EFFECT OF 12 HOURS OVERNIGHT FASTING ON BRAIN PERFUSION USING Tc-99m ETHYL CYSTEINATE DIMER SPECT/CT WITH SCENIUM ANALYSIS IN SMOKERS

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by

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LIST OF SYMBOLS

keV	kiloelectron volt
s/projection	second per projection
s	second
mAs	milliampere-seconds
kV	kilovolt
mm	millimetre
mCi	millicurie
MBq	megabecquerels
mL	milliliter
◦c	degree celcius
p.m.	post meridiem (12 hours clock format that falls in the afternoon)

LIST OF ABBREVIATIONS

WHO	World Health Organization
CBF	cerebral blood flow
rCBF	regional cerebral blood flow
SPECT	Single Photon Emission Computed Tomography
CT	Computed Tomography
99mTc-ECD	Technetium-99m-ethyl cysteinate dimer
99mTc	Technetium-99m
CMRO2	cerebral metabolic rate of oxygen
MRI	magnetic resonance imaging
fMRI	functional magnetic resonance imaging
HUSM	Hospital Universiti Sains Malaysia
HKL	Hospital Kuala Lumpur
IC	inhibitory control
rIFC	right inferior frontal cortex
MRA	magnetic resonance angiography
BREOS	Brain rewards-executive oversight system
AD	Alzheimer disease
99mTc-HMPAO	Technetium 99m-hexamethylpropyleneamine oxime
SUV	Standardized uptake value
M1	increased uptake
M0	reduced uptake
Me	equivocal uptake
KRK	Klinik Rawatan Keluarga
FTND	Fagerstrom Test Nicotine Dependence

TIA	transient ischemic attack
Na 99mTcO4	sodium pertechnetate
NaCl	sodium chloride
SPSS	Statistical Package for the Social Sciences
SD	standard deviation
p-value	asymptote significant
df	degree of freedom
L	left
R	right
SFG	superior frontal gyrus
n	number of populations
PCC	posterior cingulate cortex
SCC	cue-induced subcallosal
SPL	superior parietal lobule
GM	gray matter
SMA	supplementary motor area
preSMA	presupplementary motor area
PFC	prefrontal cortex
mSFG	middle superior frontal gyrus
DLPFC	dorsolateral prefrontal cortex
BP	brain perfusion
PET	positron emission tomography
NMI	nuclear medicine imaging
I.V.	intravenous
FDG	fluorodeoxyglucose

ROI	region of interest
BOLD	blood oxygen level dependent
EC	electronic cigarette
TCG	tobacco cigarette
CO	carbon monoxide
TTF	the time till the first cigarette of the day
CDD	the number of cigarettes smokes per day
EEG	electroencephalography
MEG	magnetoencephalography
fNIRS	functional near infrared spectroscopy
fUS	functional ultrasound imaging
CAT	computed axial tomography
DALYs	disability adjusted life years
MOH	ministry of health
FCTC	framework convention on tobacco control
SPET	single photon emission tomography
RCP	radiochemical impurity
TLC	thin layer chromatography
MPC	mini paper chromatography
HPLC	high performance liquid chromatography
IQR	interquartile range
ECD	ethyl cysteinate dimer
NAc	nucleus accumbens
OFC	medial orbitofrontal cortex
ACC	anterior cingulate cortex

BDNF	brain derived neurotrophic factor

KESAN PUASA SEMALAMAN 12 JAM TERHADAP PERFUSI OTAK MENGGUNAKAN Tc-99m ETHYL CYSTEINATE DIMER SPECT/CT DENGAN ANALISIS SCENIUM DALAM KALANGAN PEROKOK

ABSTRAK

Merokok bukanlah satu tabiat umum yang berlaku di Malaysia sahaja, tetapi juga di seluruh dunia. Tabiat ini biasanya berlaku di kalangan dewasa, tetapi kini remaja juga tergolong dalam golongan yang ketagih kepada menghisap rokok. Bahan merosakkan dalam rokok seperti nikotin boleh mengurangkan aktiviti dalam limbik, cingulate posterior, korteks prefrontal, dan meningkatkan aktiviti dalam cuneus dan precuneus. Kajian ini akan mendaftarkan subjek yang berumur 18 tahun ke atas dan memenuhi kriteria penerimaan dan pengecualian, iaitu perokok (n=18) dari Klinik Rawatan Keluarga (KRK) dan bukan perokok (n=11) dari kumpulan sukarelawan. Perfusion otak antara kedua-dua kumpulan akan dibandingkan dengan menggunakan Pengesan Tomografi Pembebasan Foton Tunggal-Tomografi berkomputer (SPECT/CT) di Hospital Universiti Sains Malaysia (HUSM) dengan menggunakan technetium-99m-ethyl cysteinate dimer (99mTc-ECD) sebagai radiotracer untuk keadaan berpuasa dan tidak berpuasa, dan perisian Scenium digunakan untuk interpretasi imej kuantitatif otak. Perokok dikategorikan sebagai perokok ringan (n=17), sederhana (n=1) dan perokok berat (n=0) berdasarkan tahap ketagihan soal selidik Fagerstrom. Oleh itu, objektif kajian ini adalah untuk menentukan perfusi otak antara perokok dan bukan perokok, membandingkan perbezaan dalam perfusi otak di kalangan perokok antara keadaan berpuasa dan tidak berpuasa, dan membandingkan perbezaan dalam perfusi otak di kalangan bukan perokok antara keadaan berpuasa dan tidak berpuasa. Hasil ujian U Mann Whitney untuk objektif pertama menunjukkan bahawa terdapat perbezaan yang signifikan dalam lobus frontal superior (SFG)

dorsolateral dan orbital sebelah kiri dan kanan. Ujian Wilcoxon Signed Rank digunakan untuk objektif kedua dan ketiga, dan terdapat perbezaan yang signifikan dalam amigdala sebelah kiri di kalangan perokok dan tiada perbezaan yang signifikan dalam kawasan perfusi otak diperhatikan di kalangan bukan perokok selepas berpuasa semalaman (12 jam).

EFFECT OF 12 HOURS OVERNIGHT FASTING ON BRAIN PERFUSION USING Tc-99m ETHYL CYSTEINATE DIMER SPECT/CT WITH SCENIUM ANALYSIS IN SMOKERS

ABSTRACT

Cigarette smoking is not only a common habit that occurs in Malaysia but also the whole world. This habit has been common among adults but nowadays teenagers are also addicted to smoking cigarettes. The destructive substances in cigarettes such as nicotine can reduce activity in limbic, posterior cingulate, prefrontal cortex and increase activity in their cuneus and precuneus. This study recruited subjects that are 18 years old and above and fulfill inclusion and exclusion criteria, smokers (n=18) from Klinik Rawatan Keluarga (KRK) and non-smokers (n=11) from volunteer poster. The brain perfusion between both groups was compared by using Single Photon Emission Computed Tomography-Computed Tomography (SPECT/CT) in Hospital Universiti Sains Malaysia (HUSM) by using technetium-99m-ethyl cysteinate dimer (99mTc-ECD) as a radiotracer for both fasting and non-fasting state and Scenium software was use for quantitative brain image interpretation. Smokers were categorized into mild (n=17), moderate (n=1) and severe smoker (n=0) based on level of addiction fagerstrom questionnaire. Thus, this study objectives were to determine brain perfusion between smoker and non-smoker, to compare the difference in brain perfusion among smoker between fasting and non-fasting state and to compare the difference in brain perfusion among non-smoker between fasting and non-fasting state. The result of Mann Whitney U test for the first objective shows that there is significant difference in left and right of superior frontal gyrus (SFG) dorsolateral and orbital. Wilcoxon Signed Rank test was used for second and third objective and there is significant difference in left amygdala among smoker and no significant difference in

brain perfusion area observed among non-smokers after overnight fasting (12 hours fasting).

CHAPTER 1

INTRODUCTION

1.1 Background of study

According to the Global Tobacco Survey by WHO in 2017, 22.8% of adults and 17.4% of adolescents are current smokers in Malaysia (WHO, 2013). The trend is static despite continuous activity and intervention done by the government especially the Ministry of Health. Studies have revealed that smoking is a learnt behavior that starts in adolescence. In Malaysia, more than 80% of adult smokers have reported to develop their smoking habit prior to reaching 21 years old (Lim et al., 2018). There are many interventions done and planned for smokers to help in reducing and stopping their smoking habit (Hassan, Yusof & Awang Hashim, 2014). The act of burning plant material or inhaling and exhaling is known as cigarette smoking. While smoking is associated with a variety of plant materials, including hashish and marijuana, it is most commonly associated with tobacco when it is smoked in a cigarette, cigar, or pipe (Rose *at al.*, 2023). Destructive substances in cigarettes will definitely affect anyone who consumes it. It will affect physical and mental health and surely will affect brain activity. Smoking can cause ongoing problems, as well as long-term effects on our organ systems. Although smoking can raise one's likelihood of acquiring some illnesses over time, such as cancer, blood clotting issues, and glaucoma, certain effects on body function can occur abruptly (Pietrangelo A, 2019). Cognitive decline also occurs as people age. However, it begins much sooner in smokers. Delusions, apathy (losing motivation), anxiety symptoms, difficulty with visual-spatial activities, hallucinations, personality changes, and depressive symptoms are all indicators of cognitive impairment. About 7,000 men and women's cognitive data from 12 years of research were examined in 2012. Researchers discovered that smokers' cognitive loss occurred considerably more quickly than that of non-smokers. It was discovered that middle-aged men smokers were more at risk than female smokers (Pharmeas*y,* 2023). The amygdala, which is vital for behavior, emotional regulation, and learning, as well as for identifying dangers in the environment and contributing to nicotine addiction through three distinct pathways, is also impacted by nicotine. The mechanisms are as follows: first, the amygdala plays a critical role in evoking negative emotional states during early abstinence; second, it exhibits strong reactivity to stimuli related to smoking, which heightens cue-induced craving; and third, there is mounting evidence that impairs amygdala-dependent danger and risk perception, which increases uninhibited nicotine desire.

Most withdrawal symptoms require time to eliminate the drug's nicotine from their system and quit the powerful habit of smoking. Smoking cessation can cause cravings and withdrawal symptoms such as tension, difficulty sleeping, difficulty thinking clearly, and weight gain. As a result, these symptoms provide significant barriers to smoking cessation. To successfully quit smoking, smokers must deal with both of these challenges: no nicotine in the brain and no smokes throughout daily routines. However, in order to perform their religious duty of fasting throughout the month of Ramadan, smokers will abstain from food, beverages, and smoking during the daytime hours of this month. This obligation somewhat helps smokers who are determined to quit smoking from the early stages during Ramadan. Thus, most of Muslim disregard their addiction status, can simply stop smoking during daytime in Ramadhan. The supply of oxygen and nutrients to the brain is accomplished via brain perfusion, occasionally referred to as cerebral perfusion or cerebral blood flow (CBF), which is quantified as the volume of blood supplied to a unit of tissue per unit of time.

For this research, overnight fasting was used to identify the brain perfusion differences among smokers during fasting state using same Single Photon Emission Computed Tomography Computed Tomography (SPECT/CT) procedure and Technetium-99m-ethyl cysteinate dimer (99mTc-ECD) as a radiotracer. Overnight fasting means not eating or drinking (except plain water) for 12 hours, usually at night. Most people fast from 7–8 PM to 7–8 AM, which supports digestion, metabolism, and recovery (Wheeler R. B., 2022). In comparison to nonsmoking control subjects, previous researcher reported that after 12 hours of smoking cessation, they observed substantial overall declines in cerebral metabolic rate of oxygen (CMRO2) and gray-matter CBF in a group of regular smokers. Cortical atrophy was observed solely in the left cingulate cortex of smokers, according to cortical thickness analysis of magnetic resonance imaging. This suggests that cortical atrophy is not responsible for the overall decline in abstemious smokers. In contrast, a prior investigation of global brain perfusion revealed a worldwide 12% decrease in CBF and an accelerated age-dependent fall in global CBF among smokers, which was restored after 1 year of abstinence in a cross-sectional study (Vafaee *et al.*, 2015). According to recent research, fasting-induced metabolic alterations may improve brain function by promoting more neuroplasticity, improved cognitive function, and increased resilience to disease and damage. However, it is well known that metabolic disorders and overeating impair brain metabolism and exacerbate the symptoms of neurodegenerative illnesses (Brocchi *et al*, 2022).

Nuclear medicine imaging (NMI) is a sophisticated diagnostic imaging modality that has been well studied. It comprises injecting radionuclides into the body, observing the gamma rays that are released, and producing visuals that provide details about the physiological properties of organs and tissues, as well as the distribution of radionuclides. In nuclear medicine imaging, positron emission tomography (PET) and single photon emission computed tomography (SPECT) are the most often used imaging modalities. Whereas PET measures gamma rays indirectly through the annihilation of body electrons and positrons created by radionuclide decay, SPECT measures gamma rays directly from the decay of radionuclides. Generally, PET has a better detection sensitivity than SPECT because it can detect two gamma photons travelling in opposite directions concurrently without the need for a collimator in which SPECT needs. The way nuclear medicine imaging functions emphasizes how appropriate radionuclides must be used as tracers in order to achieve imaging capabilities (Ge *et al.,* 2020).

Imaging tests that assist in identifying internal health issues use radioactive tracers. In order to assist in identifying issues with organs or other structures, these tracers release particles that can be detected and converted into an image. An intravenous (I.V.) line inserted into a vein is typically used to administer the tracer. The tracer, nevertheless, can either be inhaled or consumed orally. Following that, the tracer circulates throughout the body and may assemble in a specific organ or area. Among the most commonly used radioactive tracers are tritium, carbon-11, carbon-14, oxygen-15, fluorine-18, phosphorus-32, sulfur-35, technetium-99, iodine-123, and gallium-67. Including in this research study, technetium-99m is among the most commonly utilised radioisotope in medicine, having been used in over half of nuclear medicine interventions. Open Medscience refers to technetium-99m as a key radioisotope fundamental to many diagnostic imaging procedures in nuclear medicine, highlighting its short half-life, favorable gamma emissions, and adaptability for various radiopharmaceutical applications **(**Open Medscience, n.d.**).**

Predetermined volume of 99mTc-ECD solution was used for each participant before the Single Photon Emission Computed Tomography (SPECT/CT) brain scanning. Preparation of 99mTc-ECD solution via labeling of MON.ECD KIT with Tc-99m sodium pertechnetate had been performed as mention in methodology section. Comparable to the Technetium-99m Hexamethylpropyleneamine Oxime (also known as Ceretec or Tc-99m HMPAO), the neutral lipophilic material Tc-99m ECD (Neurolite) enters the blood-brain barrier via passive diffusion. The Tc-99m ECD dose is stable for six hours after it is prepared. With a peak brain activity of 5% to 6% of the injected dose, it has a first-pass extraction of 60% to 70%. Compared to Tc-99m HMPAO, blood clearance occurs more quickly, improving brain-to-background ratios. Less than 5% of the dose is still in the blood after an hour, as opposed to more than 12% in the case of a Tc-99m HMPAO dose (Central Nervous System., 2014).

In this research, brain area images quantitatively analyzed by the Database Comparison software/Scenium (Siemens Molecular Imaging Limited, Hoffman Estates, IL, USA). For neurological evaluations using either PET/CT or single-photon emission computed tomography (SPECT/CT) imaging, the Scenium software (from the Siemens Syngo.via Neurology software package, version VB10B) is used. A database comprising scans from validated normal individuals will be used for comparison of the functional studies (PET or SPECT) of a patient. Database Comparison software (Siemens Medical Solutions, Inc.) was used to perform quantitative parametric analysis. It offers robust quantification tools for the evaluation of fluorodeoxyglucose (FDG) PET-CT, evaluates abnormal regions voxel-by-voxel, and automatically identifies anatomical regions of interest (ROIs) that are best suited for Alzheimer disease (AD) evaluation. The approach yields voxel-based statistics shown as an image volume and develops ROI statistics by comparing the corresponding estimated normal population mean to the value observed in the patient. The findings are shown for each brain region as standard deviations with respect to the reference population (Ferrari *et al.*, 2019). Scenium is a software system with a clinical focus that offers quantification tools for brain analysis. When used with PET and PET/CT imaging, the software aids doctors in diagnosing patients with neurological disorders and dementias. More sophisticated fusion methods enable automatic correlation of a study of the patient using pictures of both normal and anatomical brain regions to quickly calculate any abnormalities. It can be applied to measure hypometabolism in the brain while performing the typical visual analysis. Scenium® software was selected for this investigation since it was included with the PET/CT equipment that was purchased. Although quantitative studies were not the intended purpose of its use, clinical assessments led us to recognize that patients with well-defined lesions had equal lateralization, and the visual analysis (Coelho et al., 2017).

Few research studies have included data on chronic smoking, and those that have generally found a link between smoking and lower CBF (Elbejjani *et al*., 2019), however, these studies did not investigate regional CBF. Results from structural and functional imaging studies demonstrate smoking is variously linked to abnormalities in specific regions of the brain that have been responsible for cognitive functioning, which motivates the search for regional variations in CBF between smokers and non-smokers during non-fasting state.

1.2 Problem statement

Single Photon Emission Computed Tomography-Computed Tomography (SPECT/CT), Functional Magnetic Resonance Imaging (fMRI), and Positron Emission Tomography (PET) are the alternative diagnostic tools for brain imaging techniques and each of tool visualizes brain in a different way.

The Neuroimaging Clinics of North America emphasizes that fMRI is a costly procedure, with MRI scanners priced in the millions and requiring substantial maintenance expenses. The high cost of fMRI studies is a major limitation, reducing accessibility to this imaging technology and the technique is not easily available and causes discomfort to patients (Ablison, n.d.). Because the blood oxygen level dependent (BOLD) response is so slowly, fMRI's main drawback is its temporal and spatial resolution. In addition, a range of bodily functions irrelevant to neuronal activities might affect the BOLD signal because it only offers an indirect representation of neural activity (XUE *et al.,* 2010). Further constraints on the fMRI have been discovered, such as the neurovascular coupling phenomenon, experimental design, validity and reliability of paradigms, head motion, physiological noise, structural changes in the brain, image registration, field strength, and image statistics (false positives and false negatives, correction for multiple comparisons, power calculation, sample size, and region-of-interest) (*Functional Magnetic Resonance Imaging,* 2013).

The same is true with PET scan, PET is not readily accessible and is relatively more expensive. PET can only be carried out if the radionuclide is produced locally and can be provided promptly, as the radionuclides used in it emit radiation for a short span of time (Kocak, 2021). Nuclear medicine may not have the same resolution of body structures as other imaging modalities. If there are irregularities in the body's chemical balances, PET scanning may yield inaccurate results. Variations in blood sugar or blood insulin levels can affect the test outcomes for diabetic individuals or those who have eaten within a few hours of the examination. It is crucial that the patient shows up for the appointment and receives the radioactive material at the appointed time, since the substance decays fast and is only practical for a brief amount of time. Therefore, it could be necessary to reschedule the treatment on a different day for those who missed their appointment.

Due to the limitations of both PET and fMRI scans, SPECT/CT scan was proposed in this research study. It is cheaper than fMRI and PET scan and still relatively underutilized for studying the effect of nicotine addiction on cerebral perfusion. In Malaysia, there is still no study reported on the brain perfusion among nicotine addictions. The absence of studies in Malaysia is likely due to practical challenges such as funding limitations, research priorities, and a shortage of expertise. However, Technetium-99m-ethyl cysteinate dimer (99mTc-ECD) SPECT/CT is a non–invasive way to assess patients with smoking and enables quantitative evaluation of treatment response to medications.

1.3 Research question

1. What is the difference between blood perfusion to the brain among smokers and non-smokers?

2. Is there a difference in brain perfusion among current smokers between fasting and non-fasting state?

3. Is there a difference in brain perfusion among non-smokers between fasting and non-fasting state?

1.4 Research hypothesis

1. There is significantly lower cerebral perfusion in the salient areas among smokers compared to non-smokers.

2. There is significantly higher cerebral perfusion in the salient areas among current smokers in fasting compared to non-fasting state.

3. There is no difference cerebral perfusion in the salient areas among non-smokers between fasting and non-fasting state.

1.5 Research objective

1.5.1 General objective

To study the brain perfusion among current smokers attending HUSM.

1.5.2 Specific objectives

1. To determine brain perfusion among current smokers and non-smokers.

2. To compare the difference in brain perfusion among current smokers between fasting and non-fasting state.

3. To compare the difference in brain perfusion among non-smokers between fasting and non-fasting state.

CHAPTER 2

LITERATURE REVIEW

2.1 Normal Anatomy of Brain

The brain is one of the most remarkable evolutionary creations, and despite the astounding amount of knowledge that has been discovered, many aspects of its structure and function remain unclear (Di Ieva, 2011). The human brain is a magnificent organ that weighs three pounds and is responsible for controlling all bodily functions, processing external data, and housing the mind and soul. The brain is responsible for a wide range of processes, including emotion, intellect, creativity, and memory. The brain is composed of the brainstem, cerebellum, and cerebrum, where it is located inside the skull.

The cranial nerve nuclei can be found in the brainstem, whereby it relays sensory and motor data from the spinal cord to the brain. Through the autonomic nuclei and the ascending reticular activating system, it regulates essential functions and the sleep-wake cycle, respectively. Dysautonomia, respiratory failure, cranial nerve palsies, impaired consciousness, and sensory and motor abnormalities are all possible outcomes of brainstem dysfunction (Benghanem *et al.,* 2020).

About 75% of the brain's neurons are found in the cerebellum, which combines sensory information with motor output to produce precise movements. Because ataxia, dystonia, and tremor are brought on by abnormalities in the cerebellar circuitry, this function is essential for motor coordination and motor learning. In addition, the cerebellum plays a role in non-motor functions like memory, reward, and social interaction (Achilly *et al.,* 2021). The human cerebellum is the largest motor structure in the central nervous system, containing a greater number of neurons than the entire cerebral cortex. It is positioned astride the brain stem underneath the occipital cortex, taking up around tenth of the skull cavity (Miall R. C., 2022).

The cerebrum, which is divided into two hemispheres, accounts for approximately 85% of the total mass of the human brain. The cerebrum, which makes up the superior part of the brain, conceals and hides the diencephalon and brain stem in a manner analogous to how a mushroom cap covers its stalk. A substantial part of the cerebrum is used in the processing of somatic sensory and motor input, as well as all conscious thoughts and intellectual functions. Gray matter, which is made up of unmyelinated axons and billions of neuron cell bodies organized into six distinct layers, makes up the cerebrum's outer cortex. Despite its thin thickness of 2–4 mm, this area makes up around 40% of the entire brain mass. White matter, or tracts of myelinated axons, make up the inner area. A third basic region of the cerebrum, known as the basal nuclei, is a collection of sub-cortical gray matter located deep within the cerebral white matter. These three nuclei, the globus pallidus, putamen, and caudate nucleus are crucial modulators of skeletal muscle contraction (Advanced Human Anatomy and Physiology Western Oregon University, 2013).

Figure 2.1 illustrates the location of the four lobes that comprise the cerebral hemispheres: frontal, temporal, parietal, and occipital. The fissures in each hemisphere differ. Again, each lobe can be further subdivided into regions with highly specific tasks. It's critical to realize that no brain lobe operates in isolation. The connections between the left and right hemispheres and between the lobes are extremely intricate (Anatomy_of_the_Brain, 2022).

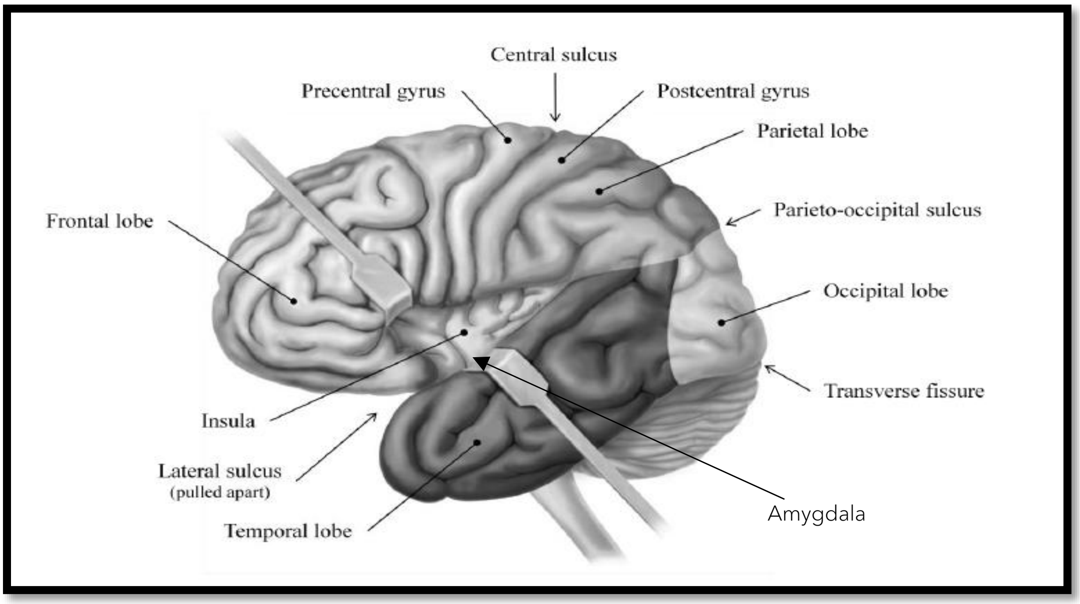

Figure 2.1 Lobes, sulci, and fissures of the cerebral hemispheres, Marieb & Hoehn (Human Anatomy and Physiology, 9th ed.)

Observed brain regions, amygdala, cuneus, precuneus, posterior cingulate and superior frontal gyrus in this research study located in the four lobes of the brain. The amygdala is a cluster of almond-shaped nuclei located near the center of the telencephalon. A previous study examined the amygdala's structure and role in the limbic system, highlighting its importance in emotions, memory, and sensory processing, as well as its link to mental health disorders (Nikolenko et al., 2020). Another previous study state that limbic system includes the vital parts of hippocampus, thalamus, hypothalamus, basal ganglia, and cingulate gyrus (Guy-Evans & Mcleod, 2024). Additionally, the amygdala plays a key role in fear conditioning and is central to the brain's fear learning and expression. Through behavioral methods, such as fear conditioning experiments, a conceptualization of the amygdala's involvement in processing negative emotions has emerged (Keifer *et al.,* 2015).

The occipital lobe, a wedge-shaped region at the rear of the brain, contains the cuneus, also referred to as the cuneate gyrus. It may be observed on the inner surface of the occipital lobe, where the parietooccipital sulcus is in front of it and the calcarine sulcus is behind and below it (Snell & R.S, 2010; Gupta *et. al,* 2022). Situated on the inner side of the cerebral hemisphere, the precuneus is a portion of the superior parietal lobule. The paracentral lobule surrounds it from the front, while the parietooccipital sulcus divides it from the occipital lobe's cuneus region in the rear (Snell & R.S, 2010). The precuneus/cuneus is a major contributor to cognitive impairment and may be a crucial integrative brain area for emotional and cognitive coordination (Wang *et al.,* 2023). The superior frontal gyrus is located above the superior frontal sulcus and extends to the medial surface of the hemisphere. The region on the lateral surface of the hemisphere is segregated into an upper and a lower half by the paramedial sulcus, an antero-posterior sulcus. Nevertheless, bridging gyri frequently interrupt this antero-posterior sulcus (Imada *et al*., 2021).

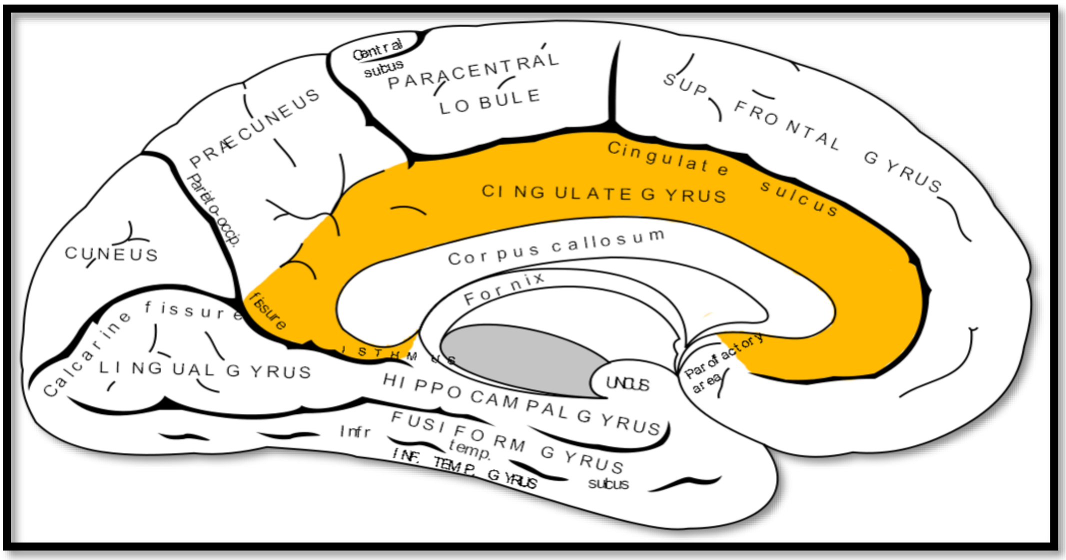

Figure 2.2 Cuneus, precuneus, cingulate gyrus and superior frontal gyrus located in the human brain

2.2 Smoking and Grading of Fagerstrom Score

Tobacco smoking is the leading cause of early death worldwide, causing over 8 million deaths annually. More than 7 million of these are due to direct tobacco use (Lim *et al.,* 2022). The Fagerstrom Test for Nicotine Dependence (FTND) was designed to offer an ordinal assessment of nicotine dependence related to cigarette smoking due to the addiction of smoking habit. It consists of six tests that gauge cigarette intake, motivation to smoke, and dependency. It can be used to test for nicotine dependency as well as to assign a severity assessment for treatment planning and prognosis. The FTND is recommended for measuring smoking dependence and determining patient smoking status (Salhi et al., 2021).

The previous study resulted the study subjects exhibited mild nicotine dependence among cigarette smokers seeking care at oral health facilities by using FTND score, with higher dependence observed in lower socioeconomic groups and older individuals. In order to effectively communicate tailored quitting techniques, tobacco cessation counseling must first evaluate the nicotine dependency scores (Parimi *et al.,* 2022). Another previous research state that their study participants demonstrated moderate tobacco dependence. There is no single universal approach that is deemed effective for those with apparent inequalities in dependency, it is imperative to identify older age groups and those from lower socioeconomic strata as distinct groups for tobacco cessation interventions (Lingamaneni et al., 2023). Another study resulted a greater prevalence of low and moderate nicotine dependency in Raichur district, Karnataka. The efficiency and cost-effectiveness of group therapies and smoke cessation strategies can be increased by doing a baseline evaluation of nicotine dependency preceding an intervention (Njattumkalayil *et al.,* 2022). On the other hand, there was a study reported that a survey demonstrated over 50% from 200 adult smokers observed a high nicotine dependence score. The quantity of cigarettes smoked daily, the smoker's age, and the duration of their smoking all positively correlate with the nicotine dependency score; however, the degree of education has an adverse correlation (Salim et al, 2022).

The Fagerstrom Test for Nicotine Dependence (FTND) is commonly utilized as the primary scale for assessing nicotine dependency in individuals who smoke regular tobacco cigarettes. However, the FTND does not assess the subject's nicotine dependence on electronic cigarettes (ECs). With six identical questions, the modified FTND scale offers the same scores as the original scale, which range from 0 to 10. The updated scale classified the vapers in the same way as the original FTND scale, with scores 1-3 indicating minimal nicotine dependence, 4-6 indicating medium nicotine dependence, and 7-10 indicating high nicotine reliance via EC. However, the new scale differs only slightly from the original FTND scale. The primary modifications are replacing the word tobacco cigarette (TCG) with an EC. Nearly all of the assessors highlighted that the frequency of EC usage was another modification made during the scale development phase. As opposed to TCGs, which are frequently smoked completely before they are disposed of. However, the EC can be used repeatedly over separate vaping sessions. As a result of the reviewers' comments, the investigator replaces the queries "how many tobacco cigarettes a day did you smoke" with "how many times a day do you vape." The investigator used a one-time vape session consisting of an average puff of up to 15. After a year of monitoring, the investigator assessed the subjects' nicotine status. Among 69 EC single users, 11 fully ceased taking nicotine, another 24 remained EC single users, 15 switched to dual usage, using both EC and TCGs, and 19 relapsed to TCG, as confirmed by CO level and saliva cotinine tests, respectively. Those EC single users who completely discontinued nicotine cessation after the one-year period had nicotine dependence values between 1 and 3 measured by the modified FTND scale at the baseline (Rahman *et al*., 2020).

As a conclusion, the Fagerstrom Test for Nicotine Dependence (FTND) is the most well-known test in terms of history, having been used in clinical and research settings. The FTND score has been a key tool for assessing and measuring nicotine addiction for decades (Lim *et al.,* 2023).

2.3 Imaging Modalities in Assessing Brain Perfusion

The use of imaging modalities in nuclear medicine to assess some element of brain function, frequently with the goal of studying the relationship between activity in certain brain areas and specific mental activities, is known as functional neuroimaging. It is extensively used in studies on social neuroscience, cognitive psychology, neuropsychology, and cognitive neuroscience. Commonly used techniques in functional neuroimaging include Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), functional Magnetic Resonance Imaging (fMRI), Electroencephalography (EEG), Magnetoencephalography (MEG), Arterial Spin Labelling (ASL), Single-Photon Emission Computed Tomography (SPECT), and functional Ultrasound Imaging (fUS). Functional brain imaging connects human cognition and behavior to local neural activity using noninvasive radiological methods. One of the approach's primary benefits is its ability to produce continuous three-dimensional maps that localise function throughout the entire brain (Grafton, 2014).

MRI provides detailed anatomical images using different sequences like T1-Weighted Imaging (T1WI), T2-Weighted Imaging (T2WI), Fluid-Attenuated Inversion Recovery (FLAIR), Diffusion-Weighted Imaging (DWI) and Susceptibility-Weighted Imaging (SWI). MRI scanners use higher field strengths for better image quality and faster scans. However, these high-field machines are costly to install and maintain, making them less common outside wealthy countries and big cities (Arnold *et al.*, 2023). The fMRI detects brain activity by tracking blood flow changes. Blood Oxygen Level Dependent (BOLD) fMRI maps active brain areas with high detail, without needing contrast, but is sensitive to motion and only measures blood flow, not direct neural activity. Resting-State fMRI (rs-fMRI) studies brain connections without tasks, useful for unconscious patients, but its data is harder to interpret and affected by breathing and heartbeat noise (Soares *et al.*, 2016). Arterial Spin Labelling (ASL) is an MRI technique that measures cerebral blood flow without contrast. It has three types which are Pulsed ASL (PASL) which is useful for stroke and dementia imaging but has low signal quality, Continuous ASL (CASL) which offers better image clarity but requires more energy and Pseudo-Continuous ASL (pCASL), a balanced approach widely used in research and clinics but more complex to implement. ASL is a valuable, non-invasive method for assessing brain perfusion (Ferreira & Bastos-Leite, 2024).

According to earlier research, positron emission tomography (PET) and single photon emission computed tomography (SPECT), when used as imaging probes with high-affinity and high-specificity molecular radioactive tracers, have a special effect in the field of molecular imaging modalities for visualizing and quantifying physiological processes in intact living brains (Lu & Yuan, 2015). A nuclear medicine imaging method termed Single Photon Emission Computed Tomography (SPECT) uses gamma rays to generate various 2-D visuals, sometimes known as projections, from different viewpoints. The SPECT scan's utility stems from its capacity to provide extensive physiological information about the tissues. This is accomplished by the development and application of radioactive tracer chemicals, often known as probes. These probes generally consist of a biologically active ligand specific to the tissue being studied (using a gamma camera) coupled with a detectable radioactive isotope (Yandrapalli & Puckett, 2022). A 3D data set is then produced by using a computer to apply a tomographic reconstruction method to the various projections. Following that, this data set may be processed to generate thin body slices along any desired axis, just as those produced by other tomographic methods, such as magnetic resonance imaging (MRI).

The SPECT scan was used as an alternative diagnostic tool in this research study, in addition to functional Magnetic Resonance Imaging (fMRI), to observe blood flow in a patient's brain because of its advantages, while also considering its disadvantages. It was cheaper and more readily available than other diagnostic tools. Additionally, the SPECT scan had good sensitivity compared to other diagnostic tools, allowed images to be merged and information to be combined, and provided information about brain function through nuclear scanning. During the SPECT scan procedure, the patient's body was exposed to radiation, but only in limited amounts.

SPECT and PET scanners use photons emitted by different radiopharmaceuticals to detect and gather three-dimensional data. SPECT cameras pick up photons that are directly emitted by SPECT radiopharmaceuticals. When radiotracers are used in PET, the positrons they release annihilate nearby electrons, releasing two photons in opposite directions (Kaechele & Chakko, 2023).

Most contemporary SPECT systems can be used in conjunction with an X-ray Computed Tomography subsystem (CT). In a multimodal SPECT/CT system, the SPECT and CT subsystems are often deployed sequentially, connected to a similar gantry, and using the same patient bed. SPECT/CT overcomes two challenges of SPECT imaging: assigning the shape of radiopharmaceutical uptake to morphologic structures and accounting for attenuation of released gamma photons, which is required for absolute quantification (Ritt, 2022). A computed tomography scan (CT scan), originally known as a computed axial tomography scan or CAT scan, is a medical imaging technology that produces comprehensive inside images of the body. CT scan technicians are known as radiographers or radiology technologists. CT scanners employ a revolving X-ray tube and a row of detectors mounted on a gantry to measure X-ray attenuation by various tissues within the body. Tomographic reconstruction techniques are then applied on a computer to analyze the many X-ray measurements obtained from different angles, producing tomographic (cross-sectional) images (virtual “slices”) of a body. When magnetic resonance imaging (MRI) is not an option for patients with iron implants or pacemakers, a CT scan can be performed in its place (CT scan, 2024). Compared to regular X-rays, CT scan pictures were more informative. Although there were many uses for a CT scan, one of its most valuable uses was the prompt examination of individuals who may have had internal damage from trauma or traffic accidents. A CT scan was used to diagnose illness or damage, arrange medical, surgical, or radiation treatments, and examine almost every part of the body. CT images were often stored as electronic data files and viewed on a computer screen. A radiologist interpreted these images and reported back to the doctor (Mayoclinic, 2022).

To assess cerebral hemodynamics, several radiopharmaceuticals have been produced. Comparative measurements of regional cerebral blood flow (CBF) can be obtained with SPECT radiotracers, such as technetium-99m ethyl cysteinate dimer (Tc99m-ECD), technetium-99m hexamethyl propylene amine oxime (Tc99m-HMPAO), and N-isopropyl-p-I-123 Iodo amphetamine (I-123 IMP). Because these radiotracers are lipophilic, they have little trouble passing through the blood-brain barrier. These substances become hydrophilic once the blood-brain barrier is crossed, allowing the brain to hold onto them for extended periods of time. Tc99m-ECD has a higher uptake in the gray matter than Tc99m-HMPAO, which improves the gray-to-white matter distinction of pictures. These radiopharmaceuticals are useful in the context of epilepsy because they provide patients time to be stabilized during the acute ictal state before transferring them to the SPECT scanner for imaging (Kaechele & Chakko, 2023).

2.4 Brain Perfusion in Non-Smokers and Smokers

Smoking is the primary preventable cause of disease and death in Malaysia. An estimated 20,000 deaths are recorded there each year, and smoking-related illnesses have been found to be a significant cause of disability-adjusted life years (DALYs) in Malaysian citizens (Lim et al., 2018). In order to ensure that smoking-related diseases are no longer a public health concern by 2020, the Malaysian government has decided to halve the rate of smoking prevalence as compared to the current prevalence through the Ministry of Health. This decision was made due to the high burden and expense of treating smoking-related diseases (MOH, 2015). In 2024, the Malaysian government remains committed to reducing smoking rates. The Control of Smoking Products for Public Health Act (Act 582), effective October 1, 2024, targets a 4% reduction in smoking prevalence by the end of 2025 (Malay Mail, 2024). To accomplish the goals, a number of initiatives have been put into place, such as campaigns for healthy lifestyles. Legislative measures, including bans on smoking in public areas, age limitations on tobacco product purchases, and periodic increases in cigarette prices, have been implemented since the 1990s in an attempt to lower the incidence of smoking among Malaysians (Sumarni MG & Kuang Hock, 2020). Per

Malaysia's Framework Convention on Tobacco Control (FCTC) 2020 study, 49% of men in the 30- to 34-year-old age group smoke at present. With the exception of male adolescents between the ages of 15 and 19, who smoke at a rate of 24%, smoking prevalence rates among males are at or above 45% in all age categories. Alarmingly, 17.4% of boys and girls between the ages of 13 and 15 smoke during this time. Among boys and girls 12 years of age and under, 9.2%, or nearly one in ten, also smoke. In Malaysia, smoking is permitted up until the age of 18.

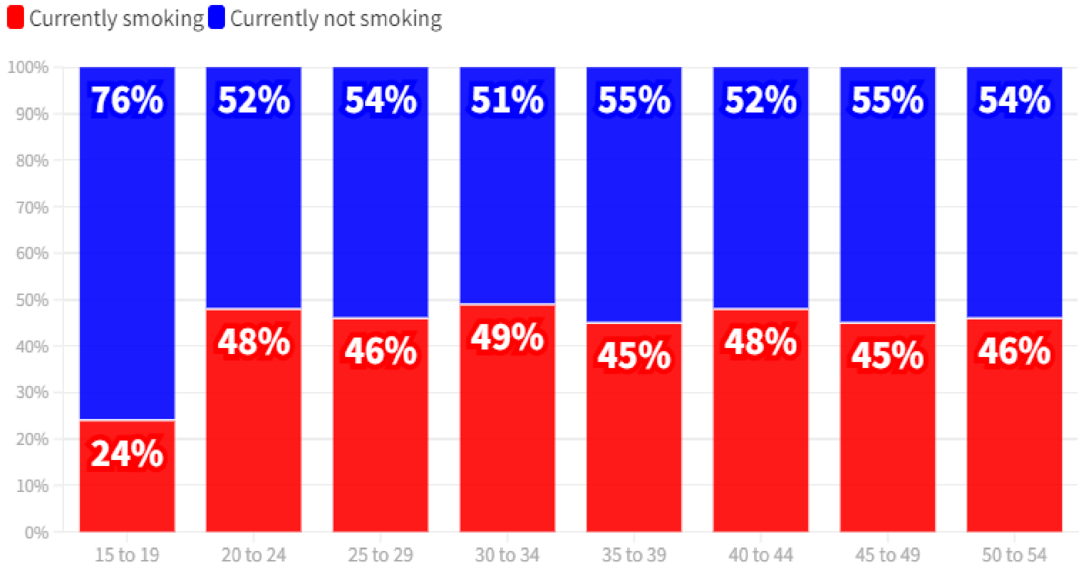

Figure 2.5 Smoking rates by age among men in Malaysia in 2019, currently smoke about 4,742,418(40.5% prevalence) ((Malaysia-2020-2021_EN., 2021;That Lingering Smoke From Malaysian Men - CodeBlue., 2022)

The act of pumping blood through a network of small blood vessels within biological tissue is known as perfusion, and it permits the passage of molecules, including oxygen, over semipermeable microvascular barriers. As a result, brain perfusion imaging techniques allow for the evaluation of regional hemodynamic conditions in extremely small blood arteries, as opposed to larger vessels examined by angiographic imaging techniques. In the therapeutic setting, brain perfusion imaging is typically conducted with X-ray computed tomography (CT) or magnetic resonance imaging (MRI). Because the vessels analyzed with perfusion imaging are too small to be seen directly, CT and MR perfusion imaging approaches rely on aggregating data from all of the vessels inside a single volume element of the brain, or voxel. This data influences the look of a single picture element, or pixel, in so-called "source images" images, which are captured using specialized techniques on a standard clinical CT or MR scanner. The perfusion source images are then postprocessed using computation to produce "maps" of various regional hemodynamic parameters. These perfusion maps are visually interpreted (Copen, Lev & Rapalino, 2016). Following brain scanning, extremely active brain regions frequently exhibit higher blood flow as well as higher oxygen and glucose consumption. You may determine which parts of your brain are most engaged by monitoring these increases. These characteristics may be less in brain regions that are not very active or damaged.

Nicotine addiction can cause significant changes inside the brain. Magnetic resonance angiography (MRA) of a chronic smoker shows reduced cerebral blood flow supplying anterior circulation of the brain (anterior cerebral and middle cerebral artery) (Song *et al.*, 2017). The majority of the frontal, parietal, and temporal lobes of the brain are supplied by this artery. This may predispose a smoker to have stroke and impaired cognitive function. A few fMRI studies show the negative impact of smoking habit. A study by Duehlmeyer and Hester (2019) reported that smokers had hyperactivity in the dorsolateral prefrontal cortex that supports poor learning from error and decreased punishment sensitivity that can limit their learning from error (Duehlmeyer & Hester, 2019). Smokers who are abstinent from smoking about 3 hours show reduced activity in the attention area (cuneus and precuneus) and high activity in craving area (limbic, posterior cingulate, prefrontal cortex) causing smoker poor attention and high craving for nicotine.

Introduction of Nicotine patch shows reduced activity in craving areas and improves their attention (Liberman *et al.*, 2018). Nicotine withdrawal also can cause anhedonia and reduce cognitive function especially during the first week of abstinence (Reed & Difranza, 2017). Anhedonia causes smokers to experience low pleasure on common reward and reduction of cognitive function causes them to be less concerned about their health. They also have a higher discount rate preferring small rewards immediately compared to large rewards later. This finding contributes to high relapse rate among smokers.

Regional CBF showed statistically significant correlations with arterial nicotine level, with the cerebellum and occipital cortex experiencing the largest increases in relative blood flow (Song *et al.*, 2017). Additionally, mesolimbic dopaminergic system injuries reduce nicotine self-administration and the effects of nicotine on promoting locomotion. Numerous researchers have observed that nicotine has an impact on how the human brain functions, as seen by changes in regional cerebral blood flow (rCBF), metabolism, or blood oxygenation level. Smoking has been demonstrated to be depriving smokers of their grey matter, as evidenced by the smaller cerebral cortex smokers possessed compared to non-smokers. This is important because the thickness of the cerebral cortex, which is a part of the brain, is important for cognitive skills, such as memory and learning (Karama *et al.*, 2015).

According to epidemiologic evidence, smoking history is clearly associated with preclinical brain changes, fast cognitive decline (executive function, verbal memory, speed of processing), and an increased risk of dementia. Receptor imaging has yielded several insights on tobacco addiction in the living human brain, according to a different study by Cosgrove et al. (2015). These results ought to influence how they approach cigarette use. For instance, they are aware that the dopaminergic signature in response to cigarette use is transient but significantly reduces craving.

2.5 Effect of Overnight Fasting in Brain Perfusion in Non-Smokers and Smokers

Fasting is a ritual and action that has been practiced for centuries. It has been demonstrated that fasting helps diabetes individuals with their insulin resistance and helps them lose weight. Fasting can slow down the aging process in the brain (aging-induced neuronal dysfunction). Additionally, it can stimulate brain plasticity, lessen excitotoxic stress, and enhance learning and memory (Cerqueira, Chausse & Kowaltowski, 2017). Study in dementia patients shows that intermittent fasting can downregulate inflammatory response inside the brain thus it can improve cognitive decline in the patient (Yoon & Song, 2019).

According to a prior research, this adds to the expanding body of information about how stopping smoking affects neurocognitive performance by providing a new look at the brain substrates that underlie the disruption of inhibitory control (IC) during withdrawal. The findings of this work indicate that the right inferior frontal cortex (rIFC) became more activated upon quitting smoking, indicating a higher demand of attention processes after quitting, rather than the control or disruption of stopping processes themselves. Future research utilizing tasks that separate the attentional and behavioral control components of IC may shed more light on the mechanisms behind the deficits in IC brought on by abstinence. The current findings also point to rIFC