The Hong Kong Polytechnic University

Department of Health Technology and Informatics

Influence of Inspired Oxygen Concentration on the Assessment of Language lateralization using Transcranial Doppler Sonography

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Certificate of Originality

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

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Wong Pok Ning

Publications and Presentations Originating from the Present Study

Conference Presentation

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Abstract

Assessment of language lateralization is essential in surgical planning, for the preservation of the language area and estimation of possible functional loss after surgery. Trancranial Doppler Sonography (TCD) is one of the techniques used in the assessment of language lateralization. Relative change of blood flow velocity (BFV) in the middle cerebral arteries (MCA) has been measured for the determination of language lateralization. Any non-neural factor such as increased inspired oxygen concentration may change the lateralization assessment result. Vasoconstrictive effect of oxygen causes the reduction of BFV, which is opposite to the increase of BFV under the influence of language stimuli. The purpose of this project was to investigate the effect of oxygen on BFV of the MCA, and to investigate the influence of increased inspired oxygen concentration on the result of language lateralization assessment.

In order to study how the inspired oxygen concentration affects the language lateralization assessment, the project was divided into two studies. In Study One, the change of BFV in the MCA on both left and right sides was simultaneously recorded during 35% oxygen inhalation and during room air inhalation. We recruited 20 healthy subjects aged from 20 to 32 years (mean ± S.D. = 24 ± 2.9 years). All of

them were native Chinese speakers. The BFV in bilateral MCA was measured simultaneously in each subject using TCD. During the TCD examination, subjects were asked to breath in 35% oxygen for 40 seconds and then room air for 40 seconds. This procedure was repeated 10 times.

In the second part of the study, oxygen was applied into language lateralization assessment to investigate its influence. We included 50 Chinese volunteers: 25 males and 25 females aged from 20 to 38 years (mean ± S.D. = 23.9 ± 3.5 years). All of them were native Chinese speakers. A language task of recognizing Chinese synonyms was used in the language lateralization assessment. The BFV in bilateral MCA was measured simultaneously in each subject using TCD while they were performing the language task. The examination was performed separately during room air inhalation and during 35% oxygen inhalation.

In the Study One, a reduction of BFV of was caused by inhalation of 35% oxygen (-7.76 +/- 5.54% for left MCA; -6.96 +/- 4.36% for right MCA), and restoration of BFV to the baseline level after inhalation of room air. Continuous TCD assessment found that the change in BFV during oxygen inhalation phase was not always decreased. Mean Latent period of 3.02 seconds and mean BFV increase of 2.48% - 2.90% above the baseline was demonstrated.

In the Study Two, a significant difference of the BFV change in left and right MCA ($p<0.001$) was observed during room air inhalation. Left lateralization of language processing was identified in all the subjects (mean lateralization = 1.61 towards left). When 35% oxygen concentration was inhaled, there was a significant reduction of BFV ($p<0.001$) in both left and right MCA. The difference in BFV between left and right MCA was not significant ($p = 0.057$). The degree of lateralization was reduced from 1.61 to 0.56 with the effect of 35% oxygen inhalation.

In the present study, latent period of cerebral haemodynamic response to increased oxygen concentration was obtained in the continuous measurement of BFV in the left and right MCA. Before the vasoconstrictive effect of oxygen was reflected by reduction of BFV, there was a small increase of BFV suggesting that the effect of oxygen on MCA is faster in the distal portion than in the proximal portion. The changing pattern of BFV in oxygen inhalation is not always decreasing. These findings increase the understanding of the effect of oxygen on cerebral haemodynamics. It provides a reference of using oxygen in the assessment of cerebrovascular reactivity. At the same time, owing to the vasoconstrictive effect of oxygen, the result of language lateralization assessment was altered during the inhalation of 35% oxygen. The laterality index changed from 1.61 during room air inhalation to 0.56 during oxygen inhalation. This alerts the clinicians in interpretating

the results of language lateralization assessment using TCD when oxygen is administered to the patient. Since the determination of hemispheric language lateralization using TCD depends on the corresponding hemodynamic changes, the effect of oxygen on such assessment using any techniques involving the measurement of haemodynamic changes should not be ignored.

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Chapter One

Introduction

The brain is the control center of the central nervous system. The cerebral cortex, the outermost layer of the cerebrum, controls most of the brain functions. A wide variety of functions are controlled by the brain. It is the site of intelligence, which includes components such as cognition, perception, attention, memory and emotion. (Kandel et al., 2000).

Language is the most unique and important function of the human brain, which is distinguished from other kinds of human activities (Kandel et al., 2000; Mohr, 1976). It affects speech and cognitive function. Loss of language function results in poor cognition, difficulty in communication and living can be a great problem. In the planning of neurosurgery, preservation of language area in the brain is very important. Post-operation complication and damage of language function should be minimized. However, language processing areas can only be identified functionally, instead of anatomically. The location varies from one individual to another. The language function can be dominant to the right or the left brain.

Previous studies showed that the left and right brain were not activated symmetrically by a language task (Knecht et al., 2000a; Knecht et al., 1998a; Rushe et al., 2004; Schmidt et al., 1999; Szaflarski et al., 2002; Vingerhoets and Stroobant, 1999). Usually one side of the brain will take the major role and the other will take the minor,

with the side assuming the major language function being termed the language dominant side. This phenomenon is known as language lateralization. In most of the right handers, the language function is lateralized to the left cerebral hemisphere but it can also be lateralized to the right cerebral hemisphere in minority of the population (Floel et al., 2001; Knecht et al., 2000a). Although handedness is one factor associated with language lateralization (Knecht et al., 2000a), it can only roughly estimate the side and degree of language lateralization (Khedr et al., 2002; Pujol et al., 1999). More objective methods are therefore required for clinical application.

Chinically many modalities and techniques have been available for the determination of language lateralization. Wada test is the golden standard for this application (Bacia et al., 1998; Benke et al., 2006; Benson et al., 1999; Knake et al., 2003; Lehericy et al., 2000; Mader et al., 2004; Rihs et al., 1999; Schulze-Bonhage et al., 2004; Woermann et al., 2003). It is an invasive procedure to determine language dominance which requires transient paralysis of one cerebral hemisphere. The angiographic administration of anesthetics and the 5% mortality risk are the concerns when performing Wada test. The invasiveness and adverse effects (Mikuni et al., 2005) of Wada test provide motivation in developing other imaging modalities such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and transcranial Doppler sonography (TCD) for the assessment of language lateralization. The details of different techniques used in the assessment of hemispheric language lateralization were discussed in the Section 1.2.

Unlike Wada test and PET, TCD does not require the administration of drugs and possesses no radiation hazard (Rihs et al., 1999). TCD can be applied in patients who are

not suitable for MRI examination. They include patients who have claustrophobia or having metallic implants (Knecht et al., 1998a). TCD has less motional artifacts than MRI. The basic instrument for the TCD examination is portable, small and relatively inexpensive. It therefore offers an alternative for a noninvasive and inexpensive assessment, and can be repeated easily for the follow-ups (Knecht et al., 1998b; Martin et al., 1993; Schmidt et al., 1999).

Similar to fMRI, TCD does not directly measure the neural activity. Relative change of blood flow velocity (BFV) is measured to demonstrate the neural activity in TCD assessment. It is therefore susceptible to the influence by non-neural physiological changes in the body. Any haemodynamic changes caused by vasoconstriction or vasodilatation would affect the TCD assessment. As oxygen is usually applied to the patients in most of the clinical settings, the effects of such factors on the assessment of language lateralization using TCD should be investigated.

With the administration of oxygen, the partial pressure of oxygen (pO_2) in the blood stream is raised above normal. Higher than normal arterial blood pO_2 level will lead to vasoconstriction of the cerebral arteries. It reduces the luminal size then the BFV of the cerebral artery. (Demchenko et al., 1031; Hoffman et al., 2000; Johnston et al., 2003; Omae et al., 1998).

When oxygen is administered to the patient during language lateralization assessment. The global effect of oxygen that causes reduction in luminal size of an artery may limit the BFV increase due to brain activation by language processing, Language lateralization is derived from the difference of BFV increase between left and right MCA. This difference is likely to be reduced by the effect of oxygen. Therefore

oxygen may lead to the change of the language lateralization result. To date, however, most of the studies are using carbon dioxide (CO_2) as a challenge to study the cerebral haemodynamic effect (Kawata et al., 1998; Park et al., 2003; Pfefferkorn et al., 2001; Silvestrini et al., 2004; Ziyeh et al., 2005). Only a few human studies assessed the effect of oxygen on cerebral blood flow velocity (Demchenko et al., 1031; Gilmore et al., 2004; Ohta, 1986; Omae et al., 1998).

In the present study, the effect of oxygen on cerebral BFV was studied to examine the BFV changes under the influence of oxygen concentration. The findings in the present study increase the understanding of haemodynamic effect of oxygen. It provides a reference in the cerebrovascular reactivity study using oxygen as a challenge before it is widely used. The study of such effect of oxygen was extended to investigate the influence on the language lateralization assessment. Owing to the vasoconstrictive effect of oxygen, it also alerts the clinicians in interpretating language laterality derived in TCD examination, especially when the technique is applied on critically-ill patients with oxygen administration.

1.1 The human brain

The brain, or encephalon (Greek for "in the head"), is the control center of the central nervous system. A wide variety of functions are controlled by the brain including perception, cognition, attention, emotion and memory. It is also responsible for the control of movements and posture. It makes cognition, motor and other forms of learning possible (Kandel et al., 2000). When a neuron is stimulated, its metabolism will be increased and more energy is required. The demand of blood supply will then be increased which triggers the haemodynamic change to maintain sufficient supply of oxygen and glucose to the activated neurons. This haemodynamic change is used to reflect the neural activities.

1.1.1 Neurons and neural activity

The brain is composed of two broad classes of cells, neurons and glia, both of them contain several different cell types which perform different functions. As the basic unit of the brain, neurons are the cells that generate and conduct nerve impulse to the other cells. A neuron consists of a cell body (soma), a dendritic tree and an axon (Figure 1-1). Its diameter ranges from 4 to 135μm that is generally larger than that of the other cells in the body (Junqueira, 2003).

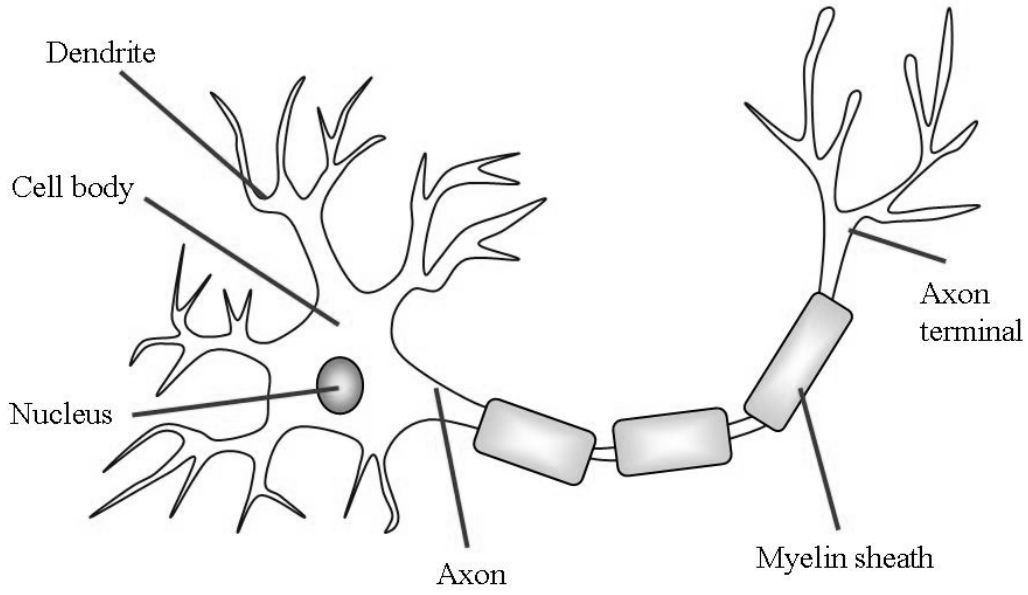

Figure 1-1. Structure of a typical neuron. Adapted from Carpenter (2003).

Neurons are able to generate and propagate electrical impulses from one part of the cell to another part along the membrane using the ion movement. If the neuron is stimulated by a strong stimulus, the membrane potential is raised, and depolarization will occur. Depolarization facilitates the opening of sodium ion (Na^+) channels, and results in the influx of Na^+ into the cell. The inflow of Na^+ further depolarizes the membrane, which opens more sodium channels. After a short delay, potassium (K^+) channels then open and K^+ is carried out of the membrane. During re-polarization, Na^+ channel closes and the membrane potential restores to resting level by continuous movement of K^+. Finally, repolarization occurs and closes the K^+ channels. The neuron

is reset to resting stage and ready for another activation (Carpenter, 2003; Somjen, 1983; Stein, 2006).

Chemical messenger may in turn trigger ion movement and is involved in communication between neurons and other cells via the synapse. In a synapse, when an action potential reaches the axon terminal, it opens the voltage-gated calcium channels, allowing calcium ions to enter the terminal. Calcium causes the release of neurotransmitter from the synaptic vesicles into the synaptic cleft. The neurotransmitters diffuse across the synaptic cleft and activate receptors on the terminal of the postsynaptic neuron (Kandel et al., 2000). There are a huge number of synapses in the human brain. Each of 100 billion neurons has on average 7,000 synaptic connections to the other neurons. It is estimated that there are about 1,000 trillion synapses in the brain of a three-year-old child. This number declines with age, stabilizing by adulthood. Estimates vary for an adult, ranging from 100 to 500 trillion synapses (Drachman, 2004). During synaptic activation, energy is consumed for active transport of ions and neural transmitter. Metabolic rate of the neuron is increased to maintain the energy supply for the neural activity. Therefore the demand of oxygen and glucose are increased in the local area of brain activation.

1.1.2 Language area of the brain

In the middle of 18th century, a French neurosurgeon, Pierre Paul Broca identified an area in left hemisphere that plays a primary role in speech production. This is a region located in the inferior region of the frontal lobe called Broca's area (Broca, 1865; Mohr, 1976; Penfield and Roberts, 1959) (Figure 1-2). Another language area called Wernicke's area was discovered by German neurologist Karl Wernicke in 1874. He found that damage to this area could result in language impairment including comprehension and speech. Wernicke's area is located at the posterior part of the superior temporal gyrus. Both of the Broca's and Wernicke's areas are mainly supplied by the middle cerebral arteries (MCA) (Papadimitriou et al., 2003; Sanders, 1998) (Figure 1-3 and 1-4). Therefore, brain activation due to language processing could be reflected in the changes of MCA blood flow.

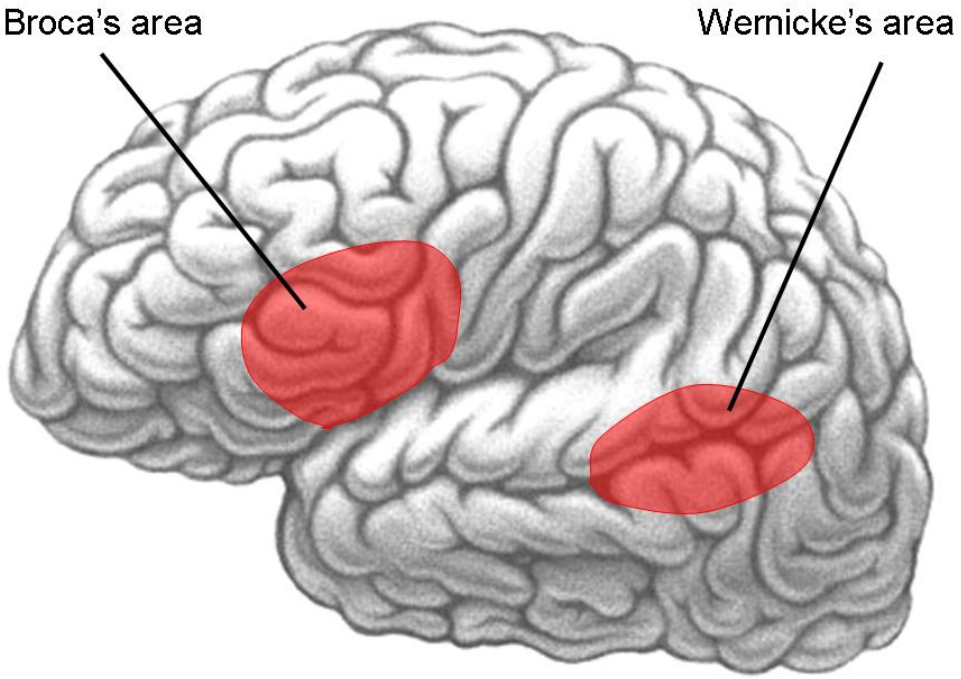

Figure 1-2. Location of the Broca's area and Wernicke's area highlighted in red. Adapted from Kandel et al. (2000).

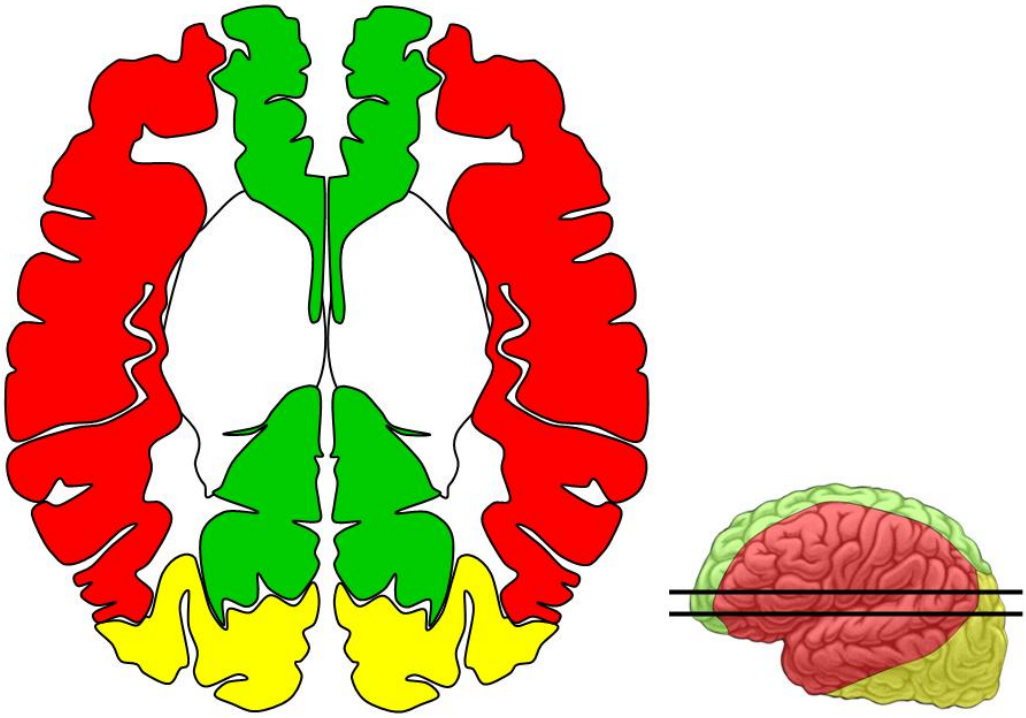

Figure 1-3. The blood supply to the cerebral cortex, by anterior (green), middle (red) and posterior (yellow) cerebral arteries. Adapted from Agur and Arthur (2005).

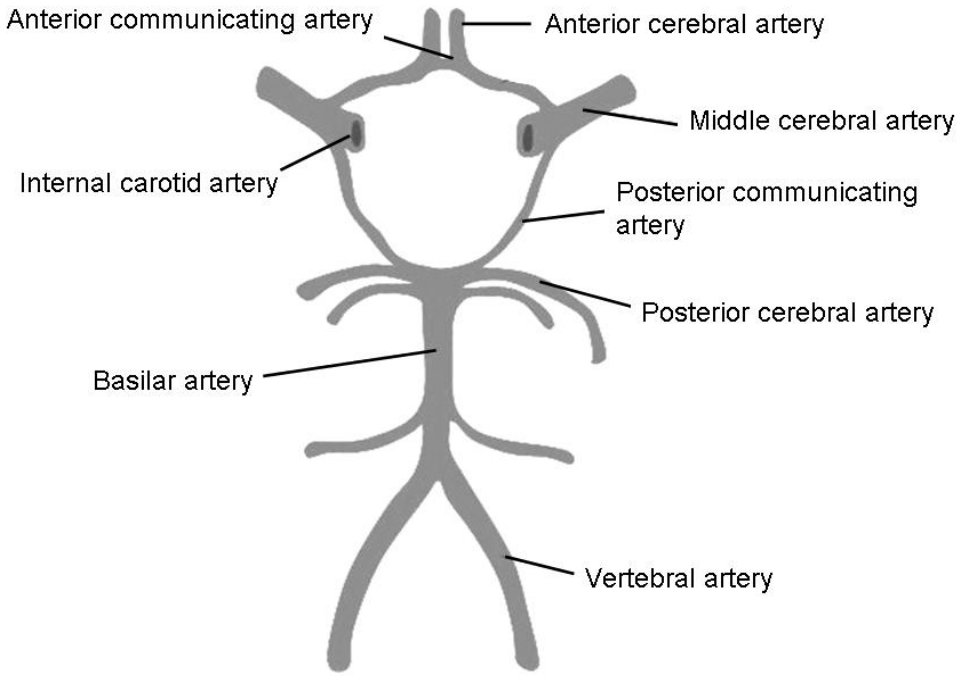

Figure 1-4 Cerebral arteries forming the Circle of Willis. Adapted from Agur and Arthur (2005).

Broca found that brain areas responsible for motor speech are not symmetrical in both left and right hemispheres. In a cadaver study of speech-impaired patients, Broca found that lesions in the left hemispheres were found to be associated with loss of speech function in most of the cases. Patients with lesions in this area showed a condition called Broca's aphasia, which makes them unable to create grammatically-complex sentences. The speech of the patients was often described as telegraphic and contained little but content words. Patients with Broca's aphasia was reactively normal in comprehension, but they could not speak properly (Broca, 1865).

Apart from retrospective correlation study of language function with the site of lesions in cadaver study, several other previous studies of healthy adults in vivo did also show the hemispheric lateralization of language processing (Knecht et al., 2000a; Knecht et al., 1998a; Rushe et al., 2004; Schmidt et al., 1999; Szaflarski et al., 2002; Vingerhoets and Stroobant, 1999). In most of the people, language is lateralized to the left cerebral hemisphere. A minor population would have their language function lateralized to the right cerebral hemisphere (Floel et al., 2001; Knecht et al., 2000a). Clinically, the degree of language lateralization is indicated by laterality index (LI) as follows (Knecht et al., 1998a; Szaflarski et al., 2002).

$$\text{Laterality Index (LI)} = [L-R] / [L+R]$$

where L represents the brain response to language stimuli in the left cerebral hemisphere and R represents brain response to language stimuli in the right cerebral hemisphere

One factor associated with language lateralization is handedness (Knecht et al., 2000a). Handedness is an attribute of human beings defined by their unequal distribution of fine motor skill between the left and right hands. Individuals who are more skillful with the right hands are called right-handed, and those who are more skillful with the left are said to be left-handed. A minority of people are equally skillful with both hands, which are known as ambidextrous. In most of the studies, it is commonly defined by the Edinburgh inventory (Anstey et al., 2004; Brito et al., 1989; Eviatar et al., 1997; Johnson et al., 1929; Oldfield, 1971; Szaflarski et al., 2002). It assesses the preference of hand

use for different activities. In Edinburgh inventory test, handedness (i.e., right, left, or ambidextrous) is determined by the hand preference in ten daily activities including writing, drawing, throwing, striking match, opening lid, using scissors, toothbrush, fork, spoon, and broom. Owing to the cultural differences, some population may replace the activity of using fork with using chopsticks. The laterality quotient (LQ) is derived using the following formula:

$$\text{Laterality quotient (LQ)} = (R - L)/(R + L) \times 100,$$

where R is the total number of activities using right hand and L is the total number of activities using left hand. Therefore, handedness is not either right or left only. It is a scale. LQ scores ranged from -100 for strong left-handedness to +100 for strong right-handedness.

1.1.3 Haemodynamic changes during language activation

Language activity involves activation of neurons. Neurons, like all other cells in our body, require energy to function. However, different from other cells, neurons have no energy storage. All the energy required for neuronal activities depends on external blood supply. This energy is supplied in the form of glucose and oxygen. The blood supply of the brain is dynamically regulated to supply more energy to the activated neuron. Therefore, there is a haemodynamic change in terms of blood flow

increase in local area during brain activation. During brain activation, glutamate, the most abundant fast excitatory neurotransmitter in the human nervous system is released from the pre-synaptic neuron. It opens the N-methyl-D-aspartic acid (NMDA) receptors on other neurons which facilitate the entry of calcium ions into the neuron. The calcium ions activate nitric oxide synthesis. Nitric oxide (NO) then diffuses out and causes relaxation of smooth muscle surrounding the local arterioles, and results in local vasodilatation. This vasodilatation allows increase the blood flow volume into the local capillaries, to increase the oxygen and glucose supply for neuronal activity (Manev et al., 1989; Nelson and Cox, 2000; Okumoto et al., 2005; Shigeri et al., 2004) (Figure 1-5).

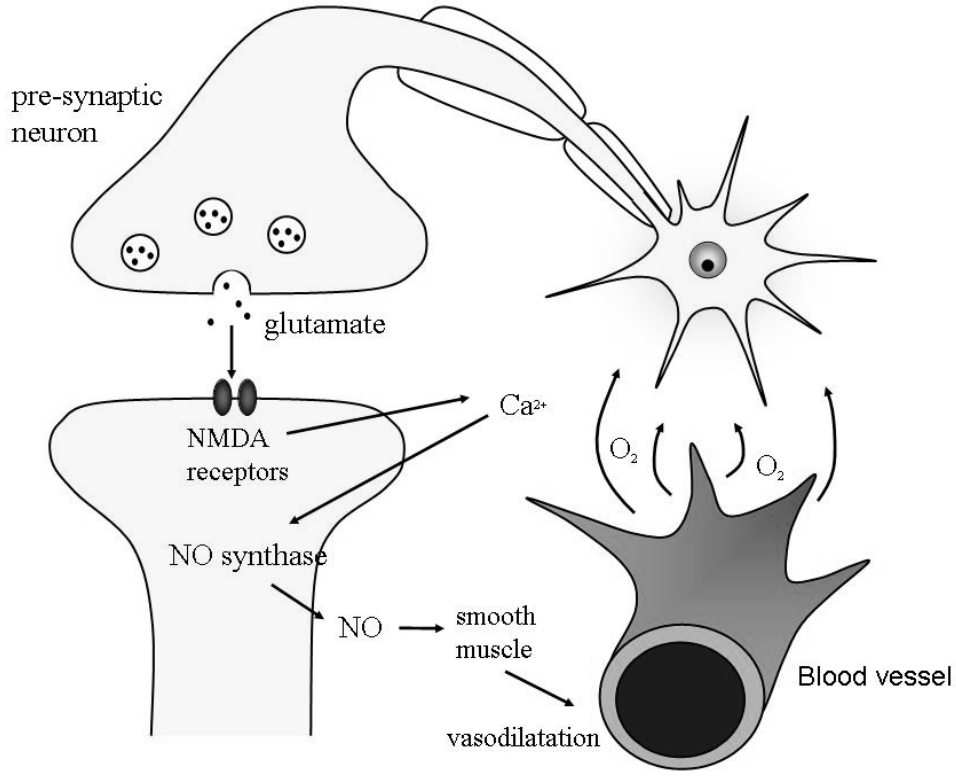

Figure 1-5. Pathway of vasodilatation induced by nitric oxide. The process is initiated by glutamate released from pre-synaptic neuron. Adapted from Carpenter (2003).

There are three mechanisms to regulate the blood supply to the brain. The first mechanism involves the humoral and neural influence over the cardiovascular system, which controls the distribution of blood flow and redirects flow from other organs to cerebral circulation when cerebral perfusion is threatened. The second mechanism is the cerebrovascular autoregulation by vasoconstriction and vasodilatation to maintain stable cerebral perfusion despite changes in arterial pressure. The third mechanism is

the functional hyperemia where blood flow to an activated brain region is increased to enhance substrate delivery and removal of by-products of metabolism. Impairment of either one of these mechanisms would lead to brain dysfunction due to ischemia (Carpenter, 2003; Kandel et al., 2000; Somjen, 1983).

1.2 Technique for the assessment of language lateralization

Previous literature showed that 95% of right-handed people are left dominant, and 76-78% of left-handed people are left dominant in language processing (Pujol et al., 1999; Szaflarski et al., 2002). The hemodynamic response to language stimuli in the left brain is much higher than that in the right brain in the majority of right-handers. Although exact neurophysiology relating to the association between language processing and handedness is not clear, handedness is sometimes used for a rough estimation of language lateralization. For an accurate determination of hemispheric dominance of language, Wada test (Bacia et al., 1998; Benke et al., 2006; Benson et al., 1999; Knake et al., 2003; Lehericy et al., 2000; Mader et al., 2004; Rihs et al., 1999; Schulze-Bonhage et al., 2004; Woermann et al., 2003), positron emission tomography (PET) (Bookheimer et al., 1997; Muller et al., 1997; Tatlidil et al., 2000), functional magnetic resonance imaging (fMRI) (Benke et al., 2006; Benson et al., 1999; Szaflarski et al., 2002; Woermann et al., 2003) and transcranial Doppler sonography (TCD) (Knake

et al., 2003; Knecht et al., 1998a; Knecht et al., 2000b; Rihs et al., 1999) would be the choices instead.

1.2.1 Wada test

Wada test is the golden standard in the clinical assessment of language lateralization. It was first described by Dr. Juhn Wada. The procedures of Wada test include cerebral angiography, hemispheric anesthetization and language assessment using language task in the awake hemisphere. During Wada test, cerebral angiography is performed by trans-femoral approach and then followed by injecting anesthetic agent (100mg sodium amobarbital) into the internal carotid artery. After that, language test is performed until the motor function returns. The language tasks can be reading words and sentences, comprehension of words and sentences and naming of objects from an image. Performance of the patient will be scored according to his/her responses to the language tasks. After the patient recovers from anesthetic condition which is approximately 30 to 45 minutes from the onset of effective anesthesia, the procedure is repeated on the opposite side of hemisphere. The degree of language dominance will then be determined by comparing the scores obtained from the responses of both left and right brain (Bacia et al., 1998; Mader et al., 2004; Woermann et al., 2003). Although Wada test is the golden standard in the assessment of language lateralization, it has many adverse effects (Mikuni et al., 2005). The cerebral angiograhic and anesthetic procedure are invasive. It has as high as 5% risk of morbidity. The scores obtained can also be influenced by the cross-flow of anesthetic agent from the punctured carotid artery to the contralateral

hemisphere via the circle of Willis (Figure 1-5) when both the carotid arteries are injected on the same day (Knecht et al., 1998a). These disadvantages make it less favorable than the other choices of techniques.

1.2.2 Positron emission tomography

Positron emission tomography (PET) is an imaging modality to detect metabolic changes (Phelps, 2006; von Schulthess, 2003; Workman, 2006). Oxygen-15 is used to label oxygen in water for the study of blood flow (Shiomi et al., 2000; Thiel et al., 2001). After the administration of oxygen-15 water tracers, it flows in the circulation and is absorbed by body tissues. When there is a regional increase in blood flow, the tracers become accumulated. PET assesses the brain activity indirectly by measuring the localized cerebral blood flow changes. The concentration of oxygen tracers in different brain regions depends on the brain activation by language stimuli (Bookheimer et al., 1997; Tatlidil et al., 2000). The pattern of oxygen tracer concentration will then be shown on images with areas of high radioactivity. Although PET can be an imaging modality for the determination of language lateralization, its radiation hazards limit its use. In addition, owing to short half-life of the oxygen-15 (approximately 2 minutes), it imposes constraints in the delivery of language tasks.

1.2.3 Functional magnetic resonance imaging

fMRI brain mapping has been used for more than a decade. Using a fast imaging technique, it detects the local haemodynamic changes (blood flow, blood volume and oxygenation) in the brain regions activated by language stimuli with blood oxygen level dependent (BOLD) effect (D'Esposito, 2006; Faro and Mohamed, 2006). It provides a non-invasive alternative for the assessment of the language lateralization (Baker et al., 2007; Benke et al., 2006; Benson et al., 1999; Fernandez et al., 2004; Lehericy et al., 2000; Pujol et al., 1999; Roux et al., 2003; Szaflarski et al., 2002; Xue et al., 2004). It is radiation free and has high spatial resolution of 3-6 millimeters to provide details of brain anatomy on its structural image underlay (Belliveau et al., 1991; Benson et al., 1999; Ogawa et al., 1990a; Ogawa et al., 1990b). However, there are some limitations of MRI which make it difficult to perform in some cases. The MRI system is bulky and very expensive. The MR images are highly affected by motional artifacts and therefore maximal cooperation is required during the scanning procedure. It cannot be applied in patients with claustrophobia, gross obesity, cardiac pacemakers and other metallic implants (Knecht et al., 1998a). Although fMRI has moderately good spatial resolution, the temporal resolution is low which cannot be comparable with the transcranial Doppler sonography.

1.3 Transcranial Doppler sonography

Clinically TCD has been used to evaluate cerebrovascular reactivity (Kassner and Roberts, 2004; Marshall et al., 2003; Pfefferkorn et al., 2001; Silvestrini et al., 1996b), to detect and follow up subarachnoid hemorrhage-induced vasospasm (Aaslid et al., 1982; Lysakowski et al., 2001; Venkatesh et al., 2002a), to detect cerebral emboli (Mackinnon et al., 2004) and in stroke screening (Akopov and Whitman, 2002). With the advanced analysis approach of the cerebral blood flow velocities from the TCD examinations, the technique has also been developed to demonstrate the brain activation by measuring the cerebrovascular response to cognitive stimulation (Akopov and Whitman, 2002; Kelley et al., 1992; Knecht et al., 1998a; Knecht et al., 1998b; Martin et al., 1993; Ringelstein et al., 1990; Vingerhoets and Stroobant, 1999). Since the functional activity of the brain is studied, the procedure is named as functional transcranial Doppler sonography (fTCD).

Transcranial Doppler sonography (TCD) is an application of Doppler sonography in the haemodynamic evaluation of the brain. It makes use of Doppler effect which is frequency change of backscattering signals of moving red blood cells. In the blood stream, red blood cell is the major source of backscattering signals for Doppler sonography. When the incident ultrasound is produced by the transducer, it transmits through the wall of blood vessels and is reflected by the red blood cells. (Figure 1-6) The frequency of the reflected ultrasound increases if the red blood cells are moving towards the transducer. On the contrary, the frequency of the reflected ultrasound decreases if the red blood cells are moving away from the transducer. Doppler shift can be measured by

comparing the frequency of the incident (f_o) and the frequency of (f) received ultrasound (Sanders, 1998).

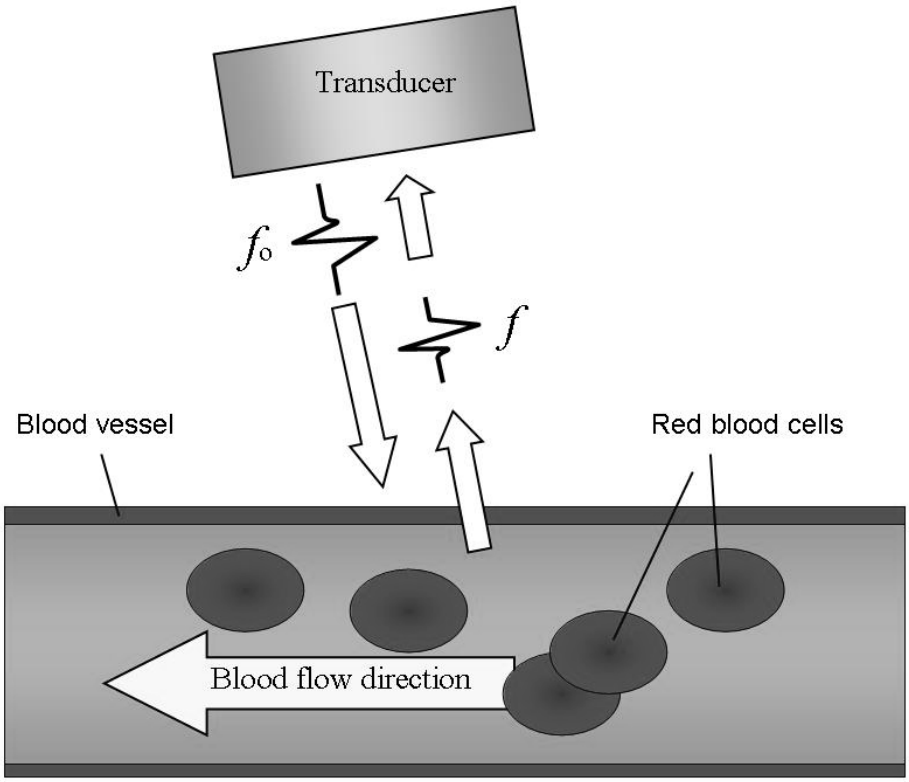

Figure 1-6. Frequency change of ultrasound waves reflected by moving object due to Doppler effect.

The Doppler shift depends on the transmitted ultrasound frequency fo, the velocity of red blood cell v, the velocity of sound in the medium c, and the angle of insonation θ.

$$\text{Doppler shift frequency } f = 2f_o (v / c) \cos \theta$$

The direction and velocity of the blood flow can be determined by the Doppler shift value which can be measured by comparing the frequency of the incident and received ultrasound (Martin et al., 1993). In practice, the Doppler shift frequency is converted and presented in velocity.

$$\text{Velocity of the red blood cells } v = fc / 2fo \cos \theta$$

Clinically, cerebral region is a relative blind spot in ultrasonography. Ultrasound imaging of intracranial soft tissues using conventional ultrasound machine is difficult as the bony skull obscures the incident ultrasound (White et al., 1967; White et al., 1978). Therefore, low operating frequency (2 MHz) is used in transcranial Doppler sonography, to reduce the attenuation. In addition to the modification in operating frequency, a region with thinner bony structure should be used as an insonation window for the acquisition of signals. For this reason, recording is performed in the temporal region above the zygomatic arch (Martin et al., 1993; Ringelstein et al., 1990; White et al., 1967; White et al., 1978). In 1982, assessment of intracranial arteries became possible using low frequency and transtemporal technique, to detect and follow up subarachnoid hemorrhage-induced vasospasm (Aaslid et al., 1982).

1.3.1 Functional transcranial Doppler sonography

Dual 2 MHz transducers has been used in most of the functional transcranial Doppler sonography (fTCD) measurements (Floel et al., 2001; Knecht et al., 1998a; Knecht et al., 2000b; Mackinnon et al., 2004; Schmidt et al., 1999; Silvestrini et al., 1996b; Vingerhoets and Stroobant, 1999). The transducers are fixed at the temporal region by an immobilization device such as a head band. The cerebral artery is located by adjusting the scanning depth and angulation until signal is obtained. Fine adjustment of angulation and Doppler gain is performed to obtain maximal value of signal with minimal noise. Measurement starts when a steady signal is obtained.

In the assessment of language lateralization, brain language areas are activated by language stimuli such as words or sentences. During such language tasks, oxygen consumption in the language area increases due to neuronal activity. In order to maintain a constant supply of oxygen and nutrients to the neurons, perfusional changes occur and results in increased blood flow (Iadecola, 2004; Mohrman and Heller, 2006; Takano et al., 2006). These changes in blood flow can be measured in term of blood flow velocity (BFV), as BFV changes in the MCA have been found to correlate with changes in cerebral blood flow. Such changes in bilateral MCA can be simultaneously measured by TCD and hemodynamic response function can then be obtained. These changes in MCA flow velocity reflect changes in cerebral metabolism due to brain activation (Floel et al., 2001; Vingerhoets and Stroobant, 1999). (Figure 1-7.) The cerebral language response can be measured in terms of BFV changes while one is performing language task. By

comparing the bilateral BFV changes, the degree of language lateralization can be obtained.

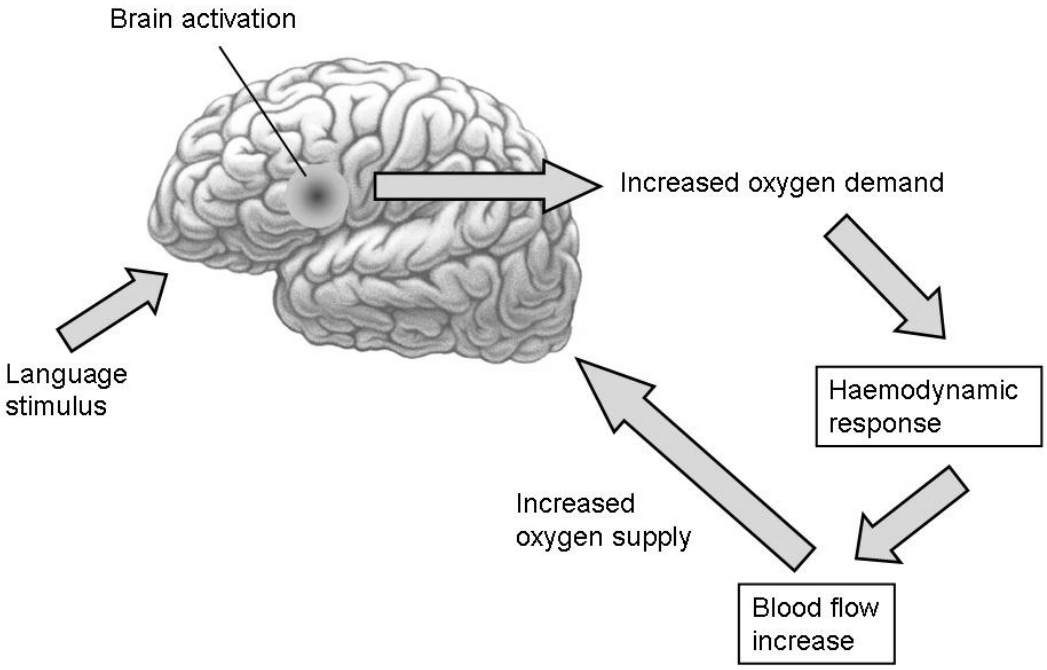

Figure 1-7 Cerebral metabolism of the brain activity while performing language tasks. 1. Language stimulus activates language processing area. 2. Increased oxygen demand to the activated area. 3. Increased blood flow increase the supply of oxygen and glucose to the activated area.

1.3.2 Advantages and limitations of transcranial Doppler sonography in the assessment of language lateralization

Unlike Wada test and PET, TCD is noninvasive and possesses no radiation hazard. Its performance has been confirmed by Wada test (Rihs et al., 1999). Compared with fMRI, TCD also succeeded in discriminating significant lateralization of blood flow induced by a complex cognitive visuospatial task (Schmidt et al., 1999). TCD can reveal dynamic changes in cerebral arteries that may not be able to be identified easily in MR angiography (Akopov and Whitman, 2002). TCD can be applied in patients who are not suitable for MRI examination such as patient with metallic implants and claustrophobia (Knecht et al., 1998a). It has less motional artifacts than MRI. The basic instrument for the TCD examination is portable, small and relatively inexpensive. TCD therefore offers a possibility for the noninvasive, inexpensive assessment and can be repeated easily for the longitudinal study and bedside assessment (Knecht et al., 1998b; Martin et al., 1993; Schmidt et al., 1999).

However, like the BOLD signal of MRI, BFV measured in TCD is only an indirect assessment of neural activity, and is therefore susceptible to the influence by non-neural changes in the body such as carbon dioxide and oxygen. In TCD assessment, the brain activity is reflected by blood flow velocity (BFV) changes. The BFV value is prone to be altered by other factors such as oxygen. This can be vasoconstrictive effect of oxygen or the reduced oxygen demand from the activated brain region owing to the increased oxygen concentration in general circulation. The result of TCD language lateralization assessment is then affected. Therefore, the changing pattern of BFV in the

condition of increased oxygen concentration is crucial for the understanding of how the increased oxygen concentration affects on the determination of language lateralization using TCD.

1.4 Organization of the artery and haemodynamic changes with stimulus

Arteries are not rigid pipes. The luminal size can be changed by vasoconstriction and vasodilatation regulated by both neurological and chemical mechanisms (Mohrman and Heller, 2006). The ability of changing the luminal size is facilitated by muscular and elastic layer of the artery.

1.4.1 The organization of artery and arteriole

Artery and arteriole are made of three concentric layers: the tunica adventitia, the tunica media and the tunica intima (Figure 1-8). The outermost layer of an artery is the tunica adventitia. It is made of fibrous connective tissue that holds the artery in position. The middle layer is the tunica media, which is the thickest layer of the arterial wall. The tunica media is made of smooth muscle and elastic protein (elastin), which provides elasticity for expansion and contraction of the arterial lumen during each wave of systolic blood flow. The innermost layer of an artery is the tunica intima, which is also known as endothelium. It is a smooth layer with one cell thick only. The tunica intima

contacts with the blood directly and prevents the passage of plasma protein getting out of the vessel (Mertz, 2004; Mohrman and Heller, 2006).

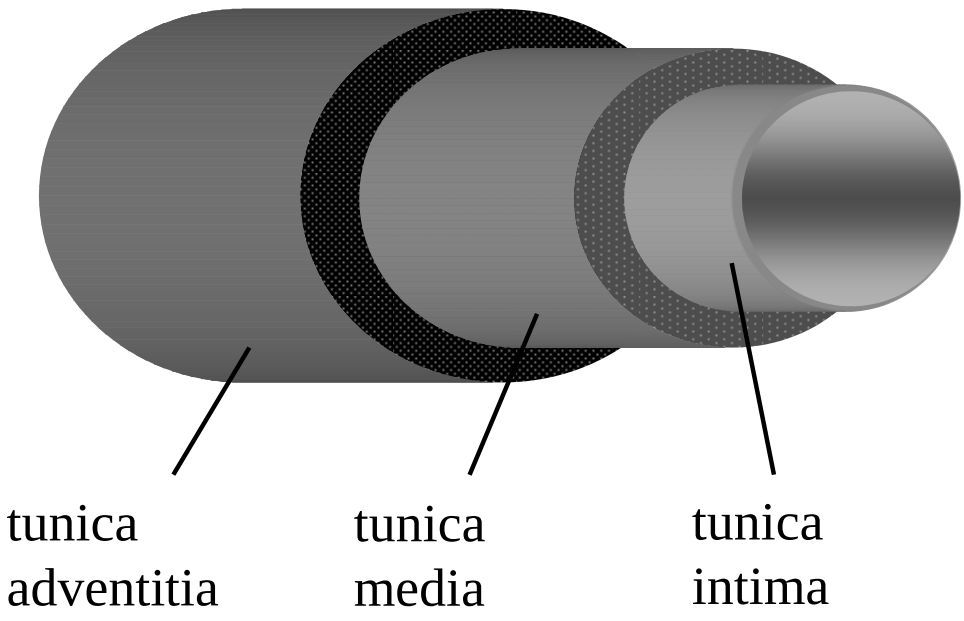

Figure 1-8. Three layers of artery and arteriole. Adapted from Mertz (2004) and Mohrman and Heller (2006).

The internal diameter of the artery is called the lumen, which can be adjusted by the mechanism of vasoconstriction and vasodilatation. Vasoconstriction and vasodilatation are facilitated by contraction and relaxation of the smooth muscle in the tunica media. Such vascular changes are to control the rate of blood flow to the tissues. Vascular smooth muscle cells are small (about 5μm x 50 μm), spindle-shape and have single nuclei (Mohrman and Heller, 2006). The cells themselves are arranged in sheets or bundles and connected by gap junctions. Unlike the striated muscle, the filaments are

not arranged in regular and in terms of repeated sarcomere unit. Therefore striation characteristic is not visible in the micrograph of smooth muscle cells. However there is an organized actin filaments attach to the inner surface of the cell. The actin filaments of smooth muscle cells are much longer than those in striated muscle. Myosin filaments are interspersed betweens the actin filaments.

1.4.2. Contractile mechanism of the artery

Like the striated muscle cells, the force of smooth muscle in artery is developed by the shortening of smooth muscle fibres, resulting from the cross-bridge interaction between actin and myosin filaments. The smooth contraction is adjusted by intracellular free calcium ions (Ca^{2+}) level, ranging from about 10^{-6} mol during maximum contraction to 10^{-7} mol during relaxation. During smooth muscle contraction, Ca^{2+} first forms a complex with protein calmodulin. The complex then activates myosin light chain kinase, a phosphorylating enzyme. This enzyme causes phosphorylation of the myosin chain protein to form cross-bridge. Phosphorylation breaks down adenosine triphosphate (ATP) and energy is released. Tension is developed as repeated cycle of phosphorylation to build up vascular tone. Once the tension is developed, the contraction of smooth muscle can be maintained at very low energy costs (Mohrman and Heller, 2006).

In smooth muscle contraction, the intracellular free Ca^{2+} level can be changed either by electromechanical or pharmacomechanical coupling mechanism (Figure 1-9). Electromechanical coupling is initiated by membrane depolarization. It facilitates the

opening of voltage operated channels (VOC) for Ca^{2+} and then leads to smooth muscle cells contraction. Conversely, smooth mucles relaxation is caused by membrane hyperpolarization. The voltage operated Ca^{2+} channels of the vascular smooth muscles are partially activated by low resting membrane potential and therefore develops a basal contractile stage. Pharmacomechanical coupling is controlled by chemicals such as neurotransmitters. It induces contraction of smooth muscle without the need of membrane potential change. As illustrated in Figure 1-9, the binding of vasoconstrictor agonist (such as norepinephrine) with specific membrane receptor (such as α_1-adrenergic receptor) induces the opening of the receptor-operated calcium channel (ROC). This leads to an influx of Ca^{2+} from extraccellular fluid. In addition, the activated receptor may induce the formation of second messenger, inositol trisphosphate (IP_3). IP_3 opens Ca^{2+} channels in sacroplasmic reticulum that release Ca^{2+}. In both electromechanical and pharmacomechanical pathways, the intracellular Ca^{2+} level raises and induces vascular smooth muscle contraction.

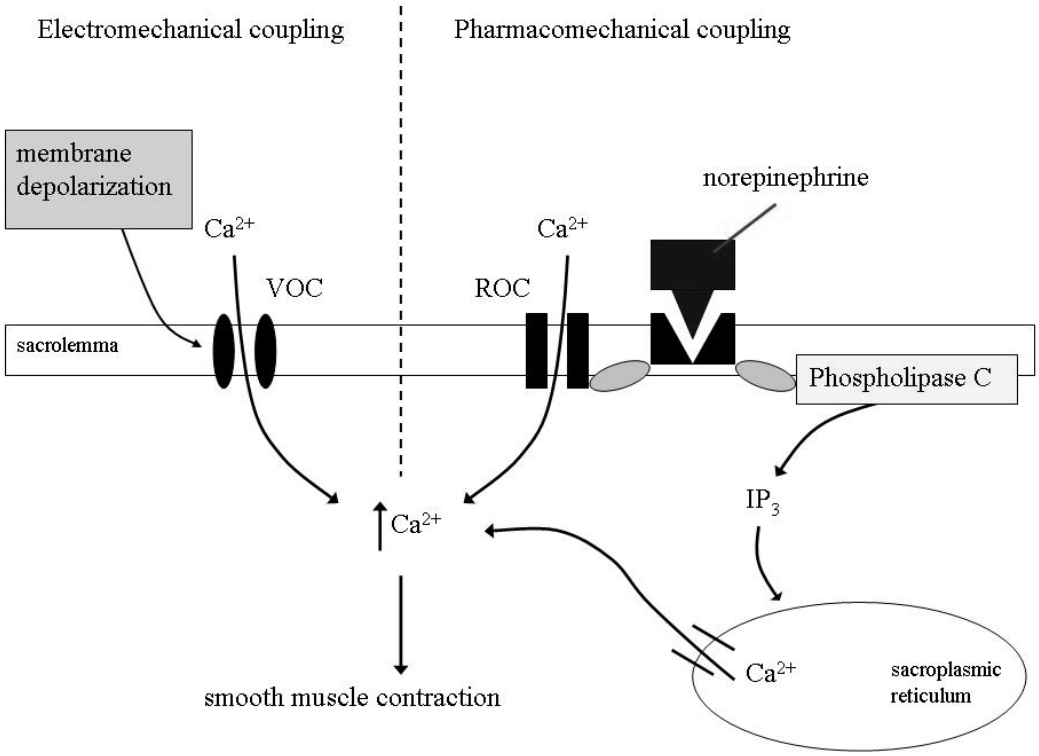

Figure 1-9. Electromechanical coupling and pharmacomechanical coupling mechanisms in vascular smooth muscle contraction. VOC, voltage operated Ca^{2+} channel. ROC, receptor-operated Ca^{2+} channel. G, GTP-binding protein. Adapted from Mohrman and Heller (2006).

1.4.3 Effect of carbon dioxide on cerebral haemodynamics

Carbon dioxide is a one of the mediators of local autoregulation of blood supply by vasodilatation. Cerebral blood flow increases when the partial pressure of carbon dioxide (CO_2) in the blood is raised above normal. When CO_2 is administered, the partial pressure of CO_2 (pCO_2) increases. Increase in pCO_2 causes decrease in pH, which is an important factor to determine vascular tone and the cerebrovascular resistance (Kuschinsky and Wahl, 1978). It causes membrane depolarization and activates nitric oxide synthase which produces nitric oxide (NO). NO contributes to inhibiting vascular smooth muscle contraction by reducing the intracellular Ca^{2+} concentration. As Ca^{2+} is associated with vasoconstriction, vascular tone is reduced when Ca^{2+} concentration decreases. Vasodilatation of the cerebral arteries will then lead to blood flow increase (Dahl et al., 1992; Kirkham et al., 1986; Markwalder et al., 1984; Nishiyama et al., 1997).

1.4.4 Vascular reactivity

The vasodilatative effect of CO_2 is applied in the assessment of the response of the blood vessels to CO_2, which is known as vascular reactivity of CO_2 or CO_2 reactivity. Clinically, blood flow velocity (BFV) is usually used to represent blood flow. The pCO_2 increase can be achieved by breath-holding technique (Silvestrini et al., 2004), CO_2 inhalation (Kastrup et al., 1998a; Kastrup et al., 1997; Kodaka et al., 1996; Park et al.,

2003; Valdueza et al., 1999; Ziyeh et al., 2005) or administration of acetazolamide (Valikovics et al., 1996). Many studies have shown the direct relationship of CO_2 concentration and cerebral blood flow velocity (Kawata et al., 1998; Park et al., 2003; Pfefferkorn et al., 2001; Silvestrini et al., 2004; Ziyeh et al., 2005). The cerebrovascular reactivity of CO_2 is defined as the change of BFV in the MCA per unit change in CO_2 concentration. The cerebrovascular reactivity reflects the elastic property of the cerebral artery and the sensitivity of the auto-regulation system, which can be an indicator of cerebrovascular pathology. Clinically, cerebrovascular reactivity assessment reveals the severity of diseases in patients with different pathological conditions such as migraine, diabetes mellitus and peripheral vascular disease. TCD demonstrated that increased cerebrovascular reactivity to CO_2 challenge in patient with migraine (de Hoon et al., 2003; Fiermonte et al., 1995; Harer and von Kummer, 1991; Kara et al., 2003; Kastrup et al., 1997; Kastrup et al., 1998b; Schreiber et al., 2000; Silvestrini et al., 1995; Silvestrini et al., 1996a; Sugahara, 2004). Increased baseline BFV and cerebrovascular reactivity to CO_2 were demonstrated in patients with diabetes mellitus, while the cerebrovascular reactivity was decreased in patient with peripheral vascular disease (Kawata et al., 1998).

1.4.5 Effect of oxygen on cerebral haemodynamics

Oxygen has effects on blood vessels opposite to those of CO_2. It causes reduction of blood flow velocity (Demchenko et al., 2002; Hoffman et al., 2000; Johnston et al., 2003; Omae et al., 1998). When oxygen is administered by hyperbaric oxygen inhalation,

pO_2 in the blood stream is raised above normal. Higher than normal arterial blood pO_2 level leads to vasoconstriction of the cerebral arteries resulting in blood flow reduction. A few human studies assessed the effect of oxygen on the cerebral BFV and reported vasoconstriction caused by increased oxygen concentration (Demchenko et al., 2002; Ohta, 1986; Omae et al., 1998). The property of vasoconstriction such as the latency and the rate of change of BFV have not been reported. Clinically, cerebrovascular reactivity assessment using oxygen challenge is still in early developing stage, compared with CO_2 reactivity assessment. Therefore, the property of oxygen induced cerebrovascular change must be assessed before oxygen is widely used as a challenge in cerebrovascular reactivity examination. Furthermore, as increased oxygen concentration causes vasoconstriction and reduces BFV. It is likely to affect any assessment involving the measurement of haemodynamic changes. This includes the assessment of language lateralization.

1.5 Aims of the present study

In the present study, instead of CO_2, the effect of oxygen on the hemodynamic change in cerebral circulation measured by TCD from which the cerebrovascular reactivity and language lateralization were derived was focused.

We hope that the findings in the present study would increase the understanding of the effect of oxygen on the cerebral BFV. In the previous literature, electromechanical coupling and pharmacomechanical coupling mechanisms are involved in the vascular activities, (Section 1.4.2). Complex process involved in vascular smooth muscle contraction suggests that the vasoconstriction would not happen immediately after the increase of circulation oxygen. Taking the advantage of high temporal resolution of TCD, we would like to study the oxygen effect on the BFV changes in the middle cerebral arteries with more details by measuring the BFV in MCA continuously since the onset of oxygen inhalation. The findings would help in developing cerebrovascular reactivity examination using oxygen as a challenge. The findings in Study One would also serve as a reference to extend an investigation of the oxygen effect on the language lateralization assessment in Study Two.

In language lateralization assessment, relative change of BFV in the MCA has been measured to reflect the neural activation due to language processing. Hypothetically, the lateralization index is susceptible to be changed by increased inspired oxygen concentration. In this part of the study, we would like to investigate the effect of inspired oxygen concentration on the language lateralization assessment. The findings in the present study should increase the understanding of the effect of increased

inspired oxygen concentration on language lateralization assessment. It serves as a reference in interpretating results of language lateralization assessment from those patients requiring oxygen administration.

1.6 Hypothesis to be tested

1) We hypothesized that: there would be a continuous changing pattern of blood flow velocity in the middle cerebral arteries showing the vasoconstrictive effect of oxygen.

2) The vasoconstrictive effect of oxygen would constraint the increase of blood flow velocity in the middle cerebral arteries induced by language stimuli. The result of language lateralization assessment would be affected by increased inspired oxygen concentration.

Chapter Two

Study One

Effect of oxygen on the cerebral blood flow velocity measured by transcranial Doppler sonography

2.1 Introduction

Like all of the body tissues, brain requires sufficient supply of blood to transport oxygen and glucose. However, there is no energy storage in neuron. Continuous blood supply with auto-regulatory mechanism is maintained for the high level energy consumption of neurons. To maintain the brain perfusion, the higher the brain activation, the more the blood supply to the local area. Therefore, brain activation is indirectly reflected as change in local blood supply. TCD is a clinical tool using BFV to reflect brain activation. Without neural factors, haemodynamic changes can be caused by non-neural factors such as vasoconstrictor or vasodilator. The measurement of haemodynamic response per unit change of vasoconstrictor or vasodilator concentration is known as vascular reactivity. TCD has been used as a clinical tool for the assessment of cerebrovascular reactivity and functional activities of the brain (Dahl et al., 1992; Fiermonte et al., 1995; Harer and von Kummer, 1991; Kastrup et al., 1998a) (Section

1.3.1). TCD is superior to MRI and PET in terms of temporal resolution and absence of radiation hazards (Akopov and Whitman, 1274; Kelley et al., 1992; Knecht et al., 1998a; Knecht et al., 1155; Kodaka et al., 1996; Mackinnon et al., 2004; Ringelstein et al., 1990; Silvestrini et al., 1996b; Venkatesh et al., 2002b) respectively (Section 1.3.2).

Previous studies showed that oxygen has a vasoconstrictive effect which causes reduction in luminal size of the artery, and results in reduction of BFV (Demchenko et al., 2002; Gilmore et al., 2004; Ohta, 1986; Omae et al., 1998). In the present study, the effect of oxygen on the haemodynamic change in cerebral circulation was investigated. No neural factor such as language task was involved in this part of the study. By continuous measurement of BFV change in MCA during oxygen inhalation using TCD, the pattern of the haemodynamic change was revealed. This finding was expected to increase the understanding of the oxygen effect on the haemodynamic change in the cerebral vasculature, and the development of the cerebrovascular reactivity examination using oxygen as a challenge. This also served as a reference of oxygen concentration and experimental set up for the Study Two of this project, to investigate the effect of oxygen on the assessment of language lateralization.

2.2 Materials and Methods

2.2.1 Subjects

20 healthy subjects aged 20 – 40 years were recruited. The number of male and female subjects was almost the same to exclude gender difference. Younger age subjects were recruited to reduce the chance of arterial pathology due to degenerative changes such as aneurysm and thrombosis (Chandrasoma 1998; Mohrman and Heller, 2006). Another reason to recruit younger age subjects is better penetration of incident ultrasound through the temporal window, leading to better signal for the BFV measurement. In contrast, more calcified squamous portion of temporal bone in the elderly increases the attenuation of incident ultrasound to the cerebral arteries. It reduces the intensity of incident ultrasound and therefore reduces the signal for the BFV measurement. Therefore, recruiting younger subjects reduce the exclusion of subjects due to poor TCD signal obtained and arterial abnormalities. Previous literature also found that cerebral blood flow reduction was higher in younger subjects than older subjects (Nakajima et al., 1983; Watson et al., 2000). Younger subjects recruited in the present study were expected to have more BFV reduction during oxygen inhalation.

Previous literature showed that 95% of right-handed people are left dominant, and 76-78% of left-handed people are left dominant for language processing (Pujol et al., 1999; Szaflarski et al., 2002). In the present study, all the subjects recruited were right handers to reduce the chance of left dominance of language processing. In TCD examination, subjects showing right dominance for language processing were excluded.

Blood pressure and pulse rate were measured in each subject to ensure that they did not have hypertension (Table 2-1) (Alpert, 1978; Cameron et al., 1999; Shulman, 1987). The subjects were explained the whole procedures of the examination. Consent was obtained from each subject before the examination (Appendix 1). The subjects were asked to rest for 15 minutes for the stabilization of haemodynamics. Subjects with any known or suspected central nervous system disease, stenosis or known cerebrovascular disease, cerebral metabolic disorders, known neurological disease, or a history of drug abuse were excluded (Heckmann et al., 1998; Kastrup et al., 1998b; Papadimitriou et al., 2003; Silvestrini et al., 2004). Moreover, subjects who were taking or had taken analgesic drugs 10 days prior to the examination (Silvestrini et al., 2004; Silvestrini et al., 1996a), or receiving any kind of chronic medication including vasoactive or psychoactive drugs (Papadimitriou et al., 2003) were also excluded. Smoking, taking or drinking any caffeine-containing food or drinks, and drinking any alcoholic drinks were prohibited 6 hours prior to the examinations. This is because all the nicotine, caffeine and alcohol may induce blood flow changes (Cameron et al., 1990; Papadimitriou et al., 2003; Terborg et al., 2002).

The study was divided into two parts: ultrasound carotid examination and TCD examination. The examinations were carried out in a quiet room with the subjects lying for the carotid examination and sitting in a comfortable position for the TCD examination. In order to keep the environmental conditions constant, all the examination records were taken in the same room. The present study was approved by the Human Subjects Ethics Sub-Committee of the Department of Health Technology and Informatics, The Hong Kong Polytechnic University.

2.2.2 Ultrasound carotid examination

All the subjects were screened by B-mode and Doppler ultrasound carotid scan using the HDI 5000 System (Philips Medical Systems Company, USA) in conjunction with a 5 – 12 MHz transducer. Gray scale B-mode, color and pulsed-wave Doppler ultrasound scan was performed in common carotid arteries, internal carotid arteries, external carotid arteries and vertebral arteries bilaterally for each subject before the examination in order to exclude the presence of carotid and vertebral arteries steno-occlusive disease, narrowing or intimal thickening (Sanders, 1998).

2.2.3 Transcranial Doppler sonography examination

TCD examination was carried out with the subjects in sitting position, using Multi-DopX4 transcranial Doppler (TCD) system (DWL Elektronische Systeme GmbH, Germany).

Dual 2-MHz pulse Doppler transducers were fixed on the temporal region bilaterally with an elastic head band, which provided fixation, adjustment of insonation site and angle (Figure 2-1). Temporal region located above the zygomatic arch was chosen as a window of insonation. The M1 portion of middle cerebral arteries (MCA) was scanned at the depth of 50-58 mm from the skin surface (Papadimitriou et al., 2003; Sanders, 1998). The transducer was manipulated slightly forward and upward to identify the MCA. Angle of insonation was adjusted until the maximal value of the pulsative

signal with minimal noise was obtained. The flow direction in MCA was towards the transducer. Subjects were instructed to keep still in order to reduce the motional artifacts and brain responses at the motor areas. Simultaneously blood flow velocity in both left and right MCA was acquired. Subjects took normal depth of breathing with room air and 35% oxygen at the flow rate of 5 liter per minutes alternatively during the TCD measurement. 35% concentration of oxygen was used because it is relatively safe to the subject. It was expected to have significant effect on BFV because significant BFV change was obtained in the pilot study using as low as 30% oxygen. Although as high as 100% oxygen was used in some of the previous studies (Nakajima et al., 1983; Watson et al., 2000; Omae et al., 1998), and the higher the oxygen concentration, the more the BFV change induced, safety is still the main concern in the present study.

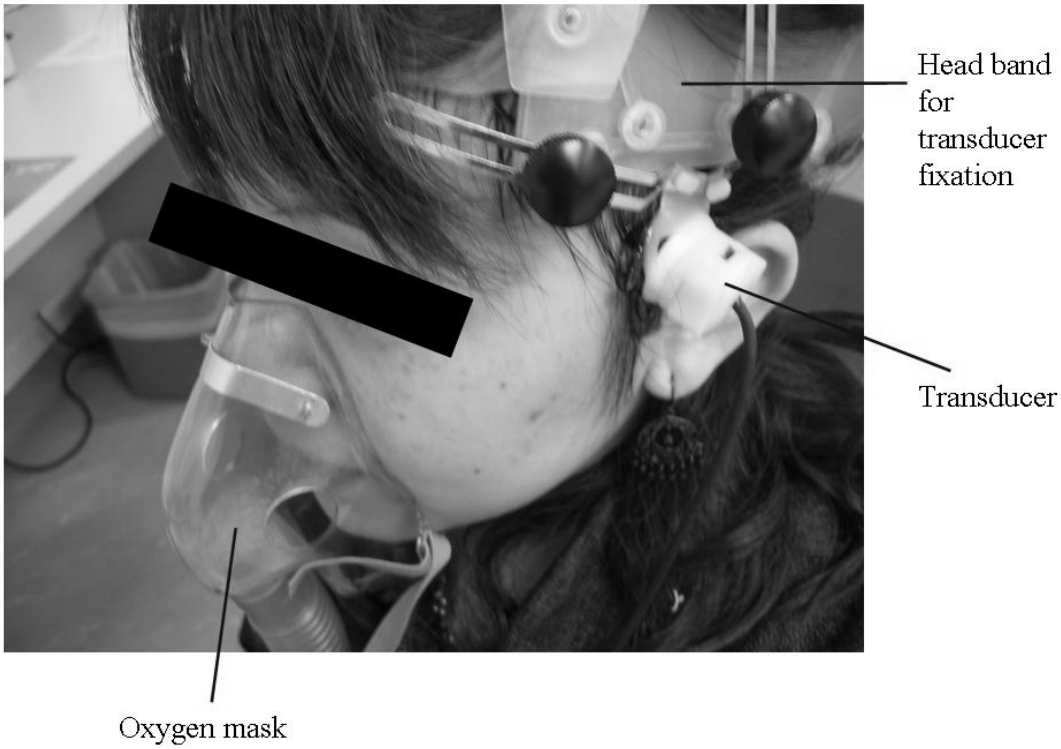

Figure 2-1 TCD set up including the transducers and oxygen mask. Dual 2-MHz pulse Doppler transducers were fixed on the temporal region bilaterally with an elastic head band. Oxygen mask was used for 35% oxygen delivery to the subject.

2.2.4 Oxygen challenge paradigm

The oxygen challenge paradigm composed of 10 epochs of 40 second oxygen inhalation and 10 epochs of 40 second room air inhalation (Figure 1). The software E-PRIME (Psychology Software Tools, Inc. Pittsburgh, USA) was used for the presentation of oxygen/room air inhalation tasks and time markers were sent to synchronize the acquisition of blood flow velocity on the TCD system. During the oxygen inhalation periods, subjects were instructed to have normal breathing with the administration of 35% of oxygen at the flow rate of 5 liters per minute through the face mask. During the room air inhalation periods, subjects were instructed to have normal breathing. Face mask was removed by the operator instead of the subject during room air inhalation phase. It is because any motion of the subject during the examination may generate motor activation of the brain. This test was repeated twice on each subject to increase the signal-noise ratio on averaging. The blood flow velocities values and time markers were recorded for offline analysis.

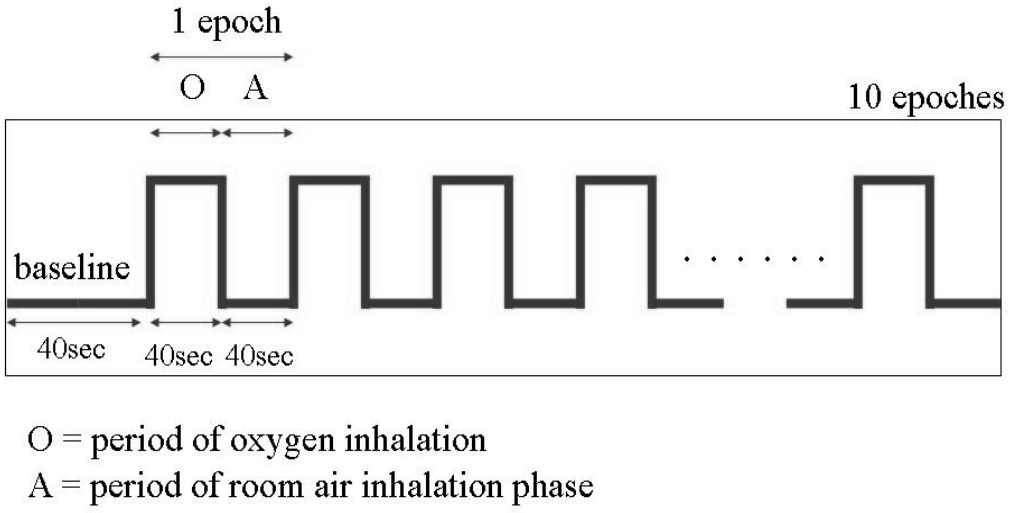

Figure 2-2. Oxygen challenge paradigm composed of 10 epochs of 40 second oxygen inhalation (O) and 10 epochs of 40 second room air inhalation (A).

2.2.5 Data analysis

In the present study, relative percentage changes of BFV were used instead of actual BFV changes. It is because the actual BFV change can be varied by other factors such as baseline BFV and the Doppler angle (Martin et al., 1993). As the Doppler angle for each measurement was not the same, the BFV change can be varied without the effect of oxygen. Therefore the baseline BFV and the BFV change were converted to relative change for standardization and inter-subject comparison. To obtain the relative changes of normalized BFV and minimizing of noise, BFV in the left and right MCA with time markers were analyzed by the software AVERAGE (Deppe et al., 1997). The analysis procedures included standardization of the sample rate, modification of time marker, heart rate calculation, respiratory component filtering, heart cycle integration,

velocity normalization and baseline correction. The normalized BFV in the dataset was divided into activation and resting epochs according to the markers which represented the time interval of oxygen and room air inhalation periods respectively. Averaging was done on activation epochs and resting epochs respectively. After those processes, the continuous changes of BFV within activation and resting period were obtained. Paired t-test was used to compare the left and right BFV changes; and also to compare the BFV in activation and resting phase. Any comparisons with p values smaller than 0.05 were considered to be significantly different.

2.3 Results

A total of 20 healthy subjects (11 males, 9 females) aged 20-32 years (mean ± S.D. = 24 ± 2.9 years) were included in the present study. Two subjects were excluded. One subject had insufficient penetration of ultrasound beam through temporal bones and another had noisy TCD signals. Demographic information such as blood pressure (BP) and pulse rate of all the subjects was shown in Table 2-1.

Subject Number	Sex	Age (years)	Left BP (mmHg)	Right BP (mmHg)	Pulse Rate (beats/minute)
1	F	20	98/66	97/66	70
2	M	22	104/65	101/63	76
3	M	23	115/72	110/72	67
4	M	23	99/62	97/60	60

5	F	25	98/63	98/62	70
6	F	21	93/54	91/55	71
7	M	24	114/73	112/77	70
8	M	21	116/71	116/68	80
9	M	20	90/57	97/60	70
10	F	28	100/63	100/65	68
11	F	25	96/59	96/63	70
12	F	23	101/68	100/70	76
13	F	24	97/59	99/59	66
14	M	23	100/65	103/66	65
15	M	23	97/60	99/60	68
16	F	32	113/75	118/76	75
17	F	25	100/61	103/66	66
18	M	23	96/60	98/60	68
19	M	23	115/75	113/75	72
20	M	28	99/62	102/62	75

Table 2-1. The demographic data of all subjects in Study One.

2.3.1 Comparison of blood flow velocity changes in the middle cerebral arteries between oxygen inhalation phase and room air inhalation phase

The changing patterns of blood flow velocity (BFV) in both left and right MCA were similar. The BFV increased initially and then decreased beyond the baseline within the oxygen inhalation period (Figures 2-3 and 2-4). Once the subjects started to inhale room air, the BFV increased gradually and reached the baseline value by the end of the air inhalation phase.

At the beginning of the oxygen inhalation, a short latent period of 3.02 seconds was followed by a significant increase of blood flow velocity above the baseline (2.90 % +/- 3.95% for left MCA; 2.48 +/- 3.56% for right MCA). After 7.78 seconds from the starting point of oxygen inhalation, the BFV decreased below the baseline (Figure 2-3). 38.70 seconds are taken to achieve minimum BFV level (-7.76 +/- 5.54% for left MCA; -6.96 +/- 4.36% for right MCA) during the oxygen inhalation phase. When the subjects started to inhale room air, the blood flow velocity increased gradually and restored to baseline level after 38.05 seconds. The maximum (P1) and minimum (P2) percentage change of BFV were identified. Time required for the percentage change of BFV (T1) increased from baseline to P1 was found. T2 was the time for the percentage change of BFV from P1 to minimum value. T3 was the time for the percentage change of BFV from minimum value to the baseline value (Table 2-2).

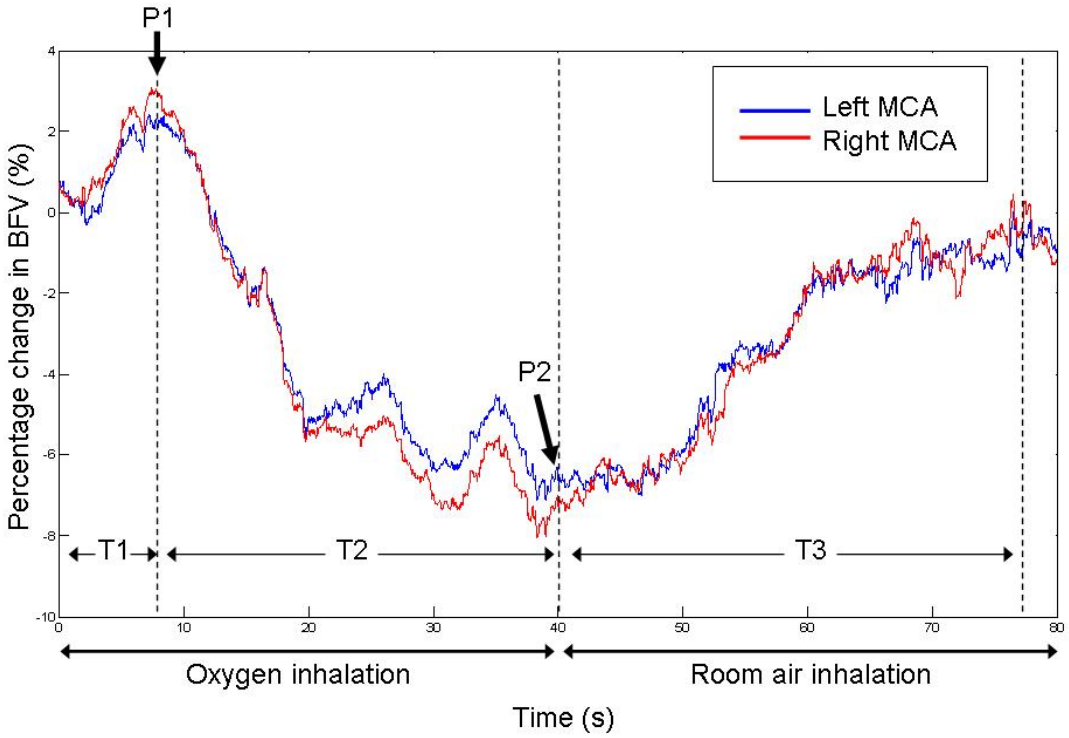

Figure 2-3. Percentage change of bilateral MCA flow velocity when breathing oxygen (0 – 40th second) and room air (40th – 80th second). Red line represents left MCA and blue line represents right MCA.

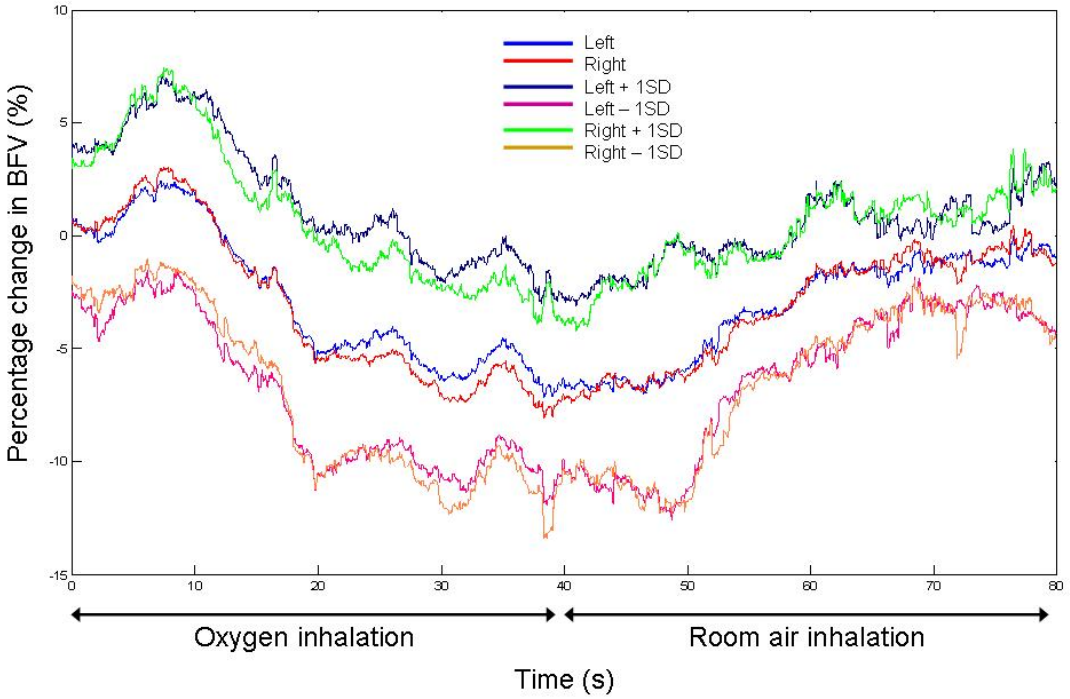

Figure 2-4. Percentage change of MCA flow velocity when breathing oxygen (0 – 40th second) and room air (40th – 80th second) and standard deviation

	The first Peak (P1)			Minimum BFV value (P2)			Time for resumption of BFV to baseline value (T3) (seconds)
	(T1) (seconds)	(% change in BFV)		(T2) (seconds)	(% change in BFV)		
		Left MCA	Right MCA		Left MCA	Right MCA	
Average	7.78	2.90	2.48	37.80	-7.76	-6.96	38.05
SD	2.28	3.95	3.56	6.18	5.54	4.36	6.89

Table 2-2. The peaks and time of BFV change in the changing pattern when breathing oxygen and air. The mean peak amplitude, P1 and P2 represent the percentage change of BFV at the first peak and the minimum percentage change of BFV respectively. The durations, T1, T2 and T3, represent the time for BFV to increase from baseline to P1, the time to achieve minimum BFV, and the time required for the BFV to restore to baseline level respectively.

2.3.2 Comparing of blood flow velocity change in left and right middle cerebral arteries

The changing pattern of BFV in left and right MCA was similar (Figure 2-3). No significant difference of mean percentage change of BFV between left and right MCA was found, in both oxygen inhalation phase (p= 0.39) and air inhalation phase (p= 0.61) (Table 2-3).

	Mean BFV in oxygen inhalation phase		Mean BFV in recovery phase (room air inhalation)	
	Left MCA	Right MCA	Left MCA	Right MCA
Average	-2.83	-3.11	-3.24	-3.12
SD	3.37	2.93	2.29	2.05
Pair t-test	p = 0.39		p = 0.61	

Table 2-3. The mean BFV of bilateral MCA when the subject breathing oxygen and room air.

2.4 Discussion

The aim of this study was to investigate the effect of altered oxygen concentration on the cerebral BFV. By continuous assessment and simultaneous recording of BFV changes in bilateral MCA using TCD, the characteristics of haemodynamic changes under the influence of oxygen in terms of rate and duration were found.

Previous studies reported that an increase in inspired oxygen concentration would reduce the luminal size of the cerebral artery by vasoconstriction, resulting in the reduction of cerebral BFV (Demchenko et al., 2002; Hoffman et al., 2000; Johnston et al., 2003; Omae et al., 1998). However, none of them has reported the changing pattern of cerebral BFV during increased oxygen concentration. They also did not report the changing pattern of CBFV when increased oxygen concentration was changed back to baseline value. It is because the BFV reduction during the saturation of oxygen effect was used for assessment of vascular reactivity of oxygen in most of the studies. Without continuous TCD measurement, the changing pattern of BFV from the onset of the vascular response to the saturation of the oxygen effect cannot be found.

For other methods, it is quite difficult for real time measurement of fast BFV change. The temporal resolution of fMRI and PET is not high enough to quantify the BFV changes. In contrast, TCD has high temporal resolution which enabled us to non-invasively capture the fast BFV change of human subjects in the present study. To our knowledge, the present study was likely the first to evaluate the changing pattern of cerebral BFV in human subjects during oxygen inhalation, with the continuous TCD assessment.

2.4.1 Changing pattern of blood flow velocity during 35% oxygen inhalation

In the oxygen inhalation phase, the changing patterns in both left and right MCA were similar where the increase in relative BFV was followed by a decrease beyond the baseline within the oxygen inhalation period (Figure 2-3 and 2-4). When oxygen was administered to the subject, 3.02 seconds time delay of haemodynamic effect was recorded. This may be the time required to build up the pO_2 level in the blood through the transport of oxygenated blood from the lung. After the delay, the haemodynamic effect of oxygen leading to an increased BFV of 2.48% - 2.90% above the baseline was first obtained. This is believed to be the vasoconstrictive effect on cerebral vasculature caused by an increase in partial pressure of oxygen (pO_2) (Mohrman and Heller, 2006) (Figure 2.5). Instead of decreased BFV, such sudden decrease in arterial diameter leads to an increase in BFV (P1). When the partial pressure of oxygen increased, all the arteries might not have been constricted at the same time. It is because the electromachanical and pharmacomechanical coupling would not happen immediately after the increased of circulatory oxygen level. At that moment, the luminal size of the distal portion of MCA reduced while the proximal portion was not yet constricted. Therefore the BFV in the constricted portion (V_1) was increased to the value above the baseline value (V_0).

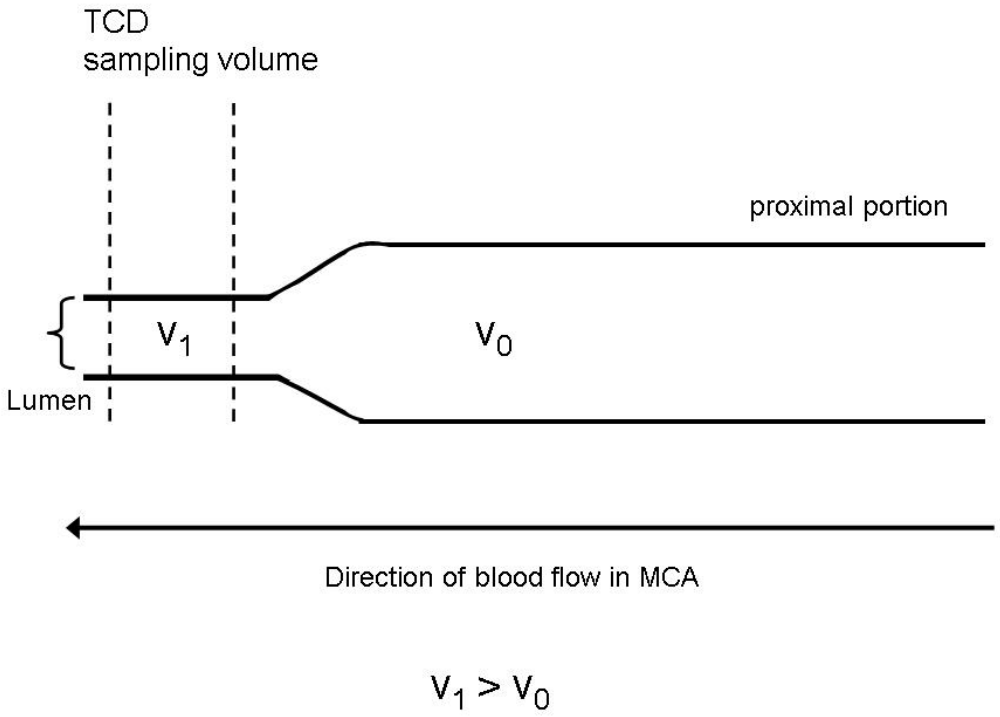

Figure 2.5 The longitudinal profile of MCA when the subjects were breathing oxygen at the 3.02nd second). Inhalation of oxygen caused vasoconstriction in the distal portion of MCA while the proximal portion was not yet constricted. The BFV of the constricted portion (V_1) was increased above the baseline value (V_0).

After the P1, the BFV began to drop and continued further below the baseline. It reached the maximum vasoconstriction and a trough of BFV of -7.76 - -6.96 was obtained. This is because the whole MCA including both the proximal and distal portions were constricted. The reduction of arterial diameter reduced the of blood flow into the lumen (Mohrman and Heller, 2006). This led to decreased BFV value (Figure 2-6).

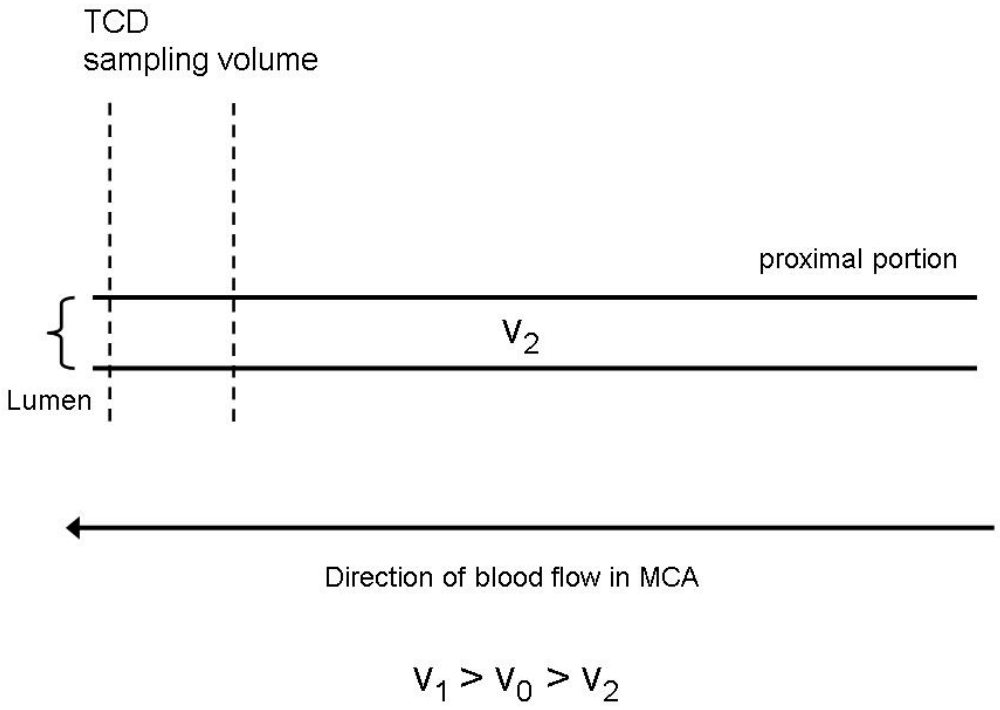

Figure 2.6 The longitudinal profile of MCA when the subjects breathing oxygen at the 8.19th second. Both the proximal and distal portions of MCA were constricted. The reduction of arterial diameter reduced the of blood flow in the MCA leading to BFV decreased (V_2) below the baseline level (V_0).

The results show that 37.80 +/- 6.18 seconds (T2) were required for the reduction of BFV to minimum level in the subjects. After the duration T2, the percentage change in BFV became flattened, as maximum vasoconstriction was achieved in the condition of 35% oxygen inhalation. No significant difference was found in MCA flow velocity between left and right sides in the conditions of oxygen and air inhalation. This implies

that the cerebral vasculature had the same response to oxygen concentration on both left and right sides.

2.4.2 Changing pattern of blood flow velocity during room air inhalation phase

Following the oxygen inhalation phase, Subject breathed in room air instead of oxygen. A continuous change of BFV in bilateral MCA was recorded. During the room air inhalation phase, the changing patterns of both left and right MCA were similar which increased and restored to baseline level at the 38.05^{th} second from the starting point of air inhalation (Figures 2-3 and 2-4). The pO_2 in the blood stream was reduced to the baseline oxygenation. The reduction of arterial blood pO_2 level decreased the vasoconstriction effect on the cerebral arteries. Reduced vasoconstriction caused the restoration of luminal diameter and allowed increased blood flow in the MCA. The BFV value finally returned to the baseline level. The mean duration for the restoration of BFV to baseline level (T3) was 38.05 +/- 6.89 seconds.

The rate of BFV decrease during oxygen inhalation was faster than the rate of BFV increase during room air inhalation (Figure 2-7). It reflects the property of the vascular smooth muscle, which has shorter time for constriction and longer time for relaxation (Levick, 2003). It implies that the vasodilatation process was relatively slow.

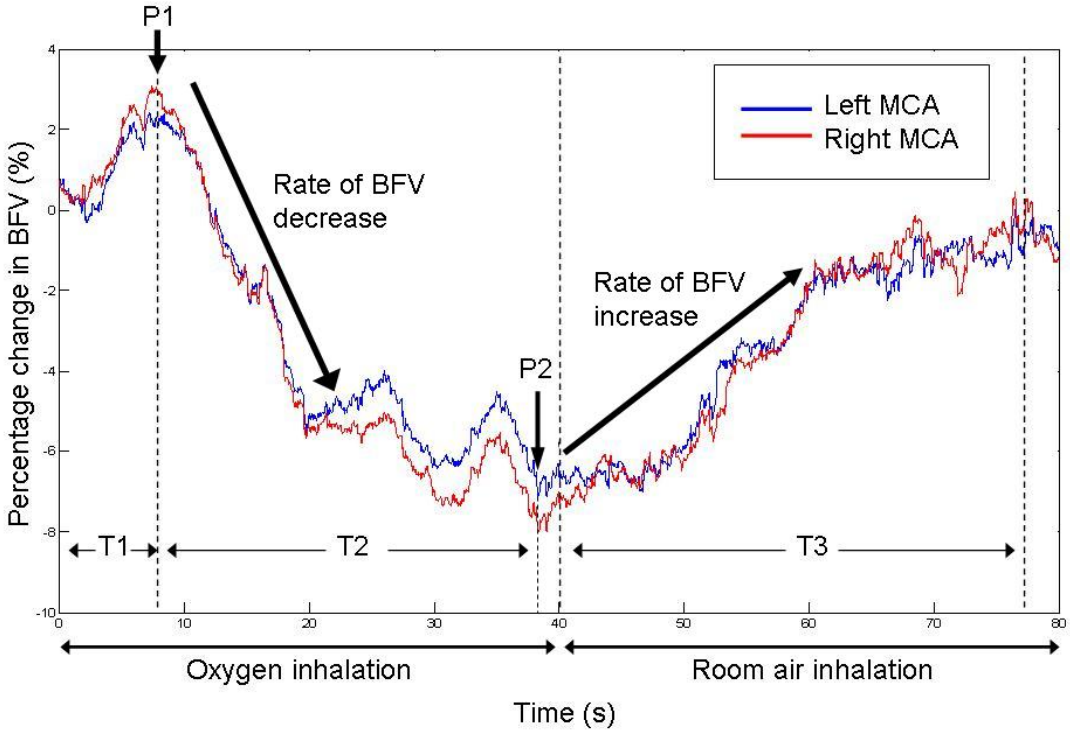

Figure 2-7. Rate of change of BFV in oxygen inhalation and air inhalation.

2.4.3 Clinical significance

The present study was likely to be the first to evaluate the changing pattern of cerebral BFV in human subjects during oxygen inhalation. In the present study, the change of BFV during oxygen inhalation is not simply decreasing. The increase in relative BFV was followed by a decrease beyond the baseline within the oxygen inhalation period (Figures 2-3). Latent period of the BFV change (3.02 seconds) was the time required to initiate the haemodynamic change responding to the increased blood

oxygenation. The time of the latent period may give us information about the sensitivity of the cerebral artery to the change of oxygen concentration.

Before the reduction of BFV, there was an increase of BFV to 2.48% - 2.90% above the baseline (P1). This is opposite to our concept that oxygen causes reduction of BFV. What we obtained from the vascular reactivity studies was the higher the oxygen concentration, the more the CBFV reduction. The early change of cerebral haemodynamics was ignored in the conventional methods of data collection. However, useful information can be obtained as it reveals the healthy status such as elastic property of the artery. It is valuable in the diagnosis of vascular disease such as migraine (Fiermonte et al., 1995) and stroke (Akopov and Whitman, 2002) as it provides additional information which cannot be obtained in the conventional methods. The findings of present study increase the understanding of effect of oxygen on BFV. Single BFV measurement over a period used in the previous studies may mask some valuable information such as the changing pattern, the latency and the rate of change of the haemodynamics for diagnosis of vascular disease. It alerts the clinicians in interpretating the BFV over the periods of oxygen inhalation. The present study also provides a guideline for setting up oxygen reactivity study using TCD. The cerebrovascular reactivity of oxygen can be derived by maximum BFV reduction per unit change of inspired oxygen concentration. The present study measured the time required for maximum BFV reduction in 35% oxygen (37.8 seconds), and the time required for recovery of BFV to the baseline level after room air was inhaled (38.05 seconds). To ensure the contraction status of vascular system was restored to baseline level, the time 40 seconds was suggested to be the minimum time interval between oxygen inhalation

events. Otherwise, the cumulative effect of oxygen did not allow the arterial lumen restored to the baseline level. It reduces the difference of relative BFV between oxygen inhalation and room air inhalation. The vascular reactivity will therefore reduced. Furthermore, the finding of this study was a reference for Study Two in this project. As there was a significant reduction of BFV during 35% oxygen inhalation in Study One, this concentration was applied into Study Two to investigate the influence of oxygen on the language lateralization assessment.

2.4.4 Summary

The purpose of the present study was to investigate the effect of oxygen on cerebral BFV in bilateral MCA in Chinese subjects, using TCD for continuous assessment. In summary, the present study showed that BFV reduction caused by inhalation of 35% oxygen, and the restoration of BFV to the baseline level after inhalation of room air. Continuous TCD assessment found that the BFV was not always decreased during oxygen inhalation phase. Latent period and early peak of BFV increase were found. The findings of this study increase the understanding of effect of oxygen on the cerebral BFV. Single BFV measurement may mask some valuable information such as the changing pattern, the latency and the rate of change of the BFV for diagnosis of vascular disease. It alerts the clinicians in interpretating the BFV over the periods of oxygen inhalation. It also provides a reference in setting up the study to investigate the effect of oxygen on the language lateralization assessment.

Chapter Three

Study Two

Influence of inspired oxygen concentration on the assessment of language lateralization using transcranial Doppler sonography

3.1 Introduction

Evidence has been well documented on the hemispheric dominance of language processing. Majority of people are left dominant in language processing. There are many methods to assess the language lateralization include Wada test, PET, fMRI and TCD (Bacia et al., 1998; Benke et al., 2006; Benson et al., 1999; Knake et al., 2003; Lehericy et al., 2000; Mader et al., 2004; Rihs et al., 1999; Schulze-Bonhage et al., 2004; Woermann et al., 2003). TCD is superior to fMRI and PET in terms of its high temporal resolution and easy accessibility. The advantage and disadvantages of these modalities for language lateralization assessment have been discussed in Section 1.2 and 1.3.

For language lateralization assessment using TCD, the neural activity is not directly measured. Relative change of BFV is measured to reflect the neural activity. Therefore the findings are susceptible to the influence of non-neural changes in the body such as oxygen and CO_2 concentration. In Study One of this project, the findings showed that

inhalation of 35% oxygen led to 7.8% BFV reduction. This finding is consistent with those in previous literature which showed reduction of blood flow / BFV when oxygen concentration increased (Demchenko et al., 2002; Hoffman et al., 2000; Johnston et al., 2003; Omae et al., 1998). Although the BFV reduction obtained in the present study (7.8%) was not as higher than that obtained by Omae (1998) (20% reduction) using 100% oxygen. The present study demonstrated the effect of relative small increase in oxygen concentration on the BFV and this situation can be commonly occurred in clinical practice.

As BFV is the parameter to reflect the neuronal activity of language processing in TCD assessment of language lateralization, such reduction of BFV may alter the lateralization index in such assessment. The findings were expected to increase the understanding of the oxygen effect on the language lateralization assessment. If the result of language lateralization assessment using TCD can be changed by increased oxygen concentration, other cerebral haemodynamic based technique for the assessment of language lateralization can also be influenced by increased oxygen concentration.

In clinical settings, application of oxygen is common for emergency, patients with difficulty in breathing and patients with poor lung function. Since the result of language lateralization assessment directly affects the pre-surgical planning, the effect of increased inspired oxygen concentration on the assessment of language lateralization was investigated using TCD in the present study.

3.2 Materials and Methods

3.2.1 Subjects

The present study recruited 50 healthy subjects aged 20 – 40 years. The number of male and female subjects was almost the same to exclude gender difference. All the subjects were native Chinese speakers and did not understand Korean. Furthermore, all the subjects were right handers. Subjects showing right dominant in language processing were excluded. The subjects were explained the whole procedures of the examination. Consent was obtained from each subject before any examination (Appendix 1). The subjects were asked to rest for 15 minutes to stabilize the haemodynamics. Subjects with any known or suspected central nervous system disease, stenosis or known cerebrovascular disease, cerebral metabolic disorders, known neurological disease, or a history of drug abuse were excluded (Heckmann et al., 1998; Kastrup et al., 1998b; Papadimitriou et al., 2003; Silvestrini et al., 2004). Moreover, subjects who were taking or had taken analgesic drugs 10 days prior to the examination (Silvestrini et al., 2004; Silvestrini et al., 1996a), or receiving any kind of chronic medication including vasoactive or psychoactive drugs (Papadimitriou et al., 2003) were also excluded. Smoking, taking or drinking any caffeine-containing food or drinks, and drinking any alcoholic drinks were prohibited 6 hours prior to the examinations. This is because all the nicotine, caffeine and alcohol may induce blood flow changes (Cameron et al., 1990; Papadimitriou et al., 2003; Terborg et al., 2002).

The study was divided into two parts: ultrasound carotid examination and TCD examination. The examinations were carried out in a quiet room with the subjects lying for carotid examination and sitting in a comfortable position for TCD examination. In order to keep the environmental conditions constant, all the examination records were taken in the same room.

3.2.2 Ultrasound carotid examination

All the subjects were screened by B-mode and Doppler ultrasound carotid scan using the HDI 5000 System (Philips Medical Systems Company, USA) in conjunction with a 5 – 12 MHz transducer. Gray scale B-mode, color and pulsed-wave Doppler ultrasound scan was performed in common carotid arteries, internal carotid arteries, external carotid arteries and vertebral arteries bilaterally for each subject before the examination in order to exclude the presence of carotid and vertebral arteries steno-occlusive disease, narrowing or intimal thickening (Sanders, 1998).

3.2.3 Transcranial Doppler sonography examination

TCD examination was carried out with the subjects in sitting position, using Multi-DopX4 transcranial Doppler (TCD) system (DWL Elektronische Systeme GmbH, Germany).

Dual 2-MHz pulse Doppler transducers were fixed on the temporal region bilaterally with an elastic head band, which provided fixation, adjustment of insonation site and angle (Figure 2-1). Temporal region located above the zygomatic arch was chosen as a window of insonation. The M1 portion of middle cerebral arteries (MCA) was scanned at the depth of 50-58 mm from the skin surface (Papadimitriou et al., 2003; Sanders, 1998). The transducer was manipulated slightly forward and upward to identify the MCA. Angle of insonation was adjusted until the maximal value of the pulsative signal with minimal noise was obtained. The flow direction in MCA was towards the transducer. Subjects were instructed to keep still in order to reduce the motional artifacts and brain responses at the motor areas. Simultaneously blood flow velocity in both left and right MCA was acquired.

Subjects were instructed to look at the screen where the visual language stimuli were presented. While the subjects were performing the language task, they took normal depth of breathing with room air and 35% oxygen at the flow rate of 5 liters per minutes respectively in separate runs.

3.2.4 Language task paradigm

The language task composed of epochs for each of the three conditions: Chinese synonyms and non-synonyms, identical and non-identical Korean characters, and crosshair. The task paradigm was shown in Figure 3-1. The software E-PRIME (Psychology software Tools, Inc. Pittsburgh, USA) was used for the presentation of the

language task and time markers were sent to synchronize the acquisition of BFV on the TCD system. When a pair of Chinese characters was presented, subjects were asked to recognize whether the Chinese characters were synonyms or not (Figure 3-2). When the Korean characters were presented, they were asked to recognize whether the Korean characters were identical or not (Figure 3-3).

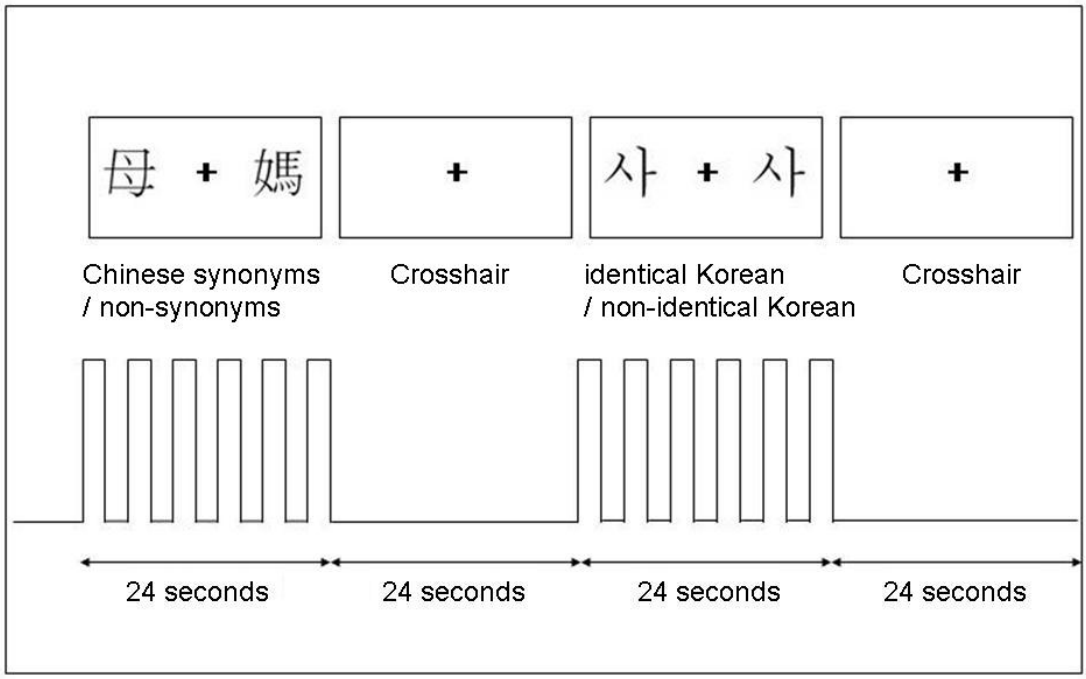

Figure 3-1. Language task paradigm consisting of Chinese synonyms and non-synonyms, identical and non-identical Korean characters, and crosshair. Each epoch lasted 24 seconds and was repeated four times in the paradigm..

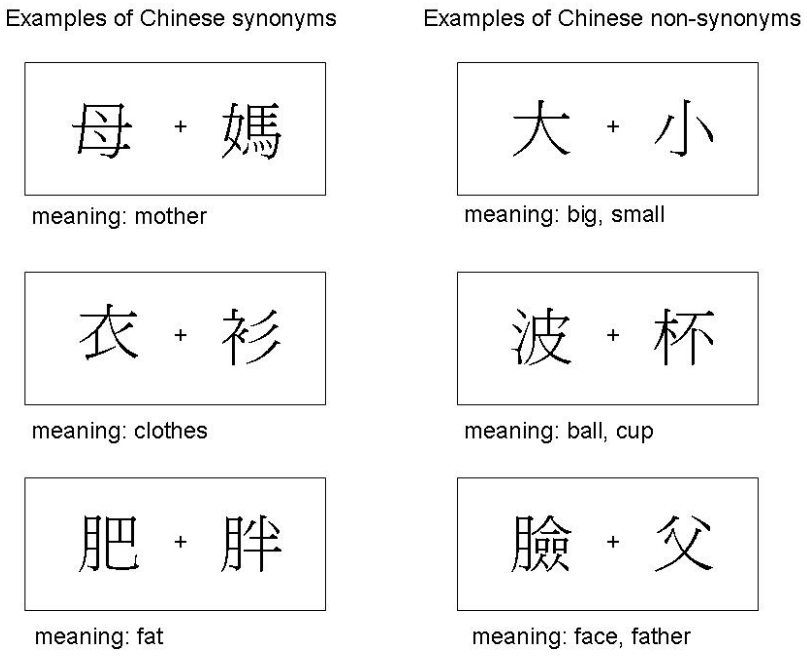

Figure 3-2 Examples of Chinese synonyms and non-synonyms.

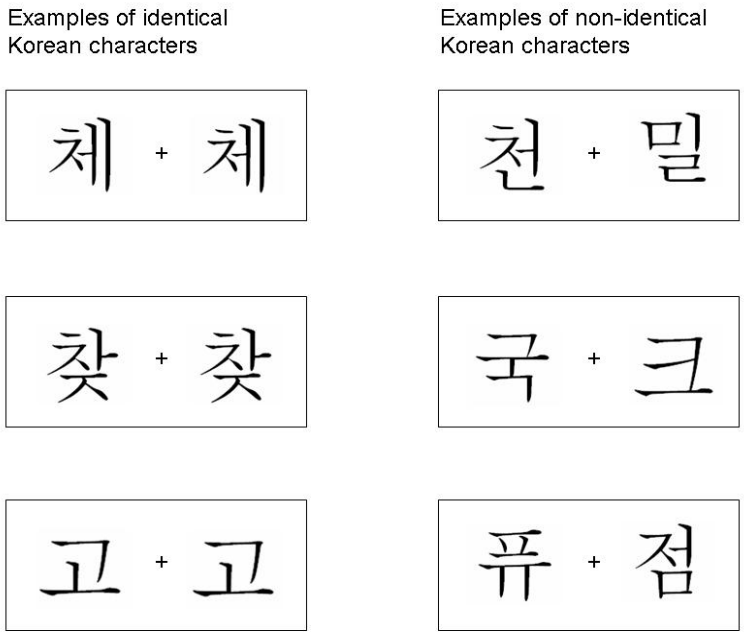

Figure 3-3 Examples of identical Korean and non-identical Korean characters.

During the TCD examination, subjects were asked to focus on the crosshair between two characters to reduce the oculomotor activation. When the crosshair was presented only, subjects were asked to focus on it. The BFV in bilateral MCA were measured while the subjects were performing language task in two conditions: 35% oxygen and room air inhalation. During the oxygen inhalation, normal depth of breathing with the administration of 35% of oxygen at a flow rate of 5 liters per minute through a face mask was performed by the subjects. During the room air inhalation, normal depth of breathing of room air was performed by the subjects. Each condition was repeated two times on each subject to increase signal - noise ratio by averaging (Figure 3.1). A total of four data sets were acquired.

3.2.5 Data analysis

Same as Study 1, BFV in the left and right MCA with time markers were analyzed by the software AVERAGE (Deppe et al., 1997). The analysis procedures included standardization of the sample rate, modification of time marker, heart rate calculation, respiratory component filtering, heart cycle integration, velocity normalization and baseline correction. Relative changes of normalized BFV were obtained. For the dataset acquired in the condition of room air inhalation, the epochs of Chinese synonyms/non-synonyms, and the identical/non-identical Korean characters were averaged separately, and then compared. For the dataset acquired in the condition

of 35% oxygen inhalation, the epochs of Chinese synonyms/non-synonyms, and the identical/non-identical Korean characters were also averaged separately, and then compared. Paired t-test was used to compare the left and right BFV changes; and also to compare the BFV change in Chinese synonym/non-synonym and identical/non-identical Korean tasks. Any comparisons with p values smaller than 0.05 were considered to be significant different.

3.3 Results

A total of 50 normal subjects (25 males, 25 females) aged 20 – 38 years (mean = 23.86 +/-3.45) were included in the present study. Three subjects were excluded. Two subjects had insufficient penetration of ultrasound beam through temporal bones and the remaining one had noisy TCD signals. As all the subjects were found to be left dominant for language processing, no subject was excluded due to right dominance for language processing. Demographic information such as blood pressure (BP) and pulse rate of all the subjects was shown in Table 3-1. As the assessment of language lateralization included repeated measurement, reliability test was done to ensure the variation of the measured value was not mainly due to technical error. Intra-class correlation coefficient (ICC) test was used (Appendix 3). The results showed high reliability in repeated measurement in room air inhalation, and moderate to high reliability in repeated measurement in room air inhalation, implying that the repeated measurements by TCD in the present study are reliable.

Subject No.	Sex	Age (years)	Left BP (mmHg)	Right BP (mmHg)	Pulse Rate (beats per minute)
1	M	38	131/81	128/82	77
2	F	22	117/76	116/76	70
3	F	28	98/62	102/67	70
4	F	21	93/54	92/57	75
5	F	24	97/53	96/53	65
6	M	27	104/68	119/71	70
7	M	25	97/59	96/59	60
8	F	26	103/71	102/71	70
9	F	25	97/60	96/60	70
10	F	20	119/83	122/84	86
11	F	19	95/59	99/61	70
12	M	21	116/71	116/68	80
13	M	20	107/69	107/73	70
14	F	21	109/72	109/68	75
15	M	21	103/65	110/71	75
16	M	21	100/62	100/63	62
17	F	22	98/58	109/64	77
18	M	20	99/57	103/58	70
19	M	24	106/67	106/68	75

20	M	20	103/64	102/65	65
21	M	24	99/62	97/60	60
22	M	24	103/63	109/63	75
23	M	21	117/80	119/81	65
24	M	21	90/57	97/60	70
25	F	26	104/63	104/64	75
26	M	21	113/72	109/75	75
27	M	21	112/77	112/78	70
28	M	26	110/76	116/74	62
29	F	22	105/66	105/66	72
30	F	23	100/60	105/63	74
31	F	22	107/66	105/66	70
32	F	25	110/72	112/75	70
33	M	26	100/61	100/60	64
34	F	23	106/62	109/60	76
35	F	24	100/62	100/65	77
36	F	24	99/59	98/59	66
37	M	30	99/75	97/70	61
38	M	23	96/60	98/60	68
39	M	23	100/60	101/60	65
40	F	26	109/66	109/66	68

41	F	32	105/62	108/65	75
42	M	25	100/62	100/63	61
43	M	24	103/66	100/63	65
44	F	22	96/58	99/60	78
45	F	22	114/73	115/72	72
46	M	30	110/72	110/70	75
47	M	25	113/70	113/65	70
48	M	23	97/60	99/60	68
49	F	24	102/60	103/60	70`
50	F	26	107/65	107/67	72

Table 3-1. The demographic data of all subjects in Study Two.

3.3.1 Change of blood flow velocity in bilateral middle cerebral arteries while performing language task in room air condition

In the condition of room air inhalation, when the subjects were recognizing Chinese synonym, the mean percentage change of BFV was 3.58% in the left MCA, and 2.01% in the right MCA. There was significant difference of BFV between left and right MCA in recognizing Chinese synonym ($p < 0.001$), indicating a significant left side lateralization during visual language task. (Table 3-2)

When the subjects were identifying Korean characters, the mean percentage change of BFV was 1.05 in left MCA, and 1.10 in right MCA. There was no significant difference of BFV between left and right MCA in identifying Korean characters ($p = 0.63$) (Table 3-2).

The mean lateralization index in the condition of room air inhalation was 1.62 +/-0.45.

	Room air inhalation				
	Chinese synonyms		Korean characters		Lateralization
	Mean % change in left BFV	Mean % change in right BFV	Mean % change in left BFV	Mean % change in right BFV	Lateralization index
mean	3.58	2.01	1.05	1.10	1.61
SD	1.56	1.55	2.64	2.63	0.45
Pair t-test	p = 0.00016 *		p = 0.63		

* Significant difference of BFV change between left and right MCA

Table 3-2 Comparison of the BFV in left and right MCA with the stimuli of Chinese synonym/non-synonym and the stimuli of identical/non-identical Korean characters, also the laterality, in the condition of room air inhalation.

3.3.2 Change of blood flow velocity in bilateral middle cerebral arteries while performing language task in an increased oxygen condition

In the condition of 35% oxygen inhalation, when the subject were recognizing Chinese synonyms, the mean percentage change of BFV was 0.89% in the left MCA, and 0.56% in the right MCA. There was no significant difference of BFV between left and right MCA in recognizing of Chinese synonyms ($p = 0.057$), indicating that there was no hemispheric lateralization of language processing (Table 3-3). When the subjects were identifying Korean characters, the mean percentage change of BFV was 0.43 in the left MCA, and 0.56 in the right MCA. There was no significant difference of BFV between left and right MCA in identifying Korean characters ($p = 0.20$) (Table 3-3).

The mean lateralization index in the condition of 35% oxygen inhalation was 0.56 +/- 0.32.

	35% oxygen inhalation				
	Chinese synonyms		Korean characters		Lateralization
	Mean % change in left BFV	Mean % change in right BFV	Mean % change in left BFV	Mean % change in right BFV	Lateralization index
mean	0.88	0.56	0.43	0.56	0.56
SD	2.67	2.86	2.45	2.13	0.32
Pair t-test	p = 0.057		p = 0.20		

* Significant difference of BFV change between left and right MCA

Table 3-3. Comparison of the BFV in left and right MCA with the stimuli of Chinese synonym/non-synonym and the stimuli of identical/non-identical Korean characters, also the lateralization, in the condition of 35% oxygen inhalation.

3.3.3 The changing pattern of lateralization index in room air and increased oxygen concentration

Similar shape of changing pattern of lateralization index was obtained in 35% oxygen inhalation and room air inhalation. However, lateralization index was low in the condition of breathing 35% oxygen relative to the condition of breathing room air (Figure 3-4).

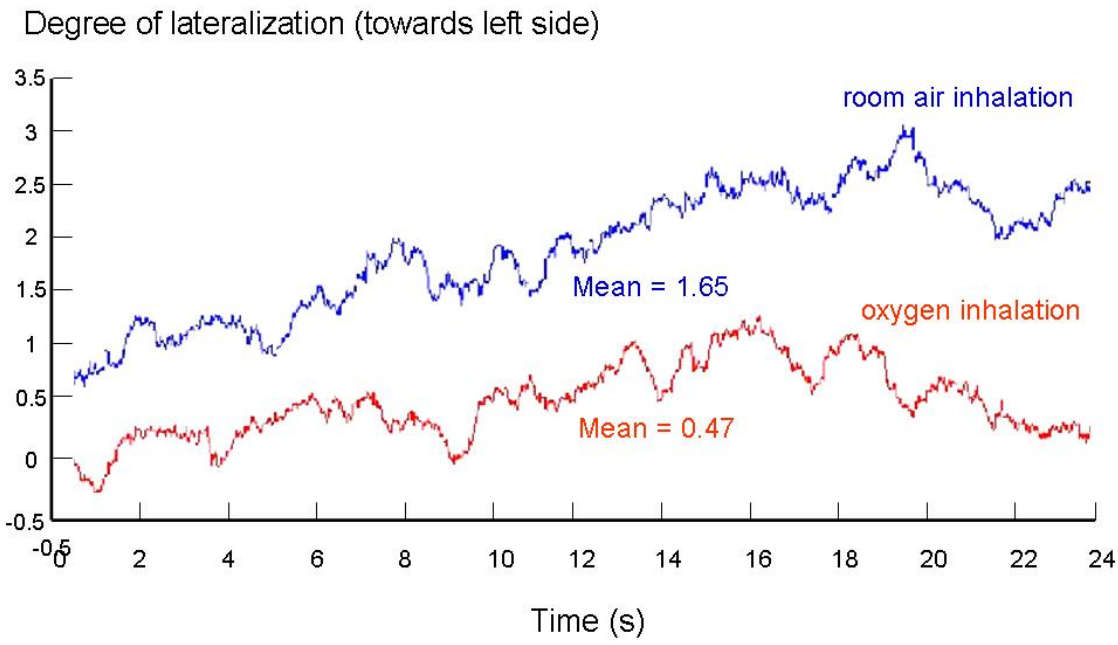

Figure 3-4. The changing pattern of the lateralization index when the subjects were recognizing Chinese synonyms (24 seconds) in room air inhalation (blue line) and 35% oxygen inhalation (red line).

3.4 Discussion

Language lateralization was determined by measuring BFV in bilateral MCA while the subjects were performing language task. Based on the findings in Study One, the lateralization index for language is likely to be altered by increased inspired oxygen concentration. In the present study, the influence of inspired oxygen concentration on the assessment of language lateralization was investigated. The findings are expected to increase the understanding of the effect of oxygen on language lateralization assessment. It also alerts the clinicians in interpretating the lateralization index of cerebral haemodynamic based assessment.

3.4.1 Effect of language stimuli on the cerebral blood flow velocity

In the present study, Chinese synonyms/non-synonyms and identical/non-identical Korean characters were used in the language lateralization assessment. Recognizing Chinese synonym was the activation phase. The identification of identical/non-identical Korean characters was used as a control.

When the subjects were recognizing Chinese synonyms, increase of BFV in left (3.58%) and right (2.01%) MCA was obtained in room air inhalation (Table 3-2). This implies brain activation of language processing was caused by recognizing Chinese synonyms. The language activation increased the oxygen demand of the neurons. The BFV was increased to increase the blood supply to the activated areas. The increase of

BFV in the left MCA (3.58%) was higher than that in the right MCA (2.01). This implies that the language function was dominant to the left for the right handers in the present study. This is consistent with the findings in the previous fMRI and fTCD studies (Sections 1.2.3 and 1.3.1).

In the present study, identifying Korean characters serves as a control for the comparison of brain activation by recognizing Chinese synonyms. No significant difference of BFV increase was found between the left and the right MCA in the condition of breathing room air. Based on the previous fMRI studies, only visual area was activated when a native Chinese speaker was reading Korean characters.

3.4.2 Effect of oxygen on language lateralization

A left language lateralization was found when subjects breathing room air. When 35% oxygen was inhaled by the same group of subjects, the BFV change in the left and the right MCA was reduced relatively to the condition of breathing room air. The difference of left (0.87% +/- 2.67%) and right (0.56% +/- 2.86%) BFV was not significant (p= 0.057). The lateralization index was reduced to 0.56, comparing with 1.62 in room air condition. It implies that the vasoconstrictive effect of oxygen causes BFV reduction. Vasoconstriction caused by oxygen reduced the arterial diameter that reduced the BFV increase in language processing. Therefore, the differences of BFV increase in the left and right MCA become less significant in 35% oxygen. In general,

the lateralization index was low in the condition of breathing 35% oxygen relative to the condition of breathing room air (Figure 3-4).

3.4.3 Clinical significance

The present study was likely to be the first of measuring the language lateralization during the condition of increased oxygen concentration in human subjects. In the condition of 35% oxygen inhalation, the result of language lateralization assessment using TCD was altered. The result of other cerebral haemodynamic sensitive techniques such as fMRI and PET can be affected by increased inspired oxygen concentration. The present study alerts the clinicians in interpretating the lateralization index derived by BFV change in cerebral vasculature. Caution is required when oxygen is administered to the patients during the language lateralization assessment by cerebral haemodynamic sensitive techniques. As TCD is not able to perform localization of brain activation due to language processing, only left-right dominance determination can be performed. Further study using fMRI or PET in assessment of language lateralization during oxygen inhalation was suggested to localize the activated brain areas. More information of the effect of oxygen on functional brain mapping can be obtained.

3.4.4 Summary

The purpose of the present study was to investigate the effect of increased oxygen concentration on language lateralization. In summary, this study showed reduced lateralization index from 1.62 in room air inhalation to 0.56 in 35% oxygen inhalation. The findings increase the understanding of effect of oxygen on the language lateralization assessment using TCD. This alerts the clinicians in interpretating the lateralization index when oxygen is administered to the patient. As oxygen may change the result of language lateralization assessment using TCD, it may also change the result of other cerebral haemodynamic sensitive techniques such as fMRI and PET.

Conclusion

In the present project, two hypotheses were addressed. The first hypothesis is that there would be a continuous changing pattern of blood flow velocity in the middle cerebral arteries showing the vasoconstrictive effect of oxygen. The second is that the vasoconstrictive effect of oxygen would constraint the blood flow velocity induced by language stimuli, and therefore the language lateralization result would be affected by increased inspired oxygen concentration.

The basis of the two hypotheses is the vasoconstrictive effect of oxygen causing reduction of BFV. As complex processes are involved in vascular smooth muscle contraction, it suggests that the vasoconstriction does not happen immediately after the increase of inspired oxygen concentration. There is little information on the changing pattern of cerebral blood flow velocity during oxygen inhalation. In the previous studies, single measurement was performed in transcranial Doppler sonography examination and

the changing pattern of blood flow velocity was ignored. The present study aims to find more details of the changing pattern of the cerebral blood flow velocity during 35% oxygen inhalation taking the advantage of the high temporal resolution of transcranial Doppler sonography technique. Latent period of cerebral haemodynamic response to increased oxygen concentration was obtained in the continuous measurement of BFV in the left and right MCA. Before the vasoconstrictive effect of oxygen was reflected by reduction of BFV, there was a small increase of BFV suggesting that the effect of oxygen on MCA is faster in the distal portion than in the proximal portion. The changing pattern of BFV in oxygen inhalation is not always decreasing. These findings increase the understanding of the effect of oxygen on cerebral haemodynamics. It provides a reference of using oxygen in the assessment of cerebrovascular reactivity. At the same time, owing to the vasoconstrictive effect of oxygen, the result of language lateralization assessment is susceptible to increased inspired oxygen concentration. The present study aims to investigate the effect of oxygen on the lateralization index for language derived from the change of blood flow velocity. The findings show that the lateralization index was altered during the inhalation of 35% oxygen. The lateralization index changed from 1.61 during room air inhalation to 0.56 during 35% oxygen inhalation. This increases the understanding of oxygen effect on language lateralization assessment and alerts the clinicians in interpretating the lateralization index when oxygen is administered to the patient. Since the determination of hemispheric language lateralization using TCD depends on the blood flow velocity changes, the effect of oxygen on such assessment using any techniques involving the measurement of blood flow velocity should not be

ignored. As oxygen may change the result of language lateralization assessment using TCD, it may also change the result of other cerebral BFV sensitive techniques.

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Appendix 1

Research Consent Form

Protocol Title: Influence of Inspired Oxygen Concentration on the Assessment of Language Lateralization Using Transcranial Doppler Sonography

Chief Supervisor: Suk-tak Chan, Ph.D.

Student Investigator: Pok-ning Wong, B.Sc.

Co-investigator(s)/Study Staff: Kenneth K. Kwong, Ph.D.

Description of Subject Population: Right-handedness native Cantonese speakers

PURPOSE

We would like permission to enroll you as a participant in this research study. The purpose of this study is to determine the vascular reactivity in middle cerebral arteries by varying the concentration of inspired oxygen in right-handed native Chinese speakers. Brain activity response to language task can be detected. We believe that the findings will increase the understanding of the effect of oxygen to measurement of language lateralization. You are being asked to participate as a research subject because you are native Chinese speaker.

STUDY CONTACTS

The investigator in charge of this study is Dr. Suk-tak Chan who can be reached at 2766 6095. You are advised to contact Mr. Pok-ning Wong at 2766 4500 for the details of the study.

PROCEDURES

This study will require your participation in one transcranial Doppler sonography (TCD) examination and one carotid ultrasound sessions, and each session will last for approximately one hour. Both sessions will be performed at the Radiography Clinic, The Hong Kong Polytechnic University.

During the TCD examination, you will sit on a chair and small amount of gel will be applied to a small area of your head (just anterior to the ears). Two ultrasound probes will be fixed on an elastic headband and placed respectively on the left and the right temporal windows of your head. Cerebral blood flow velocity (BFV) will be monitored continuously and simultaneously. You will be asked to keep stable when you are scanned by ultrasound. During the scanning procedures, you will be asked to inhale

oxygen and/or perform a simple visual language task. A practicing session will be given to you before the scanning.

For the carotid ultrasound session, you will lie down on a bed and facing upward. A small amount of gel will be applied on the skin of your neck. You will be asked to keep stable when you are scanned by ultrasound. The blood vessels supplying the head on both left and right sides of your neck will be checked for any narrowing using a ultrasound machine

All the procedures do not require the administration of contrast agent or any other drugs.

COSTS
This is a purely research study with no direct costs to you.

RISKS AND DISCOMFORTS
Ultrasound carries no radiation hazard and there will be no discomforts during the procedures. Diagnostic ultrasound has been in use for over 30 years. The benefits and efficacy for medical diagnosis, including the use during human pregnancy, have also been recognized. There is no evidence of adverse effects produced by diagnostic ultrasound in humans, which was stated by the American Institute of Ultrasound in Medicine (AIUM) in October 1982.[1]

REFERRAL FOR INCIDENTAL FINDINGS
In the event of an additional abnormality being identified on the ultrasound images, measures will be taken to refer you to an appropriate clinician for further investigation.

BENEFITS
You will not receive direct benefits from the present study. However, the present study is expected to advance the knowledge in language lateralization.

CONFIDENTIALITY
Any information and data produced by this study will not be given to anyone other than the research team of the present project in a form that could identify you without your written consent. It is possible that your medical and research record, including sensitive information and/or identifying information, may be inspected by the Research Committees in the Hong Kong Polytechnic University. If your record is inspected, your privacy and the confidentiality of your information will be protected with reasonable efforts.

[1] American Institute of Ultrasound in Medicine. Bioeffects and safety of diagnostic ultrasound. Maryland: American Institute of Ultrasound in Medicine, 1993.

REFUSAL OR WITHDRAWAL OF PARTICIPATION

You do not have to participate in this study. If you decide to participate, you can change your mind and withdraw from the study at any time without affecting your present or future care in the Radiography Clinic of the Hong Kong Polytechnic University.

This study was approved by the Human Subjects Ethics Sub-Committee of the Department of Health Technology and Informatics, The Hong Kong Polytechnic University. However, if you think there are any procedures that seem to violate your welfare, you may explain in writing to professor Iris Benzie, Chair of the Departmental Research Committee, Department of Health Technology and Informatics, The Hong Kong Polytechnic University.

SIGNATURE

I confirm that the purpose of the research, the study procedures and the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Procedures of withdrawing my participation in the study have also been discussed. All my questions have been answered. I have read this consent form. My signature below indicates my willingness to participate in this study.

_____ _____

Subject/Patient Date

I have explained the purpose of the research, the study procedures, identifying those that are investigational, the possible risks and discomforts as well as potential benefits and have answered any questions regarding the study to the best of my ability.

_____ _____

Study Representative Date

研究同意書

擬訂主題：氧氣對多普勒超聲波語言功能測試的影響

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副研究員/其他研究員：鄺健民博士

研究目標：以廣東話爲母語並慣用右手的成年人

目的

我們須得到你的允許參與是項研究，本研究目的是透過腦血管多普勒超聲波機能測試腦血管對氧濃度改變的反應，論証腦部於不同氧濃度下的語言使向性。對象爲以廣東話爲母語並慣用右手的成年人。

研究聯絡人

本研究由陳淑德博士負責，聯絡電話爲 2766 6095，你亦可致電 2766 4500 聯絡學生研究員黃博寧先生有關研究的詳情。

研究程序

本研究須得到你於一次腦血管多普勒超聲波機能測試的參與和協助，需時約一小時。研究會在香港理工大學放射診斷診所進行。

腦血管多普勒超聲波機能測試：你會坐於實驗用的電腦前，然後研究員會在你的頭顱兩側近太陽穴位置繫著兩個超聲波探測器用以探測腦血管血流量的情況。在掃描過程中，你必須保持穩定，接受一個簡單的視覺及聽覺功能測試。全部程序不需注射任何顯影劑或藥物。

頸動脈超聲波素描：用以素描出你的頸動脈，以確保當中沒有收窄的情況出現。如一般超聲波素描，在過程當中你需要以仰臥的姿勢臥在床上，我們會在你的頸部塗上一層素描用的水溶性膠狀物質，用以素描你頸部兩邊動脈。

費用

由於這個是科學研究，所以你毋須付上任何費用。

研究風險和不安的感覺

超聲波並不是有害輻射，這項研究不會對你做成危險及不適。診斷用的超聲波已被使用逾三十年，其對醫學診斷的好處和效用，包括在懷孕期使用亦被承認。在許多研究中，沒有找到診斷用的超聲波對人類生理影響的証據。在一九八二年十月，美

國醫學超聲波學會(AIUM)通過一項臨床使用診斷用的超聲波作訓練、研究和診斷的安全報告。[2]

偶爾性發現的轉介

假若在超聲波影像中發現異常的情況，你將被轉介到適當的醫生作詳細檢查。

利益

在這項研究中，你不會得到任何直接的利益。

資料保密

任何資料包括個人資料是不會未經你的書面同意，以可辨認你身分的形式，交予本研究無關的人士，你的病歷或研究記錄包括一些敏感性的資料和身分資料，有可能會被香港理工大學的研究委員會查閱，如你的記錄須被查閱，你的私隱和個人資料會盡量以合理方法保護。

拒絕和退出參與是項研究

你不一定需要參與是項研究，如你決定參與，你可隨時改變決定和退出是項研究，這樣不會影響你現在或將來在香港理工大學眼科視光學診所所接受的護理。

本研究是得到香港理工大學醫療科技及資訊學系道德倫理委員會批准，如你覺得任何程序侵犯你的個人權益，你可以書面與香港理工大學醫療科技及資訊學系研究委員會主席 professor Iris Benzie 釋述。

簽署

本人確定是項研究的目的、程序、可能性風險、不安的感覺、以及潛在的利益，已向本人清楚解釋，退出參與是項研究的程序亦已討論，所有問題已被回答，我已詳細閱讀這同意書，本人下方的簽署表示本人願意參與這項研究。

_____

參與者病人　　日期

本人已解釋是項研究的目的、程序、可能性風險、不安的感覺、以及潛在的權益和回答所有問題。

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研究代表　　日期

[2] American Institute of Ultrasound in Medicine. Bioeffects and safety of diagnostic ultrasound. Maryland: American Institute of Ultrasound in Medicine, 1993.

Appendix 2
Information Sheet for the subjects

The Hong Kong Polytechnic University
Department of Health Technology and Informatics

Information Sheet

Project Title: Influence of Inspired Oxygen Concentration on the Assessment of Language Lateralization Using Transcranial Doppler Sonography

I. Personal Particulars:
Name: _____ Gender: _____ Age: _____
Education level: _____ Nationality: _____

II. Background Information:
Do you know Korean? YES / NO
Do you have any known or suspected central nervous system disease? YES / NO
Do you have any known or suspected psychiatric symptoms? YES / NO
Do you have any known or suspected auditory symptoms? YES / NO

III. Handedness Assessment:
Please indicate your preferences in the use of hands in the following activities by putting + in the appropriate column. Where the preferences is so strong that you would never try to use the other hand unless absolutely forced to, put ++. If in any case you are really indifferent put + in both columns.

Items		LEFT	RIGHT
1	Writing		
2	Drawing		
3	Throwing		
4	Scissors		
5	Toothbrush		
6	Chopsticks		
7	Spoon		
8	Broom (Upper Hand)		
9	Striking Match		
10	Opening Lid		

Laterality Quotient (LQ) = _____

Appendix 3

Reliability test for the repeated measurement of the hemispheric lateralization of language processing using Intra-class correlation coefficient test

Introduction

Reliability of repeated runs is very important to ensure that the variation of the measured value is not due to technical error. Reliability test was done to separate out possible intra-operator variation from the influence of the variables of interest: (1) lateralization of BFV due to language processing, (2) the effect of change in oxygen concentration. Twenty-five male and twenty five female subjects aged from 20 to 38 years were included in the present study. Two subjects were excluded. One subject had insufficient penetration of ultrasound beam through temporal bones and another had noisy TCD signals. None of the subjects had a history of central nervous system disease, stenosis or known cerebrovascular disease, cerebral metabolic disorders, known neurological disease, or a history of drug abuse. None of the subjects had been smoking, taking or drinking any caffeine-containing food or drinks, and drinking any alcoholic drinks 6 hours prior to the examinations. No gross abnormalities were found such as carotid and vertebral arteries steno-occlusive disease, narrowing or intimal thickening in the B-mode ultrasound screening. The blood pressure for all of the subjects was within

the normal range, with systolic pressure not greater than 140 mmHg and diastolic pressure not greater than 90 mmHg.

For each subject, two measurements were taken of the MCA on both the right and left sides. Subjects were instructed to look at the screen where the visual language stimuli were presented. While the subjects were performing the language task, they took normal depth of breathing with room air and 35% oxygen at the flow rate of 5 liters per minutes respectively in separate runs. All of the BFV changes were measured by the same TCD system, Multi-DopX4 transcranial Doppler system (DWL Elektronische Systeme GmbH, Germany). The procedure of measuring BFV change was described in Chapter 3.

For each subject, four values of lateralization index due to language processing was calculated which included two measurements in room air inhalation and two measurements in oxygen inhalation. Intra-class correlation coefficient (ICC) test was done on these two pairs of lateralization index to assess the reliability of repeated measurement in same oxygen concentration. The ICC is a reliability index that is calculated using variance estimates obtained through an analysis of variance (Porteny and Watkins 1993). One-way analysis of variance (ANOVA) model was used for the ICC test. The reason is that the subject variance represents the only systemic source of variance (McGraw 1996). ICC model with repeated measures analysis of variance was used because the test raters in the present study, the operator of TCD were considered the only raters of interest. Therefore this model is appropriate for testing intrarater reliability in the present study (Porteny and Watkins 1993). The ICC ranges between 0.00 and 1.00. Values closer to 1.00 representing stronger reliability, with minimal error

variance. Porteny and Watkins (1993) suggested that ICC values above 0.75 indicate good reliability, and below 0.75 indicate moderato to poor reliability.

Results

The ICC of the measurement of the hemispheric lateralization due to language processing was studied for all 50 subjects aged from 20 to 38 years (mean, 23.86 years). The ICC values for average measures were shown in Table A3, which represent the reliability between the first and second measurement of lateralization index in room air inhalation and 35% oxygen inhalation respectively. For measurements in room air inhalation, ICC value of 0.765 ($p<0.001$) indicated good reliability between repeated measurements. ICC value of 0.707 ($p<0.001$) indicated moderate to good reliability between repeated measurements of BFV change for assessment of language lateralization.

Intraclass Correlation Coefficient

	Intraclass correlation coefficient	F value	p-value
Room air inhalation	0.765	4.215	<0.001
Oxygen inhalation	0.707	3.411	<0.001

Table A3: The ICC value for the first measurement vs the second measurement of lateralization index in room air inhalation and 35% oxygen inhalation.

Discussion

In the condition of room air inhalation, ICC value of 0.765 indicated good reliability between repeated measurements. Subject fatigue in the second measurement is suggested as a source of error. Decrease of attention when the subjects were looking at the visual language task is believed to reduce the brain activation due to language processing. Increasing the resting time between measurements can improve the problem of subject fatigue but the trade-off is to increase the total examination time.

In the condition of oxygen inhalation, ICC value of 0.707 indicated moderate to good reliability between repeated measurements. Although the reliability in oxygen inhalation was lower than that it room air inhalation, it still acceptable.

In summary, the result of the reliability test showed that TCD is a suitable and robust tool for longitudinal measurement of language lateralization in both the condition of oxygen inhalation and room air inhalation.