fMRI STUDY OF PAIN THRESHOLD IN THE PRESENCE AND ABSENCE OF THE LOVED ONE

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KAJIAN PENGIMEJAN RESONANS MAGNET KEFUNGSIAN KE ATAS TAHAP KESAKITAN DENDAN KEHADIRAN DAN KETIDAKHADIRAN ORANG TERSAYANG

oleh

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LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
AMA	American Medical Association
AMY	Amygdala
BMS	Bayesian Model Selection
BOLD	Blood-Oxygenation-Level-Dependence
CNS	Central Nervous System
DCM	Dynamic Causal Modelling
ECR-RS	Experience Closed Relationship - Relationship Structure
EPI	Echo Planar Imaging
FFX	Fixed Effect
FID	Free Induction Decay
fMRI	functional Magnetic Resonance Imaging
FOV	Field of View
FWE	Family Wise Error
GLM	General Linear Model
HIP	Hippocampus
HUSM	Hospital Universiti Sains Malaysia
IASP	International Association for the Study of Pain

INS	Insula
MCC	Middle Cingulate Cortex
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
OFC	Orbito-frontal Cortex
PCC	Posterior Cingulate Cortex
PET	Positron Emission Tomography
PHG	Parahippocampal Gyrus
RF	Radio Frequency
RFX	Random Effect
SE	Standard Error
SII	Secondary Somatosensory Cortex
SMA	Supplementary Motor Area
SPM	Statistical Parametric Mapping
SPSS	Statistical Package for Social Sciences
TE	Echo Time
THA	Thalamus
Th: YAG	Thulium: Yttrium-Aliminium Garnate
TR	Repetition Time

USM	Universiti Sains Malaysia
USMaP-i	Universiti Sains Malaysia Personality Inventory
VLPFC	Ventrolateral Prefrontal Cortex

LIST OF SYMBOLS

ω_o	Larmor frequency in unit Hz
γ	Gyromagnetic ratio in unit MHz/T
A	Intrinsic connectivity
B	Modulation of connectivity
B_o	External magnetic field strength in unit Tesla (T)
C	Direct input
D	Nonlinear input component
G1	Group which presented more pain in the presence of the love one (indicates *Love Hurts*)
G2	Group which presented less pain in the presence of the love one (indicates *Love Heals*)
H1	Higher energy in laser stimuli
H2	Highest energy in laser stimuli
L1	Lower energy in laser stimuli
L2	Lowest energy in laser stimuli
M	Mean energy in laser stimuli
mJ	miliJoule
mm	milimeter
mm^2	milimeter square

ms	milisecond
T	Tesla
T1	Longitudinal relaxation time
T2	Transverse relaxation time
$T2^{*}$	Transverse relaxation time for gradient echo field
u_1	input stimulus (laser energy)
u_2	input modulator (presence of the loved one)
Z_1	Neuronal dynamic of ACC
z_1	variable in ACC
Z_2	Neuronal dynamic of MCC
z_2	variable in MCC
Z_3	Neuronal dynamic of PCC
z_3	variable in PCC
α	significant level
μm	micrometer

KAJIAN PENGIMEJAN RESONANS MAGNET KEFUNGSIAN KE ATAS TAHAP KESAKITAN DENGAN KEHADIRAN DAN KETIDAKHADIRAN ORANG TERSAYANG

ABSTRAK

Kesakitan yang disebabkan oleh haba laser dan teknik pengimejan resonans magnet kefungsian (fMRI) telah digunakan untuk mengkaji respons terhadap modulasi kesakitan di dalam dua keadaan. Keadaan pertama ialah apabila peserta tidak ditemani orang tersayang (keadaan Bersendiri) dan keadaan kedua ialah apabila peserta ditemani oleh orang tersayang (keadaan Sokongan) semasa stimulasi kesakitan diberikan di dalam gantri MRI. Laser jenis Th:YAG digunakan untuk stimulasi kesakitan dan alur cahayanya disasarkan ke dorsum tangan kanan. Paradigma eksperimen menggunakan kaedah blok dihasilkan terlebih dahulu untuk imbasan fMRI. Sebanyak 17 subjek perempuan telah dipilih (min umur 20.59, SD 2.85 tahun) dan tahap kesakitan ditentukan terlebih dahulu sebelum imbasan. Soal selidik Pengalaman Hubungan Akrab - Struktur Hubungan (ECR-RS) dan ujian personaliti USM (USMaP-i) diberikan kepada subjek sebelum imbasan. Pemetaan Statistik Berparameter (SPM) versi 8 dengan pendekatan pemodelan dinamik penyebab (DCM) digunakan untuk mengkaji kehubungan di antara kawasan-kawasan pengaktifan dan akhirnya satu model kehubungan optimum ditentukan. Kajian ini mendapati tindak balas individu terhadap kesakitan boleh dibahagikan kepada dua kategori. Kumpulan yang mempunyai ahli keluarga sebagai orang tersayang mempunyai tahap kesakitan yang lebih rendah di dalam keadaan Sokongan, mewakili kes *Cinta Menyakitkan*; manakala kumpulan yang ditemani oleh pasangan mereka mempunyai tahap kesakitan yang lebih tinggi di dalam keadaan

yang sama, mewakili kes *Cinta Menguatkan*. Ciri-ciri personaliti seperti Ekstraversi didapati mengurangkan tindak balas kepada rangsangan kesakitan apabila ditemani oleh orang tersayang. Semua peserta menunjukkan pengaktifan di dalam kawasan otak yang berkaitan dengan pemprosesan kesakitan. Di dalam keadaan Bersendiri, ACC, MCC, INS, AMY, VLPFC dan HIP didapati teraktif, manakala di dalam keadaan Sokongan, INS, VLPFC, SII, THA dan girus supramarginal didapati teraktif. Analisis DCM menunjukkan *Cinta Menyakitkan* melibatkan pengaktifan dalam THA, PHG dan HIP manakala *Cinta Menguatkan* melibatkan pengaktifan di semua bahagian korteks singulat. BMS menunjukkan *Cinta Menguatkan* boleh diwakili oleh rangkaian kortikal yang melibatkan kehubungan intrinsik ACC → PCC → MCC dan ACC → MCC. Kesimpulannya, kajian ini mendapati kehadiran orang tersayang berdekatan individu memodulasikan kesakitan secara berbeza dan bergantung kepada keperibadian seseorang individu serta jenis perhubungannya dengan orang tersayang. Lebih menarik lagi, kajian ini mendapati satu kemungkinan rangkaian korteks yang baru untuk mekanisma *Cinta Menguatkan*.

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ABSTRACT

Laser-induced heat pain and functional magnetic resonance imaging (fMRI) techniques were used to investigate the modulation of pain response under two different conditions. One condition was when the participants were not accompanied by their loved ones (Alone condition) and the other condition was when the participants were accompanied by their loved ones (Support condition) during pain stimulus delivery inside the MRI gantry. Th:YAG laser was used as pain stimuli with its light beam targeted onto the dorsum of the right hand. An experimental paradigm utilizing block design was first developed for the fMRI scan. 17 female subjects participated (mean age 20.59; SD 2.85 years) and the pain threshold was determined prior to scanning. The Experience Closed Relationship - Relationship Structure (ECR - RS) test and USM personality inventory questionnaires (USMaP-i) were given prior to fMRI scanning. Statistical Parametric Mapping (SPM) version 8 with Dynamic Causal Modelling (DCM) approach was used to investigate the connectivity between activated regions and one optimum connectivity model was identified finally. It was found that individual responses to pain may be divided into two categories. The group accompanied by a family member as the loved one have lower pain threshold in Support condition, representing *Love Hurts*; while the group accompanied by a partner have higher pain threshold in the same condition, representing *Love Heals*. Extraversion personality was found to reduce the response to pain stimulation when accompanied by the loved one. All participants showed activations in areas associated with pain processing. In Alone condition, ACC, MCC,

INS, AMY, VLPFC and HIP were activated, while in Support condition, INS, VLPFC, SII, THA and supramarginal gyrus were activated. DCM analysis revealed that *Love Hurts* involved activations in THA, PHG and HIP while *Love Heals* involved activations in all parts of cingulate cortex. BMS showed that *Love Heals* could be represented by a cortical network involving the intrinsic connectivity of ACC → PCC → MCC and ACC → MCC. In conclusion, the present study revealed that having a loved one nearby modulates pain differently depending on the personality of the individual and the type of relationship with the loved one. More interestingly, this study discovers a new possible cortical network for *Love Heals*.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Functional magnetic resonance imaging (fMRI) is one of the techniques to image the brain in vivo and is capable of correlating psycho-physiological processes. This technique records neuronal activity by measuring changes in blood flow (Brooks & Tracey, 2005). fMRI provides information related to brain function by capturing the blood oxygenation level dependent (BOLD) contrast which is sensitive to changes in the state of oxygenation of the hemoglobin (Westbrook *et al.*, 2005).

fMRI has become a tool to understand the physiology of pain. Pain is not a simple and straightforward sensory experience because it does not have one specific cortical area like other sensations such as vision and auditory. Using fMRI with an appropriate experimental paradigm and a suitable analysis, the pain-related brain regions can be investigated. For more advanced findings, this method is not only used to explore the pain network but also to investigate connectivity between brain regions associated with pain in relevant situations and its relation with personality and feelings.

Research reveals that pain perception is influenced by two aspects: a sensory-discriminative and an affective-motivational aspect of pain (Brooks & Tracey, 2005; Aurav *et al.*, 2010; Ahmad & Abd. Aziz, 2014). The sensory-discriminative component provides information about the intensity, modality and location of pain (Ohara *et al.*, 2005) while the affective-motivational component is subjective to personal perception and involves psychological variables such as attention, anxiety,

emotional responses and personality (Ohara *et al.*, 2005; Ahmad & Abd. Aziz, 2014). While pain is subjective, an analysis of connectivity can be done to investigate how the two pain components as well as the related brain regions are connected to each other. A method called Dynamic Causal Modelling (DCM) which generates the model of brain network (Friston *et al.*, 2003; Stephan *et al.*, 2010) can be used to explain the connection between all related areas in one interaction.

1.2 Problem statement

Pain is multidimensional and is subjective to personal experience and perception. A similar type of pain stimulus may be perceived differently by different individuals. The perception of pain maybe influenced by a person's surrounding or maybe modulated by the personality or a person's past experiences. Considering these factors, medical practitioners or therapists may face problems in treating patients' pain.

Pain research is not only limited to patients but is also performed in healthy individuals. Healthy individuals' reaction to pain varies and can be related to their psychological characteristics. For example, 'emotionally fragile' individuals are unable to bear much pain. Without the knowledge of the factors that enable a person to feel more pain or less pain, it is difficult to predict a person's reaction to pain and difficult to plan for any treatment.

Most people feel comfortable and have positive emotion when their loved ones are near them. However, emotions are not always helpful. Emotions can hurt as well as help us (Gross, 2008). For instance, upon receiving the stimulus of pain, some people

will feel more pain even while receiving support from their loved ones. This phenomenon is called *Love Hurts*. On the other hand, some people will tolerate pain more. In this work, a new phrase *Love Heals* is introduced to explain such group. Taking these conditions into account, several questions arise such as:

1. Are there any differences in the response to pain based on the specific conditions during the stimulus delivery?
2. Are the same areas of the brain involved in the different responses to pain?
3. What is the relationship between different pain reactions and the connectivity of areas in the brain that are activated during pain processing?

1.3 Objective of the study

The objectives of the study are:

1. To study the individual responses to acute pain using laser heat stimulation.
2. To study whether the individual's responses to acute pain is modulated by the presence of a loved one.
3. To map the brain activation to laser heat pain in two conditions: alone in the experiment room and in the presence of support by a loved one.
4. To investigate the possible model of connectivity based on Bayesian selection technique that may explain the individual's pain response.

In general, the expected outcome of this study is to obtain the functional brain map of pain signal based on laser heat pain stimuli under two conditions (1) alone and (2) accompanied by a loved one. This study tries to find evidence to prove whether *Love Hurts* or *Love Heals*. Note that *Love Hurts* means the situation where a person reports more pain in the presence of support from a loved one, while *Love Heals*

means the resulting response of feeling less pain in the presence of a loved one while receiving pain.

Bayesian technique is a method of selecting and proving that there exists the possibility of an optimal connectivity model which may be used to represent an interaction. Bayesian Model Selection (BMS) applies a Bayes approach in estimating and choosing the most optimal model among the competing models (Stephan *et al.*, 2010). It is to be noted that the distance between points of connectivity should not be too far.

1.4 Scope of study

This study is firstly, to focus on the influences of a loved one on the person's response to pain stimulation. Secondly, it is to determine the brain map of regions that are activated upon receiving the stimulus. The connectivity study is not meant to build a full pain model but rather to expect the connectivity of activated brain regions for different responses to pain by different groups.

The study is focussed to consider only Malay-right-handed-female participants, with no history of brain injuries and critical illnesses or mental disorder. All participants must be MRI compliant and not pregnant. The main focus to the study is to investigate only the preoccupied type of relationship between participants and their loved ones.

1.5 Benefits of the study

The study is expected to contribute to the knowledge of physiology of pain, focusing on the effect of the presence and absence of a significant figure near the patient during receiving pain. This study may lead to a new method or assessment on patients by medical practitioners or therapists in handling patients. The outcome of the study is expected to contribute to the knowledge of brain connectivity.

1.6 Outline of the thesis

In Chapter 2, the relevant studies are reviewed. This chapter reviews some research that had been done by other researchers which have some relatedness with this study and also some theoretical framework to explain the basic idea. Chapter 3 outlines the methodology of the study. This chapter focuses on the experimental design, the data acquisition and method of analyses. Chapter 4 presents the results obtained from the study. Chapter 5 explains the discussion of the results. Lastly, the conclusion and suggestions for further work are summarised in Chapter 6.

CHAPTER 2

LITERATURE REVIEW

2.1 Pain

The perception of pain is complex and subjective. Even though pain can be defined as unbearable sensation arising from specific parts of the body, it is evident that pain is not experienced by different individuals in the same way (Ohara *et al.*, 2005). The International Association for the Study of Pain (IASP) widely used definition stated that "pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP, 1994a); while the American Medical Association defined pain as "the sensory and emotional experience of discomfort, which is usually associated with actual or threatened tissue damage or irritation" (AMA, 2003).

2.1.1 Pain pathway

Studies of pain reveal that the perception of pain comprises two major components. First, a sensory-discriminative component of pain which processes the information of pain modality, location and the quality of pain (Ohara *et al.*, 2005; Vogt, 2015; Ahmad & Abd. Aziz, 2014). Secondly, an affective-motivational aspect of pain which is responsible in processing the cognitive factors which modulate pain perception such as emotion, attention, anxiety, fear, expectation and anticipation (Opisov *et al.*, 2010; Ahmad & Abd. Aziz, 2014).

In general, the pain pathway starts from the periphery, the site where the pain stimulation is received before transmitting the pain information from periphery to the cerebral cortex of the brain and translating it (Ahmad, 2004). Pain signals are then

carried by two types of afferent peripheral fibers: A-delta and C fibers. C fibers transmit impulses involved in diffuse dull, burning, or aching pain sensations while A-delta fibers transmit sharp or pricking pain (Tortora & Grabowski, 2003; Sarafino & Smith, 2011). Signals from A-delta and C fibers follow different paths when they reach the brain where A-delta signals go to motor and sensory areas in the brain, while C fiber signals terminate mainly in the forebrain (Sarafino & Smith, 2011).

2.1.2 Pain related regions

Pain does not have a specific cortical area as it has diverse dimensions. However, many studies show that basically the pain-related brain areas especially the pain associated to skin acute pain involves the activation in primary and secondary somasosensory cortex (Chen *et al.*, 2002; Bingel *et al.*, 2003; Apkarian *et al.*, 2005) , thalamus (Baumgartner *et al.*, 2010; Ploner *et al.*, 2010; Yen *et al.*, 2013) and insula (Ploner *et al.*, 2010; Wiech *et al.*, 2014b). The cognitive affective aspect of pain may involve several unspecified brain regions such as anterior cingulate cortex (Ohara *et al.*, 2005; Roy *et al.*, 2009), amygdala (Bornhovd *et al.*, 2002; Weich & Tracey, 2013), orbitofrontal cortex (Roy *et al.*, 2009) and ventrolateral prefrontal cortex (Ahmad, 2011; Wiech *et al.*, 2014a, b). These are not fixed regions in the process of pain perception and depends on the cognitive modulations.

2.1.3 Pain modulations

Pain perception is not only modulated by the nociceptive inputs such as the intensity, quality and location of pain (Ohara *et al.*, 2005; Ahmad, 2011) but also by the affective and cognitive factors (Aurav *et al.*, 2010; Wiech & Tracey, 2013) which constitute more subjective psychological variables comprising attention, anxiety,

expectation, depression, stress and anticipation (Valente *et al.*, 2009; Wiech & Tracey, 2013; Ahmad & Abd Aziz, 2014). In recent studies, the pain perception is found to be modulated by several other factors like emotion, personality, and the condition at the time the individual receives the pain (Roy *et al.*, 2009; Cheng *et al.*, 2010; Cameron, 2011; Martinez *et al.*, 2011; Inakagi & Eisenberger, 2012; Tamam *et al.*, 2014).

Emotion, such as love, like, hate, sad, happy and etc, may either be positive or negative modulates the pain perception of the individual. The emotion of individual is, in turn, modulated by the personality characteristics. Another factor that contributes to modulation of pain is the presence or absence of a significant other at the time of receiving pain (Cheng *et al.*, 2010). According to a study by Montoya *et al.* (2004), the social support through the presence of a significant other can influence pain processing at the subjective behavioral level as well as the central nervous system level; and this has been proven true by Cheng *et al.* (2010) and Tamam *et al.*, (2014). However how this factor influences the neuronal hemodynamic responses is still under investigation.

2.2 Pain studies

Laboratory studies of pain need to pay attention to several aspects which would help to obtain the precise findings other than just to minimize the confounding factors or analysis imperfection due to too many variables. For instance, pain studies should consider what type of stimulus to be used and what type of pain would it produced. The experimental design should take into account some precautions to avoid the confounding effects which result in an unwanted outcome.

2.2.1 Quality of pain

Human skin may sense pain with many different qualities. For instance, a sensation of stabbing or pricking might be described as “sharp” while others may be felt as “dull” pain (Sarafino & Smith, 2011). A sensation of heat may not always be perceived as warm or hot, but sometimes may be perceived as sharp pain when a targeted area is very small.

2.2.2 Laser heat pain

A study by Agostino *et al.*, 2000 revealed that a pinprick sensation can be produced using a small laser beam. Laser stimuli with a diameter of 2.5mm, irradiating an area of approximately $5mm^2$ produces a sharp pain like a pinprick (Agostino *et al.*, 2000). Laser heat is used in pain studies since it selectively activates nerves under the skin which evoke brain responses and a variety of sensations (Arendt-Nielsen & Bjerring, 1988). The Th:YAG (Thalium: Yttrium-Aluminium-Garnate) laser with a wavelength of 1.96μm, spot diameter of 5mm is widely used in laser pain studies and is able to penetrate the human skin up to 360μm in depth (Bornhovd *et al.*, 2002). The pinprick sensation created by laser stimulation elicits the activation of thinly myelinated A-delta and unmyelinated C-fibres (Bornhovd *et al.*, 2002) with no damage to epidermis or subcutaneous tissue (Spiegel *et al.*, 2000)

2.2.3 Sensitivity to pain

Pain studies should take into account several things that affect the sensitivity to pain such as gender, location of stimulation, sensitisation and habituation.

2.2.3.1 Gender differences in pain response

A number of studies have been carried out to investigate the differences in pain response between men and women. Shah *et al.* (2012) found that pain parameters are influenced by gender when they found that females have lower pain threshold compared to males. This statement is supported by the function of testosterone in males which helps men to release more endorphin than women (Pednedkar & Mulgaonker, 1995). The increases of quantity of endorphins in men will greatly increase the pain threshold (Crafts, 1998). On the other hand, in women, progesterone increases excitability of spinal neurons, thus decreases the pain threshold (Hashami & Davis, 2009). In terms of brain activation, Paulson *et al.*, 2007 revealed that both men and women activate pain-related areas such as thalamus, somatosensory cortex, cingulate cortex and insula cortices.

2.2.3.2 Location of stimulation

The selection of the location of stimulation is an important consideration in designing a pain study. This is because pain can be detected all over the body but varies considerably in terms of intensity and quality. The main aim is to find the location that would give the optimum pain effect, so that the pain-brain activation is easily and reliably captured by fMRI.

Based on previous studies, the cheek is a known site to have high sensitivity to heat (Moulton *et al.*, 2012). However in this research, cheek is not a good choice to receive a series of laser heat due to aesthetic and ethical issues. Targeting laser onto the participant's cheek would also make them feel terrified and afraid to receive the subsequent stimulation. Furthermore, the resulting response may be distorted by the

extreme fear. Other sites that are chosen for pain stimulation include ventral surface of forearm, wrist and foot (Coghill *et al.*, 1994; Davis *et al.*, 1997; Kong *et al.*, 2006; Ploner *et al.*, 2010a,b).

In this study, the dorsum of hand is chosen since many laser pain studies uses this site to deliver laser stimuli (Becerra *et al.*, 1999; Bornhorvd *et al.*, 2002; Watson *et al.*, 2002; Baumgartner *et al.*, 2010). Laser energy mediates pinprick sensation on the skin (Agostino *et al.*, 2000; Bornhovd *et al.*, 2002). Basically, the pinprick threshold is significantly increased with the increase of distance from the brain (Agostino *et al.*, 2000). This means the nearer the location of stimulation from brain, the lower the pinprick pain threshold. For instance, targeting the laser heat onto the hand may result in a lower pain threshold compared to targeting the laser heat onto the foot. However, the thickness of epidermis and conduction distance may also influence the laser threshold. The skin thickness is similar in the forehead, upper arm, thigh, cheek, hand and ankle (Agostino *et al.*, 2000). Taking all factors into consideration, the dorsum of hand is selected for the current research due to the skin thickness and easy access. When the participant is placed in the MRI gantry, it is much easier to deliver the laser via the fibre optic cable to the dorsum of hand compared to other locations such as cheek, forehead and arm.

2.2.3.3 Sensitisation and habituation

According to the IASP taxonomy, sensitisation is defined as "Increased responsiveness of nociceptive neurons to their normal input, and/or recruitment of a response to normally subthreshold inputs." (IASP, 1994b); while habituation in

relation to pain may occur when greater tolerance for persisting pain is reported or the person reports the pain as not as troubling as it had been (Nicholas, 2013).

Sensitisation may affect the experimental result by increasing the responsiveness due to repeated application of pain stimuli. It may occur typically with high-intensity stimuli which make the body become more sensitive to the stimuli. Sensitisation is usually temporary and will recover in a short time by giving a resting state during the interstimulus interval.

Habituation can also be understood as the ability to discontinue the response to highly repetitive stimuli. This condition may decrease the strength of behaviour and can be short or long-term, depending on the presentation and interval between stimuli. Habituation may result in significant signal attenuation (Becerra *et al.*, 1999).

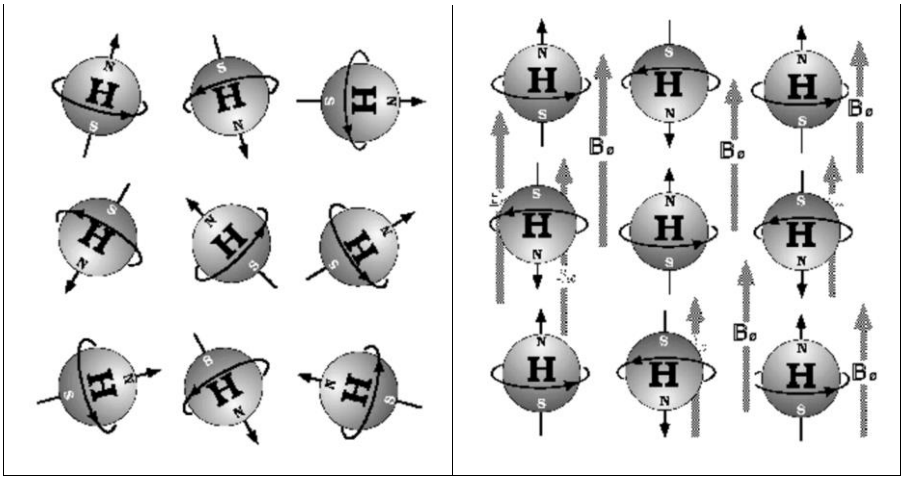
In order to minimise the sensitisation and/or habituation, the stimuli can be rotated among several locations with a duration of presentation at each site followed by a duration of rest (Coghill *et al.*, 1994). Bornhovd and Agostina slightly moved the stimulation site after each pain stimulus to avoid sensitisation, habituation and tissue damage (Agostina *et al.*, 2000; Bornhovd *et al.*, 2002). Using and rotating different types of stimulations also help in reducing the sensitisation and habituation as done by Chen *et al.*, 2002. These investigators started the imaging session with tactile runs and followed by thermal runs. The current study follows the method used by Bornhovd and Agostino.

2.3 fMRI pain studies

The fMRI offers sufficiently great benefits for the study of pain over other brain imaging modality such as positron emission tomography (PET) especially in its ability to capture the time course of a physiological response (Becerra *et al.*, 1999; Ahmad & Abd. Aziz, 2014). fMRI technique allows us to understand the central nervous system (CNS) changes related to pain experiences (Wise, 2010). The advantages of fMRI include lack of exposure to ionising radiation, good anatomical localisation, sensitive to many different types of contrast (Huettel *et al.*, 2003) and able to image pain in individual patients (Becerra *et al.*, 1999). The technique that forms the basis for nearly all fMRI studies and creates data associated with brain function is called blood-oxygenation-level-dependent (BOLD) contrast imaging (Huettel *et al.*, 2003; Amaro & Barker, 2006).

2.4 MRI: Basic principles

The MRI relies on basic physical principles which involve directional magnetic field, or moment of charged particles in motion (Mackiewich, 1995; Huettel *et al.*, 2003). The human body consists of abundant hydrogen nuclei which are also known as single protons. The proton is a charged particle where when it spins, generates an electrical current and induces a torque called magnetic moment. Because proton has an odd number of atomic mass i.e. 1, the spin results in an angular momentum. Both magnetic moment and angular momentum are the important characteristics for a nuclei to be useful for MRI. When the protons are placed in an external magnetic field, they change their orientation and initiate a gyroscopic motion known as precession (Huettel *et al.*, 2003).



(a) (b)

Figure 2.1 (a) In the absence of magnetic field, protons are in random orientation. (b) When a strong magnetic field is applied, the protons precess about the direction of the magnetic field (B_o). Source: Simply Physics, http://www.simplyphysics.com.

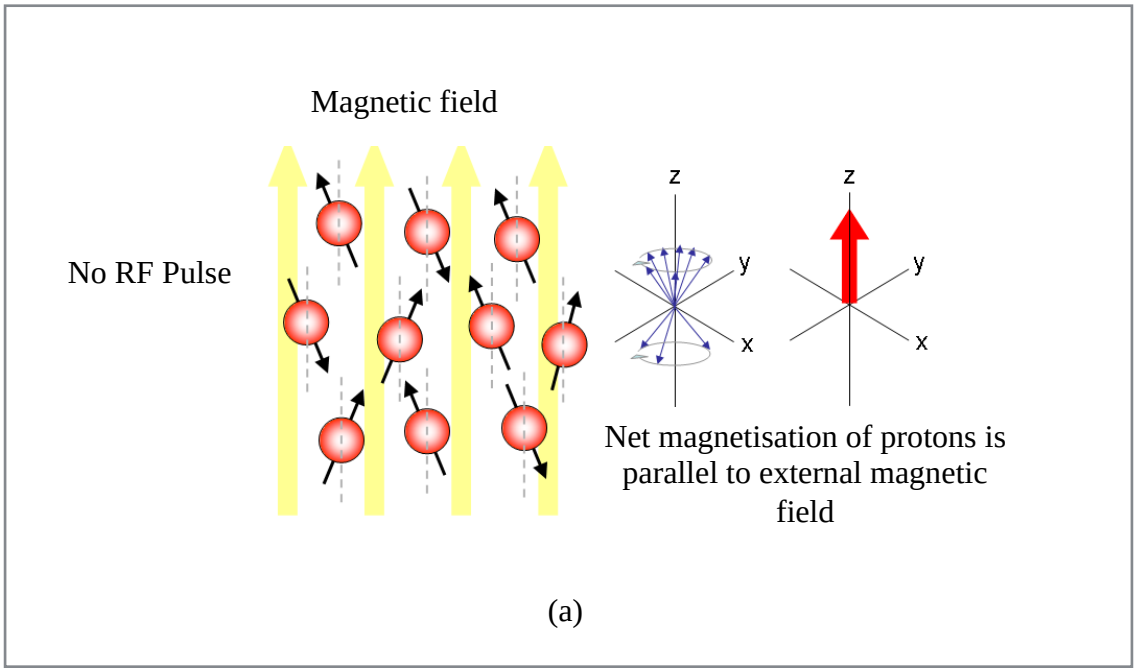
At the point when the protons are placed in a large magnetic field, they align themselves with the direction of the magnetic field and precess about the magnetic field direction. This behaviour is called Larmor precession (Mackiewich, 1995). The frequency of Larmor precession is proportional to the external magnetic field strength (B_o). This frequency is called Larmor frequency. Larmor equation is shown as following (eq. 2.1):

$$\omega_{ø} = \gamma B_{ø} \quad \text{..... (2.1)}$$

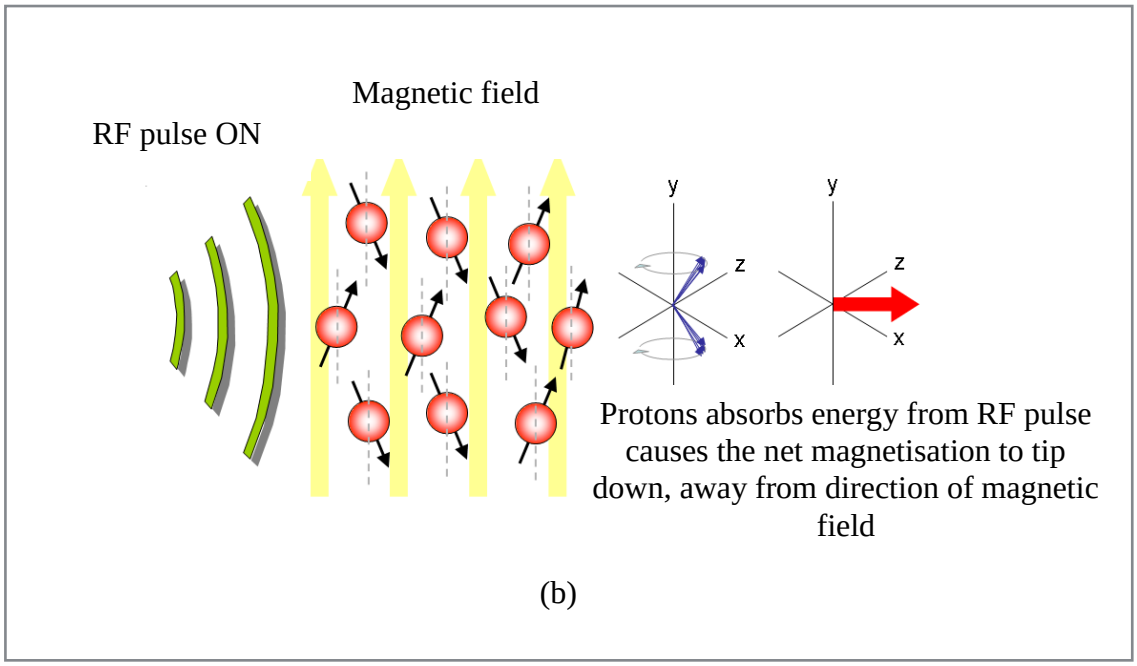
Where the ω_o is the Larmor frequency in unit Hz, the Ω is the gyromagnetic ratio in MHz/T, and B_o is the external magnetic field strength in Tesla (T). The gyromagnetic ratio (γ) is a constant, 42.56 MHz/T for Hydrogen (proton) (Mackiewich, 1995; Huettel *et al.*, 2003; Simply Physics, n.d.).

2.4.1 MRI signal detection

In order to obtain an MR image, the body is placed in a uniform magnetic field, B_o. This causes the abundant hydrogen nuclei in the human body align with the magnetic field and create a net magnetic moment, M_o, parallel to B_o. Next the radio frequency (RF) pulse at a Larmor frequency is applied perpendicular (90°) to B_o. At the time the RF pulse is applied at Larmor frequency, the protons absorb the energy thus excite to a higher energy state causing the M_o to tip down. When the RF pulse is off, the nuclei return to equilibrium, and their net magnetic moment, M_o, is again parallel with B_o. This is referred to as relaxation. On their way to relaxation, the nuclei loses energy, emitting their own RF signal (Figure 2.2). This signal is referred to as the free-induction decay (FID) response signal. The FID signal is measured by a conductive field coil placed around the body that is being imaged, and the measurement will be reconstructed to produce MR greyscale images.



(a)



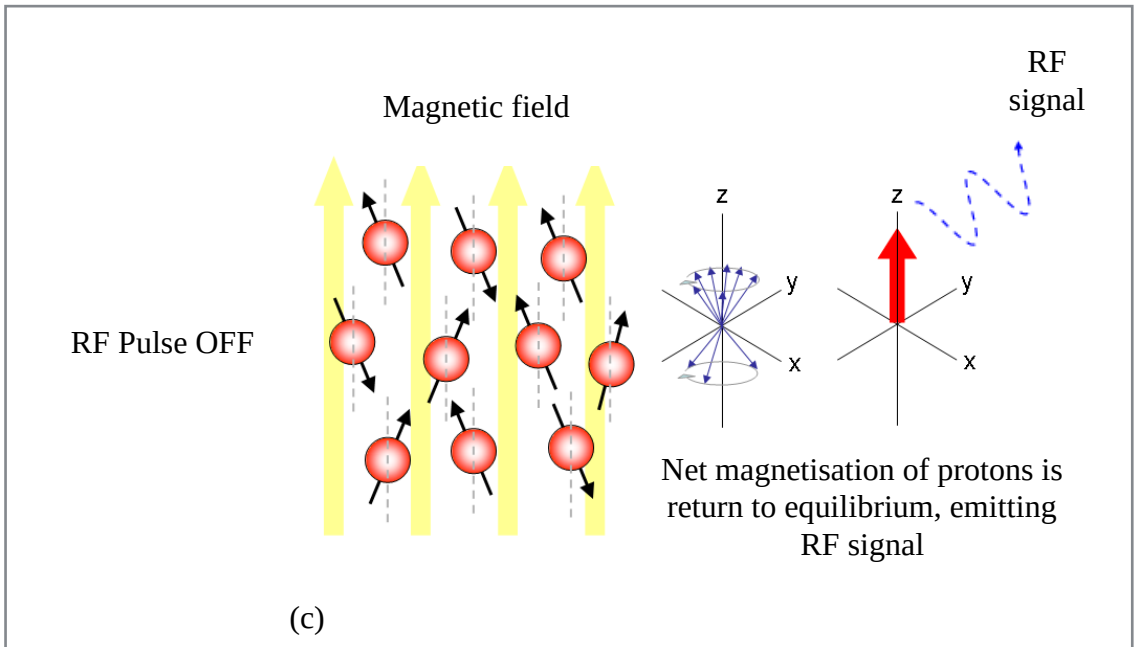


Figure 2.2 The MRI signal detection is explained by the process involving the change of direction of net magnetisation of the protons when the RF pulse is turned on and off. (a) The equilibrium state of protons with an absence of RF pulse. The net magnetisation is parallel to the direction of magnetic field strength. (b) The RF pulse is sent perpendicular to the magnetic field resulting in the net magnetisation to change the angle of precession of the protons and directed away from the magnetic field. (c) When the RF pulse is turned off, the net magnetisation of protons return to its equilibrium state causing the emission of RF signal which will be detected by a detector coil as MR signal. Source: Amiya Sarkar, 2010.

2.4.2 T1 recovery

When the RF pulse is turned off, not all of the emission energy is detectable as an RF pulse. Some of the energy is used to heat up the surrounding tissues, called as lattice. The spin system gradually loses energy causing the excited spins to go back to their original low energy state. This results in longitudinal relaxation or spin-lattice relaxation due to loss of energy to the surrounding or lattice of nuclei. The recovery rate of growing magnetisation is characterised by the time content T1, which is unique to every tissue. At a time t = T1 after the RF is turned off, 63.2% of the magnetisation has recovered its alignment with B_o. The relaxation time is shown by figure 2.3.

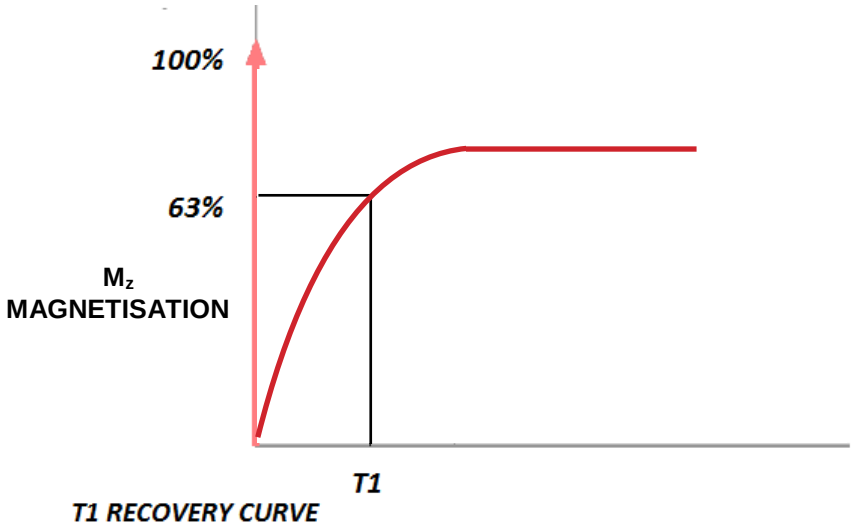


Figure 2.3 The T1 recovery curve showing that at time t = T1 after the excitation pulse, about 63% of the M_z magnetisation has recovered alignment with B_o. Source: mrimaster.com

2.4.3 T2 decay

In T2 decay, the signal decays resulting from transverse or spin-spin relaxation. The T2 value is the time for a signal to decay after the excitation and reduces signal to 36.8% of its original value. Note that this value is opposite of T1 where 63.2% of magnetisation is recovered in a duration of T1. The decay time of T2 is also known as transverse relaxation since it involves the decay of the magnetisation in XY plane. It is also called spin-spin relaxation due to the gradual loss in spins coherence resulting in an out of phase (Huettel *et al.*, 2003). The T2 decay curve is shown in figure 2.4.

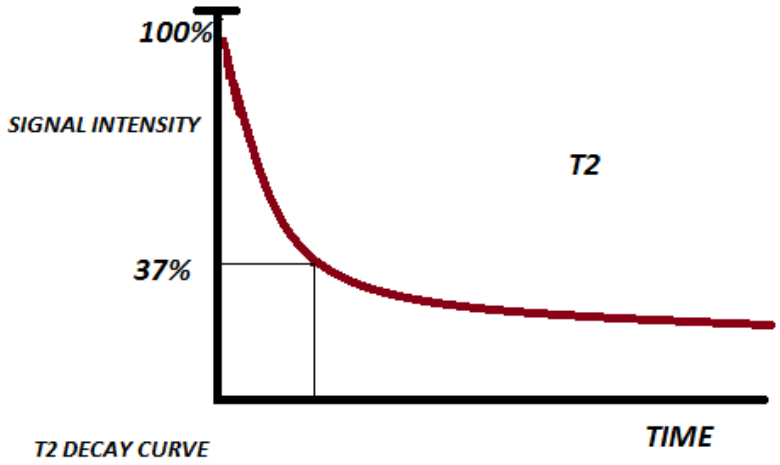


Figure 2.4 The T2 decay curve showing that the signal is lost about 37% of its original signal intensity in T2 period. Source: mrimaster.com

2.4.4 $T2^*$ decay

The T2 decay occurs due to the interaction between spins nuclei. However, this is not the only factor that contributes to the loss of signal. Although the magnetic field is assumed as homogenous, in reality, there are many factors that create imperfections

in the homogeneity of a magnetic field. The combined effects of spin-spin interaction and field inhomogeneity lead to signal loss known as $T2^*$ decay. $T2^*$ is always shorter than T2 decay time because this type of decay is considered an additional factor of field inhomogeneity. The $T2^*$ decay is an essential concept in forming the basis for BOLD-contrast fMRI.

2.4.5 Blood Oxygenation Level Dependent (BOLD)

BOLD effect is the basis of fMRI imaging. The BOLD sensitivity of MR signal is due to deoxyhemoglobin which alters the magnetic susceptibility of blood. The hemoglobin molecule has magnetic properties that differ depending upon whether or not it is bound to oxygen. Oxygenated haemoglobin is diamagnetic, it has no unpaired electron and zero magnetic moment; while deoxygenated hemoglobin is paramagnetic, it has both unpaired electron and a significant magnetic moment (Heuttel *et al.*, 2003).

Introducing an object with magnetic susceptibility into a magnetic field causes spin dephasing, resulting in a decay of transverse magnetisation that depends on the time constant $T2^*$ (Huettel *et al.,* 2003). When a stimulus is given, the neuronal will response to it and triggering a hemodynamic response. At this time, changes in blood oxygenation level occurs thus changing the magnetic susceptibility. Because blood-deoxygenation affects magnetic susceptibility, MR pulse sequences is sensitive to $T2^*$ (Arthurs & Boniface, 2002). The MR signal is high where blood is highly oxygenated and less MR signal where blood is highly deoxygenated (Huettel *et al.*, 2003). The deoxyhemoglobin increases the magnetic susceptibility but decreases the T2* thus decreases the MR signal. Figure 2.5 explains the whole process in brief.

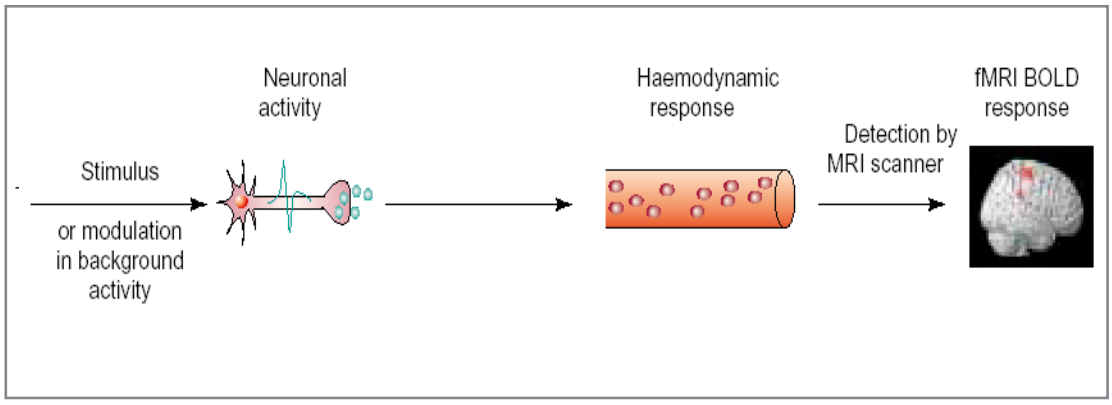


Figure 2.5 The neurons respond to a stimulus and trigger a hemodynamic response by changing the blood oxygenation level as well as the magnetic susceptibility along the blood flow. The decrease in magnetic susceptibility (high oxygenation) will increase the T2*, therefore increase the MR signal. Source: Arthurs & Boniface, 2002.

2.5 fMRI experimental paradigm

In fMRI study, the experimental design is crucial in order to capture the neuronal activity or the BOLD signal within the brain. There are several types of designs that can be used to carry out an fMRI experiment such as blocked design, event-related and sparse design. Whatever the experimental paradigm used, the most important factor that should be considered as a precaution while designing an fMRI experiment is the method to reduce the confounding factors. The confounding factor may 'disturb' the findings of the study and may result in invalid data. An approach that can help to prevent the confounding factor is to vary the stimulation sequence in the experiment randomly. This is called randomisation. However, sometimes there are factors that cannot be completely random. So we need to try to make sure that a potential confound is equally present for all conditions, which is called counterbalancing (Huettel *et al.*, 2003).

The blocked design is a simple yet extremely powerful method in detecting significant fMRI activity (Huettel *et al.*, 2003) and is used in many fMRI studies (Baumgartner *et al.*, 2010; Cheng, 2010; Ahmad Nazlim *et al.*, 2011; Brodersen *et al.*, 2012; Wiech *et al.*, 2014). Moreover, the blocked design increases statistical power and produces relatively large BOLD signal change related to baseline (Amaro *et al.*, 2005). Based on these benefits, the present study choose to use the blocked design to capture the brain activation associated with pain stimuli.

2.6 fMRI data processing

An fMRI data consists of a 3D matrix of volume elements (voxels) that are repeatedly sampled over time. A straightforward way of analysing the fMRI data set would be to extract the raw time course for each voxel and compare each of these time course to a hypothesis using a test of significance. Prior to the statistical testing, a computational procedure or preprocessing step is a crucial part of fMRI data processing. The preprocessing has two major goals. Firstly is to remove any unwanted artefacts from the data and secondly to prepare the data for statistical analysis (Huettel *et al.*, 2003).

There are many aspects in preprocessing step to be considered. However, the most familiar and often used are the step of realignment, normalisation and smoothing. In fMRI, the aim of realignment is primarily to remove movement artefacts (Ashburner *et al.*, 2011). The artefact such as head motion can be subtracted through the realignment analysis. Next is the normalisation which is performed by matching the whole of the head (including the scalp) to the template. The template images supplied with SPM conform to the space defined by the ICBM, NIH P-20 project,

and approximate that of the space described in the atlas of Talairach and Tournoux (1988) (Ashburner *et al.*, 2011). Lastly the smoothing in the analysis is used to suppress noise and effects due to residual differences in functional and gyral anatomy during inter-subject averaging (Ashburner *et al.*, 2011). Figure 2.6 shows the schematic figure of the fMRI preprocessing analysis.

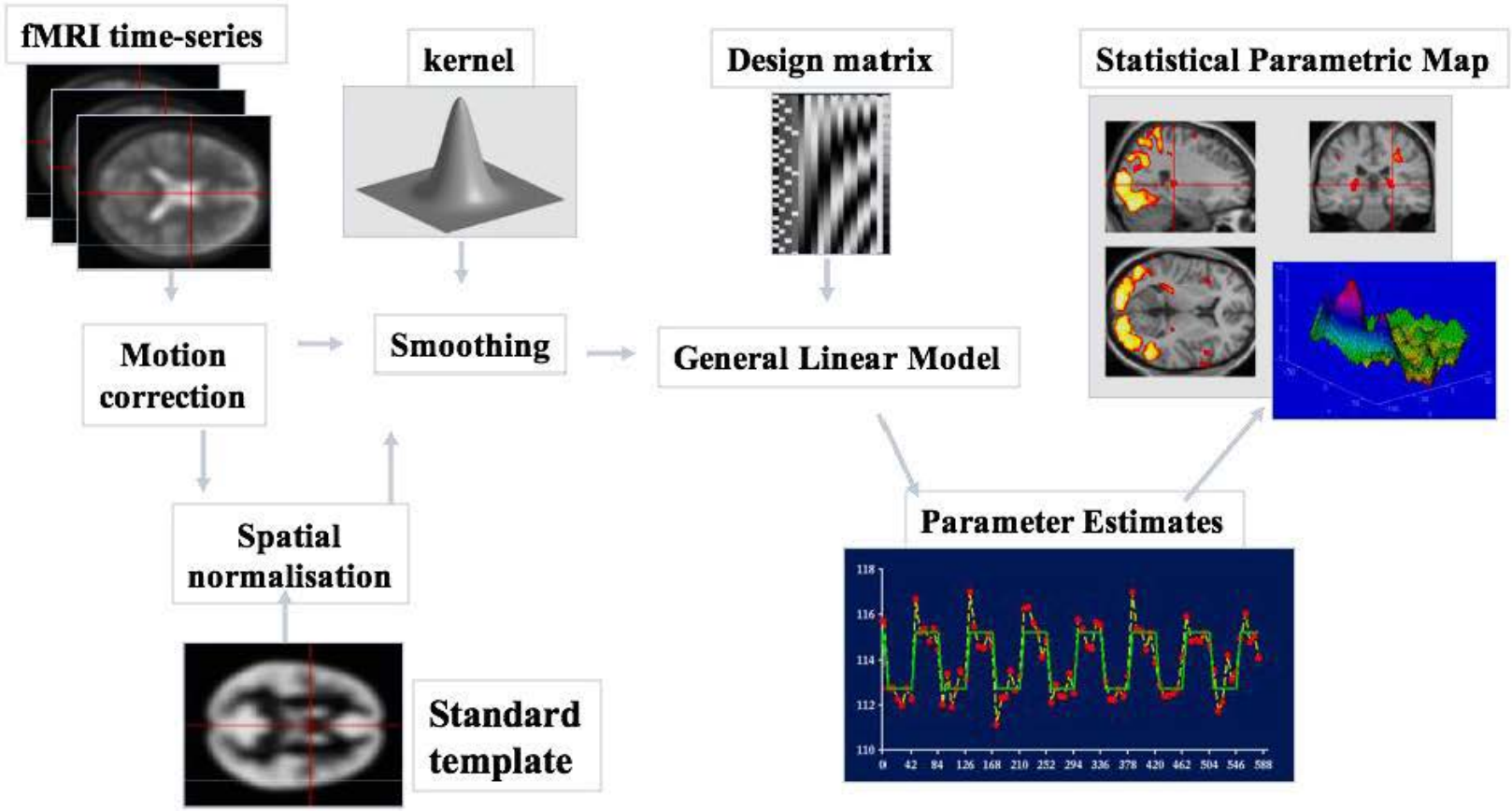


Figure 2.6 The preprocessing step of fMRI data. Source: Friston *et al*., 2003.

2.6.1 fMRI: Statistical analysis

The statistical analysis is carried out by considering the voxels of fMRI images. When evaluating whether a voxel has different mean signal levels in two experimental conditions, the common t-test is appropriate (Huettel *et al.*, 2003). The t-test can be done using specific analysis software like the one used in the current study, Statistical Parametric Mapping (SPM). SPM utilise the standard statistical inference to translate the activations in the brain during the fMRI task session (Friston 2004).

In SPM, the statistical analysis is done based on the general linear model (GLM). The GLM is a class of statistical tests which assume that the experimental data are composed of the linear combination of different model factors, along with uncorrelated noise (Huettel *et al.*, 2003). GLM model the hemodynamic stimulus through a design matrix (Friston, 2003; Aini, 2011). Note that the design matrix is the specification of how the model factors change over time (Huettel *et al.*, 2003). The design matrix created by GLM is applicable for single subject analysis as well as for group analysis. The additional analyses such as fixed effect (FFX) and random effect (RFX) are only applicable upon group analysis (Aini, 2011).

Whatever the analysis approach used, a problem in fMRI studies is that most of the statistical tests result in a false-positive finding. The standard corrections like the Bonferroni method are too strict and may eliminate significant activations. Therefore the Gaussian random field is found to be more reliable in fMRI statistical analysis because it deals with the properties of smooth, spatially extended data compared to Bonferroni correction (Huettel *et al.*, 2003; Friston, 2003)