STUDY ON KRATOM USAGE EFFECTS AT RESPIRATORY CENTRE (BRAIN STEM) BY FUNCTIONAL MRI

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Table of contents Page

LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

BOLD	Blood oxygenation level-dependent
CNS	Central nervous system
fMRI	Functional magnetic resonance imaging
HUSM	Hospital Universiti Sains Malaysia
PACS	Picture archiving and communications system
SD	Standard deviation
SPM12	Statistical Parametric Mapping software 12
DICOM	Digital Imaging and Communications in Medicine

LIST OF FIGURES **PAGE**

ABSTRAK

Latar belakang: *Mitragyna speciosa* (Kort) atau lebih dikenali sebagai ketum adalah tumbuhan asli yang biasa ditemui di Asia Tenggara dan penggunaan tradisional sebagai ubat herba untuk pelbagai penyakit, biasanya digunakan sebagai ubat penahan sakit dan untuk memerangi kepenatan. Selain penggunaannya untuk tujuan perubatan, ia juga digunakan sebagai ubat rekreasi dan sebagai pengganti opioid. Walaupun penggunaan ketum semakin meningkat di seluruh dunia, potensi kesan sampingan penggunaan ketum yang berpanjangan pada struktur otak pada masa kini tidak jelas terutamanya di bahagian pernafasan.

Metod: 22 orang pengguna ketum dewasa dan 20 orang bukan pengguna ketum (kumpulan kawalan) yang menjalani functional MRI untuk penyelidikan terdahulu telah dimasukkan dalam kajian keratan rentas ini. Pengesahan kesan kepada bahagian otak dikenalpasti dalam bacaan fMRI images dimana ia ditukar kepada format lain dan dikira menggunakan software MATlab SPM12 untuk mendapatkan keputusan.

Keputusan: Purata bagi jumlah activation dalam kalangan pengguna ketum dewasa adalah 4487 (Z : 6.78) sewaktu P< 0.05 dan apabila mengubah P< 0.001 didapati kawasan yang dikesani adalah sebanyak 10300(Z : 6.78). Bagi subjek bukan pengguna ketum, didapati kawasan yang dikenali menunjukkan pengesanan adalah 5 (Z :5.2) pada P< 0.05 dan apabila mengubah P<0.001, tiada perubahan pengesanan dapat dikenal pasti di bahagian batang otak (brainstem). Kesimpulannya, jumlah pengesahan didapati dalam bahagian batang otak adalah tinggi di kalangan pengguna ketum berbanding

dengan di kalangan bukan pengguna ketum. Ini menunjukkan kesan negatif terhadap sistem pernafasan di kalangan pengguna ketum.

Kesimpulan: Kajian kami menunjukkan bahawa penggunaan ketum secara berpanjangan dikaitkan dengan kesan sampingan ke atas bahagian otak khas untuk pernafasan(batang otak), namun kesannya tidak begitu ketara dalam analysis yang dijalankan di kalangan bukan pengguna ketum. Walaubagaimanapun, kajian lanjut diperlukan untuk mewujudkan lebih banyak data untuk kegunaan ketum dan kesan berkaitan dengan aktiviti otak yang lain.

Kata kunci: Mitragyna speciosa, ketum, magnetic resonance imaging brain, opioid

ABSTRACT

Background: *Mitragyna speciosa* (Korth.) or kratom is an indigenous medicinal plant native to Southeast Asia, a tropical tree *(Mitragyna speciosa)* that contains compounds that can have psychotropic effects. Kratom leaves can be freshly chewed or by decoction preparation for herbal use. Kratom has effects similar to both opioids and stimulants. Despite increasing usage across the globe, the potential side effects and respiratory depression due to prolonged use of kratom are still unclear. Functional magnetic resonance imaging (fMRI) is a non-invasive imaging method for measuring brain activity by detecting neurophysiological changes in cerebral blood flow to predict neuronal activation in the brain.

Methods: 22 regular kratom adult kratom users and 20 non-kratom users who underwent fMRI for prior research were included in this cross-sectional study. A non-invasive neuroimaging technique measuring brain activity by detecting neurophysiological changes in the cerebral blood flow and thus predicting neuronal activation within the brain. The activation on fMRI images were transferred to SPM format and calculated using Mathlab SPM12.

Results: Area of brain activation referred to kratom group was calculated $P<0.05$, at least, 4487 (Z value 6.78) and at $P< 0.001$ noted 10300(Z value 6.78). While, area of brain activation in non-kratom user group was calculated, noted at $P< 0.05$ only 5 (Z value 5.2) and at $P<0.001$, no activation was depicted at brainstem. This suggests respiratory depression in the brainstem noted within Kratom users.

Conclusion: Overall study shows long-term consumption of kratom is associated with respiratory depression affecting the brainstem within Kratom users as we noted significant activation- higher cluster level seen within brainstem. On the other hand, minimal activation noted within the brainstem in non Kratom users. However, further studies are needed to establish more data on use of kratom and its effects on brain function using more invasive and diagnostic methods like analysing blood gas parameters for more accurate results.

keywords: Mitragyna speciose, kratom, magnetic resonance imaging brain, opioid, BOLD

CHAPTER 1: BACKGROUND

1.1 Introduction

Mitragyna speciosa (Korth.) or kratom is a native medicinal plant of Southeast Asia, a tropical tree (Mitragyna speciosa) that contains compounds that may have psychotropic effects. Kratom leaves can be chewed fresh or prepared as a decoction for use as a medicinal plant. Kratom can produce effects similar to opioids and stimulants. Two compounds in kratom leaves, mitragynine and 7-α-hydroxymitragynine, act as agonists at the mu and delta opioid receptors and at the alpha-2 and 5-HT2A adrenergic receptors in the brain, producing potent analgesic effects(Hill, R., Kruegel, A. C., Javitch, J. A.,Lane, J. R., & Canals, M. (2022). These are the same receptors that are affected by opioids and some other drugs. It can produce sedation, pleasure, and pain relief. Like other drugs with opioid-like effects, kratom can be addictive and cause physical withdrawal symptoms when overdosed.

The effects of kratom last for about 30 minutes to an hour. The duration of the analgesic effect is about 4-5 hours, and the stimulant effect is 2-5 hours. The peak effect of kratom occurs within an hour or two after ingestion. (Singh, Darshan; Narayanan, Suresh; Vicknasingam, Balasingam; et al.2012)

Functional magnetic resonance imaging (fMRI) is a non-invasive imaging method for measuring brain activity by detecting neurophysiological changes in cerebral blood flow to predict neuronal activation in the brain (Baert et al. l, 2007). 1.5 or 3 Tesla (T) MRI scanners are widely available, and the techniques for determining which parts of the brain are activated (e.g., during movement of a finger, a decision, or an inspiration) are easy to implement in a standard clinical setting.

When an MRI scanner detects changes in the regional distribution of blood flow to tissue with higher oxygen demand due to increased neural activity, human brain

mapping is achieved. Blood oxygenation level-dependent (BOLD) effect is measured when MRI scanner is equipped with echo-planar imaging capability depends on image intensity results from various tissue contrast mechanisms (e.g., proton density, T1 and T2 relaxation rates, in-flow in blood plasma protons) and show functional sensitivity used noninvasively detect functional changes in the human brain.

Advances in current brain imaging technology have provided researchers with the platform to study regional brain activity while subjects are in various emotional states or performing cognitive and motor tasks (Cohen and Bookheimer 1994). One fMRI has been conducted to study the specific neural actions of drugs abuse (Kleinschmidt et al. 1999). These studies are designed to investigate regional changes in neural activation in the presence or absence of a specific drug during a particular activational state, which includes the performance of a specific cognitive or motor task, or simple exposure to a specific visual or auditory stimulus.

Several previous studies have explored the effects of stimulant drugs, such as cocaine or amphetamine, on brain activity during auditory or visual stimulation (Howard et al. 1996; Gollub et al. 1998), during the presentation of a drug-related visual cue (a stimulus used to induce craving (Maas et al. 1998)), or during the performance of the Wisconsin Card Sorting Task (a measure of executive function (Daniel et al. 1991)). The results of these studies indicate that stimulant drugs may increase neural activity in specific brain regions when subjects are engaged in particular tasks, and the particular brain regions are activated corresponding to its functional neuroanatomy. Thus, the fMRI technique makes it possible to understand brain function and study the mechanisms whereby psychoactive drugs may alter behaviour.

FMRI can detect blood oxygen level–dependent (BOLD) changes in the MRI signal due to changes in neuronal activity following a change in brain state produced by

a stimulus or task. It is well established that an increase in neural activity in a region of the cortex stimulates an increase in the local blood flow to accommodate the demand for oxygen and other substrates. The change in blood flow exceeds the needs at the capillary level, thus there is a net increase in the balance of oxygenated arterial blood to deoxygenated venous blood. Essentially, the change in tissue perfusion exceeds the additional metabolic demand, so the concentration of deoxyhemoglobin within tissues decreases. This decrease directly affects the signals used to produce magnetic resonance images. The result of having lower levels of deoxyhemoglobin in blood in a region of brain tissue is therefore that the MRI signal from that region decays less rapidly and so is stronger when recorded in a typical magnetic resonance image acquisition. This small signal increase is the BOLD signal recorded in fMRI as activation.

The aim of this study is to determine if there is significant respiratory depression in the brainstem of regular adult kratom users compared to non-kratom users. The focus will be on assessing overall brain activation in specific areas related to the respiratory system. We hope that this could be a first step in determining the effect of kratom and using kratom as a substitute for opioids.

1.2 Objectives

1.2.1 General Objective

To explore the kratom effects on respiratory centre on regular kratom user's brain using fMRI.

1.2.2 Specific Objectives

1. To determine the areas of brain activation healthy subjects and among regular kratom users using BOLD activation by fMRI.
2. To compare the intensity of activation (respiratory depression) at respiratory centre(brainstem) in kratom and healthy subjects by low frequency fluctuations(LFF) analysis in fMRI images using software Mathlab.

1.3 Hypothesis

1. There is significant brain activation between the regular kratom user and non-kratom users.

2. There is significant respiratory depression at brainstem on regular kratom user compared with non-kratom users.

1.4 Research Question

1. Does kratom cause respiratory depression as other opiods by affecting respiratory centres in the brain regions?
2. Are there any differences in brain activation at respiratory centre between the kratom users and healthy controls using fMRI ?

CHAPTER 2: LITERATURE REVIEW

2.1 Kratom and its effects:

Mitragyna speciosa (Korth.) or kratom is a native medicinal and spice plant from Southeast Asia. Kratom leaves can be chewed fresh or used as a decoction as an herb. It is usually used to increase energy to work harder, increase physical stamina (Ahmad & Aziz, 2012; Singh et al., 2018), increase sexual desire (Ahmad & Aziz, 2012), for relaxation (Ahmad & Aziz, 2012; Cinosi et al., 2015), socialisation (Singh et. al, 2014; Singh et al, 2018), substitute for other drug addiction (Ahmad & Aziz, 2012; Vicknasingam et. al 2010) and stress management (Singh et al, 2018). Kratom belongs to the Rubiaccecae family, which also includes the genus Coffea (Eisenman 2014).

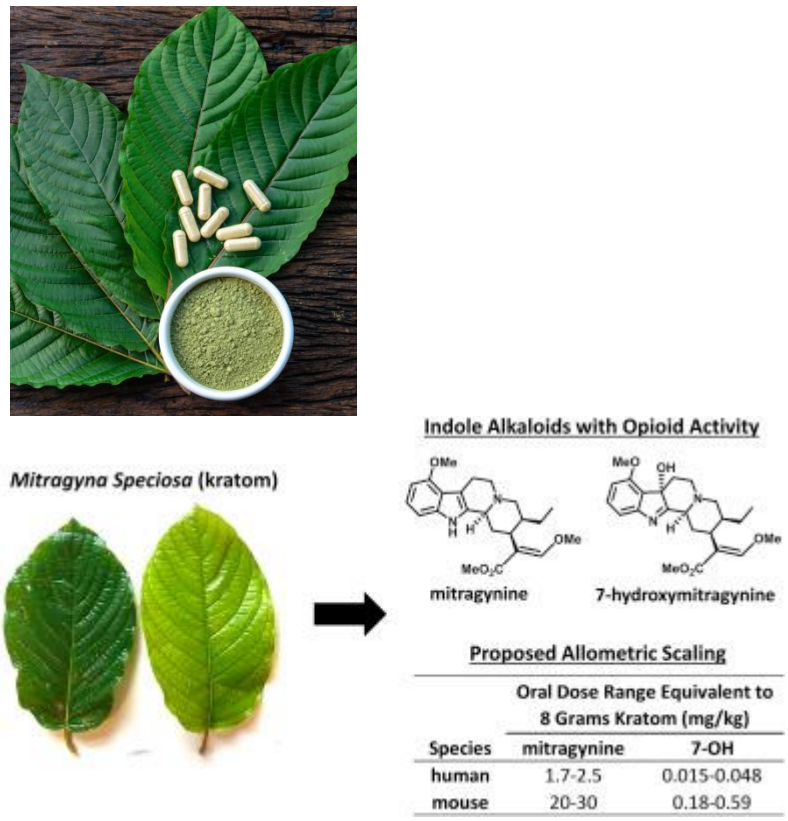

Figure 1: *Mitragyna speciosa* and chemical structure (source: adapted from Wikipedia)

Kratom has been used for centuries in Southeast Asia for its medicinal properties, but its use is still not widely accepted and controversial in many countries around the world. Some studies (Singh et. al, 2014; Singh et al, 2018) have shown that kratom and its alkaloids have a range of therapeutic effects, including pain relief, mood enhancement, and increased energy. However, there is still limited research on the safety and efficacy of kratom. It also has the potential for abuse and dependence.

Kratom's effects are dose-dependent, with lower doses producing stimulant-like effects and higher doses producing opioid-like effects. Some studies have suggested that kratom may be effective for managing opioid withdrawal, but more research is needed to confirm this(Ahmad & Aziz, 2012; Vicknasingam et. al 2010). Kratom is also commonly used for self-treatment of mental health conditions such as depression and anxiety, but the safety and efficacy of its use have not been thoroughly studied.

Despite its use as a traditional medicine and its potential therapeutic benefits, kratom is not approved by the US Food and Drug Administration (FDA) for use and is considered a drug of concern. The FDA has issued warnings about the potential dangers of kratom use, including the risk of addiction, overdose, and even death. It is important to note that the regulation on the use of kratom varies by country.

In summary, while kratom is a promising therapeutic agent, more research is needed to fully understand its effects and potential risks. People should be cautious when using kratom and seek medical advice before use.

2.2 Kratom studies:

There are few studies investigating the side effects of regular kratom use on the respiratory system, especially using functional MRIs and medications. Most of the studies have been conducted on animals. Recently, one study has shown that there is evidence of respiratory depression from kratom consumption. (Hill, R., Kruegel, A. C., Javitch, J. A.,Lane, J. R., & Canals, M. (2022). Oral mitragynine was studied because mitragynine is the only active alkaloid in many drugs and kratom products. Many products have low or undetectable concentrations of other alkaloids that may also contribute to respiratory and other effects (Chakraborty et al. 2021; Sharma et al. 2019; Sharma and McCurdy 2021). Furthermore, unlike opioids and other substances widely used recreationally, which are intravenous, insufflated or inhaled (e.g. O'Brien 2015), kratom is taken orally as dried kratom leaf powder in the form of teas, food or drink, or capsules filled with leaf powder (Cinosi et al. 2015; Henningfield et al. 2018, 2022; Ramanathan and McCurdy 2020; Singh et al. 2016).

Neuroimaging findings and studies show that chronic opiate abuse affects the prefrontal cortex (PFC) (Parvaz et al., 2011), temporal insula and thalamus (Goldstein and Volkow, 2002), nucleus accumbens (Noel and Gratton, 1995), amygdala (Baxter et al., 2000) and sensorimotor cortexes (Liu et al., 2009). However, the evidence to date is not yet sufficient to capture the integrity of physiological events and psychological events as brain functions and behaviours in kratom users.

Earlier studies concluded that mitragynine has low respiratory depressant potential compared to morphine or other μ-opioid agonists. Macko et al (1972) measured respiratory rate in cats and dogs after administration of morphine and/or codeine or mitragynine and found lower effects of mitragynine. Another recent study (Hill et al. 2022) compared 3 to 90 mg/kg mitragynine with 3, 10 and 30 mg/kg morphine administered to mice in plethysmography chambers that allowed measurement of respiratory minute volume as designed. The morphine administered resulted in a dose-dependent decrease in respiratory minute volume. The maximum effect of 90 mg/kg mitragynine was needed to produce an effect of 10 to 30 mg/kg morphine. The most important finding of the study was that mitragynine did not produce signs of respiratory depression at doses many times higher than those used in humans. In contrast, oxycodone/morphine produced the expected dose-dependent respiratory depressive effects, consistent with its potent morphine opioid (i.e. μ-opioid) receptor-mediated action. These results are consistent with the pharmacology of mitragynine, including partial μ-opioid receptor agonism with low recruitment of the respiratory depressant activating β-arrestin pathway (Kruegel et al. 2016, 2019; Váradi et al. 2016). Both of the aforementioned studies conducted in mice, Macko et al. (1972) and Hill et al. (2022) found no respiratory depressant effect at 3 mg/kg, but noted some respiratory depression at 10 mg/kg, which they reported did not change at higher doses. However, it is unclear whether this difference from the present finding is due to the mice, the measurement methods or some other factor. In addition, Váradi et al. (2016, p. 7) reported that a mitragynine analogue had a lower risk of respiratory depression compared to the opioid morphine, in contrast to the dose-dependent respiratory depressive effects of morphine. The human equivalent doses (HED) of the doses tested in this rat study are based on estimated body surface area, with rat doses divided by 6.2 according to the study (Nair

and Jacob 2016). Manufacturers of mitragynine-containing kratom extract state that about 25 mg per serving, or about 0.35 mg/kg for a 70 kg human, is the minimum amount accepted by regular consumers. In a study conducted in Malaysia involving 293 male regular kratom users, they were found to use kratom as a substitute for alcohol and narcotics in cocktails (D Singh et al., 2018). However, the use of kratom has been reported to carry the risk of addiction and unpleasant withdrawal symptoms (Singh et al., 2014; Swogger & Walsh, 2018), sometimes causing severe adverse effects such as seizures and coma (Nelsen et al., 2010). Some studies show that kratom does not affect social functioning (Singh et al. 2015) and cognitive functioning (Singh et al., 2018). Furthermore, there was a study on human subjects by Singh et. al, 2018 which concluded that there was no significant difference in brain volume, diffusion tensor imaging metrics, fractional anisotropy (FA) and mean diffusivity (MD) between kratom users and controls.

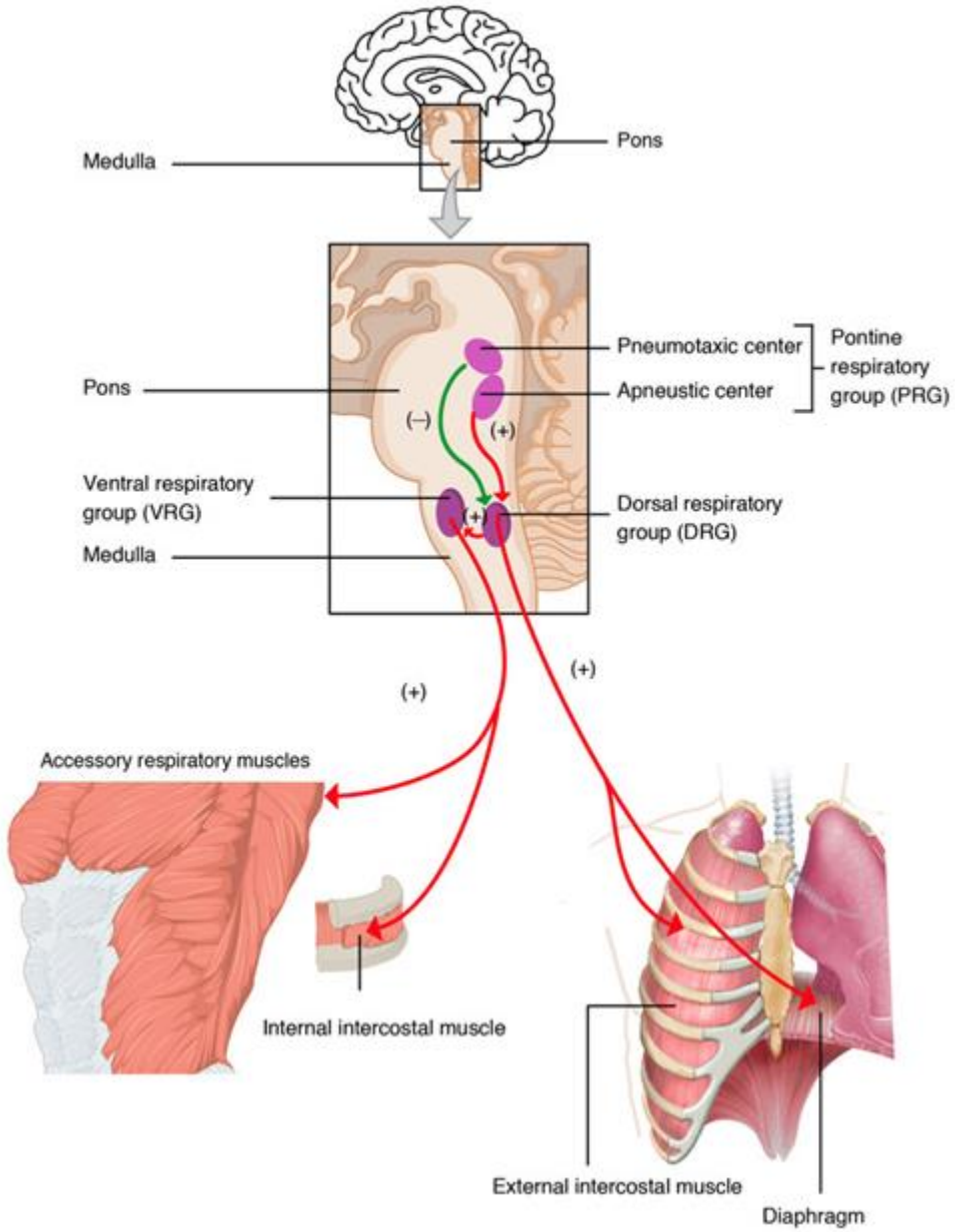

Figure 2: Respiratory activity in brainstem illustrated (source:Levitzky, Michael G. (2002)

2.3 Respiratory centre in the brainstem

The respiratory rate (minute volume) is primarily controlled and determined by the carbon dioxide content in the blood, which is determined by the metabolic rate. The central chemoreceptors are located on the ventrolateral surface of the medulla oblongata and detect changes in spinal pH. The apneustic (stimulating) and pneumotaxic (limiting) centres of the pons work together to control respiratory rate. The spinal cord sends signals to the muscles that initiate inhalation and exhalation, and controls other reflexes such as coughing and sneezing. The ventral respiratory group in the medulla oblongata anterior and lateral to the DRG (consisting of the nucleus ambiguus and the nucleus retro ambiguus) controls voluntary forced expiration and increases the force of inspiration. The dorsal respiratory group in the upper part of the spinal cord (consisting of the nucleus tractus solitarius) mainly controls inspiratory movements and speed. The aim of this study is to localise and investigate respiratory depression in kratom addicts using FMRI. Respiration is thought to modulate the signal from BOLD primarily by inducing changes in carbon dioxide (CO2) levels, a potent vasodilator (Birn et al., 2006). This assumption is supported by several reasons:(1) partial pressure of arterial CO2 (PaCO2) is a function of ventilation, defined as the product of depth and rate of respiration (Berne and Levy, 1993); (2) inhalation of elevated CO2 concentrations triggers cerebral vasodilation, leading to increased cerebral blood flow (CBF) and corresponding BOLD signal changes (Bandettini and Wong, 1997; Kastrup et al., 1999; Kwong et al, 1995; Li et al, 1999; Nakada et al, 2001; Rostrup et al, 2000; Stillman et al, 1995; Vesely et al, 2001; Wise et al, 2007). This was implicated in the previous study (USM/JEPEM/19010053) when fMRI images were acquired for kratom and non-kratom users at rest and during inspiration.

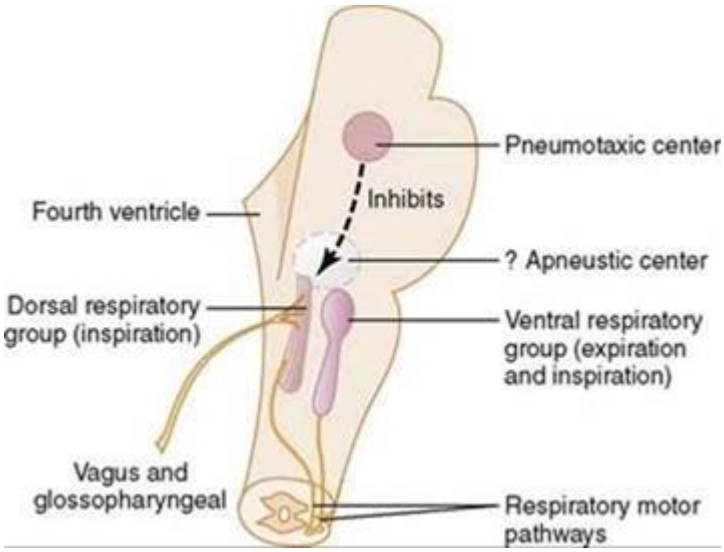

Figure3: Brainstem illustration.(Adapted from Levitzky, Michael G. (2002))

2.4 Functional MRI and BOLD

Functional magnetic resonance imaging (fMRI) allows identification of the location, pattern and activity of the human brain without intravenous contrast agents or radioactive tracers. FMRI is a non-invasive neuroimaging technique that indirectly measures brain activity by detecting neurophysiological changes in cerebral blood flow and is able to detect neuronal activation in the brain (Baert et al. l, 2007). Functional imaging of the human brain is now widely available as MRI scanners with 1.5 or 3Tesla (T) are universally available. Moreover, the techniques for determining which parts of the brain are activated during a task are easy to implement in a standard clinical setting. "Brain mapping" of the human brain is achieved by adjusting the MRI scanner to detect changes in the regional distribution of blood flow to tissue with higher oxygen

demand due to increased neuronal activity in the region. There are several methods to achieve this; the most common technique uses the blood oxygenation dependent effect (BOLD). For this, the MRI scanner should be equipped with the capability of echoplanar imaging. The method is based on physiological contrast effects, where the intensity of the magnetic resonance image results from different tissue contrast mechanisms (e.g. proton density, T1 and T2 relaxation rates, influx of protons in the blood plasma). Importantly, some of these tissue contrast mechanisms have functional sensitivity (cortex, precortex and brainstem) and can therefore be used to detect functional changes in the human brain non-invasively.

Haemodynamic response, defined as an increase in cerebral blood flow (CBF), cerebral blood volume and cerebral capillary and venous oxygen saturation (because the increase in CBF results in a greater supply of oxygenated blood to the tissues than is required by the increased metabolic demand). Because deoxyhaemoglobin disrupts the uniformity of the MRI scanner's magnetic field more than oxyhaemoglobin itself, the increased venous oxygen saturation leads to an increase in the MR signal and thus a different image intensity in the region of neuronal activation.

The BOLD effect occurs because the microvascular magnetic resonance signal on T2- and T2*-weighted images is strongly influenced by the oxygen content of the blood. The iron content in haemoglobin is used as a tool in a magnetic susceptibility-induced T2*-shortening intravascular contrast agent, which serves as a local indicator of functional activation. Oxygenated blood contains oxygenated haemoglobin, which is diamagnetic and has a low magnetic susceptibility effect. Deoxyhaemoglobin is significantly more paramagnetic and thus alters the local magnetic field, B0. The local T2*, which is critical in fMRI, is calculated based on the ratio of deoxygenated to