFC tract strength showed less

impulsive behaviour (smaller discount rates) (van den Bos et al., 2015). Thus, the dlPFC was chosen as ROI for the current study.

2.4.4 Adolescents

Adolescence is the period of transition from childhood to adulthood. The age range of adolescence differs between countries and cultures (van Duijvenvoorde et al., 2016). The adolescence period was historically acknowledged between the age of 12 to 18 years old and this period roughly corresponds to the time when puberty begins to that of guardian independence (Dahl, 2004). This time period undeniably often co-occurs with puberty which is characterized by a rapid rise in gonadal hormones (Blakemore et al., 2010; Sawyer et al., 2018; Walker et al., 2017). However, recent studies on the brain have expanded the term adolescence at the age of 10 up to 25 years which is almost the age of young adulthood (Arain and Johal, 2013; Jaworska and MacQueen, 2015; Sawyer et al., 2018; van Duijvenvoorde et al., 2016). This is to cover the period where neural changes within the adolescents' brain which still occurs beyond the age of 18 (Fuhrmann et al., 2015; Jaworska and MacQueen, 2015; Sawyer et al., 2018). Molecular imaging and functional genomics research have found that the adolescents' brain actively undergoes development throughout the adolescence period (Arain and Johal, 2013; Demidenko et al., 2020; Fryt, 2017; Fryt et al., 2021; R. Li, 2017). Adolescents are constantly associated with a tendency for higher risk-taking behaviours as well as an increase of emotional reactivity in comparison to other age groups (Arain and Johal, 2013; Fryt, 2017; Steinberg et al., 2018; van Duijvenvoorde et al., 2016). Hence, adolescence is a unique period that should be studied in order to obtain further understanding of the inner workings of the adolescent brain.

In the current study, only female Malay adolescents were analyzed. This is due to the fact that past studies have found significant sex differences between male and female white matter microstructure (Damme et al., 2017; Karlsgodt et al., 2015; Menzler et al., 2011; Van Hemmen et al., 2017). An example would be the diffusion MRI study on a reward structural connectivity between the NAcc and OFC called the accumbofrontal tract by Karlsgodt and colleagues in 2015 (Karlsgodt et al., 2015). They cross-sectionally assessed age-related change in fractional anisotropy (FA) of the accumbofrontal tract from childhood to adulthood and found that the change was significant. This is shown by the early peak at the age 14.8 (1.76) and was followed by a rapid decrease which then levelled out. However, there was a significant sex difference of the age-related change in FA as it was shown that males had a higher and earlier peak at age 13.9 (6.85) during adolescence. In comparison to females, their peak was shown at a much later adolescence period which was at the age of 18.6 (3.79) years (Karlsgodt et al., 2015).

Another study on reward structural connectivity of adolescents also found a significant sex difference in microstructural indices. The study investigated the NACC-mOFC and the NAcc-amygdala tracts which were also the tracts included in the current study (Damme et al., 2017). Firstly, even though it was non-significant, the male was shown to score higher in Hypomanic Personality score (HPS) compared to female. Other than that, they found significantly higher FA in both the NAcc-amygdala and the NAcc-mOFC tracts. Hence, from these recent studies on reward-related connectivity, the current study chose to focus on analysing female Malay adolescents.

2.2 Diffusion Magnetic Resonance Imaging

2.4.1 Magnetic Resonance Imaging (MRI)

The MRI enables the production of detailed anatomical images through the detection of hydrogen proton signals from the water molecules which is abundant in the tissues of the body. The general principles of the echo-planar imaging were first introduced by Sir Peter Mansfield of the University of Nottingham (Lavrakas, 2008). Only in 1980 was the first clinical magnetic resonance images produced in Nottingham and Aberdeen (Hawkes et al., 1980; F. Smith et al., 1981). Nowadays, the MRI is a tool often used in both the research and clinical field.

In the human body, the atomic nuclei of hydrogen (single proton) within the water molecule possess a quantum property called "spin" and has a net positive charge (Fisher, M., & Radzihovsky, 2018; Grover et al., 2015; Ilisca, 2021). The constant spinning induces a magnetic moment which generates a small magnetic field hence behaving like a bar magnet with north and south poles. If a strong external magnetic field is applied to the proton, it would align the protons either in a parallel or perpendicular position to the external field. The MRI scanners currently use cryogenic superconducting magnets more commonly in the range of 1.5 Tesla (T) or 3T which acts as the main magnet coil (Grover et al., 2015; Muench et al., 2018; Murray et al., n.d.; Pujol et al., 2021; Tse et al., 2020). There are also low-filed 0.5T scanners which may not give enough detail while 3T systems can improve signal-to-noise ratio. Application of the strong external magnetic field (B_0) from the main magnet coil within MRI is able to align the protons in the body. The protons would undergo precession at the same frequency. MR signals are able to be localized due to the use of gradient coils where the gradient of magnetic fields can be applied in any orthogonal direction (x, y and z) (Chilla et al., 2015; Grover et al., 2015; Holmes et al., 2018; Ping et al., 2016).