PREFRONTAL CORTEX ACTIVITY DURING RESTING AND TASK STATES AS MEASURED BY FUNCTIONAL NEAR-INFRARED SPECTROSCOPY

by

Ling-Yin Liang

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biomechanics and Movement Science

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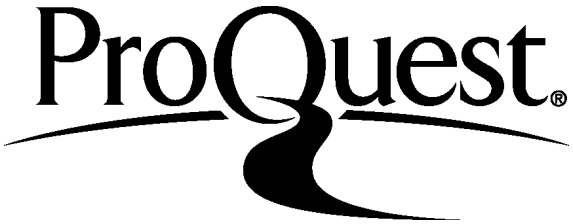

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LIST OF ABBREVIATION

Δoxy-Hb = Relative concentration changes of oxygenated hemoglobin

Δdeoxy-Hb = Relative concentration changes of deoxygenated hemoglobin

ASD = autism spectrum disorders

BOLD = blood oxygenation level-dependent

Deoxy-Hb = deoxygenated hemoglobin

DCD = developmental coordination disorder

DMN = default mode network

EF(s) = executive function(s)

fNIRS = functional near-infrared spectroscopy

Oxy-Hb = oxygenated hemoglobin

PFC = prefrontal cortex

TD = typically developed

Total-Hb = total hemoglobin

ABSTRACT

The prefrontal cortex (PFC) is involved in executive functions (EFs) and is important for high-order cognition. Executive functions have been examined by task-induced activation in the PFC. Within neuroimaging results of goal-oriented task study, a consistent task-induced deactivation has been observed in the PFC. The medial PFC shows spontaneous activity during resting states which contributes to the default mode network (DMN). The DMN activates during task-free resting states and deactivates by performing goal-oriented tasks. Functions of the DMN include monitoring environment and processing self-relevant information. The DMN also interacts with high-order cognitive functions and has influences on behavioral performance. The disruption of the DMN could be an underlying mechanism of neurodevelopmental disorders such as autism spectrum disorders (ASD). Structural and functional abnormalities in the PFC have been observed in ASD. In this dissertation, activity in the PFC during resting and task states was examined in children and adults. Measuring brain activity during resting states provides information about the baseline focal activation, which is critical during the interpretation of task-induced activity. The intrinsic property in the brain, regulated by the DMN, can be analyzed as well. In addition, activity in the PFC during an EF task was compared to a simple motor task and a resting state in adults to obtain the model of changes among tasks in a mature system. Then, changes of the PFC activity in children with ASD were compared to typically developed children. Study 1: The activation levels in the PFC during resting states between eyes open and eyes closed conditions were compared in order to

provide a rationale for proper baseline selection. A significant higher activation in eyes closed condition compared to eyes open condition suggested eyes open condition may be a better baseline condition for goal-oriented task study. Study 2: Regional differences of activation in the PFC were examined to investigate the relationship between the medial PFC and the lateral PFC. The effect of eyes condition was observed in the left and medial PFC. However, the study failed to identify regional differences in the PFC. Study 3: Activity in the PFC was measured during resting, a simple motor task, and an EF task in adults. A significant higher activity during the EF task was found compared to the resting state. Study 4: Activity in the PFC among 3 conditions was measured in children with ASD and age- and gender-match typically developed children. There was no significant difference in oxygenated hemoglobin and deoxygenated hemoglobin among conditions. There was no interaction of group and conditions on concentration of hemoglobins. However, children with ASD had poor performance of Tower of Hanoi indicated deficits in the PFC. Based on the finding in Study 1, eyes open condition was used as the baseline condition in Studies 3 and 4. When examined the effects of task conditions on the activation, all channels were averaged for comparison since there was no significant difference among regions in the PFC according to the results in Study 2. We found a significant effect of task condition on the activation and the involvement of the PFC during an EF task was verified in Study 3. Due to limited sample size and high variation in our participants, we did not observe a main effect of task condition on the PFC activation in children with and without ASD. Future study needs to focus on participants in specific age ranges in order to explore effects of maturation on EF functions and PFC activity.

Chapter 1

INTRODUCTION

The prefrontal cortex (PFC) is involved in executive functions (EF) which include planning, decision making, cognitive flexibility, and inhibition control, with working memory playing a critical role (Ozonoff & Strayer, 2001). Working memory is a temporary storage system that maintains task-relevant information during the performance of a cognitive task, and is considered the basis of complex cognition. Along with other EFs, the PFC is generally seen as the center of high-order cognitive functions (Barendse et al., 2013). Prefrontal cortex dysfunctions have been linked to various neurodevelopment disabilities such as autism spectrum disorders (ASD). Deficits in structural and functional development may lead to abnormal behaviors seen in individuals with ASD (Shalom, 2009).

While the development of the PFC peaks around age of 12, the maturation of the EF is a multistage process with different functions reach adult-like level at different ages (Anderson et al., 2001; Giedd et al., 1999). The development of EF has been examined using both behavioral and neuroimaging approaches. In neuroimaging studies, task-induced activation in the PFC is linked to EF-related tasks. However, the PFC is activated not only during goal-oriented tasks but also during resting states. Raichle and colleagues (2001) observed consistent task-induced deactivations in several regions in the brain including the medial PFC and identified a default mode network (DMN) in resting states. The magnitude of the DMN during resting states is important to interpretation of task-induced activity. Currently, most studies related to

the DMN have focused on functional connectivity examining the interactions among regions in the DMN. Thus, information related to the focal activation in the DMN is very limited.

The brain activation can be quantified by hemodynamic changes. Several instrumentations have been used to measure hemodynamic changes including electroencephalography (EEG), functional magnetic resonance imaging (fMRI), and functional near-infrared spectroscopy (fNIRS). Each of these instrumentations quantifies brain activity based on different physiological events triggered by neuronal activation. As a consequence, different activation patterns between eyes open and eyes closed conditions during resting states have been observed using different modalities. The representation of brain activity can easily have different interpretations by using different baseline conditions so it is critical to define a proper baseline condition for a specific modality.

Of the aforementioned modalities, fMRI is one of the most popular neuroimaging instrumentation. However, fMRI imaging is very expensive and highly susceptible to motion artifacts. Head movement less than 1mm is enough to compromise fMRI images. Thus, most of motor function research using fMRI adopts a simple motor task like finger tapping (Field et al., 2000). Moreover, loud and closed testing environment of fMRI scanning limits its feasibility in clinical research. Recently, more neuroimaging studies have been conducted using fNIRS. fNIRS is relatively low-cost and more tolerant to motion artifacts. fNIRS also provides a balance between spatial and temporal resolution compared to fMRI and EEG. The fNIRS is easy to apply so participants who are unfit for traditional neuroimaging environment such as children with ASD can perform tasks in a more natural setting.

The high tolerance to motion artifacts of fNIRS allows brain activation during larger movement such as daily activity to be examined. Based on current literature, there are some gaps need to be filled before we apply the fNIRS to study the PFC activations during different tasks.

1.1 Literature Review

The Prefrontal Cortex

The prefrontal cortex is one of three primary functional regions in the frontal lobe located at the anterior part of the frontal lobe in front of the motor and premotor cortices. The PFC involved in 'metacognitive' EFs which included decision making, working memory, inhibition, planning, problem solving, and cognitive flexibility, as well as 'emotional/motivational' EFs, which are responsible for emotion regulation and social reasoning through inhibitory control of behaviors (Ardila, 2008; Griebling et al., 2010; Miller & Wang, 2006; Riva et al., 2013). The PFC integrates primary sensorimotor processes and modulates higher-order cognitive functions. The disruption of development of this region has been suggested to be the underlying reason for the behavioral symptoms of childhood mental disorders such as ASD and developmental coordination disorder (DCD) (Amaral et al., 2008; Barendse et al., 2013; Carper & Courchesne, 2005; Courchesne et al., 2011; Debrabant et al., 2013; Girgis et al., 2007).

The PFC is usually seen as the center of cognitive functions and does not reach full maturity until early adulthood. The development of PFC peaks around age 12 with females reaching the peak earlier than males (Giedd et al., 1999; Gogtay et al., 2004; Sowell et al., 2001). In a longitudinal neuroimaging study, a linear increase in cortical white matter of the frontal lobe was identified when a nonlinear change was seen in

the gray matter in the frontal lobe with the developmental curve peaking at about age of 12. The gray matter in the frontal lobe continues to increase from early childhood to pre-adolescence and then declines during post-adolescence with a maximum size occurring at 12.1 years for males and 11.0 years for females (Giedd et al., 1999). A loss in cortical density related to increased synaptic pruning and myelination was also noticed during maturation (Sowell et al., 2004). It has been suggested that the regressive activity in gray matter (i.e., synaptic pruning) results in more effective synaptic transmission and therefore, enhanced functional performance (Cowan et al., 1984; Kharitonova et al., 2013; O'Donnell et al., 2005).

Structural changes in the PFC were examined with behavioral performance of EF functions. Development of EF is a multistage process that involves different skills emerging at different stages of life and maturation occurring at different rates. Development of EF starts as early as 6 months and continues to develop through early adulthood (Anderson et al., 2001). A study with participants ranging from 8 to 64 years found that cognitive flexibility is matured even in the youngest participant while working memory capacity, planning, and problem solving keep developing in teenager and young adults (De Luca et al., 2003). A preliminary cross-sectional data showed that the development of some EFs starting to plateau around 10 to 12 years old but other EFs showed linear increase from 6 years old to 18 years old (Waber et al., 2007). In addition, basic inhibitory functions develop earlier than more complex functions such as planning, selective attention, and self-regulation (Klenberg et al., 2001).

The PFC exhibits different activation patterns during different stages of maturation. Casey and colleagues (1997) examined the activation of the PFC during performance of an inhibition task and found that the location of activation in the

prefrontal cortex was the same between children and adults, with children having a greater volume of activation than adults. Immature brain systems in children may require more activated regions to process the task while adults can perform the task more efficiently due to an increase in neural selectivity. It is reasonable to suspect that younger children would have a more diffuse activation and older children will have a higher focal activation because individuals can control tasks more specifically and efficiently as they mature (Tamm et al., 2002).

The PFC plays an important role in cognitive functions and dysfunctions of the PFC may be an underlying mechanism of neurodevelopmental disorders such as ASD. The development of volume in the PFC peaks around age of 12 but the EF continues to develop into early adulthood. Behavioral performance was correlated to task-induced activation. The results showed that a more matured system can perform tasks in a more efficient manner.

The Default Mode Network

The term 'default mode network' was proposed by Raichle et al. (2001) when they located areas in the brain, including the medial PFC, that consistently deactivate during task-induced activities. Increased neural activity and increased blood flow in the brain is expected when performing goal-directed tasks. However, decreases in brain activity were also observed in comparing the activities in active conditions to passive conditions. The default mode network (DMN) is the spontaneous activity in the brain during task-free resting states. The brain accounts for 20% of the total oxygen consumption of the body during task-free resting state implying significant functionality in the resting state (Clark & Sokoloff, 1999). High amounts of oxygen consumption suggest the importance of brain activity during resting conditions. One

function of the DMN is to monitor the external environment and prepare for unexpected future events. The DMN supports a broad low-level focus of attention of the external world during resting state by continuously gathering information and reallocating attention to previously unattended input as needed (Gilbert et al., 2007; Gusnard & Raichle, 2001; Hahn et al., 2007; Hampson et al., 2006; Leech et al., 2011). In addition, the DMN contributes to self-generated thoughts and constructs the internal mentation. The representations of states of the self can then be used on inferences to others' mental states (Gusnard et al., 2001; Kelley et al., 2002; Mitchell et al., 2006). The DMN also interacts with high-order cognitive function and has influence on behavioral performance (Bonnelle et al., 2011; Duan et al., 2012).

The DMN was previously deemed to emerge in parallel with the development of specific cognitive functions (Gao et al., 2009, Smyser et al., 2010). Gao et al. (2009) acquired magnetic resonance (MR) images in healthy pediatric participants between 2 weeks and 2 years of age while they were in natural sleeping. The results showed that a primitive and incomplete DMN was present in 2-week-olds and the network became similar to that observed in adults by the age of two. However, Doria and colleagues (2011) believed that the DMN emerges with the neural growth before the normal time of birth at term, so they examined resting state network in preterm infants. The results showed that fragments of resting state networks were detectable at 30 weeks of postmenstrual age and complete networks were present by term (around 40 weeks of postmenstrual age). A similar result has been found in another study that local connectivity was detected in preterm babies and there is no significant difference between preterm infants and full term infants (Lee et al., 2013). Presence of the DMN has been detected throughout the developmental stages (Gordon et al., 2011; Power et

al., 2010; Zielinski et al., 2010). De Bie et al. (2011) detected immature DMN in 5 to 8 year-old children in task-free state using magnetic resonance imaging (MRI). Regions of the DMN identified in children are similar to adults including the PFC. However, regions involved in higher-order cognitive functions such as the medial PFC is in an immature state (Thomason et al., 2008). The DMN in adults is commonly represented in one single component. However, the DMN seems to be fragmented into several sparsely connected components in early school age children (Fair et al., 2008). In addition, the connectivity within the DMN is weaker in children compared to adults (Fair et al., 2008; Marsh et al., 2006; Supekar et al., 2010). Another feature of the DMN at young ages is that activation in a region tends to have strong correlations with nearby regions (Lee et al., 2013). As children develop into adolescence, correlations with nearby regions are weakened, but correlations with more distant regions are increased (Power et al., 2010).

There is plenty of evidence supporting the existence of the DMN at birth and throughout developmental stages. The DMN in children is weaker, but by adulthood, the DMN becomes more focalized. Most of the current studies have focused on the connectivity in the DMN, which investigate the interaction between different regions in the DMN. Studies relating to focal activation in the PFC during resting states in children are limited. While localized task-induced activity is usually compared to regional blood flow during resting states, it is important to examine the baseline focal activation in the DMN during resting states.

The Prefrontal Cortex and the Default Mode Network in ASD

The prefrontal cortex and EF are essential to complex cognition. Disruptions in the PFC and resultant issues with EF could be the underlying reasons for mental

disorders, including ASD, leading to abnormal behaviors (Carper & Courchesne, 2005; Courchesne et al., 2011). There are several lines of evidence that suggest the PFC may be functioning abnormally in individuals diagnosed with ASD. Previous studies show that individuals with lesions in the PFC had behavioral impairments that are similar to abnormal behaviors seen in individuals with ASD (Anderson, 1999). Poor judgment in decision-making related to personal realms and disturbances in social activity have been found in patients with PFC lesions (Bar-On et al. 2003). Ridley (1994) hypothesized that executive dysfunction renders individuals with ASD unable to plan and control their behaviors in the usual manner, resulting in restricted and repetitive behaviors.

The abnormality of PFC in ASD is supported by both structural and function neuroimaging results. Enlargement of the PFC in young children with ASD (age 2 to 5 years old) was found compared to control group (Carper & Courchesne, 2005; Carper et al., 2002). A global increase in gray matter was also observed in older children (age 7 to 15 years) (Palmen et al., 2005), as well as in teenagers and adults with ASD (Hazlett et al., 2006). Results of fMRI studies showed that adolescents and adults with ASD had a greater activity in the medial PFC when performing EF-related tasks including alphabet task, random generation task, and Theory of Mind tasks, (Gilbert et al., 2008; White et al., 2014). However, a lower activation in the PFC during an inhibition task was found in adults with ASD using near-infrared spectroscopy (NIRS) (Ishii-Takahashi et al., 2014).

Abnormal task-induced activation in the PFC was found in adolescents and adults with ASD (Gilbert et al., 2008; Ishii-Takahashi et al., 2014; White et al., 2014). Abnormalities in the DMN were also observed in individuals with ASD in different

age ranges. Functions of the DMN are related to EF and high-order cognition. It is also believed that the DMN serves in other functions as well. First, the DMN monitors the external environment and prepares for unexpected future events. In contrast to attention-demanded tasks that require focused attention, the DMN supports a broad low-level focus of attention of external world during resting state (Gilbert et al., 2007; Hahn et al., 2007). Children with ASD do not have a broad focus of attention that can help them identify the information coming from the environment. Instead, they are focus in their own world. Second, the DMN involves to self-referential thoughts and constructs the internal mentation. Activation of the medial PFC was found when individuals make self-relevance judgments (Kelley et al., 2002; Mitchell et al., 2006). Impairments in processing self-related information seen in ASD could be attributed to dysfunctional DMN.

Several neuroimaging studies have examined functional connectivity and the majority of the studies reported reduced connectivity in individuals with ASD. Cherkassky et al. (2006) analyzed resting-state network in 57 participants with ASD (mean age 24 ± 10.6 years) using data from fMRI studies. They found similar activation organization in ASD and control groups in multiple regions of interest but the functional connectivity of the resting network in ASD was lower than control group. Underconnectivity was also found between major regions of DMN, medial PFC and posterior cingulate cortex. Reduced connectivity was observed in participants with ASD (mean age 15.7 ± 3.0 years) and symptom severity can be predicted by the level of connectivity (Assaf et al., 2010). The results found that increased symptom severity was associated with decreased connectivity. Another research found poor social

functioning was correlated to weak connectivity between the posterior cingulate cortex and the superior frontal gyrus (Monk et al., 2009).

Most of the DMN studies found reduced functional connectivity in individuals with ASD. Even though the findings are consistent, there are some limitations in application. First, ages of participants were all older than 12 years old. In typically developed children, the volume of the PFC peaks at age of 12 with a part of EF maturing before that (Waber et al., 2007). The developmental trend in the DMN is not clear without information from children younger than 12 years old. Second, previous studies in the DMN all focused on the functional connectivity investigating interaction between regions. It would be more meaningful to measure focal activation during task-induced activation. Currently, no information is available regarding to baseline focal activation in children with ASD.

Physiological Changes during Brain Activation

Neuroimaging modalities measure brain activity by detecting different physiological events triggered by neuronal activation. Electroencephalography (EEG) and magnetoencephalography (MEG) detect magnetic field changes induced by post-synaptic ionic current, while magnetic resonance imaging (MRI) and fNIRS assess hemodynamic changes related to energy usage and oxygen consumption (Riera et al., 2008; Sizonenko et al., 2013). Brain activity requires glucose and oxygen consumption. Increased neural activity exhibits an initial deoxygenation phase, which is attributed to the rapid increase in local deoxygenated hemoglobin (deoxy-Hb) (Hu et al., 1997). The reduction in glucose and oxygenation results in increased cerebral blood flow. The changes in cerebral blood flow evolve over a period of 10 to 12 seconds, carrying more glucose and oxygen to the area. However, the increased

oxygen supply usually exceeds oxygen utilization, results in increased blood flow oxygenation in active areas (Fox et al., 1988; Malonek & Grinvald, 1996; Obrig & Villringer, 2003). Thus, an increase in total hemoglobin (total-Hb) and oxygenated hemoglobin (oxy-Hb) with a decrease in deoxygenated hemoglobin (deoxy-Hb) are expected to be observed in active area in brain.

fNIRS and the Quantification of Brain Activity

fNIRS detects hemodynamic changes induced by brain activity based on optical properties of oxy-Hb and deoxy-Hb. Biological tissues are relative transparent to light in the near-infrared range between 700 to 900 nm because the absorbance of main components of human tissue are relatively small within this wavelength (Jobsis, 1977). Fortunately, the absorbance of oxy-Hb and deoxy-Hb are significantly different within this range (Rolfe, 2000). Oxy-Hb and deoxy-Hb absorbing different amounts of photons in different wavelengths allows the separation of these two compounds to be possible using a few sample wavelengths (Horecker, 1943). Photons introduced into the head are either absorbed or scattered. A photodetector placed a certain distance away collect the scattered photons that are not absorbed and thus follow a banana-shaped path back to the surface of the scalp. The concentration of oxy-Hb and deoxy-Hb can be obtained from the changes of optical density at two wavelengths using the modified Beer-Lambert Law (Bunce et al., 2006; Izzetoglu et al., 2007; Villringer & Chance, 1997).

fNIRS is a noninvasive neuroimaging technique. It is relatively inexpensive and can be more feasible than fMRI and EEG in clinical studies. Although EEG has great temporal resolution, EEG signals are easily contaminated by head movements (O'Regan et al., 2012; Uchida et al., 1996). fMRI also provides excellent spatial

resolution but the accuracy of fMRI results is significantly affected by movement (Im et al., 2007; Kellman et al., 2009; Li et al., 2011). fNIRS provides a balance between temporal and spatial resolution for monitoring local changes of oxygenation in the cerebral cortex (Table 1.1). The temporal resolution of fNIRS can be up to hundreds of hertz which is better than the temporal resolution of fMRI that is limited at a minimum of one hertz (Weishaupt et al., 2008). fNIRS can also reach a better spatial resolution of one centimeter than standard EEG that show a low spatial resolution of 5 to 9 cm (Babiloni et al., 2009; Chance et al., 1998; Dieler et al., 2012; Ferree et al., 2001). Moreover, fNIRS allows more motion artifacts with various methods that can be applied to remove the noise of signals (Cooper et al., 2012; Lloyd-Fox et al., 2010; Robertson et al., 2010; Sweeney et al., 2011).

Table 1.1: The comparison of fNIRS, fMRI, and EEG.

	fNIRS	fMRI	EEG
Temporal resolution	2 – hundreds of Hz	0.5 – 1 Hz	100-3333Hz
Spatial resolution	5 to 10 mm	1 to 3 mm	5 to 9 cm
Motion artifacts tolerance	High	Low	Low

High correlation indices has been reported between the temporal course of the changes in the concentrations of oxy-Hb and total hemoglobin measured by fNIRS and blood oxygen level dependent (BOLD) signal recorded by fMRI (Strangman et al., 2002; Huppert, et al., 2006). Concurrent validation has also been established between task-induced activation of fNIRS and fMRI in motor, visual, and cognitive tasks (Cui et al., 2011; Kleinschmidt et al., 1996; Moosmann et al., 2003; Obrig et al.,

2000; Okamoto et al., 2004). fNIRS demonstrates sufficient test-retested reliability in both event-related and resting state measurements (Plichta et al., 2006; Plichta et al., 2007; Sato et al., 2006; Schecklmann et al., 2008; Zhang et al., 2011).

Differences of Brain Activity in Resting States between Eyes Open and Eyes Closed Conditions

Measuring brain activity in resting states not only helps to identify the DMN but also provides basic information about baseline conditions of brain activity. The choice of baseline condition is critical to interpreting task-induced brain activities. Both eyes closed and eyes open conditions are commonly used baseline conditions. However, with the same external environment, mere transition from eyes closed to eyes open can cause different activation patterns in different brain regions (Table 1.2) (Brismar, 2007, Hufner et al., 2009; Marx et al., 2003; Marx et al., 2004; McAvoy et al., 2008; Wu et al., 2010; Zou et al., 2009).

It has been found that the brain showed greater spontaneous BOLD oscillations in the eyes closed than the eyes open conditions (McAvoy et al., 2008). A higher functional connectivity in the eyes closed conditions were also reported in other fMRI studies (Wu et al., 2010; Zou et al., 2009). However, the opposite result has also been reported that functional connectivity in the DMN is lower with eyes closed as opposed to eyes open condition (Van Dijk et al., 2010). The medial PFC showed a significant decrease in functional connectivity in eyes-closed condition compared to eyes-open conditions (both with and without fixation). Between two different eyes-open conditions, the medial PFC had a higher functional connectivity with a fixation compared to eyes-open without a fixation. The same trend was found in the amplitude of low frequency fluctuation in the medial PFC suggesting that the regional activity is

higher in eyes-open with a fixation condition compared ein yes-open without a fixation and both eyes-open conditions had a higher activity compared to eyes-closed condition (Yan et al., 2009).

In EEG studies, a higher level of alpha hemodynamic responses was found in the eyes closed condition versus the eyes open condition (Wu et al., 2010; Yang et al., 2010). The alpha band is localized mainly in the posterior regions of brain suppressed by visual stimulation, thus decreased alpha activity was found in eyes open condition (Wu et al., 2010). The alpha band is not the only band that differs between eyes closed and eyes open condition. Focal reductions were also seen in other bands (delta, theta, and beta) from eyes closed to eyes open condition. The reduction of delta and theta bands is associated with stimulus processing and indicated by increased activity. Beta reduction in posterior region was accompanied by increased activity in frontal regions, which is compatible with increased mental efforts (Barry et al., 2007; Barry et al., 2009).

Although inconclusive, these results suggest that modulation of neuronal activity and local hemodynamic responses are sensitive to environment changes that do not involve mental effort. The representation of brain activity can easily have different interpretations by using different baseline conditions. Therefore, it is important to differentiate the resting brain activation patterns between eyes open and eyes closed conditions in order to choose appropriate baseline condition for specific task conditions.

Table 1.2: The differences between eyes open (EO) and eyes closed (EC) in healthy participants.

	Modality	Comparison	Results
Barry et al., 2007	EEG	power of alpha, delta, theta, and beta	EC > EO
Barry et al., 2009	EEG	power of alpha, delta, theta, and beta	EC > EO
McAvoy et al., 2008	fMRI	spontaneous blood oxygenation level-dependent (BOLD) oscillations	EC > EO
Van Dijk et al., 2010	fMRI	functional connectivity	EO > EC
Wu et al., 2010	EEG fMRI	alpha hemodynamic responses functional connectivity	EC > EO
Yan et al., 2009	fMRI	functional connectivity	EO > EC
Yang et al., 2010	EEG	alpha power and alpha-band phase-synchronization	EC > EO
Zou et al., 2009	fMRI	functional connectivity	EC > EO

1.2 Specific Aims

My long-term goal is to understand the neural basis of motor control and learning in children with and without disabilities. The aims of the current study, which served as the first step toward attainment of this long-term goal, were to understand developmental changes of the baseline activity in the PFC and to measure the changes of activity in the PFC during different tasks in children with and without ASD.

Aim 1: To investigate the effect of resting conditions, eyes open and eyes closed, on brain activation in the PFC in different age groups.

Hypothesis 1.1: The oxygenation of the PFC will be different between eyes open and eyes closed conditions.

Hypothesis 1.2: The oxygenation of the PFC will be different among age groups.

Aim 2: To assess the regional activation and identify developmental changes of the DMN in the PFC.

Hypothesis 2.1: The oxygenation of PFC will be different among regions.

Hypothesis 2.2: The regional changes in the PFC will be different among age groups.

Aim 3: To compare the activity in the PFC during resting, a motor task, and an EF task in adults.

Hypothesis 3: The oxygenation of PFC will be higher during the EF task compared to the oxygenation during resting and the motor task.

Aim 4: To compare the activity in the PFC during resting, a motor task, and an EF task in children with and without ASD.

Hypothesis 4.1: The oxygenation of PFC will be higher during the EF task compared to the oxygenation during resting and the motor task.

Hypothesis 4.2: The changes of oxygenation in responds to the EF task will be different in children with ASD compared to children without ASD.

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Chapter 2

BASELINE ACTIVITY IN THE PREFRONTAL CORTEX IN HEALTHY PARTICIPANTS: THE EFFECTS OF EYE CONDITION.

Abstract

The prefrontal cortex (PFC) plays an important role in cognitive process related to executive functions (EFs). As the center of cognitive function, the PFC tends to mature more slowly than other parts in the brain. The modulation of brain activity in children is not the same from the modulation presented in adults (Colonnese & Khazipov, 2012). The PFC activates not only when individuals perform EF-related tasks but also in resting states. Brain activity during resting states provides a baseline for interpreting task-induced brain activities. Both eyes open and eyes closed condition are commonly used baseline conditions. However, brain activity in eyes open condition is different from activity in eyes closed condition. In EEG studies, alpha band activity was in a higher level in eyes closed condition versus eyes open condition (Wu et al., 2010; Yang et al., 2010). The level of baseline activity is critical to interpretation of task-induced activity. The aim of this study was to examine differences in brain activity between eyes open and eyes closed conditions during resting states to provide a rationale of proper selection of baseline condition. The development changes of the resting state activation were compared in three maturation stages. Total of 36 participants in three age groups were recruited in this study including twenty-four adults, five 12-15 years old children, and seven 8-11 years old children. Relative changes of concentrations of oxygenated hemoglobin (Δoxy-Hb)

and deoxygenated hemoglobin (Δdeoxy-Hb) were obtained using functional near-infrared spectroscopy (fNIRS) in eyes closed and eyes open conditions, 3 minutes each. Contrasts were tested to compare the differences of Δoxy-Hb and Δdeoxy-Hb between eyes open and eyes closed conditions. A significant higher Δoxy-Hb was found in eyes closed condition indicated a higher activity in the PFC during resting state if individuals had their eyes closed. The higher activity seen in eye closed condition was inhibited when individuals opened their eyes resulted in a lower activation level. Therefore, eyes open condition may be a better choice for baseline condition since it has less activity in the PFC that could interfere with interpretation of task-induced activity.

2.1 Introduction

The prefrontal cortex (PFC) is one of three primary functional regions in the frontal lobe located at the anterior part of the frontal lobe. The PFC is involved in executive functions (EF) which included cognitive processes such as decision making, working memory, planning, inhibition of responses, and cognitive flexibility. It also plays a key role in self-regulation and contingency-based learning (Gusnard et al., 2001; Kelley et al., 2002; Mitchell et al., 2006). The PFC integrates primary sensorimotor processes and modulates higher-order cognitive functions (Bonnelle et al., 2011; Duan et al., 2012). Increased activity in the PFC has been found when individuals performing EF-related tasks such as Go/No Go tasks (Stevens et al., 2009), Verbal Fluency Tests (Ehlis et al., 2007; Takizawa et al., 2008), the Stroop task (MacDonald et al., 2000; Schroeter et al., 2004), N-back tasks (Abraham et al., 2013; Koike et al., 2013), and a combination of the Stroop task and an N-back task (Griffiths et al., 2013). Both eyes closed and eyes open conditions are commonly used as baseline conditions in neuroimaging studies examining tasks induced activation. However, with the same external environment, a mere transition from eyes closed to eyes open can cause different activation patterns in the brain (Brismar et al., 2007, Hufner et al., 2009; Marx et al., 2003; Marx et al., 2004; McAvoy et al., 2008; Wu et al., 2010; Zou et al., 2009).

Several instrumentations have been used to study brain activity including electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). Each of these modalities measures brain activity by detecting different physiological events triggered by neuronal activation. EEG detects magnetic field induced by post-synaptic ionic current while fMRI assesses hemodynamic changes related to energy usage and oxygen consumption (Riera et al., 2008; Sizonenko et al., 2013). Different

activation patterns during resting states were observed between eyes open and eyes closed conditions among results using different modalities. In EEG studies, alpha band rhythm, which was mainly found in the posterior part of the brain, was at a higher level in the eye closed condition when compared to the eyes open condition indicating an increase in arousal level (Wu et al., 2010; Yang et al., 2010). Focal reductions were also seen in other bands (delta, theta, and beta) from eyes closed to eyes open condition. The reduction of delta and theta bands was associated with stimulus processing indicating increased activation (Barry et al., 2007; Barry et al., 2009). In fMRI studies, greater spontaneous blood oxygenation level-dependent (BOLD) oscillations were found in eyes closed condition when compared to the eyes open condition (McAvoy et al., 2008). Regarding functional connectivity using fMRI, higher functional connectivity was reported in both eyes open and eyes closed conditions in different studies (Van Dijk et al., 2010; Wu et al., 2010; Yan et al., 2009; Zou et al., 2009). Although it is inconclusive on which condition presents a higher functional connectivity, it is clear that modulation of neuronal activity and local hemodynamic responses are sensitive to environment changes such as eyes condition that do not involve mental effort. The representation of brain activity can easily have different interpretations by using different baseline conditions. Meanwhile, each neuroimaging technique measures signals associated with specific physiological mechanism and outcomes from studies using different techniques cannot be applied interchangeably without cautions (Riera et al., 2008). Therefore, it is important to differentiate the resting brain activation patterns between eyes open and eyes closed conditions in order to choose an appropriate baseline condition for specific task conditions.

The activity during resting state is related to the intrinsic activity that activates spontaneously in several regions in the brain including the PFC. This intrinsic activity processes information continuously and helps us respond to unpredicted stimulus (Raichle, 2006). The activity during resting is also related to EFs and high-level cognitive control (Seeley et al., 2007). Duan and colleagues (2012) examined the activity during resting states in Chinese chess players. They found a broader task-induced deactivation in the master level Chinese chess players compared to the novice players. The result indicated that the activity in the resting states changes according to the level of cognitive function. It is possible that children in different development stages of cognitive functions have altered activity during resting state. The development of the PFC, both structural and functional, starts to plateau around 11years old (Giedd et al., 1999; Waber et al., 2007). We examined the brain activation in the PFC in a younger group and an older group to see the developmental changes.

Brain activation was measured using functional near infrared spectroscopy (fNIRS) in current study. fNIRS detects hemodynamic changes induced by brain activity based on optical properties of oxygenated hemoglobin (oxy-Hb) and deoxygenated hemoglobin (deoxy-Hb). Oxy-Hb and deoxy-Hb absorb different amounts of light in different wavelengths which allows for the separation of these two chromophores to be possible using a few sample wavelengths (Horecker, 1943). fNIRS is a noninvasive and low-cost neuroimaging technique making it more feasible than fMRI and EEG in clinical studies. fNIRS provides a balance between temporal and spatial resolution for monitoring local changes of oxygenation in the cerebral cortex compared to EEG and fMRI. EEG has great temporal resolution which can be thousands of hertz but a low spatial resolution of 5 to 9 cm (Minagawa-Kawai et al.,

2008). fMRI provides excellent spatial resolution but the temporal resolution of fMRI is limited at a minimum of one hertz (Weishaupt et al., 2008). Also, the accuracy of fMRI results is significantly affected by movement. fNIRS is less sensitive to action artifacts that various methods can be applied to remove the noise of signals (Cooper et al., 2012; Lloyd-Fox et al., 2010; Robertson et al., 2010; Sweeney et al., 2011). This advantage of fNIRS allows more movement during measurement so participants can actually perform the tasks in more natural settings.

The aim of this study was to investigate the effect of resting conditions, eyes open and eyes closed, on brain activation in the PFC in different age groups. Our hypotheses were: 1): the oxygenation of the PFC will be different between eyes open and eyes closed conditions, and 2) the oxygenation of the PFC will be different among age groups.

2.2 Method

All research was performed in the Biosignal Lab at the National Cheng Kung University, Tainan, Taiwan. The study was approved by the Institutional Review Board at the University of Delaware (Appendix A) and the National Cheng Kung University (Appendix B).

Participants

A total of 36 participants in three age groups, 8 to 11 years old (younger children, 4 males and 3 females, age = 9.96 ± 1.38 years old), 12 to 15 years old (older children, 1 male and 4 females, age = 13.33 ± 1.30 years old), and 20 to 35 years old (adults, 11 males and 13 females, age = 25.89 ± 3.08 years old), were recruited in the study (Table 2.1). Participants were recruited from local universities and communities by word-of-mouth. To be included in the study, participants were: 1) right-handed, 2)

physically and mentally healthy, and 3) without a family history of any mental disorders. The healthy participants of all age groups were recruited based on these criteria. A questionnaire of exclusion criteria was given to participants or their parents (Appendix C & D) to confirm that they met the inclusion criteria and did not have any of following exclusion criteria: 1) previous head injury of any type or severity, 2) a seizure disorder, 3) open wound on the forehead, 4) unable to stay in sitting position and rest for 6 minutes, and 5) allergic to rubbing alcohol. The study was approved by the Institutional Review Board at the National Cheng Kung University. Inform consent from adult participants and parents of minor participants were obtained before the study.

Table 2.1: Participant demographic information.

	Adults	Older Children	Younger Children
Number	24	5	7
Males/Females	11M/13F	1M/4F	4M/3F
Age (years)	25.89 ± 3.08	13.33 ± 1.30	9.96 ± 1.38
Height (cm)	167.20 ± 8.36	153.00 ± 13.83	139.38 ± 9.46
Weight (kg)	62.78 ± 12.24	42.90 ± 15.69	31.00 ± 7.46

Procedures

Data were collected in the Biosignal Lab at the National Cheng Kung University. Upon the arrival of participants, the procedures of the study were explained thoroughly by the experimenter in lay language to ensure that they understood the study. Participants sat in front of a monitor (19" VG1921wm LCD

monitor, ViewSonic) for data collection. The chair was adjusted to the height of participants so that they could reach the ground and the visual angle to the center of the monitor was within ±15 degrees to the horizontal line of sight. Example power point slides, including the 5 second countdown, a starting page, a transition page, and an ending page was given to all participants before data collection to ensure they understood the procedure.

The experimenter cleaned their forehead with alcohol swab in order to remove skin tissues that might interfere light scattering and absorption. A plastic custom-made holder of a plastic cap with holding spots for light sources and detectors was placed on the forehead of participants. Then, eight light sources and three detectors of the fNIRS system were mounted to the cap (Figure 2.1). The lights in the room were dimmed during data collection due to the requirement of the instrumentation. One 6-minute trial with 3 minutes of eyes open and 3 minutes of eyes closed was recorded for each participant. The order of conditions was randomly assigned. They were instructed to relax and stare at a cross on the screen during eyes open condition and close their eyes during eyes closed condition. The instruction given was: 'Relax. Don't think of anything. All you need to do is to close or open your eyes, but don't fall asleep.' A 5-second count down was given and the trial started with an auditory signal. The second auditory signal was given after 3 minutes to notify the change of eyes condition and then the final auditory signal at the end of the trial.

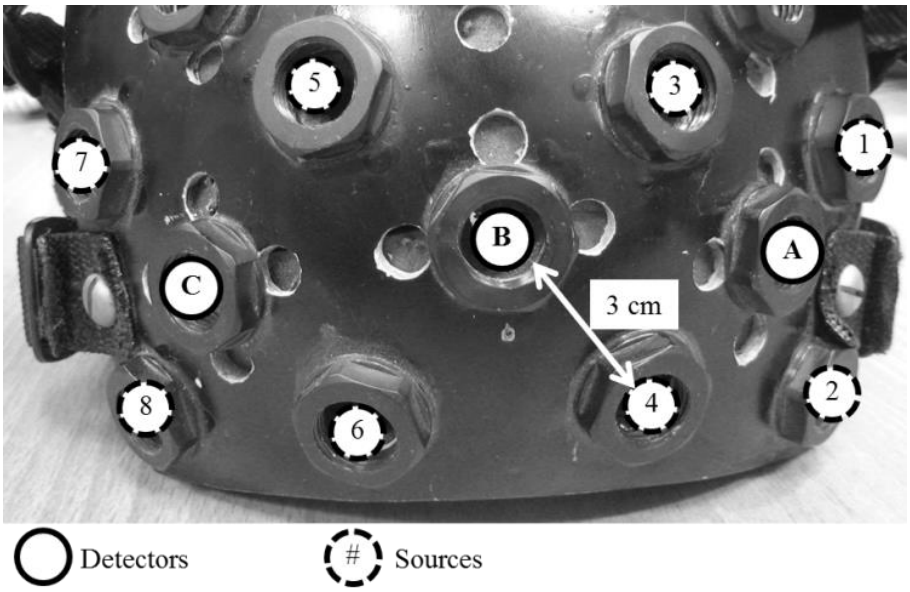

Figure 2.1: The custom-made holding cap and arrangement of light sources and light detectors.

fNIRS Measurements and Data Processing

Raw light signals were acquired using a multi-channel frequency-domain fNIRS system and ISS BOXY software package (Imagent; ISS Inc., Champaign, IL). The system has 4 light detectors and 16 light sources. The wavelengths of light sources were 690 nm and 830 nm. Twelve channels configured by three detectors and eight sources were recorded (Figure 2.2). The distance between pairs of source-detector was 3 cm which allows the light to penetrate 2 to 3 cm in depth from the scalp and reach the surface of cortex (Okada & Delpy, 2003a; Okada & Delpy, 2003b). The midpoint of middle two sources at the lower row was located roughly at the Fpz position in 10-20 international system. This recording area covered bilateral superior frontal gyrus (BA 9, 10) including dorsolateral PFC and anterior PFC (Homan et al., 1987; Okamoto et al., 2004). Data were collected at a sampling rate of 12.5 Hz.

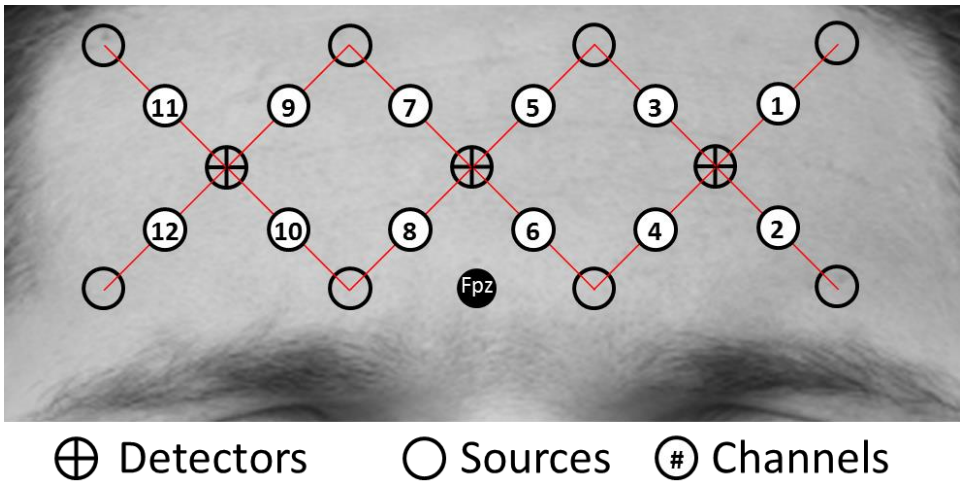

Figure 2.2: Location of optodes and channels.

Relative changes of concentration of oxygenated hemoglobin (Δoxy-Hb) and deoxygenated hemoglobin (Δdeoxy-Hb) were calculated from changes of light intensity using the modified Beer-Lambert Law (Delpy et al., 1988; Villringer & Chance, 1997):

$$\text{Light extinction} = -\log\left(\frac{I_0}{I_I}\right) = \mu_a \cdot c \cdot L \cdot B + G$$

where I_O is the light intensity detected, I_I is the input light intensity, μ_a is absorption coefficient, c is substance concentration, L is the distance between light source and light detector, B is differential pathlength factor, G is scattering loss due to an unknown geometry dependent factor. Without the knowledge of exact differential pathlength factor and scattering loss, it is not possible to measure absolute concentration of hemoglobin (Floume et al., 2008; Hoshi, 2003).

Δoxy-Hb and Δdeoxy-Hb were calculated with estimated age-dependent differential pathlength factors (Duncan et al., 1996). A third order Butterworth band-pass filter of 0.002 to 0.5 Hz was applied to remove physiologically irrelevant data and equipment noise (Ayaz et al., 2011; Izzetoglu et al., 2007; Lin et al., 2012). A principal component analysis was then performed to remove large motion artifacts (Huppert et al., 2009). The data were processed using MATLAB (MathWorks, Natick, Massachusetts) and an open source software HomER ("PMI Lab —HomER," http://www.nmr.mgh.harvard.edu/PMI/resources/homer/home.htm).

The first and the last 15 seconds of each condition were removed to prevent unstable state at the beginning of the test and the effect from previous activity (Izzetoglu et al., 2007). The remaining 1,875 data points were averaged in each channel. Z scores were then calculated using mean and standard deviation of all 12 channels in each participant using $z = \frac{x-mean}{SD}$. Channels with extreme values (Z scores above 2 or less than -2) were removed as outliers (Van Selst & Jolicoeur, 1994). Numbers of channels removed were listed in Table 2.2. Most of channels with extreme values were channels located at the edge of the holding cap (channels 1, 2, 11 and 12), where light signals might be affected by hair and resulted in extreme values.

Table 2.2: Numbers of channels removed.

Channel	1	2	3	4	5	6	7	8	9	10	11	12
oxy_open	7	4	1	0	0	0	0	0	1	0	1	6
oxy_closed	8	5	0	0	0	0	0	0	0	1	2	5
deoxy_open	4	7	1	0	0	0	0	0	1	0	2	4
deoxy_closed	5	2	1	1	0	1	0	0	0	0	2	4

After removed the extreme values, the average of remaining channels for each condition in each participant was calculated. Besides Δoxy-Hb and Δdeoxy-Hb, the variations of each type of hemoglobin between eyes open and eyes closed was compared among groups. We calculated variation in Δoxy-Hb by subtracting the Δoxy-Hb in eyes open condition from the Δoxy-Hb in eyes closed condition. The variation in Δdeoxy-Hb was calculated using the same method. If variation score was positive, which meant the concentration of the hemoglobin had a higher concentration in eyes closed condition. If variation score was negative, which meant the concentration of the hemoglobin had a higher concentration in eyes open condition.

Statistical Analysis

The data were not normally distributed with positive skewness in Δoxy-Hb in eyes closed and Δdeoxy-Hb in eyes open conditions but negative skewness in Δoxy-Hb in eyes open and Δdeoxy-Hb in eyes closed conditions (Table 2.3). There was no appropriate transformation for data sets with skewness in different directions. The variance for Δoxy-Hb and Δdeoxy-Hb were both significantly different among three age groups, $F(2,69) = 6.623$, $p = .002$ and $F(2,69) = 14.385$, $p < .001$, respectively. Due to violations of assumptions, contrasts were tested with Welch correction to compare the differences of concentrations of Δoxy-Hb and Δdeoxy-Hb between eyes open and eyes closed condition in each group and all groups combined (Table 2.4). All data were analyzed using Statistical Package for Social Sciences (SPSS) software (version 17, SPSS, Inc, Chicago, IL, USA). The level of significance was set at .05.

Table 2.3: Skewness of Δoxy-Hb and Δdeoxy-Hb in eyes open and eyes closed conditions.

		Skewness		
		Statistic	Standard Error	Z score
Oxy-Hb	Eyes Open	-1.756	.393	-4.47
	Eyes Closed	1.844	.393	4.69
Deoxy-Hb	Eyes Open	3.199	.393	8.14
	Eyes closed	-.253	.393	-0.64

Table 2.4: Contrast table of paired comparisons of differences between eyes open and eyes closed conditions.

	Eyes open			Eyes closed		
	YC	OC	Adults	YC	OC	Adults
YC	-1	0	0	1	0	0
OC	0	-1	0	0	1	0
Adults	0	0	-1	0	0	1
All groups	-1	-1	-1	1	1	1

YC = Younger children (8-11 years old); OC = Older children (12-15 years old); Adults = Adults (20-35 years old).

2.3 Results

Oxygenated Hemoglobin

The result of contrast tests showed that Δoxy-Hb was higher in eyes closed condition compared to eyes open condition when all groups were combined ($t(17.268) = 3.021$, $p = .008$). When comparing Δoxy-Hb between eyes conditions in each group, a significant difference was found only in younger children ($t(9.459) = 2.734$, $p = .022$). There were no significant differences in Δoxy-Hb between eyes conditions in older children ($t(6.095) = .798$, $p = 455$), and adults ($t(45.858) = 1.738$, $p = .089$) (Figure 2.3).

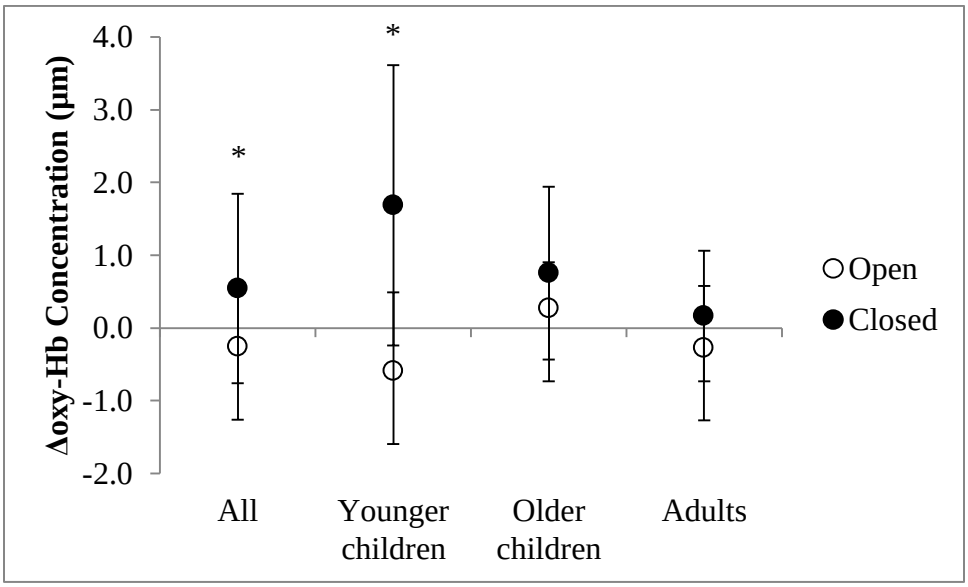

Figure 2.3: Δoxy-Hb in three groups and all groups combined during eyes open and eyes closed conditions (bars represent standard deviation) (* $p < .05$).

Deoxygenated Hemoglobin

No significant differences were found in Δdeoxy-Hb between eyes open and eyes closed conditions in each group and all groups combined (Figure 2.4).

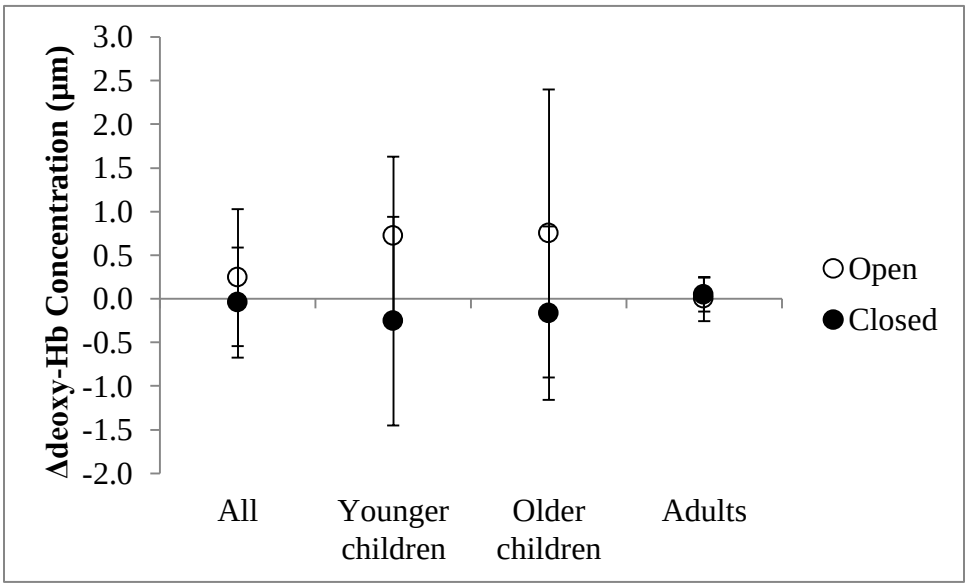

Figure 2.4: Δdeoxy-Hb in three groups and all groups combined during eyes open and eyes closed conditions (bars represent standard deviation).

Variation Between Eyes Open and Eyes Closed

No significant differences were found in the variations of Δoxy-Hb among groups (Figure 2.5). No significant differences were found in the variations of Δdeoxy-Hb among groups either (Figure 2.6).

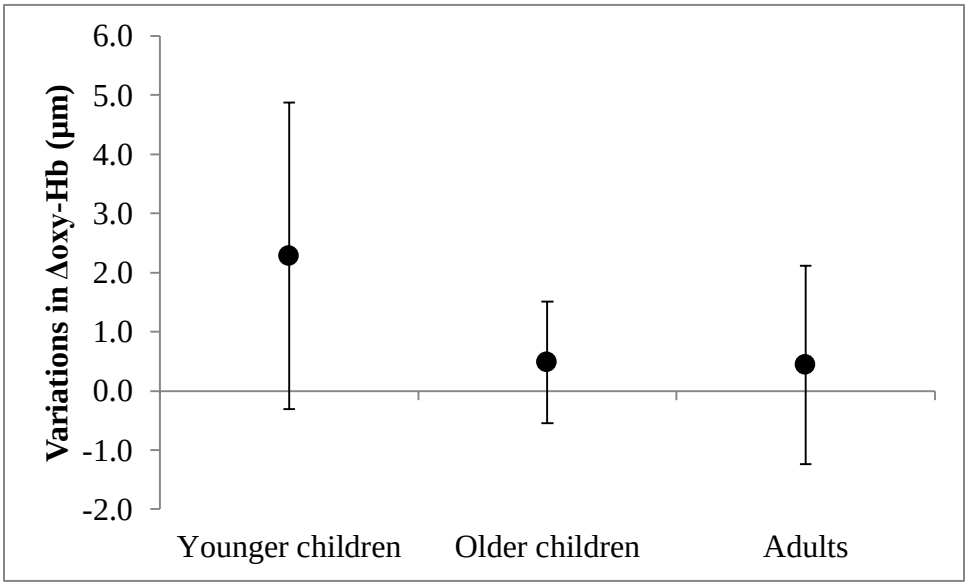

Figure 2.5: Variations in Δoxy-Hb between eyes closed condition and eyes open condition (eyes closed - eyes open) (bars represent standard deviation).

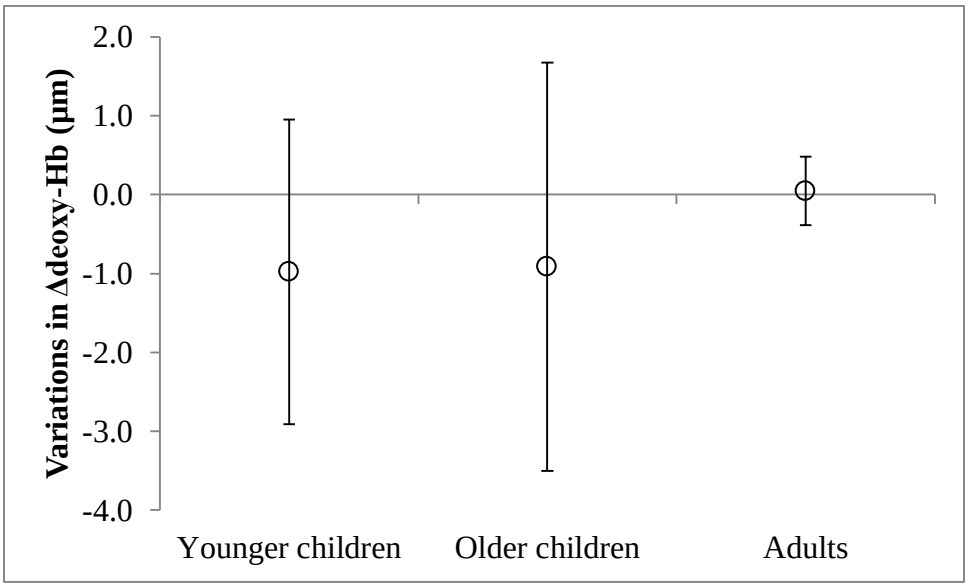

Figure 2.6: Variations in Δdeoxy-Hb between eyes closed condition and eyes open condition (eyes closed - eyes open) (bars represent standard deviation).

2.4 Discussion

The study was conducted to examine brain activity during resting states by measuring the Δoxy-Hb and Δdeoxy-Hb in the PFC. The results found that younger children had a significant higher Δoxy-Hb in the PFC during eyes closed condition compared to eyes open condition. The results indicated the brain activity was greater in the PFC during eyes closed condition compared to eyes open condition in younger children. The same trend was observed when all groups were combined, however, no significant differences were found in older children and adults groups.

McAvoy and his colleagues (2008) have reported greater spontaneous BOLD oscillations during eyes closed condition. Our results provided similar trend that a higher activity in eyes closed condition compared to eyes open condition. However, McAvoy et al. (2008) saw higher activities in the sensory and paralimbic cortex located in the posterior part of the brain, while the present study found a higher activity in the PFC located in the front part of the brain. The PFC plays a key role in EFs. It is also part of the default mode network (DMN) that activates during resting states and deactivates when performing goal-oriented tasks (Raichle et al., 2001). The DMN is related to self-reflective thoughts and constructs the internal mentation (Gusnard et al., 2001; Kelley et al., 2002; Mitchell et al., 2006). It also interacts with high-order cognitive function and has influence on behavioral performance (Bonnelle et al., 2011; Duan et al., 2012). Although eyes open with fixation is regarded as a passive fixation task which requires minimum mental efforts, it decreases the activation found in the PFC during eyes closed condition. It is possible that the DMN has a higher activity and processes more information when individuals close their eyes rather than keep their eyes open.

Eyes closed condition has been used in most of the functional connectivity studies using fNIRS (Duan et al., 2012; Lu et al, 2010; Zhang et al., 2010). However, the rationale of the selection of testing condition was not provided in those studies. Our results provided a possible explanation of their choices with using eyes closed condition. The greater activity of the DMN in the PFC during eyes closed condition indicates that more information related to the DMN may be available if researchers use eyes closed condition for data collection. On the other hand, a higher activity during baseline condition such as resting with eyes closed may interfere with the interpretation of task-induced activations. It is easy to identify task-induced activation if baseline condition has zero or minimum activation. Increases of activation found in task conditions can be attributed to mental efforts toward the requested tasks. With certain amount of activity in baseline condition, it is harder to differentiate task-induced activity from baseline activity. Theoretically, activation found in the DMN decreases during goal-oriented tasks. However, reductions of deactivation in the DMN have been reported in specific populations such as individuals with ASD and their unaffected siblings (Kennedy et al., 2006; Spencer et al., 2012). Activation seen in task conditions can be a summation of task-induced activation and the residuals of the DMN that lack deactivation. Therefore, a baseline condition with eyes open, which has a lower activation, is a better choice in order to minimize the effects of the activation found in resting state.

When comparing differences between eyes open and eyes closed conditions, a significant difference was found only in younger children but not older children or adults. Previous study has reported different responsiveness to emotional stimulus in the PFC in young children and adults. Todd and colleagues (2012) found a greater

response in the left frontal regions when participants processing emotional stimulus in younger children compared to an inhibition task. However, no difference in activation was found in adults between response of emotional stimulus and the inhibition task. In addition to different responsiveness to emotional stimulus, opposite response patterns to visual stimulus were also observed in younger children and adults. Born and colleagues (1998) reported a decrease in MRI signals in the younger children during visual stimulation in the occipital cortex, whereas an increase in MRI signals was found in adults during visual stimulation in the occipital cortex. The response to visual stimulation was not limited to the occipital cortex. Event-related activations in the PFC has also been observed in infant's brain implies that the PFC may plays a role in processing visual signals (Taga et al., 2003). Young children and adults have different activation patterns in the PFC responded to emotional stimulus and different activation patterns in the occipital cortex responded to visual stimulus. Our results showed different activation patterns in the PFC in response to visual stimulation which suggested that the PFC contributed to processing of visual information, especial in younger children.

The differences between eyes open and eyes closed condition in all groups were observed in oxy-Hb but not deoxy-Hb in our study suggested oxy-Hb is a more sensitive parameter to regional cerebral blood flow. Regional brain activation requires glucose and oxygen consumption. The reduction of glucose and oxygenation results in increased blood flow to the region. The increased oxygen supply usually exceeds regional oxygen demands, resulting in increased oxygenation in the area (Fox et al., 1988; Malonek & Grinvald, 1996; Obrig & Villringer, 2003). An increase in oxygenation is presented in two patterns: 1) with an increase in total hemoglobin

(Total-Hb), and 2) with no changes in Total-Hb (Hoshi and Tamura, 1997). In pattern one, an increase of oxy-Hb with an increase or no changes in deoxy-Hb are observed with increases in Total-Hb. In pattern 2, increases in oxy-Hb with reciprocal decreases in deoxy-Hb are observed with no changes in Total-Hb. While increases in oxy-Hb are always observed in activated areas, deoxy-Hb can be increasing, decreasing, or present with no changes (Hoshi, 2003). Less sensitivity observed in deoxy-Hb can be attributed to its variations in changing directions.

2.5 Conclusion

Resting state is commonly used baseline condition in study of brain activity during goal-oriented tasks. The selection of baseline activity is critical to interpretation of task-induced activity. Our results showed that eyes open condition with a lower activity in the PFC may be a better baseline condition compared to eyes closed condition for EF tasks.

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Chapter 3

REGIONAL DIFFERENCES IN BRAIN ACTIVATION IN THE PREFRONTAL CORTEX DURING RESTING STATES

Abstract

The medial prefrontal cortex (PFC) is part of the default mode network (DMN) that activates during task-free resting states. The functions of the DMN include monitoring environment and preparing for future events, processing self-relevant information, and interacting with high-order cognitive function to regulate behavioral performance. The disruption of the DMN could be the underlying cause for neurodevelopmental disorders such as autism. The aim of this study was to examine the DMN by comparing regional activations of the PFC. Three age groups were recruited in the study including younger children, older children, and adults. Resting brain activity was measured under both eyes open and eyes closed condition using functional near-infrared spectroscopy. Concentrations of oxygenated hemoglobin and deoxygenated hemoglobin were quantified in three regions, the left PFC, the medial PFC, and the right PFC. There were no significant differences among regions. However, younger children presented with asymmetry activation responses during the transition between eyes open and eyes closed in the PFC.

3.1 Introduction

The default mode network (DMN) is the spontaneous activity in the brain during task-free resting states. Functions of the DMN include monitoring the external environment and preparing for unexpected future events. The DMN supports a broad low-level focus of attention of the external world during resting state, continuously gathers information and reallocates attention to previously unattended input as needed (Gilbert et al., 2007; Gusnard & Raichle, 2001; Hahn et al., 2007; Hampson et al., 2006; Leech et al., 2011). In addition, the DMN contributes to self-reflective thoughts and constructs the internal mentation. The representation of the state of the self can then be used during inferences to others' mental states (Gusnard et al., 2001; Kelley et al., 2002; Mitchell et al., 2006). Previous studies have shown that the DMN is important to learning, attention, and high-level of cognitive functions (Bonnelle et al., 2011; Duan et al., 2012). Disruptions to the DMN could be the underlying causes of mental disorders including autism spectrum disorder (ASD) that lead to abnormal behaviors (Kennedy et al., 2006; Raichle, 2006).

Evidence shows that neural growth in the DMN is present prior to full term birth. Fragments of the DMN have been detected in pre-term infants and a complete network template was present by term (Doria et al., 2011; Lee et al., 2013). It is believed that the DMN develops in parallel with the development of related cognitive functions and changes of the DMN have been detected from infancy through adolescence (Gordon et al., 2011; Power et al., 2010; Zielinski et al., 2010). Although regions of the DMN in children are similar to adults, regions involved in higher-order cognitive functions such as the medial prefrontal cortex (PFC) have not fully matured in children ages 7 to 12 years (Thomason et al., 2008). The PFC involved in executive functions (EFs) that include: decision making, working memory, inhibition, planning,

problem solving, and cognitive flexibility. It is seen as the center of learning and plays a critical role in high-order cognitive functions (Ardila, 2008; Miller & Wang, 2006; Riva et al., 2013). The PFC tends to mature more slowly than other brain regions, which usually reach full maturity in late adolescence (Lebel et al., 2008). Both structural and functional development of the PFC continue to progress until early adulthood (Sowell et al., 1999).

In the PFC, a consistent decrease has been observed in the medial PFC during goal-oriented tasks from the baseline (Raichle et al., 2001). The medial PFC is a subsection of the DMN, has a higher activity during task-free resting states compared to activity during performing goal-oriented task conditions. While previous research have examined large-scale functional connectivity in the DMN using whole brain functional magnetic resonance imaging (fMRI), we were interested in regional activation in the PFC. We expected to see a higher activation in the medial PFC compared to the lateral PFC. Therefore, the purpose of our study was to examine activations in different regions of the PFC between the immature state of children and the fully maturated brain of adults using functional near infrared spectroscopy (fNIRS).

3.2 Method

Participants

Thirty-six participants were recruited for this study. Seven participants were in younger children group between 8 to 11 years old (4 male, 3 female, age: 9.96 ± 1.38 years). Five participants were in older children group between 12 to 15 years old (1 male, 4 female, age: 13.33 ± 1.30 years). Twenty-five participants were in adult group between 20 to 35 years old (11 male, 15 female, age: 25.89 ± 3.08 years) (Table 3.1). They were all right-handed and healthy with no family history of any mental disorders.

Participants with following exclusion criteria were excluded: 1) a previous head injury of any type or severity, 2) a seizure disorder, 3) open wound on the forehead, 4) unable to stay in sitting position and rest for 6 minutes, and 5) allergic to rubbing alcohol. A questionnaire of exclusion criteria was given to participants or their parents to confirm that they met the inclusion criteria and did not have any exclusion criteria.

Table 3.1: Participant demographic information.

	Younger Children (8-11 years)	Older Children (12-15 years)	Adults (20-35 years)
N	7	5	24
Male/Female	4M/3F	1M/4F	11M/13F
Age (years)	9.96 ± 1.38	13.33 ± 1.30	25.89 ± 3.08
Height (cm)	139.38 ± 9.46	153.00 ± 13.83	167.20 ± 8.36
Weight (kg)	31.00 ± 7.46	42.90 ± 15.69	62.78 ± 12.24

Procedures

All research was conducted in the Biosignal Lab at the National Cheng Kung University. The study was approved by the Institutional Review Board at National Cheng Kung University. All participants or their parents provided informed consent before the study. The primary investigator cleaned the foreheads of participants with an alcohol wipe in order to remove substances on the skin that might interfere light scattering and absorption. A custom-made holding cap with three detectors and eight light sources was placed on the forehead and connected to the fNIRS system. Participants sat in front of a monitor during data collection. They were instructed to relax and stare at a cross on the screen during the eyes open condition and close their eyes during eyes closed condition. One 6-minute trial with 3 minutes of eyes open and

3 minutes of eyes closed was recorded for each participant. The order of conditions was randomly assigned.

fNIRS Measurements

A frequency domain system and ISS Boxy Software package was used for data collection (Imagent; ISS Inc., Champaign, IL). Twelve channels configured by three detectors and eight light sources with the interoptode distance of 3 cm were used (Figure 3.1). Each light source contains two laser diodes emitting at two wavelengths at 690 nm and 830 nm. The midpoint of the middle two sources at the lower row was located roughly at the Fpz position in the 10-20 international system (Figure 3.2). This recording area covered the superior frontal gyrus (BA 9, 10) including the dorsolateral PFC and the anterior PFC (Homan et al., 1987; Okamoto et al., 2004). Data were collected at a sampling rate of 12.5 Hz.

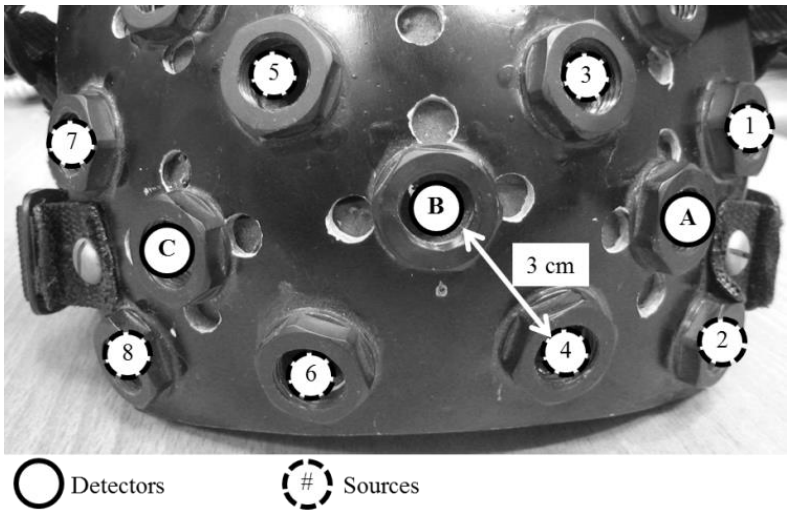

Figure 3.1: The custom-made holding cap and arrangement of light sources and light detectors.

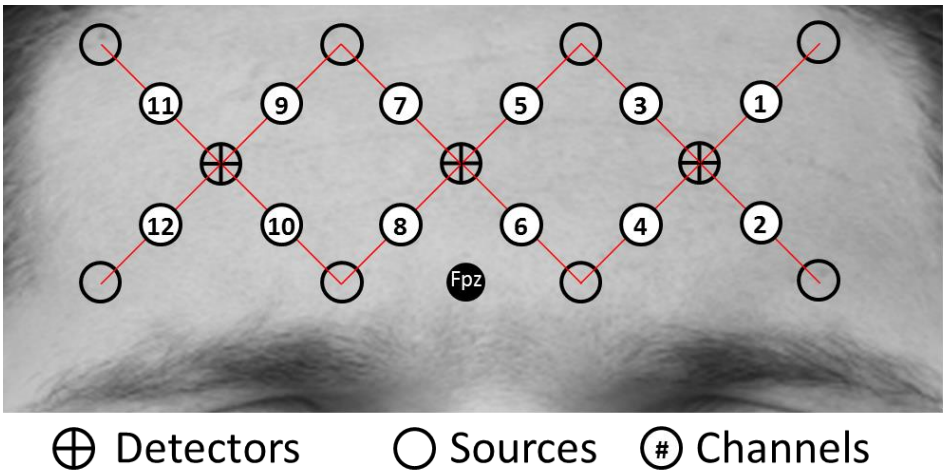

Figure 3.2: Location of optodes and channels.

Data Processing

Relative changes of concentration of oxygenated hemoglobin (Δoxy-Hb) and deoxygenated hemoglobin (Δdeoxy-Hb) were calculated using the modified Beer-Lambert Law (Delpy et al., 1988; Villringer & Chance, 1997). A third order Butterworth band-pass filter of 0.002 to 0.5 Hz was applied to remove physiologically irrelevant data and equipment noise (Ayaz et al., 2011; Izzetoglu et al., 2007; Lin et al., 2012). A principal component analysis was then performed to remove large motion artifacts (Huppert et al., 2009). The data were processed using MATLAB (MathWorks, Natick, Massachusetts) and an open source software HomER ("PMI Lab —HomER," http://www.nmr.mgh.harvard.edu/PMI/resources/homer/home.htm).

The first and the last 15 seconds of each condition were removed to omit the unstable state at the beginning of the test and the effect from the previous activity (Izzetoglu et al., 2007). The remaining 1,875 data points were averaged in each channel. Z scores were then calculated using the mean and standard deviation of all 12

channels in each participant using Z = (x-mean)/SD. Only channels with Z scores between 2 and -2 were retained for further analysis. Channels with extreme values (Z scores above 2 or less than -2) were removed as outliers (Van Selst & Jolicoeur, 1994).

Twelve channels were grouped in three regions: channels 1 to 4 = left PFC, channels 5 to 8 = medial PFC, channels 9 to 12 = right PFC. Averages of Δoxy-Hb and Δdeoxy-Hb in each region for each participant were calculated.

Variation between Eyes Open and Eyes Closed

The variations of each type of hemoglobin in the same region between eyes open and eyes closed conditions were calculated. We calculated variation in Δoxy-Hb by subtracting the Δoxy-Hb in eyes open condition from the Δoxy-Hb in eyes closed condition. The variation in Δdeoxy-Hb was calculated using the same method. If variation score was positive, which meant the concentration of the hemoglobin had a higher concentration in eyes closed condition. If variation score was negative, which meant the concentration of the hemoglobin had a higher concentration in eyes open condition.

Regional Variation

Variations between regions in the same condition were calculated for both Δoxy-Hb and Δdeoxy-Hb in each group. We calculated regional variation between the medial and the left PFC (M – L) by subtracting the concentration of the hemoglobin in the left PFC from the medial PFC. Regional variation between the right PFC and the medial PFC (R – M) by subtracting the concentration of the hemoglobin in the medial PFC from the right PFC. Regional variation between the right PFC and the left PFC (R – L) by subtracting the concentration of the hemoglobin in the left PFC from the right

PFC. If the value of regional variation was positive, which meant the region on the right side in the comparison had a higher concentration (the right PFC > the left PFC, the right PFC > the medial PFC, or the medial PFC > the left PFC). If the value of regional variation was negative, which meant the region on the left side in the comparison had a higher concentration (the left PFC > the right PFC, the left PFC > the medial PFC, or the medial PFC > the right PFC).

Statistics Analysis

The regional Δoxy-Hb, $D(216) = .298$, $p < .001$, and deoxy-Hb, $D(216) = .298$, $p < .001$, were both significantly non-normal. The variances were significantly unequal among groups for both Δoxy-Hb, $F(2,213) = 19.287$, $p < .001$, and Δdeoxy-Hb, $F(2,213) = 29.569$, $p < .001$. Since the assumptions of normality and homogeneity were not met, contrasts were tested for paired comparisons among regions in each group instead of parametric tests. Welch correction was applied for unequal variance. Three regions in the PFC of left PFC, medial PFC, and right PFC were compared in pairs in each group (Table 3.2). The differences between eyes open and eyes closed conditions in each region were compared in each group (Table 3.3). Variations between eyes open and eyes closed in each region were compared as a function of age (Table 3.4). Variations between any two regions were compared as a function of age as well (Table 3.5). Δoxy-Hb and Δdeoxy-Hb were compared separately.

All data were analyzed using Statistical Package for Social Sciences (SPSS) software (version 17, SPSS, Inc, Chicago, IL, USA). The level of significance was set at .05.

Table 3.2: Contrast table of paired-comparisons among regions.

	Eyes open			Eyes closed		
	LPFC	MPFC	RPFC	LPFC	MPFC	RPFC
Contrast 1	-1	1	0	0	0	0
Contrast 2	0	-1	1	0	0	0
Contrast 3	-1	0	1	0	0	0
Contrast 4	-1	2	-1	0	0	0
Contrast 5	0	0	0	-1	1	0
Contrast 6	0	0	0	0	-1	1
Contrast 7	0	0	0	-1	0	1
Contrast 8	0	0	0	-1	2	-1

LPFC = the left prefrontal cortex, MPFC = the medial prefrontal cortex, RPFC = the right prefrontal cortex.

Table 3.3: Contrast table of paired-comparisons between eyes conditions in each region.

	Eyes open			Eyes closed		
	LPFC	MPFC	RPFC	LPFC	MPFC	RPFC
Contrast 1	-1	0	0	1	0	0
Contrast 2	0	-1	0	0	1	0
Contrast 3	0	0	-1	0	0	1

LPFC = the left prefrontal cortex, MPFC = the medial prefrontal cortex, RPFC = the right prefrontal cortex.

Table 3.4: Contrast table of paired-comparisons of variations between eyes open and eyes closed conditions (eyes closed – eyes open) as a function of age.

	Younger children			Older children			Adults		
Variation in	LPFC	MPFC	RPFC	LPFC	MPFC	RPFC	LPFC	MPFC	RPFC
Contrast 1	-1	0	0	1	0	0	0	0	0
Contrast 2	0	-1	0	0	1	0	0	0	0
Contrast 3	0	0	-1	0	0	1	0	0	0
Contrast 4	-1	0	0	0	0	0	1	0	0
Contrast 5	0	-1	0	0	0	0	0	1	0
Contrast 6	0	0	-1	0	0	0	0	0	1
Contrast 7	0	0	0	-1	0	0	1	0	0
Contrast 8	0	0	0	0	-1	0	0	1	0
Contrast 9	0	0	0	0	0	-1	0	0	1

LPFC = the left prefrontal cortex, MPFC = the medial prefrontal cortex, RPFC = the right prefrontal cortex.

Table 3.5: Contrast table of paired-comparisons of variations between regions as a function of age. ΔoxyHb and Δdeoxy-Hb in each eyes condition was examined separately.

	Younger children			Older children			Adults		
	M – L	R – M	R – L	M – L	R – M	R – L	M – L	R – M	R – L
Contrast 1	-1	0	0	1	0	0	0	0	0
Contrast 2	-1	0	0	0	0	0	1	0	0
Contrast 3	0	0	0	-1	0	0	1	0	0
Contrast 4	0	-1	0	0	1	0	0	0	0
Contrast 5	0	-1	0	0	0	0	0	1	0
Contrast 6	0	0	0	0	-1	0	0	1	0
Contrast 7	0	0	-1	0	0	1	0	0	0
Contrast 8	0	0	-1	0	0	0	0	0	1
Contrast 9	0	0	0	0	0	-1	0	0	1

M – L = medial PFC – left PFC;
R – M = right PFC – medial PFC;
R – L = right PFC – left PFC.

3.3 Results

Regional Differences in Each Group

No significant differences in Δoxy-Hb and Δdeoxy-Hb were found in any paired-comparison among regions in younger children (Table 3.6), older children (Table 3.7), or adults (Table 3.8).

Variation between Eyes Open and Eyes Closed in Each Region

Younger children. A significant higher Δoxy-Hb ($t(11.807) = 3.316$, $p = .006$) and a significant lower Δdeoxy-Hb ($t(10.295) = -3.034$, $p = .012$) were found in eyes closed condition compared to eyes open condition in the left PFC (Figure 3.3). The medial PFC also had a significant lower Δdeoxy-Hb in eyes closed condition compared to eyes open condition ($t(11.960) = -2.615 = .023$). No significant differences were found in other comparisons (Table 3.9).

Older children. No significant differences were found in the paired-comparisons of variations between eyes open and eyes closed in any region in either Δoxy-Hb or Δdeoxy-Hb (Figure 3.4) (Table 3.9).

Adults. No significant differences were found in the paired-comparisons of variations between eyes open and eyes closed in any region in either oxy-Hb or deoxy-Hb (Figure 3.5) (Table 3.9).

Variation between Eyes Conditions and Regional Variation as a Function of Age

No significant differences were found in the variations between eyes conditions among groups (Table 3.10). There were no significant differences in any variation between regions among groups either (Table 3.11).

Table 3.6: Paired-comparison results of Δoxy-Hb and Δdeoxy-Hb among regions in each eyes condition in younger children.

		Δoxy-Hb			Δdeoxy-Hb		
		t	df	p	t	df	p
Eyes open	Left vs. Medial	1.167	10.062	.270	-.617	10.393	.474
	Medial vs. Right	1.633	10.263	.133	-1.339	9.067	.213
	Left vs. Right	.633	11.987	.539	-.738	11.416	.475
	Medial vs. Lateral	-.170	5.329	.871	1.974	8.665	.081
Eyes closed	Left vs. Medial	-.572	11.990	.578	.393	11.980	.701
	Medial vs. Right	.258	11.941	.801	1.061	7.240	.323
	Left vs. Right	-.298	11.980	.771	1.231	7.344	.256
	Medial vs. Lateral	.127	9.254	.902	-.508	6.000	.630

* $p < .05$.

Table 3.7: Paired-comparison results of Δoxy-Hb and Δdeoxy-Hb among regions in each eyes condition in older children.

		Δoxy-Hb			Δdeoxy-Hb		
		t	df	p	t	df	p
Eyes open	Left vs. Medial	.307	4.970	.771	.189	7.636	.855
	Medial vs. Right	-1.968	6.934	.090	-.295	7.376	.776
	Left vs. Right	-1.394	6.089	.212	-.461	6.508	.660
	Medial vs. Lateral	-1.180	4.101	.302	-.747	4.994	.489
Eyes closed	Left vs. Medial	-.444	5.790	.673	-.057	7.349	.956
	Medial vs. Right	1.423	4.556	.220	1.538	6.122	.174
	Left vs. Right	1.330	4.132	.252	1.129	5.214	.308
	Medial vs. Lateral	1.185	4.063	.301	.472	7.341	.651

* $p < .05$.

Table 3.8: Paired-comparison results of Δoxy-Hb and Δdeoxy-Hb among regions in each eyes condition in adults.

		Δoxy-Hb			Δdeoxy-Hb		
		t	df	p	t	df	p
Eyes open	Left vs. Medial	-.033	45.913	.973	-.362	45.916	.719
	Medial vs. Right	-.167	44.823	.868	-.793	41.950	.432
	Left vs. Right	-.134	45.354	.894	-.479	42.840	.634
	Medial vs. Lateral	-1.972	5.424	.101	.533	10.046	.606
Eyes closed	Left vs. Medial	.244	40.387	.808	-.089	43.595	.929
	Medial vs. Right	.104	45.969	.918	.239	40.643	.813
	Left vs. Right	.371	40.975	.713	.186	35.289	.853
	Medial vs. Lateral	1.345	4.161	.247	.257	7.413	.804

* $p < .05$.

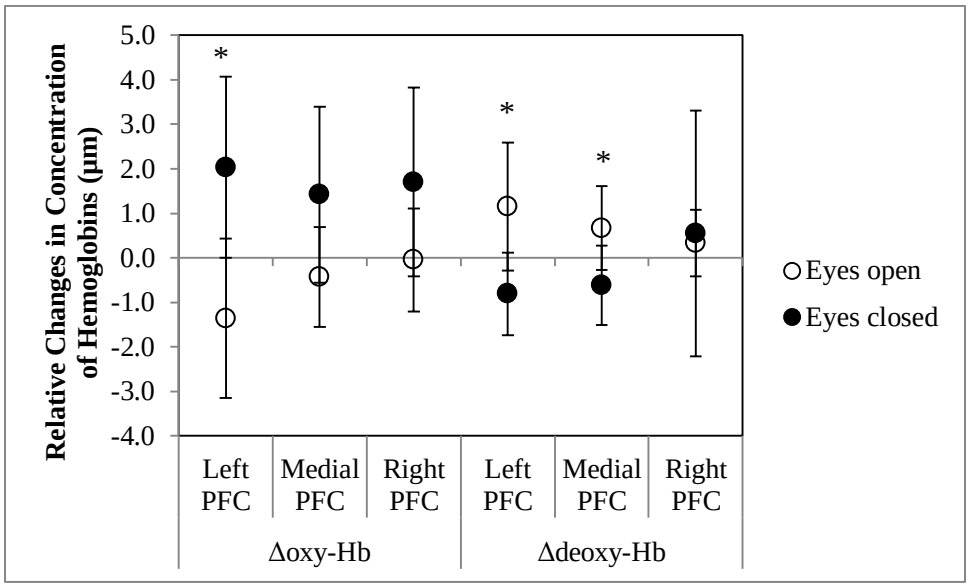

Figure 3.3: Regional concentration of Δoxy-Hb and Δdeoxy-Hb during eyes open and eyes closed conditions in younger children (bars represent standard deviation) (* $p < .05$).

Table 3.9: Paired-comparison results of variations between eyes open and eyes closed in each region in each group. Δoxy-Hb and Δdeoxy-Hb were compared separately.

	Δoxy-Hb			Δdeoxy-Hb		
	t	df	p	t	df	p
Younger children						
Left PFC	3.316	11.807	.006*	-3.034	10.295	.012*
Medial PFC	2.159	9.483	.058	-2.615	11.960	.023*
Right PFC	1.917	9.277	.086	.199	6.883	.848
Older children						
Left PFC	-1.270	6.828	.245	-1.129	7.975	.292
Medial PFC	-.984	5.650	.365	-1.374	6.702	.214
Right PFC	1.677	4.723	.158	.009	6.303	.993
Adults						
Left PFC	1.689	45.283	.098	.297	40.298	.768
Medial PFC	1.607	43.912	.115	.581	44.291	.564
Right PFC	1.780	45.739	.082	1.060	45.572	.295

* $p < .05$.

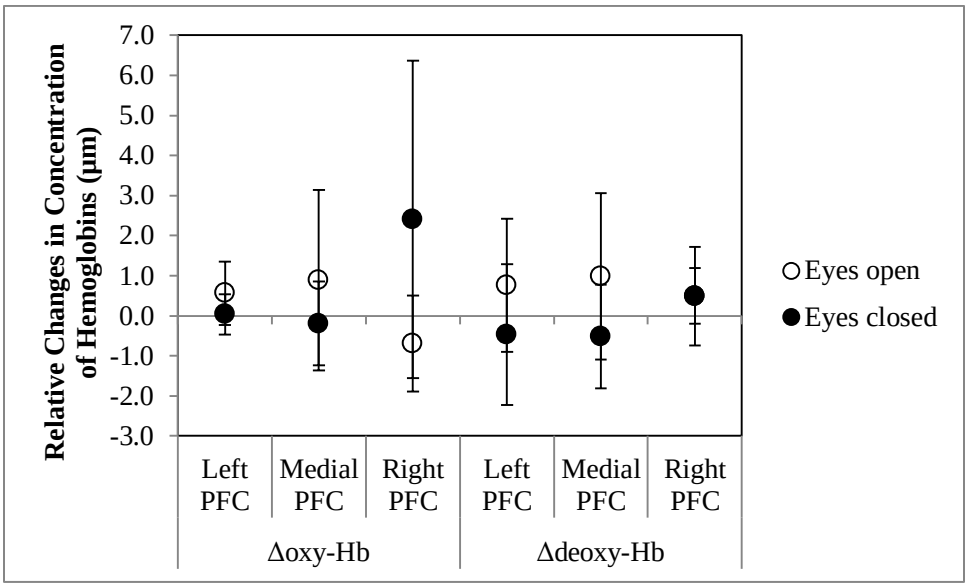

Figure 3.4: Regional concentration of Δoxy-Hb and Δdeoxy-Hb during eyes open and eyes closed conditions in older children (bars represent standard deviation).

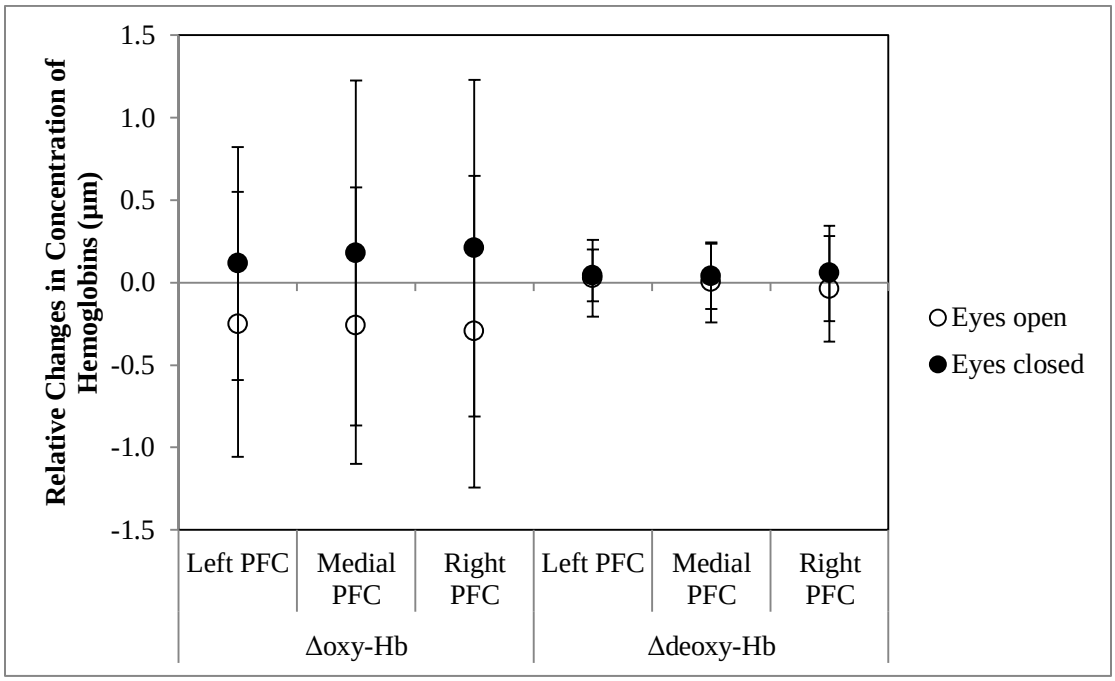

Figure 3.5: Regional concentration of Δoxy-Hb and Δdeoxy-Hb during eyes open and eyes closed conditions in adults (bars represent standard deviation).

Table 3.10: Paired-comparison results of variations between eyes conditions as a function of age in Δoxy-Hb and Δdeoxy-Hb.

		Δoxy-Hb			Δdeoxy-Hb		
		t	df	p	t	df	p
YC vs. OC	LPFC	-.891	11.238	.392	0.671	10.868	0.516
	MPFC	-1.018	10.007	.333	1.437	10.218	0.181
	RPFC	-.076	11.492	.941	1.071	8.417	0.314
YC vs. Adults	LPFC	-.368	4.825	.728	-0.131	8.000	0.899
	MPFC	1.546	4.326	.192	0.763	5.038	0.480
	RPFC	1.542	6.718	.169	0.941	5.047	0.389
OC vs. Adults	LPFC	.149	43.950	.883	0.178	44.493	0.859
	MPFC	.282	43.603	.779	0.543	37.560	0.590
	RPFC	.123	45.983	.903	0.378	41.783	0.707

LPFC = variations in the left prefrontal cortex between eyes open and eyes closed, MPFC = variations in the medial prefrontal cortex between eyes open and eyes closed, RPFC = variations in the right prefrontal cortex between eyes open and eyes closed, YC = younger children, OC = older children.

Table 3.11: Paired-comparison results of variations in each region in the PFC as a function of age.

		Δoxy-Hb, open			Δdeoxy-Hb, open			Δoxy-Hb, closed			Δdeoxy-Hb, closed		
		t	df	p	t	df	p	t	df	p	t	df	p
M – L	YC vs. OC	-.811	9.421	.437	.353	11.839	.731	1.564	11.594	.145	.958	6.842	.371
	YC vs. Adults	.407	11.404	.692	-.593	10.234	.566	.420	11.773	.682	.972	6.594	.365
	OC vs. Adults	1.148	8.270	.283	-.875	9.542	.403	-.981	10.900	.348	.126	11.647	.902
R – M	YC vs. OC	-1.159	5.815	.292	-1.666	5.732	.149	1.366	4.133	.242	1.356	4.992	.233
	YC vs. Adults	-1.385	7.706	.205	-1.791	7.926	.111	1.405	4.167	.230	1.108	4.720	.321
	OC vs. Adults	.189	6.529	.855	.508	6.054	.629	-.084	7.896	.935	-.049	7.794	.962
R – L	YC vs. OC	-.321	45.998	.749	-.400	45.942	.691	-.251	25.296	.804	.520	45.005	.605
	YC vs. Adults	-.371	44.118	.713	-.849	40.538	.401	.185	45.425	.854	.288	38.733	.775
	OC vs. Adults	-.085	44.012	.932	-.542	39.724	.591	.549	25.875	.588	-.080	35.417	.936

M – L = medial prefrontal cortex – left prefrontal cortex; R – M = right prefrontal cortex –medial prefrontal cortex; R – L = right prefrontal cortex – left prefrontal cortex; YC = younger children; OC = older children.

3.4 Discussion

This study was conducted to examine developmental changes of regional brain activation in the PFC during resting states by measuring Δoxy-Hb and Δdeoxy-Hb. More specifically, activation in the left PFC, medial PFC, and right PFC was examined. The medial PFC is a subsection of the DMN, the spontaneous activation during resting states. We expected to see a higher activation in the medial PFC compared to the lateral PFCs during resting states in all groups. However, our results did not find any regional differences. In addition, no differences were seen when comparing the medial PFC to the lateral PFC (the left and the right PFC were combined) either. However, significant differences between the eyes open and eyes closed conditions were found in the left and the medial PFC in younger children.

One of the possible explanations of the differences we found in the left and medial PFC but not the right PFC is functional asymmetry in the brain hemispheres. It is well known that the left hemisphere is more involved in the processing of verbal information, whereas the right hemisphere is more involved in spatial functions (Nebes, 1974). Similar functional asymmetry is also found in the PFC. During information processing, activation of the PFC is left-lateralized during memory encoding but right-lateralized during memory retrieval (Nyberg et al., 1996; Tulving et al., 1994). The PFC is lateralized to stimulus type as well. The left PFC is preferred in processing verbal stimuli but the right PFC is preferred for nonverbal or image materials (Kelley et al., 1998). Our study examined the brain activity under task-free resting states in which no information processing was involved. But the image provided during eyes open condition may perceived as a visual image to younger children who had less experience in a computer, resulted in an increased activity in the

right PFC whereas the activity in the left PFC decreased. Therefore, an asymmetrical response in the left and right PFC were seen in younger children.

Another possible explanations of the differences we is the existence of fluctuations in hemoglobin in the PFC. Hoshi and Tamura (1997) have reported fluctuations in blood flow and oxygenation during resting states in the PFC. Different fluctuation patterns have been observed in different regions in the PFC. A significant change in Δoxy-Hb was found in left lateral frontal region while no changes were observed in the left medial PFC (Hoshi and Tamura, 1997). This could explain the differences seen between the left PFC and the medial PFC during eyes open condition. Combined with the more fragmented DMN and more widespread activation in children (Fair et al., 2008; Gaillard et al., 2000), the greater fluctuation in blood flow in the left PFC resulted in a larger change in Δoxy-Hb and Δdeoxy-Hb in younger children.

While brain activation was significantly affected by the different eyes conditions, our results failed to find regional differences in the PFC. We expected to see a higher activation in the medial PFC because it is part of the DMN and the DMN has a higher activation during resting states. However, the DMN was first noticed from the consistent task-induced deactivation in certain region. A higher activity in resting states compared to task states does not necessary mean a higher activation in resting states compared to other non-DMN regions. However, the sample size of our study was too small and our participants were too heterogeneous to allow a final conclusion.

3.5 Conclusion

The results of current study showed asymmetry activation responses in the PFC in younger children with a higher activation in the eyes closed condition compared to the eyes open condition. Brain activation in the PFC had no regional differences in older children and adults.

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Chapter 4

BRAIN ACTIVATION IN THE PREFRONTAL CORTEX DURING MOTOR AND COGNITIVE TASKS IN ADULTS

Abstract

The prefrontal cortex (PFC) plays an important role in cognitive function. It is involved in executive functions (EFs) including planning, working memory, and inhibition. Tower of Hanoi is a commonly used tool to assess EF and has demonstrated sensitivity to PFC dysfunction. However, limited neuroimaging evidence is available to support the contribution of the PFC in the Tower of Hanoi. In the current study, we use functional near infrared spectroscopy (fNIRS) to examine hemodynamic response associated with neural activity in the PFC in adults during the Tower of Hanoi. We compared changes in cerebral oxygenation during resting, a motor task (tapping), and the Tower of Hanoi in a total of 17 adults. fNIRS data was taken throughout each condition, and relative changes in concentration of oxygenation (Δoxy-Hb) and deoxygenation (Δdeoxy-Hb) were measured. Performance on the Tower of Hanoi was measured by number of moves used to complete each level and the highest level of successful performance (3, 4, or 5 disks). We found a significant higher value of Δoxy-Hb during the Tower of Hanoi. An opposite change was observed in Δdeoxy-Hb that the value of Δdeoxy-Hb during the Tower of Hanoi was the lowest among 3 conditions.

4.1 Introduction

The prefrontal cortex (PFC) is involved in executive functions (EFs) that include cognitive processes such as decision making, working memory, planning, inhibition of responses, and cognitive flexibility. It is seen as the center of cognitive functions. Nevertheless, the PFC also contributes to motor functions. The activation in the PFC has been linked to motor timing, movement selection, and control of gait (Bortoletto & Cunnington, 2010; Koenraadt et al., 2014; Kram et al., 1998). Motor timing is related to the decision on when to start a movement and determining the appropriate time for movement initiation (Bortoletto & Cunnington, 2010). Movement selection helps to suppress automatically triggered response and prepare for proper reaction to the task (Kram et al., 1998). Examining the activity in the PFC can help us to understand neural basis of motor control and the knowledge can be applied to population with motor dysfunctions. In current study, task-induced activity in the PFC was measured during a simple motor task and an EF task to examine the role of the PFC in each task.

Many tasks have been used to assess EFs of the PFC. Tower of Hanoi is one of commonly used tools to assess EFs (Goel & Grafman, 1995). The game consists of three vertical pegs and a number of disks graduated in size with the largest at the bottom. The object of the game is to move all the disks from a start state to a goal state while following two rules: 1) only one disk may be moved at a time, and 2) a disk may never be placed on top of a disk smaller than itself. The completion of the Tower of Hanoi requires complex EFs including inhibition, working memory, and planning (Welsh et al., 1999; Welsh & Huizinga, 2005).

Due to the EF elements required in completion of the Tower of Hanoi, it has been used in patients with PFC lesions (Goel & Grafman, 1995). Patients with PFC

lesions performed worse than the control group but the strategy they used to solve the puzzle was similar to the one used by the controls. The finding suggested that these patients had deficits in inhibition control rather than planning. Nonetheless, the contribution of the PFC during the Tower of Hanoi has been verified by studies using fMRI (Anderson et al., 2005; Fincham et al., 2002) and EEG (Guevara et al., 2012; Guevara et al., 2013). Increased prefrontal-parietal intrahemispheric correlation was observed during Tower of Hanoi task in both left and right hemispheres (Guevara et al., 2012). Changes in absolute power of EEG activity in the PFC were also observed during the Tower of Hanoi (Guevara et al., 2013). The left prefrontal regions have been associated with the process of the Tower of Hanoi in right-handed participants (Anderson et al., 2005). However, limited resources can be found on using functional near-infrared spectroscopy (fNIRS) to examine brain activity during the Tower of Hanoi.

fNIRS measures the hemodynamics changes based on the optical properties of hemoglobins. It provides a balance between spatial and temporal resolution for monitoring local and instance changes of oxygenation in cerebral cortex (Babiloni et al., 2009; Chance et al., 1998; Dieler et al., 2012; Lloyd-Fox et al., 2010; Weishaupt et al., 2008). In addition, fNIRS is more tolerant to motion artifacts making it more feasible for clinical studies, especially in motor tasks (Ayaz et al., 2010; Izzetoglu et al., 2010). The aim of current study was to compare the activation in the PFC during resting, a simple motor task, and the Tower of Hanoi to investigate the involvement of the PFC in an EF task. We expected to see a higher activity in the PFC during the Tower of Hanoi.

4.2 Methods

Participants

A total of 18 participants were recruited in the study. All participants were students of the University of Delaware. They were healthy and without family history of mental disease. Exclusion criteria included: 1) previous head injury, 2) open wound on the forehead, 3) seizure disorder, and 4) allergy to rubbing alcohol. Data of one participant was removed because she fell asleep during the study, leaving a total of 17 adult participants (8 females and 9 males, 22.3 ± 3.1 years old) in the study.

Procedures

The study has been approved by the IRB at the University of Delaware (Appendix A). Informed consents were obtained from participants (Appendix F). After participants given the informed consents, the primary investigator explained the tasks to participants. There were two three-minute resting conditions, eyes open and eyes closed, and two two-minute tasks on an iPad, the Tower of Hanoi and a tapping task. Participants completed resting conditions first then the tasks on an iPad after one-minute break. Another one-minute break was provided between two iPad tasks. Participants practiced the Tower of Hanoi on the iPad until they understood the rules and was able to complete a 3-disk task. The sensor pad was applied on the forehead of participants after the practice. During eyes open resting condition, participants stared at a '+' sign (1 cm * 1 cm) in the middle of a monitor on a 15.6" laptop (Satellite L755-S5351, Toshiba). An auditory sound was provided to notify participants the start and the end of each condition. Participants were instructed to keep quiet and minimize their movement during data collection. The instructions for the resting tasks were: 'Try to relax and do nothing' and 'Do not fall asleep.' Data collection was completed within 40 minutes for all participants.

Tower of Hanoi. The Tower of Hanoi is a disk-transfer task. The goal of the task is to move disks from a start state to a goal state using the fewest number of moves possible while following two rules: 1) only one disk may be moved at a time, and 2) a disk may never be placed on top of a disk smaller than itself. Tower Hanoi task in our study required participants to move disks from the leftmost peg to the rightmost peg. Participants performed the task on an iPad using Tap Towers (Madcap Studios, Inc.), starting from 3 disks then progressed to 4 disks, 5 disks, and 6 disks if time allowed (Figure 4.1). The level participants reached and numbers of moves to achieve goal states of each level were recorded.

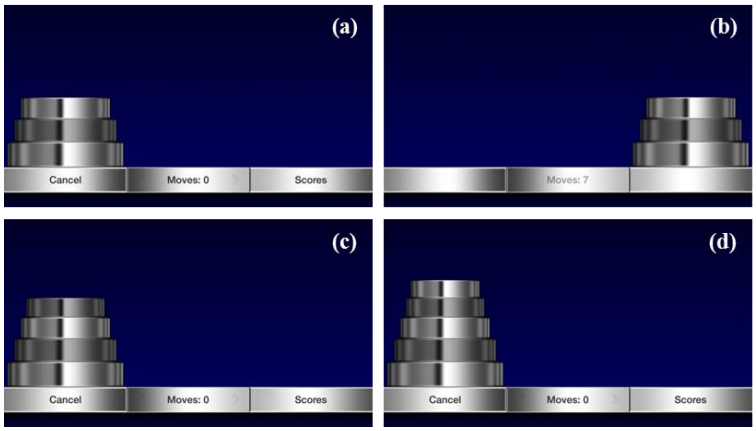

Figure 4.1: Tower of Hanoi. (a) Start state of 3-disk level. (b) End state of 3-disk level. (c) Start state of 4-disk level. (d) Start state of 5-disk level.

Tapping. Participants tapped two spots 13 cm away back and forth on an iPad at a self-selected pace for two minutes. The number of taps was recorded. This task was chosen as a simple motor task without any EF required.

Functional Near-infrared Spectroscopy (fNIRS)

The activity in the prefrontal cortex was measured by a fNIRS system (fNIR100A, Biopac System Inc.). fNIRS detects hemodynamic changes resulted from brain activation based on the general transparency of biological tissues to infrared light as well as optical properties of oxygenated-hemoglobin (oxy-Hb) and deoxygenated-hemoglobin (deoxy-Hb) (Jobsis, 1977). The fNIRS system has a sensor pad, a control box, and a computer installed with Cognitive Optical Brain Imaging (COBI) Studio software and fnirSoft for data collection and analysis. The sensor pad contains 4 light sources (LED) and 10 light detectors creating an array of 16 channels (Figure 4.2). The source-detector distance is 2.5 cm and the depth of penetration is approximately 1.5 cm beneath the scalp (Okada & Delpy, 2003a; Okada & Delpy, 2003b). The light sources and detectors are embedded in a flexible pad made with medical grade silicone. The pad can be worn as a headband and match the contour of the foreheads. The light sources emit infrared light at 730 nm and 850 nm which penetrates the scalp. Part of light is absorbed by hemoglobins and the rest of light reaches detectors in a banana-shape path. Oxygenated-hemoglobin and deoxygenated-hemoglobin has different absorption coefficient. Concentrations of hemoglobin can then be calculated by the ratio of light absorbed at different wavelengths. LED current and detector gain were adjusted before prior to the study to prevent signal saturation. Baseline measurement was obtained then the fNIRS signal was recorded at 2 Hz.

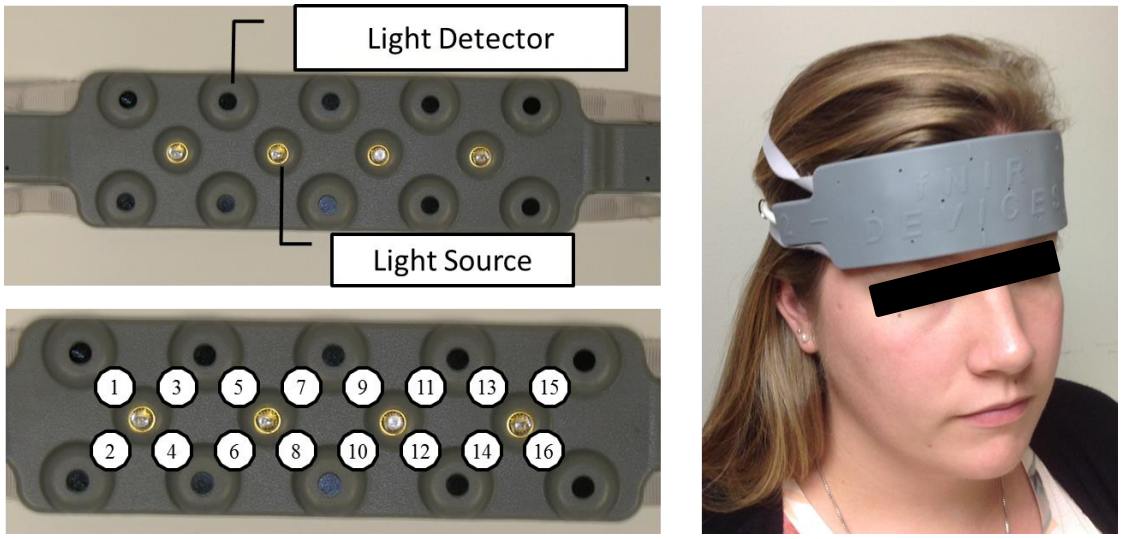

Figure 4.2: The sensor pad with 8 light detectors and 4 light sources (left, top) creates 16 channels (left, bottom). The sensor pad positioned on a participant (right).

Data Processing

Raw data was visually inspected and channels with saturated or very low signal were removed. Optodes on the sensor pad may not contact with the skin completely and resulted in saturated channels. Low signal was due to interference with light transmission from hair. Motion artifacts were excluded manually. Raw light intensity was filtered by a low-pass FIR filter with an order of 20 and cutoff frequency at 0.1 Hz to attenuate the high frequency noise, respiration, and cardiac cycle effects (Izzetoglu et al., 2005). Relative changes of concentration in oxy-Hb (Δoxy-Hb) and deoxy-Hb (Δdeoxy-Hb) was calculated collection using modified Beer-Lambert law, with a 10-second baseline recorded at the beginning of data collection. Data was processed using fnirSoft (Ayaz, 2010). The first and the last 15 seconds of each condition were removed to prevent unstable state at the beginning of the test and the effect from previous activity (Izzetoglu et al., 2007). The remaining data points were averaged in each channel for each task. Z scores were calculated using mean and standard deviation of all 16 channels in each task of each participant using z= (x-mean)/SD. Channels with extreme values (z scores above 2 or less than -2) were removed as outliers (Van Selst & Jolicoeur, 1994). Remaining channels were averaged for each condition.

Statistics

One-way repeated measures ANOVA and Bonferroni post hoc tests were used to examine the differences in Δoxy-Hb and Δdeoxy-Hb among the resting state, tapping, and the Tower of Hanoi. The eyes open condition was used for resting state due to the finding in the chapter 2.

4.3 Results

All participants completed 3-disk level in 2 minutes (average moves 9.9 ± 4.3). Eleven of them went on to finish 4-disk level (average moves 25.1 ± 9.3) and 3 participants completed 5-disk level (average moves 39.3 ± 4.9) within the time limit. A significant difference was found in Δoxy-Hb among the resting state, tapping, and the Tower of Hanoi, $F(2,32) = 3.309$, $p = .049$, partial eta squared = .171. Post hoc analysis indicated a significant difference between tapping, and the Tower of Hanoi ($p = .033$). No significant difference was found between the resting state and tapping ($p = 1.000$). There was no significant difference between the resting state and the Tower of Hanoi ($p = .152$) (Figure 4.3). There was also a significant difference among 3 conditions in Δdeoxy-Hb, $F(2,32) = 3.312$, $p = .049$, partial eta squared = .171 (Figure 4.4). However, no significant differences between conditions were found in post hoc analysis.

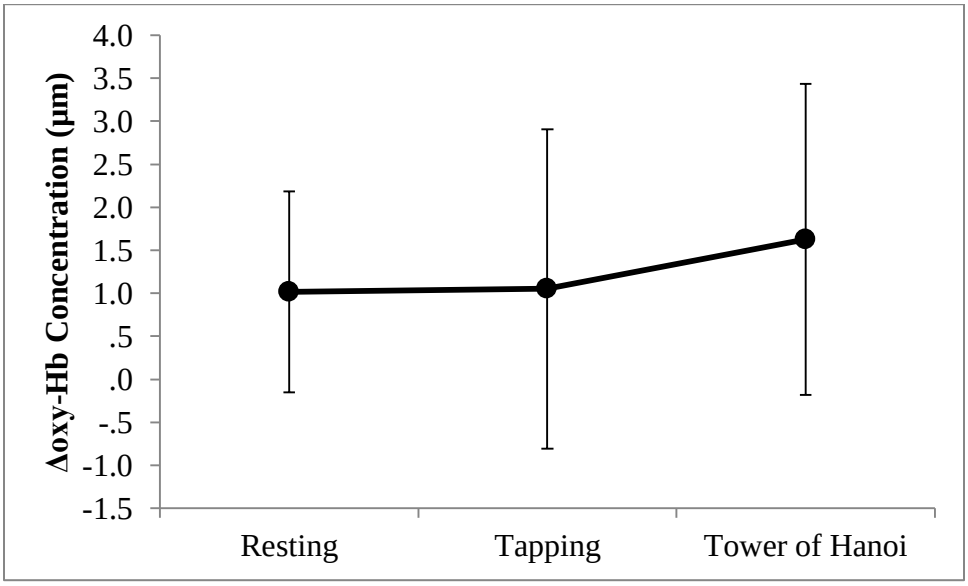

Figure 4.3: Δoxy-Hb in adults during resting, tapping, and the Tower of Hanoi (bars represent standard deviation).

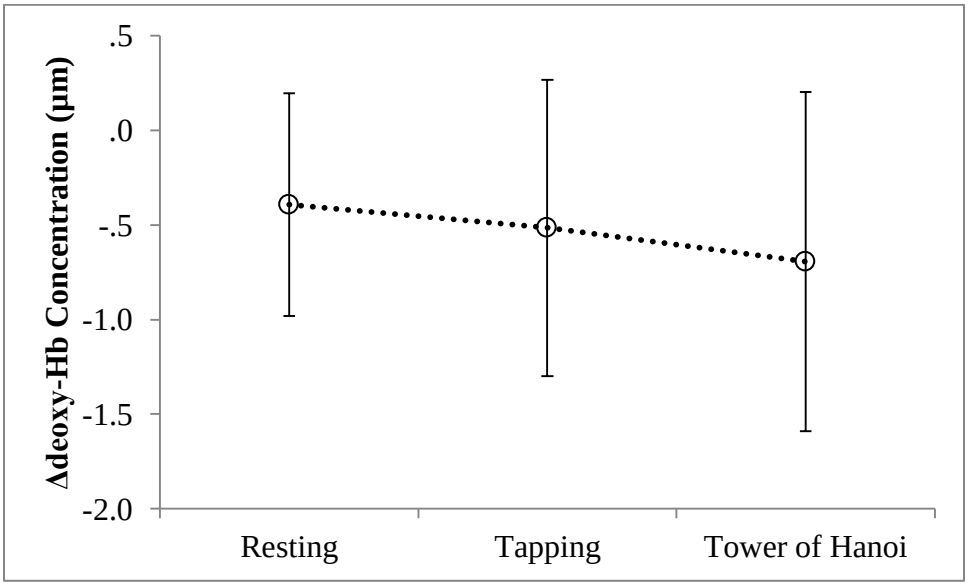

Figure 4.4: Δdeoxy-Hb in adults during resting, tapping, and the Tower of Hanoi (bars represent standard deviation).

4.4 Discussion

The aim of current study was to examine the changes of brain activity during the Tower of Hanoi. Δoxy-Hb and Δdeoxy-Hb were measured by fNIRS during resting, a simple motor task, and the Tower of Hanoi in adults. Our results found an increased Δoxy-Hb during Tower of Hanoi. An increase in Δoxy-Hb indicated increases in cerebral blood volume in response to neuronal activation (Kato et al., 1993). The finding was corresponding with previous studies exploring the involvement of the PFC in problem solving tasks (Boghi et al, 2006; Newman et al., 2003; Ruh et al., 2012; Wagner et al., 2006). An increase of activity in the PFC was observed during the Tower of Hanoi but not the simple motor task because the simple motor task did not require planning or other EFs. The motor task may induce activation in other brain regions such as motor area. However, it was not measured in our study.

While Δoxy-Hb increased with the increasing cognitive load during the Tower of Hanoi, the other variable we measured, Δdeoxy-Hb, decreased at the same time. The responses of these variables matched what we expected in hemodynamic changes associated with increased brain activity. An increase in neuronal activity induces an initial increase of Δdeoxy-Hb. Within seconds, there is an increase in cerebral blood flow (Hu et al., 1997; Malonek & Grinvald, 1996). The supply of blood flow exceeds the amount needed, results in an increase in Δoxy-Hb and decrease in Δdeoxy-Hb (Fox et al., 1988).

In current study, we examined the average amount of changes in a period of time. We did not follow the time course of changes in oxygenation. In the future, we can utilize the advantage of fNIRS in temporal resolution to examine the temporal characteristics of the hemodynamic changes in response to neuronal activity.

4.5 Conclusion

Current study found a significant increase in the PFC activation during the Tower of Hanoi, an EF task, but not a simple motor task in adults. The result confirmed the involvement of the PFC in the EF task. In addition, we found Δoxy-Hb and Δdeoxy-Hb responded differently to an increased cognitive load. Δoxy-Hb increased as the activity in the PFC increased, while Δdexoy-Hb decreased as the activity in the PFC increased.

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Chapter 5

BRAIN ACTIVATION IN THE PREFRONTAL CORTEX DURING MOTOR AND COGNITIVE TASKS IN CHILDREN WITH AND WITHOUT AUTISM

Abstract

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder characterized with deficits in social communication, repetitive behavioral patterns, and often, movement dysfunction. Researchers have linked some of these issues to atypical prefrontal cortex (PFC) activity. In the current study, we use functional near infrared spectroscopy (fNIRS) to examine hemodynamic response associated with neural activity in the PFC in boys with and without ASD. We compared changes in cerebral blood flow during an executive function task (Tower of Hanoi) and a motor task (tapping) in a total of 16 participants (10 typically developed (TD) children and 6 children with ASD). Performance on the Tower of Hanoi was measured by number of moves to complete each level and the highest level of successful performance (3, 4, or 5 disks). fNIRS data were taken throughout each condition, and relative changes in concentration of oxygenation (Δoxy-Hb) and deoxygenation (Δdeoxy-Hb) were measured. No significant difference was found in changes of Δoxy-Hb and Δdeoxy-Hb among conditions. However, the performance of Tower of Hanoi in children with ASD was worse than TD children that the ASD group finished 3-disk level with more moves. The lower performance in the Tower of Hanoi suggested the ASD group had difficulty organizing and planning during the Tower of Hanoi task.

5.1 Introduction

Autism spectrum disorders (ASD) is one of the most common developmental disabilities affecting 1 in 68 children in the U.S. (CDC, 2014). It has been estimated that costs up to $3.2 million are needed to care for an individual with ASD over his or her lifetime (Ganz, 2007). Those with ASD often present with deficits in social communication and interactions, as well as restricted, repetitive patterns of behavior, interests, and activities. Patients with prefrontal lobe lesions often exhibit social impairments that are similar to the behaviors seen in individuals diagnosed with ASD suggesting that an abnormal prefrontal lobe may lead to the deficits present in individuals with ASD (Bar-On et al., 2003). Dysfunctions in the PFC render individuals unable to plan and control their behaviors in a usual manner, resulting in restricted and repetitive behaviors. The PFC also plays an important role in processing self-relevant information and autobiographical memory, which is in line with the difficulties individuals with ASD encounter (Griebling et al., 2010). Recently, motor dysfunctions including gait abnormalities, delayed motor development, poor coordination, and decreased postural control have been found to be pervasive symptoms in individuals with ASD (Fournier et al., 2010; Jansiewicz et al., 2006; Landa & Garrett-Mayer, 2006; Vilensky et al., 1981). Deficits in the motor planning and execution have been identified in children with ASD suggesting that abnormal functioning PFC may be related to motor disturbances observed in individuals with ASD (Glazebrook et al., 2006; Rinehart et al., 2006). Examining brain activity in the PFC can lead to a better understanding of the neuronal basis of both behavioral and motor abnormalities in individuals of ASD.

Tower of Hanoi has been widely used as a diagnostic tool for evaluating executive functions (EFs) (Goel & Grafman, 1995). The game consists of three

vertical pegs and several disks graduated in size. The object of the game is to move all the disks from a start state to a goal state while following two rules: 1) only one disk may be moved at a time, and 2) a disk may never be placed on top of a disk smaller than itself. To successfully solve Tower of Hanoi puzzles requires complex EFs including inhibition control, working memory, and planning (Welsh et al., 1999; Welsh & Huizinga, 2005).

The Tower of Hanoi has been used as a tool to examine development of EFs (Ahonniska et al., 2001; Bishop et al., 2001; Klahr & Robinson, 1981). Although using different strategies from adults, preschoolers demonstrated the ability to complete the Tower of Hanoi (Klahr & Robinson, 1981). Griebling and colleagues (2010) examined the relationship between the volume of the frontal lobe and the performance of the Tower of Hanoi in children with ASD. They found that the performance of children with ASD was worse than the control group. However, no difference was found in the volumetric measurements. Also, no relationship was found between the volume of the frontal lobe and the performance of Tower of Hanoi. In our knowledge, there is no study examine the relationship between the activity in the PFC and the performance of the Tower of Hanoi in children with ASD.

The aim of current study was to examine the activation in the PFC during the Tower of Hanoi in children with and without autism. The performance of the Tower of Hanoi was also compared between groups. We used functional near infrared spectroscopy (fNIRS) to measure the hemodynamics and oxygenation changes in the PFC. fNIRS has relatively good spatial and temporal resolution for monitoring local and instance changes of oxygenation in cerebral cortex (Babiloni et al., 2009; Chance et al., 1998; Dieler et al., 2012; Lloyd-Fox et al., 2010; Weishaupt et al., 2008). In

addition, fNIRS is less vulnerable to motion artifacts making it more feasible for clinical studies, especially in children (Ayaz et al, 2010; Izzetoglu et al., 2010). One of the challenges that researchers encounter when working with younger children in neuroimaging studies is their cooperation and their ability to be stationary (Poldrack et al., 2002). Children often exhibit a certain degree of movement even with the best effort to be motionless. In fMRI studies, movement less than 1mm is enough to create false activation artifacts that cannot be corrected using postprocessing motion correction techniques (Field et al., 2000). On the other hand, various methods can be applied in fNIRS data to remove the noise of signals and reduce the rejection of trials (Cooper et al., 2012; Lloyd-Fox et al., 2010; Robertson et al., 2010; Sweeney et al., 2011). We expected to see differences in the PFC activations and the performance of the Tower of Hanoi between two groups.

5.2 Methods

Participants

Total of 21 participants were recruited in the study including 11 children with ASD and 10 age- and gender-matched typically developed (TD) children. Children with ASD were recruited in Delaware and Pennsylvania areas. Flyers were sent to Autism organizations in the area and distributed by the organizations to the families with children of autism. Participants in ASD group were also recruited through a pediatric physical therapist. TD children were recruited from boys and girls clubs and a local school. Families were contacted by principal investigator through emails and phones if they were interested in the study. They were invited to the developmental motor control lab of the University of Delaware if their children matched inclusion criteria: 1) meet Diagnostic and Statistical Manual of Mental Disorders, Fourth

Edition (DSM-IV) criteria for ASD and have previous diagnosis from physicians (for ASD group), 2) be able to understand the researchers, and 3) be able to stay in sitting position and rest for 6 minutes, and 4) be able to follow two-step directions, directions with one basic conjunction or additional phrase, e.g.: sit down on the chair and rest for 6 minutes. Participants were excluded if they had: 1) previous head injury, 2) open wound on the forehead, 3) seizure disorder, and 4) allergy to rubbing alcohol. Data was collected in local churches, boys and girls clubs, and schools for participants who were unable to visit the lab. Four children with ASD did not complete the study. One felt uncomfortable wearing the sensor pad and three were not cooperative to finish the test. Another participant of ASD was removed due to extreme values. Total numbers of participants for further analysis were 6 ASD children (all males, 11.8 ± 3.5 years old), and 10 TD children (all males, 12.3 ± 2.6 years old).

Procedures

The study has been approved by the IRB at the University of Delaware (Appendix A). Informed consents were obtained from parents of participants (Appendix G). Informed assent was obtained from participants as well (Appendix H). After participants given the informed consents or assent, the primary investigator explained the tasks to participants. There were two three-minute resting conditions, eyes open and eyes closed, and two two-minute tasks on an iPad, the Tower of Hanoi and a tapping task. Participants completed resting conditions first then the tasks on an iPad after one-minute break. Another one-minute break was provided between two iPad tasks. Participants practiced the Tower of Hanoi on the iPad until they understood the rules and was able to complete a 3-disk task. The sensor pad was applied on the forehead of participants after the practice. During eyes open resting condition,

participants stared at a '+' sign (1 cm * 1 cm) in the middle of a monitor on a 15.6" laptop (Satellite L755-S5351, Toshiba). An auditory sound was provided to notify participants the start and the end of each condition. Participants were instructed to keep quiet and minimize their movement during data collection. The instructions for the resting tasks were: 'Try to relax and do nothing' and 'Do not fall asleep.' Data collection was completed within 40 minutes for all participants.

Tower of Hanoi. The Tower of Hanoi is a disk-transfer task. The goal of the task is to move disks from a start state to a goal state using the fewest number of moves possible while following two rules: 1) only one disk may be moved at a time, and 2) a disk may never be placed on top of a disk smaller than itself. Tower Hanoi task in our study required participants to move disks from the leftmost peg to the rightmost peg. Participants performed the task on an iPad using Tap Towers (Madcap Studios, Inc.), starting from 3 disks then progressed to 4 disks, and 5 disks if time allowed (Figure 5.1). The level participants reached and numbers of moves to achieve goal states of each level were recorded.

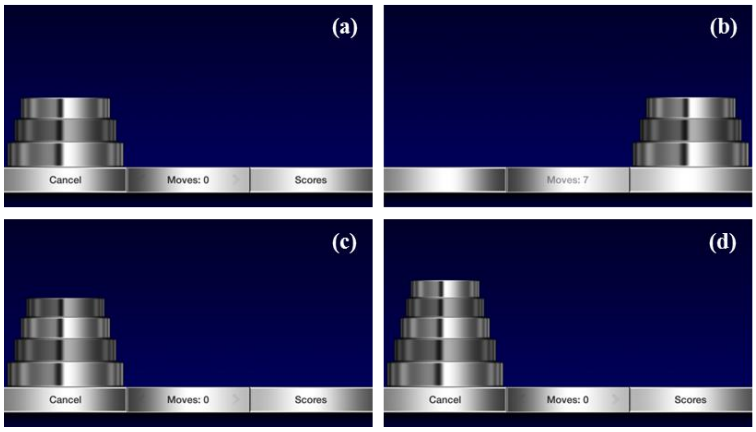

Figure 5.1: Tower of Hanoi. (a) Start state of 3-disk level. (b) End state of 3-disk level. (c) Start state of 4-disk level. (d) Start state of 5-disk level.

Tapping. Participants tapped two spots 13 cm away back and forth on an iPad at a self-selected pace for two minutes. The number of taps was recorded. This task was chosen as a simple motor task without any EF required.

Functional Near-infrared Spectroscopy (fNIRS)

The activity in the prefrontal cortex was measured by a fNIRS system (fNIR100A, Biopac System Inc.). fNIRS detects hemodynamic changes resulted from brain activation based on the general transparency of biological tissues to infrared light as well as optical properties of oxygenated-hemoglobin (oxy-Hb) and deoxygenated-hemoglobin (deoxy-Hb) (Jobsis, 1977). The fNIRS system has a sensor pad, a control box, and a computer installed with Cognitive Optical Brain Imaging (COBI) Studio software and fnirSoft for data collection and analysis. The sensor pad contains 4 light sources (LED) and 10 light detectors creating an array of 16 channels (Figure 5.2). The source-detector distance is 2.5 cm and the depth of penetration is approximately 1.5 cm beneath the scalp (Okada & Delpy, 2003a; Okada & Delpy,

2003b). The light sources and detectors are embedded in a flexible pad made with medical grade silicone. The pad can be worn as a headband and match the contour of the foreheads. The light sources emit infrared light at 730 nm and 850 nm which penetrates the scalp. Part of light is absorbed by hemoglobins and the rest of light reaches detectors in a banana-shape path. Oxygenated-hemoglobin and deoxygenated-hemoglobin has different absorption coefficient. Concentrations of hemoglobin can then be calculated by the ratio of light absorbed at different wavelengths. LED current and detector gain were adjusted before prior to the study to prevent signal saturation. Baseline measurement was obtained then the fNIRS signal was recorded at 2 Hz.

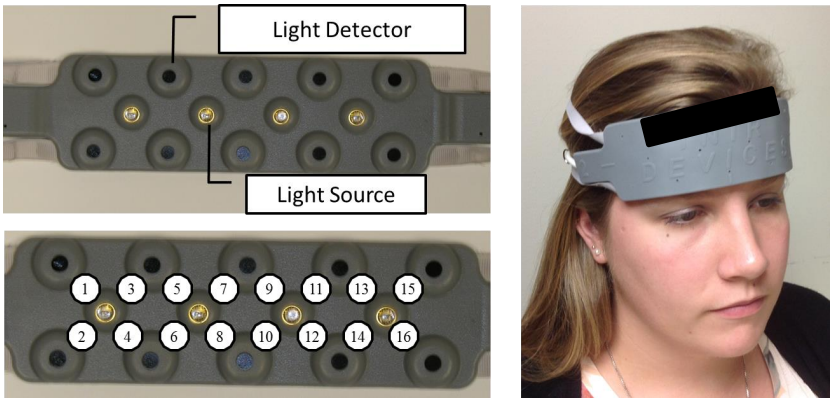

Figure 5.2: The sensor pad with 8 light detectors and 4 light sources (left, top) creates 16 channels (left, bottom). The sensor pad positioned on a participant (right).

Data Processing

Raw data was visually inspected and channels with saturated or very low signal were removed. Optodes on the sensor pad may not contact with the skin

completely and resulted in saturated channels. Low signal was due to interference with light transmission from hair. Motion artifacts were excluded manually. Raw light intensity was filtered by a low-pass FIR filter with an order of 20 and cutoff frequency at 0.1 Hz to attenuate the high frequency noise, respiration, and cardiac cycle effects (Izzetoglu et al., 2005). Concentration of oxy-Hb (Δoxy-Hb) and deoxy-Hb (Δdeoxy-Hb) was calculated collection using modified Beer-Lambert law, with a 10-second baseline recorded at the beginning of data collection. Data was processed using fnirSoft (Ayaz, 2010). The first and the last 15 seconds of each condition were removed to prevent unstable state at the beginning of the test and the effect from previous activity (Izzetoglu et al., 2007). The remaining data points were averaged in each channel for each task. Z scores were calculated using mean and standard deviation of all 16 channels in each task of each participant using z= (x-mean)/SD. Channels with extreme values (z scores above 2 or less than -2) were removed as outliers (Van Selst & Jolicoeur, 1994). Remaining channels were averaged for each condition.

Statistics

A two-way repeated measures ANOVA was used to examine the effects of group (between subject factor, TD vs. ASD) and condition (within subject factor, resting, tapping, Tower of Hanoi) on Δoxy-Hb and Δdeoxy-Hb, respectively. The eyes open condition was used for resting state due to the finding in the chapter 2. T-test was used to examine moves used to complete each Tower of Hanoi level and numbers of tapping between 2 groups. Pearson chi-square test was used to examine the relationship between group and the highest level achieved.

5.3 Results

All participants completed 3-disk level in 2 minutes. Seven TD children and 2 children with ASD completed 4-disk level as well. The performance of the Tower of Hanoi and tapping was presented in Table 5.1.

Table 5.1: Behavioral performance on the Tower of Hanoi and tapping tasks.

		TD	ASD
Tower of Hanoi			
3-disk	# of participants completed	10	6
	# of moves used	9.8 ± 3.3	21.3 ± 12.0
4-disk	# of participants completed	7	2
	# of moves used	22.4 ± 3.7	22.5 ± 4.9
Tapping #		216.7 ± 69.6	206.3 ± 69.0

A significant difference was found in moves used to complete 3-disk level, $t(14) = -2.903$, $p = .012$. Children with ASD used a significant higher amount of moves to complete 3-disk level. No significant difference was found in the moves used to complete 4-disk level among two groups, $t(7) = -.021$, $p = .984$. There was no significant association between the group and the highest level achieved, $X^2(1) = 2.049$, $p = .152$. However, the odds of completing 4-disk level were 2.1 times higher in children without ASD than that in children with ASD based on the odds ratio.

A two-way repeated measures ANOVA found no significant interaction of condition and group on Δoxy-Hb, $F(2,28) = 1.112$, $p=.343$, partial eta squared = .074. There was no main effect of condition on Δoxy-Hb, $F(2,28) = .256$, $p = .776$, partial eta squared = .018 (Figure 5.3).

There was no significant interaction of condition and group on Δdeoxy-Hb, $F(2,28) = 3.134$, $p=.059$, partial eta squared = .183. There was no main effect of

condition on Δdeoxy-Hb, F(2,28) = .173, p = .842, partial eta squared = .012 (Figure 5.4).

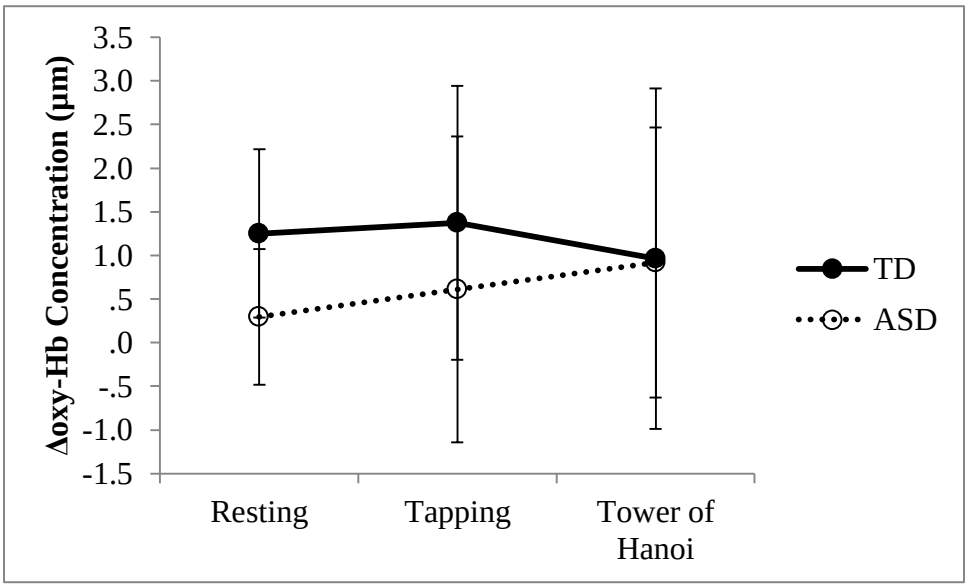

Figure 5.3: Δoxy-Hb in both groups during resting, tapping, and the Tower of Hanoi (bars represent standard deviation).

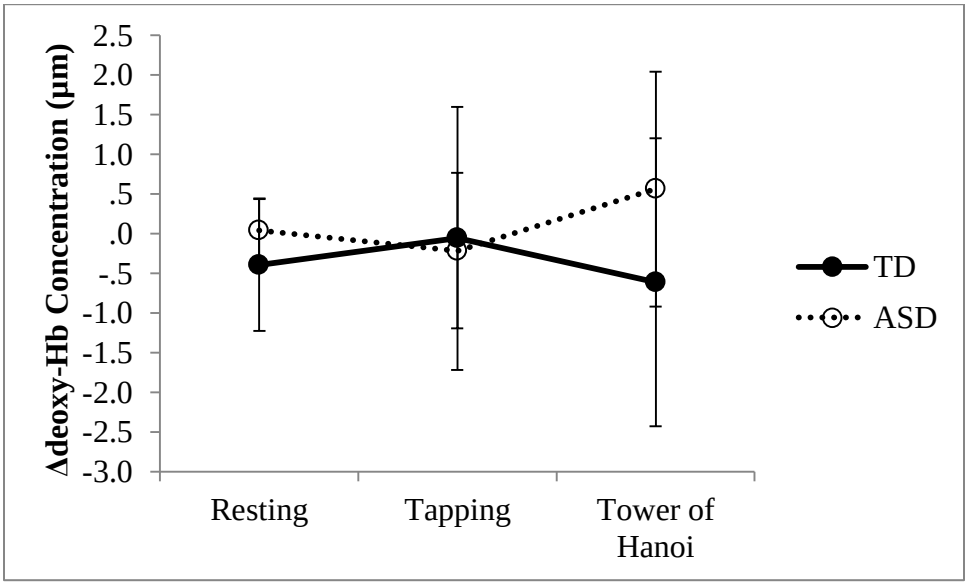

Figure 5.4: Δdeoxy-Hb in both groups during resting, tapping, and the Tower of Hanoi (bars represent standard deviation).

5.4 Discussion

The aim of current study was to examine the performance of the Tower of Hanoi and changes of brain activity during the task. Δoxy-Hb and Δdeoxy-Hb were measured by fNIRS during resting, a simple motor task, and the Tower of Hanoi, in children with and without ASD. There were no main effects of group and condition on Δoxy-Hb and Δdeoxy-Hb. However, the performance of the Tower of Hanoi in children with ASD was worse than TD children.

In our study, the performance of the Tower of Hanoi in children with ASD was not compatible with the performance of TD children. Children of ASD took more moves to finish 3-disk level and had a lower ratio in achieving 4-disk level. The Tower of Hanoi requires participants to move disks from a start state to a goal state. In order to solve the problem in the most efficient way (minimum moves), a sequence of moves needs to be planned and executed. The ability to inhibit irrelevant responses is also essential to successful performance (Borys et al., 1982; Welsh et al., 1999). Our result was corresponding with previous studies examined EFs in children with ASD using the Tower of Hanoi, in which children with ASD took significant more moves and more trials to solve Tower of Hanoi problems (Griebling 2010; Ozonoff et al., 1991). The finding confirmed the deficits of EFs such as planning, working memory, and inhibition in children with ASD (Hughes et al., 1994; Ozonoff & Jensen, 1999; Ozonoff & Strayer, 1997; Ozonoff et al., 1994). The deficits in EF could be the reason why we did not see a significant increase in oxygenation in children with ASD during the Tower of Hanoi.

One of the possible reasons that we did not observe a higher activation during the Tower of Hanoi in TD children was the variation among participants. Our participants had a broad age range of 8 to 16 years old and they were in different

stages of maturation. It is possible that the effect of maturation on the activation in the PFC outweigh the effect of the condition. Therefore, there was no significant difference in the activation in the PFC among conditions. In the future, we should recruit more participants at the same age to increase the sample size at each maturation stage in order to examine the developmental changes in the PFC.

Another issue resulted in a small sample size was the completion rate of children with ASD. We recruited 11 participants but only 7 participants followed the instructions and completed the test. One of our participants refused to wear the sensor pad and another participant tried to remove the sensor pad during the testing. Another two participants were not cooperative and did not complete the test. Children with ASD have high comorbidity rate of attention deficit hyperactivity disorder (ADHD) and often presented with hyperactivity, inattention, and impulsiveness (Matson et al., 2013; Rao & Landa, 2013; van Steensel et al., 2013). In addition, hyper-sensitivity and hyper-response to tactile stimulation has been reported as characteristics observed in those with ASD (Baron-Cohen et al., 2009; Boyd et al., 2010). These features in children with ASD are issues researchers encounter when their cooperation is important to accommodate the study protocol and experimental devices. It was not clear the reason of unwillingness to complete the study but obviously, behavior issues were huge challenges to researchers who work with this population.

A limitation of the current study was the size of the sensor pad, which resulted in a higher number of rejected channels. Our fNIRS system had the light sources and light detectors embedded in a flexible silicon pad. Easy to apply on the participants was an advantage of the sensor pad. However, the design did not allow us to adjust individual light detector or light source in order to obtain the optimal contact with the

skin. The disadvantage was prominent in children groups because the size of their foreheads. Younger children have smaller forehead volume than older children and adults (Sforza et al., 2008). There is a higher chance for part of the channels being blocked by the hair when the sensor pad applied on their foreheads. Also, it is harder to fit the adult-size sensor pad on the children to obtain optimal contact for all the optodes. The size of forehead should be considered when apply the same sensor pad in children populations.

5.5 Conclusion

The results of this study found that children with ASD had a poor performance in the Tower of Hanoi. However, there was no significant change in hemodynamic responses in the PFC during the Tower of Hanoi. Small sample size and variation among participants limited the finding of current study. Future study with a larger sample size at the same age is needed to clarify the developmental changes in EFs and activation in the PFC.

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Chapter 6

GENERAL CONCLUSIONS

In this work, brain activation in the prefrontal cortex (PFC) during resting states was examined in three age groups to explore the baseline activity in the PFC and the developmental changes of the default mode network (DMN). In the first study, we compared brain activation in the PFC between eyes open and eyes closed in different age groups. We found a significant effect of eyes condition on brain activation in the PFC. A higher activation in the PFC during eyes closed condition compared to eyes open condition was found in younger children but not older children and adults. However, the level of changes between eyes open and eyes closed condition in younger children did not differ from older children or adults. Since functional near infrared spectroscopy (fNIRS) measures relative changes in concentrations of oxygenated hemoglobin (Δoxy-Hb) and deoxygenated hemoglobin (Δdeoxy-Hb) between-subject differences cannot be compared directly by the measurements. However, we calculated regional changes in each participant and used those for between-subject comparison. To our knowledge, this is the first research of its kind examining relative oxygenation changes for direct comparison between subjects. Other data processing procedures should be explored so that between-subject differences can be detected more efficiently.

In the second study, regional differences in brain activation in the PFC during resting states were assessed. We expected to see a higher activation in the medial PFC

compared to the lateral PFC but our results did not find significant differences among regions in any group. However, asymmetric responses to eyes condition were found in younger children. The level of changes between eyes open and eyes closed conditions were higher in the left and the medial PFC compared to the right PFC. While the activation was similar in all regions of the PFC during eyes closed condition, the left PFC had a lowest activation during eyes open condition. This finding suggests that the response of PFC activation during different eyes condition is age-related. Inhibitory effects observed during the eyes open condition were similar in all regions of the PFC in older children and adults. On the other hand, inhibitory effects observed during eyes open condition had effects on the left and the medial PFC but not the right PFC in younger children.

In the third study, we used eyes open as the resting baseline condition to compare the activation in the PFC during a simple motor task and an executive function task in adults. We found a significant increase of oxy-Hb and decrease in deoxy-Hb in the PFC during the executive task in adults, which was consistent with hemodynamic changes in response to neuronal activity. Then we further examined the PFC functions in children with and without ASD in the fourth study. The activation in the PFC during an executive function task was compared to a simple motor task and resting state. The results found poor performance of the executive task in children with ASD, verified the deficits of executive function in this population. However, no significant changes in the PFC were found among different conditions. Further study with a larger sample size is needed to investigate the hemodynamic changes in response to different tasks in children population.

Appendix A

APPROVAL LETTER FROM THE IRB AT THE UNIVERSITY OF DELAWARE

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
Fax: 302/831-2828

DATE:	October 23, 2012
TO:	Ling-Yin Liang
FROM:	University of Delaware IRB
STUDY TITLE:	[383648-1] Developmental changes of the default mode network in the prefrontal cortex on children with and without autism using fNIRS
SUBMISSION TYPE:	New Project
ACTION:	APPROVED
APPROVAL DATE:	October 23, 2012
EXPIRATION DATE:	October 16, 2013
REVIEW TYPE:	Full Committee Review

Thank you for your submission of New Project materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

Appendix B

APPROVAL LETTER FROM THE IRB AT NATIONAL CHENG KUNG UNIVERSITY

文件編號：8800-4-07-001

Institutional Review Board
National Cheng Kung University Hospital
138 Sheng-Li Rd. Tainan 704, Taiwan R.O.C.
TEL: 886-6-2353535 ext.3635 FAX:886-6-2388190

國立成功大學醫學院附設醫院
第一人體試驗委員會
台灣.台南市勝利路138號
E-mail:em73635@mail.hosp.ncku.edu.tw

同意人體研究證明書

計畫名稱：利用近紅外光測量正常及自閉症族群於休息狀態及進行活動時前額葉皮質的活動變化
計畫編號/本會編號: -- / A-BR-102-035
內容/版本：
1.計畫書：版本：第 2 版 日期：2013 年 4 月 19 日
2.人體研究說明及同意書：版本：三，日期：2013 年 6 月 5 日
3.注音版兒童說明書：版本：三，日期：2013 年 6 月 5 日

下次繳交報告日期：民國 103 年 7 月 31 日
試驗機構：成功大學
計畫主持人：陳家進 老師 (醫工所)
協同研究員：梁齡尹

此計畫已於民國 102 年 7 月 30 日經本院人體試驗委員會第 A020 次大會審核通過，本會組織與執行皆遵照 ICH-GCP 規範，有效執行期限為民國 102 年 7 月 30 日至民國 103 年 9 月 30 日，特此證明。計畫主持人若未依規定於執行期限到期後三個月內繳交報告者，本會將保留審核權。若有任何不良反應亦須依藥品優良臨床試驗準則(GCP)通報；該計畫任何部份若欲更改，請於有效期限內向本會提出申請。

中 華 民 國 102 年 8 月 2 日

Human Study Approval

Date: Aug. 2, 2013

Title: Monitoring brain activity in the prefrontal cortex during resting atates and task states in individuals with and without autism using functional near-infrared spectroscopy
Protocol No/ IRB No: -- / A-BR-102-035
Content/Version:
1. Protocol: Version: 2, Date: Apr.19, 2013
2. Informed Consent Form: Version: 3, Date: Jun. 5, 2013
3. Information to subjects (young children): Version: 3, Date: Jun. 5, 2013

Next Hand-in Report Date: Jul. 31, 2014
Institute：National Cheng Kung University
Investigator: Prof. Jia-Jin Chen (Department of BioMedical Engineering)
Co-Researcher: Ling-Yin Liang

This is a certification to show that the protocol has been approved by Institutional Review Board (IRB) on Jul. 30, 2013, and the valid execution date is from Jul. 30, 2013 to Sep. 30, 2014. The Institutional Review Board of National Cheng Kung University Hospital (NCKUH) is organized and operated according to the laws and regulations of ICH-GCP. For those principal investigators who are 3 months late in submitting the final reports, the NCKUH IRB will retain the review rights. If any Adverse Event occurs, the IRB should be notified as required by GCP. If any part of the protocol needs to be altered, please submit the application to NCKUH IRB within the valid execution date.

Your sincerely,
Thy-Sheng Lin M.D.
Chairman 林勝勝 Thy-Sheng Lin
Institutional Review Board
National Cheng Kung University Hospital

Appendix C

QUESTIONNAIRE OF EXCLUSION CRITERIA (ADULTS)

Participant Information and Exclusion Criteria Questionnaire (Adult)

Date:

Participant:			
Gender:		Date of Birth:	year month day
Height (cm):		Weight (kg):	

Please circle your answer:

1. Have you ever had a head injury?

   No Yes

2. Have you ever had a seizure?

   No Yes

3. Are you allergic to alcohol?

   No Yes

4. Are you able to sit quietly for 6 minutes?

   No Yes

5. Have you ever been diagnosed with any mental disorders? If yes, please list all of them.

   No

   Yes _____

   _____

6. Have any of your family members ever been diagnosed with any mental disorders? If yes, please list all of them.

   No

   Yes _____

   _____

   _____

Appendix D

QUESTIONNAIRE OF EXCLUSION CRITERIA (MINORS, FILLED BY PARENTS)

Participant Information and Exclusion Criteria Questionnaire (Minors, Filled by Parents)

Date:

Participant:			
Gender:		Date of Birth:	year month day
Height (cm):		Weight (kg):	

Please circle your answer:

1. Has your child ever had a head injury?

   No Yes

2. Has your child ever had a seizure?

   No Yes

3. Is your child allergic to alcohol?

   No Yes

4. Is your child able to sit quietly for 6 minutes?

   No Yes

5. Have your child ever been diagnosed with any mental disorders? If yes, please list all of them.

   No

   Yes _____

   _____

6. Have you or any of your family members ever been diagnosed with any mental disorders? If yes, please list all of them.

   No

   Yes _____

   _____

   _____

Appendix E

INFORMED CONSENT APPROVED BY NATIONAL CHENG KUNG UNIVERSITY

文件編號：8800-4-03-001

NCKUH IRB
102.7.30
Approved

國立成功大學
人體研究說明及同意書

適用範圍：非醫療第 8 條所規範之人體研究、問卷、訪談及檢體採集等

（本同意書應由計畫主持人親自向受試者說明詳細內容，並請受試者經過慎重考慮後方得簽名）

您被邀請參與此研究，本說明及同意書提供您有關本研究之相關資訊，研究主持人將會為您說明研究內容並回答您的任何疑問。

計畫名稱：利用近紅外光測量正常及自閉症族群於休息狀態及進行活動時前額葉皮質的活動變化

執行單位：成功大學醫工所

主要主持人：陳家進　職稱：教授　聯絡電話：06-275-7575 ext.63423

協同研究員：梁齡尹　職稱：博士研究生　聯絡電話：0952-277-809

受試者姓名：

性別：　出生日期：

通訊住址：

聯絡電話：

一、研究簡介：

前額葉皮質除具備執行功能(executive function)外，也是預設模式網絡(Default mode network)的一部分。在進行執行功能相關之工作時，前額葉皮質的活動會增加。在休息時，前額葉皮質也有較高的活動。自閉症族群患者的前額葉皮質構造已被證實和健康族群不同，本研究將針對年輕族群進一步探討前額葉在不同工作下的活動變化。本研究將使用近紅外光來測量腦部血流血氧濃度。腦部活動增加時，血氧濃度會升高。藉由測量血氧濃度可推知腦部活動大小。

二、研究目的：

本實驗將採用近紅外光測量前額葉皮質在不同工作下之血氧濃度變化，比較正常族群以及自閉症族群之間的差異，包括休息狀態下的預設模式網絡活動及不同工作狀態下的腦部活動變化。了解自閉症患者與健康族群的差異有助於釐清其腦部活動與行為及動作缺失等症狀之間的關聯，提供有用的訊息以尋找有效的治療方式。

三、研究預計執行期間、受試者數目：

預計執行期間：IRB 核准日～ 103 年 9 月 31 日

受試者包括控制組及自閉症組，每組包含三個年齡層，8-11 歲，12-15 歲，及 20-35 歲。

每組每年齡層各十人，總共六十位受試者。

四、研究之主要納入與排除條件：

納入條件:

受試者年齡需介於 8-15 歲或是 20-35 歲之間且具備下列條件之一:

1. 健康且家族無精神疾病病史。
2. 曾被診斷為自閉症，亞斯伯格症，或是廣泛性發展障礙。

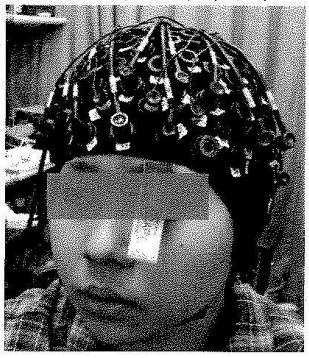
排除條件為:

1. 腦部曾經受傷。
2. 頭部有開放性傷口。
3. 有癲癇病史。

4. 無法維持靜態坐姿連續十分鐘。
5. 無法依照指示進行活動。
6. 對酒精過敏。

五、研究方法、程序及受試者應配合事項：

根據不同年齡，十六歲以上之受試者須填寫成人自閉症量表，十二至十五歲之受試者由家長填寫青少年自閉症量表，十二歲以下之受試者由家長填寫劍橋大學兒童行為及性格問卷。問卷結果將與腦部活動合併分析，探討行為與腦部活動的關聯性。另外，本實驗將使用近紅外光儀器測量受試者 1)睜眼休息狀態，2)閉眼休息狀態，3)進行電腦遊戲，以及 4)進行手部動作時的腦部活動情形。研究者會以酒精棉片清理受試者前額並為受試者戴上一個連接近紅外光儀器的帽子，受試者將輪流進行四種狀態，四種狀態的順序將以隨機決定。休息狀態下受試者將維持靜態坐姿，五分鐘開眼及五分鐘閉眼。電腦遊戲包含簡單反應時間測試及河內塔遊戲。手部動作包含鉛筆描圖，插洞板遊戲，及串珠子等，每種狀態進行三次測驗。完成全部測驗約需 90 分鐘。



六、研究資料之保存期限及運用規劃：

受試者書面資料將被保存於上鎖之資料櫃中，儀器測量所得的檔案也會以編碼命名，儲存於有密碼保護的電腦當中。所有資料將被無限期的保存，只有研究者可取得。研究結果可能會發表於國際研討會以及學術期刊，受試者個人資料將不會被提及。

七、可預見之風險及造成損害時之補救措施：

近紅外光儀器已被廣泛利用於各年齡層的人體研究，受試者涵蓋嬰幼兒、兒童、成年人至老年人。目前並無已知會對人體造成危害的可能性。如果因為測驗時間過長而感到不適，受試者可隨時要求研究人員暫停，必要時可隨時退出本研究。

八、研究預期效益：

本研究將有助於了解自閉症患者其腦部活動與健康族群的差異。然而，本研究對參與之受試者並無直接的益處。

九、損害補償與保險：

(一) 如依本研究所訂臨床研究計畫，因而發生不良反應或傷害，由成功大學負損害補償責任。但本受試者同意書上所記載之可預期不良反應，不予補償。(註：若無藥廠贊助，研究委託者改為研究機構)。

(二)如依本研究進行因而發生不良反應或傷害，成功大學 願意提供必要的協助。

(三)除前二項補償及醫療照顧外，本研究不提供其他形式之補償。若您不願意接受這樣的風險，請勿參加研究。

(四)您不會因為簽署本同意書，而喪失在法律上的任何權利。

102.7.30 Approved

十、受試者權利及個人資料保護機制：

(一)參加研究之補助

無。受試者將不會獲得補助。

(二)保護隱私

研究所得資料可能發表於學術雜誌，但不會公佈您的姓名且對受試者個人資料之隱私絕對保密，同時計畫主持人將謹慎維護您的隱私權。衛生署主管機關、研究委託者與本院研究倫理委員會在不危害您的隱私情況下，依法有權檢視您的資料。

(三) 研究過程中如有新資訊可能影響您繼續參與研究意願的任何重大發現，都將即時提供給您。

(四) 如果你(妳)在研究過程中對研究工作性質產生疑問，對身為患者之權利有意見或懷疑因參與研究而受害時，可與本院之人體試驗委員會聯絡請求諮詢，其電話號碼為：06-2353535 轉 3635 或 e-mail: em73635@mail.hosp.ncku.edu.tw 或郵寄至 704 台南市北區勝利路 138 號門診大樓 4 樓人體試驗委員會。

本同意書一式兩份，您已確實收到同意書副本，並已完整說明本研究之性質與目的。

梁齡尹 協同研究員已回答您有關研究的問題。

十一、研究可能衍生之商業利益及其應用之約定：

本研究預期不會衍生專利權或其他商業利益。

十二、研究之退出與中止：

您可自由決定是否參加本研究；研究過程中也可隨時撤銷同意，退出研究，不需任何理由，且不會引起任何不愉快或影響其日後醫師對您的醫療照顧。研究主持人亦可能於必要時中止該研究之進行。受試者於退出後可要求研究者銷毀已取得之相關資料。

十三、簽名欄：

(一) 受試者已詳細瞭解上述研究方法及其所可能產生的危險與利益，有關本試驗計畫的疑問，業經計畫主持人詳細予以解釋。本人同意接受為臨床試驗計畫的自願受試者。

受試者簽名：_____

日期：_____ 年_____月_____日

法定代理人/監護人/輔助人/有同意權人簽名(如適用)：_____

與受試者關係：_____

日期：_____年_____月_____日

(二) 見證人使用時機：

1. 受試者、法定代理人或有同意權之人皆無法閱讀時，應由見證人在場參與所有有關受試者同意書之討論。見證人應閱讀受試者同意書及提供受試者之任何其他書面資料，以見證研究主持人或其指定之人員已經確切地將其內容向受試者、法定代理人或有同意權之人解釋，並確定其充分了解所有資料之內容。
2. 受試者、法定代理人或有同意權之人，仍應於受試者同意書親筆簽名並載明日期。但得以指印代替簽名。
3. 見證人於完成口述說明，並確定受試者、法定代理人或有同意權之人之同意完全出於其自由意願後，應於受試者同意書簽名並載明日期。
4. 研究相關人員不得為見證人。

見證人簽名：_____

日期：_____年_____月_____日

102.7.30
Approved

(三) 主持人或研究人員已詳細解釋有關本研究計畫中上述研究方法的性質與目的，及可能產生的危險與利益。

主要主持人/共同主持人/研究人員簽名：_____

日期：_____年_____月_____日

Appendix F

INFORMED CONSENT (ADULTS) APPROVED BY THE UNIVERSITY OF DELAWARE

University of Delaware

INFORMED CONSENT FORM FOR PARTICIPANT IN RESEARCH PROJECT OR STUDY

Participant: _____

Investigators: Dr. Nancy Getchell, Ling-Yin Liang

Title of Project or Study: Developmental changes of the default mode network in the prefrontal cortex on children with and without autism using fNIRS

Your has been invited to join this research study because you are between 20 to 35 years old and you 1) are a healthy adult without any family history of mental disorders or 2) meet criteria DSM-IV criteria for autism spectrum disorder. Before you agree to join, it is important that you read and understand the following information. It tells how and why the study will be done. It also tells about the good things that could be learned from the study, and possible risks are described.

Please ask questions about anything that you do not understand before deciding whether or not to participate.

Initials: _____

1. PURPOSE/DESCRIPTION OF THE STUDY:

We are conducting a research project to examine the developmental changes in brain activity in resting and task conditions in children with and without autism. It is believed that our brain continues to work when we are awake and resting. The brain activity during resting state or task-free state is associated with cognitive functions and behavioral performance, such as monitoring and gathering information from the environment in order to have proper social interaction. It has been found that individuals with mental diseases have altered brain activity when they perform tasks. For example, children with severe autism have different brain activity than typically developed children during self-face recognition. However, the information about brain activity during resting state in children with autism is very limited. Also, we need to understand the brain at rest as a baseline for investigating the brain in active states. Then, we will compare task-induced activity in the brain between children with and without autism.

The purpose of this study is to identify the differences of brain activity during resting and task states between children with and without autism. In order to do that, we need to understand the development changes of resting brain in typically developed children. Brain activity will be quantified by measuring the blood oxygenation using functional near-infrared spectroscopy (fNIRS), an optical brain monitoring device, under both eyes open and eyes closed conditions.

fNIRS has light sources and light detectors. The light sources introduce near-infrared light at the scalp and the light detectors collect the light that travel through the brain and back to the skin (Figure 1a). fNIRS is like fMRI and CAT scan in that it provides the information of our brain. Instead of going in a chamber for fMRI or going through a tunnel for CAT scan to get whole brain images, the fNIRS system we will use requires participants to wear a headband-like sensor pad on the forehead and it will provide us images of the surface of the brain. The sensor pad has 4 light sources and 10 light detectors that cover the forehead with 16 voxels (Figure 1b and 1c).

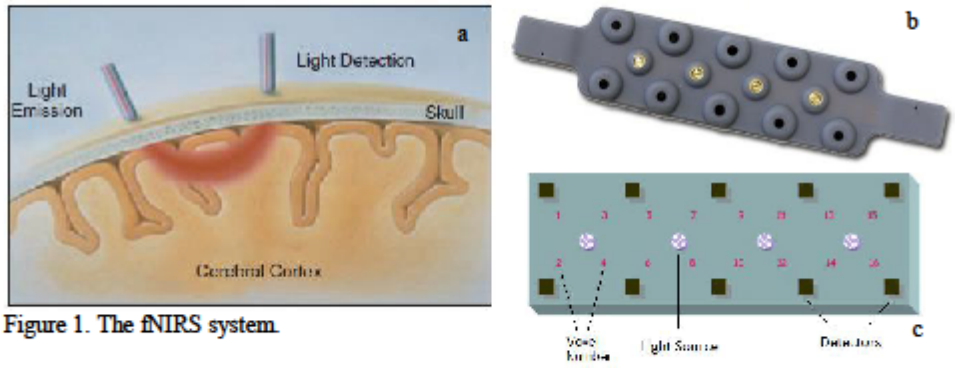


Figure 1. The fNIRS system.

There will be 3 age groups in both groups (i.e. with and without autism), 8-11 years old, 12-18 years old, and adults who are 20-35 years old. Total of 60 participants, 10 in each group, will be recruited to participate in the study. For the group with autism, we will recruit individuals with high-functioning autism who can understand the researchers and be able to follow two-step

Initials: _____

instructions, directions with one basic conjunction or additional phrase, e.g.: sit down on the chair and rest for 6 minutes.

If you give us consent to participate in the study, you will participate in at least one session of testing. We might ask you to visit for the second session if needed. During the testing session, we will ask you to sit on a chair and rest for 10 minutes before data collection. We will use an alcohol swab to clean your forehead and then a sensor pad of the fNIRS will be placed on your forehead like wearing a headband (Figure 2). During data collection, the tasks for you will be resting with your eyes closed and resting with your eyes open, 3 minutes for each condition; play a Hanoi tower game and a tapping activity on iPad, 2 minutes for each condition. The Hanoi Tower consists of three rods and different numbers of discs of different sizes depending on the difficulty. It requires moving the discs from the left rod to the right rod following specific rules. After the first testing, the sensor pad will be removed and we will ask you to rest for 30 minutes. You will complete a behavior questionnaire, The Adult Autism Spectrum Quotient, during the resting period. The result of the questionnaire will be used to investigate the relationship between your brain activity and your behavior inclination. Then, the sensor pad will be re-applied and we will repeat the data collection. After the second testing, the sensor pad will be removed. The testing will be done in 90 minutes.

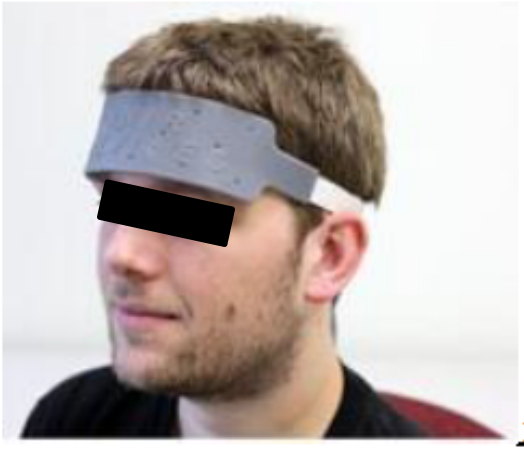


Figure 2. A participant wearing the sensor pad.

Who Can Be In The Study

To participate in the study, you must be between 20 to 35 years old. You must be 1) healthy and have no family history of mental disorders or 2) meet DSM-IV criteria for autism spectrum disorder.

Individuals Excluded From The Study

Exclusion criteria include: 1) previous head injury, 2) open wound on forehead, 3) seizure disorder, 4) unable to stay in sitting position and rest for 5 minutes, and 5) allergic to alcohol.

Initials: _____

2. CONDITIONS OF SUBJECT PARTICIPATION:

Your participation in this study will be kept confidential. No information identifying you will be released without your permission unless it is subject to a subpoena or a court order. If the results of the study are published or presented at scientific meetings, your name will not be revealed.

The paper document of your participation will be stored in locked cabinet in the Developmental Motor Control Lab at the University of Delaware for 5 years then destroyed. The data file we collect will be identified by codes only, for example: S01. No personal information will be saved in the data file. The data file we collect will be stored in password protected computers in the Developmental Motor Control Lab in the Human Performance Lab at the University of Delaware for 5 years then destroyed. The data will be available to the researchers only.

You may withdraw consent and/or stop participation in the study at any time without penalty. If you withdraw, you can ask us to destroy all previously collected data.

The risk of injury from participation in this study is very small. In the event of physical injury as a direct result of these research procedures, you will receive first aid. If you require additional medical treatment, you will be responsible for the cost. By signing this document you are not waiving any rights that you may have if injury was the result of negligence of the University or its investigators.

3. BENEFITS AND RISKS:

Benefits: There are no direct benefits for participating in this research study.

Risks: fNIRS has been used in research with adults, children and even infants. It carries minimal risks to the participants. You might feel a cold sensation after we use alcohol swabs to clean your forehead but the cold sensation should not last too long.

4. FINANCIAL CONSIDERATIONS:

There are neither costs nor compensation for participating in this study.

5. CONTACTS:

If you have questions about the study, you may contact Ling-Yin Liang (302-8318211, lliang@udel.edu) or Dr. Nancy Getchell (302-831-6682, getchell@udel.edu).

If you have questions regarding your rights in this study, you may contact the Chair, Human Subjects Review Board, University of Delaware at 302/831-2137.

Initials: _____

6. SUBJECT ASSURANCES:

You have read the above informed consent document. The nature, demands, risks, and benefits of the study have been explained to you. You understand that you may withdraw your consent and discontinue your participation in this study at any time without penalty.'

You will be advised if significant information is developed during the course of this research that may affect your willingness to continue participating.

7. CONSENT SIGNATURES:

Your signature below indicates that you have read and understand the above information, that you have agreed to participate in the study, and that a copy of this form has been given to you.

Participant's Name (printed): _____

Participant's Signature: _____ Date: _____

I certify that I have explained the purpose and procedures of this study to the parent/guardian of the potential child participant. I have explained the potential risks and benefits of this study and have answered any questions or concerns which were raised. I have witnessed the above signature and I have provided the parent with a copy of this consent form.

Principal Investigator's Signature: _____ Date: _____

Initials: _____

Appendix G

INFORMED CONSENT (PARENTS) APPROVED BY THE UNIVERSITY OF DELAWARE

University of Delaware

INFORMED CONSENT FORM FOR PARENTS/LEGAL GUARDIANS OF PARTICIPANT IN RESEARCH PROJECT OR STUDY

Participant: _____

Investigators: Dr. Nancy Getchell, Ling-Yin Liang

Title of Project or Study: Developmental changes of the default mode network in the prefrontal cortex on children with and without autism using fNIRS

Your child has been invited to join this research study because your child is between 8 to 18 years old and he/she 1) is a typically developed child without any family history of mental disorder or 2) meet criteria DSM-IV criteria for autism spectrum disorder. Before you agree to join, it is important that you read and understand the following information. It tells how and why the study will be done. It also tells about the good things that could be learned from the study, and possible risks are described.

Please ask questions about anything that you do not understand before deciding whether or not to allow your child to participate.

Parent/Guardian's Initials: _____

1. PURPOSE/DESCRIPTION OF THE STUDY:

We are conducting a research project to examine the developmental changes in brain activity in a resting condition in children with and without autism. It is believed that our brain continues to work when we are awake and resting. The brain activity during resting state or task-free state is associated with cognitive functions and behavioral performance, such as monitoring and gathering information from the environment in order to have proper social interaction. It has been found that individuals with mental disorder have altered brain activity when they perform tasks. For example, children with severe autism have different brain activity during self-face recognition compared to typically developed children. However, the information about brain activity during resting state in children with autism is very limited. Also, we need to understand the brain at rest as a baseline for investigating the brain in active states. Then, we will compare task-induced activity in the brain between children with and without autism.

The purpose of this study is to identify the differences of brain activity during resting and task states between children with and without autism. In order to do that, we need to understand the development changes of resting brain in typically developed children. Brain activity will be quantified by measuring blood oxygenation using functional near-infrared spectroscopy (fNIRS), an optical brain monitoring device, under both eyes open and eyes closed conditions.

fNIRS has light sources and light detectors. The light sources introduce near-infrared light at the scalp and the light detectors collect the light that travel through the brain and back to the skin (Figure 1a). fNIRS is like fMRI and CAT scan in that it provides the information of our brain. Instead of going in a chamber for fMRI or going through a tunnel for CAT scan to get whole brain images, the fNIRS system we will use requires participants to wear a headband-like sensor pad on the forehead and it will provide us images of the surface of the brain. The sensor pad has 4 light sources and 10 light detectors that cover the forehead with 16 voxels (Figure 1b and 1c).

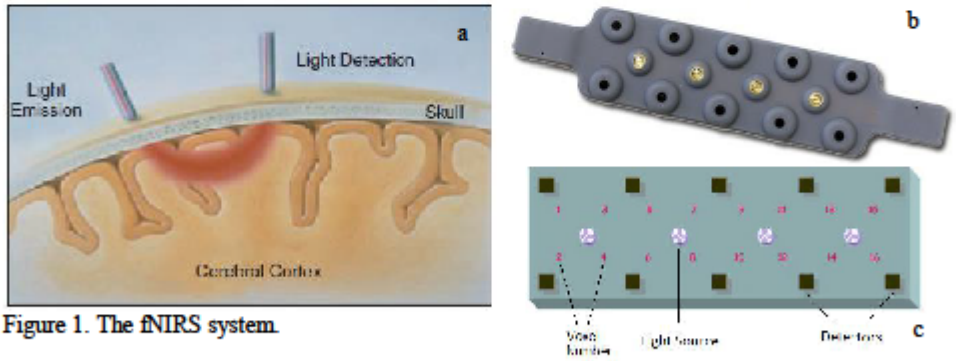


Figure 1. The fNIRS system.

There will be 3 age groups in both groups (i.e. with and without autism), 8-11 years old, 12-18 years old, and adults who are 20-35 years old. Total of 60 participants, 10 in each group, will be recruited to participate in the study. For the group with autism, we will recruit individuals with high-functioning autism who can understand the researches and be able to follow two-step

Parent/Guardian's Initials: _____

instructions, directions with one basic conjunction or additional phrase, e.g.: sit down on the chair and rest for 5 minutes.

If you give us consent for your child to participate in the study, your child will participate in at least one session of testing. We might ask your child to visit for the second session if needed. During the testing session, we will ask your child to sit on a chair and rest for 10 minutes before data collection. We will use an alcohol swab to clean the forehead of your child and then a sensor pad of the fNIRS will be placed on the forehead of your child like wearing a headband (Figure 2). During data collection, the tasks for your child will be resting with their eyes closed and resting with their eyes open, 3 minutes for each condition. And then play a Hanoi tower game and a tapping activity on iPad, 2 minutes for each condition. The Hanoi Tower consists of three rods and different numbers of discs of different sizes depending on the difficulty. It requires moving the discs from the left rod to the right rod following specific rules. After the first trial, the sensor pad will be removed and your child will rest for 30 minutes. You will complete a behavior questionnaire, The Adolescent Autism Spectrum Quotient (for 12-15 years old) or Cambridge University Behaviour and Personality Questionnaire For Children (for 8-11 years old), during the resting period. The result of the questionnaire will be used to investigate the relationship between brain activities of your child and their behavioral inclination. Then, the sensor pad will be re-applied and we will repeat the data collection. After the second testing, the sensor pad will be removed. The testing will be done in 90 minutes.

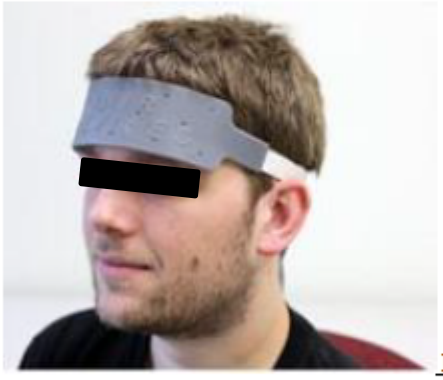


Figure 2. A participant wearing the sensor pad.

Who Can Be In The Study

To participate in the study, your child must be between 8 to 18 years old. He/she must be 1) typically developed children and have no family history of mental disorders or 2) meet DSM-IV criteria for autism spectrum disorder.

Individuals Excluded From The Study

Exclusion criteria include: 1) previous head injury, 2) open wound on forehead, 3) seizure disorder, 4) unable to stay in sitting position and rest for 5 minutes, and 5) allergic to alcohol.

Parent/Guardian's Initials: _____

2. CONDITIONS OF SUBJECT PARTICIPATION:

The participation of your child in this study will be kept confidential. No information identifying your child will be released without your permission unless it is subject to a subpoena or a court order. If the results of the study are published or presented at scientific meetings, the name of your child will not be revealed.

The paper document of your child's participation will be stored in locked cabinet in the Developmental Motor Control Lab at the University of Delaware for 5 years then destroyed. The data file we collect will be identified by codes only, for example: S01. No personal information will be saved in the data file. The data file we collect from your child will be stored on password protected computers in the Developmental Motor Control Lab of the Human Performance Laboratory at the University of Delaware for 5 years then destroyed. The data file of your child will be identified by codes only (for example: S01). All information obtained from the testing will be available only to the researchers.

You or your child may withdraw consent and/or stop participation in the study at any time without penalty. If you withdraw, you can ask us to destroy all previously collected data.

The risk of injury from participation in this study is very small. In the event of physical injury as a direct result of these research procedures, your child will receive first aid. If you require additional medical treatment, you will be responsible for the cost. By signing this document you are not waiving any rights that you may have if injury was the result of negligence of the University or its investigators.

3. BENEFITS AND RISKS:

Benefits: There are no direct benefits for participating in this research study.

Risks: fNIRS has been used in research with adults, children and even infants. It carries minimal risks to the participants. Your child might feel a cold sensation after we use alcohol swabs to clean his/her forehead but the cold sensation should not last too long.

4. FINANCIAL CONSIDERATIONS:

There are neither costs nor compensation for participating in this study.

5. CONTACTS:

If you have questions about the study, you may contact Ling-Yin Liang (302-8318211, lliang@udel.edu) or Dr. Nancy Getchell (302-831-6682, getchell@udel.edu).

If you have questions regarding your rights in this study, you may contact the Chair, Human Subjects Review Board, University of Delaware at 302-831-2137.

Parent/Guardian's Initials: _____

6. SUBJECT ASSURANCES:

You have read the above informed consent document. The nature, demands, risks, and benefits of the study have been explained to you. You understand that you may withdraw your consent and discontinue your child's participation in this study at any time without penalty.'

You will be advised if significant information is developed during the course of this research that may affect your willingness to continue participate.

7. CONSENT SIGNATURES:

Your signature below indicates that you have read and understand the above information, that you have discussed the study with your child, that you have agreed to let your child participate in the study, and that a copy of this form has been given to you.

Parent/Guardian's Name (printed): _____

Parent/Guardian's Signature: _____ Date: _____

I certify that I have explained the purpose and procedures of this study to the parent/guardian of the potential child participant. I have explained the potential risks and benefits of this study and have answered any questions or concerns which were raised. I have witnessed the above signature and I have provided the parent with a copy of this consent form.

Principal Investigator's Signature: _____ Date: _____

Parent/Guardian's Initials: _____

Appendix H

INFORMED ASSENT APPROVED BY THE UNIVERSITY OF DELAWARE

University of Delaware

INFORMED ASSENT FORM FOR MINOR

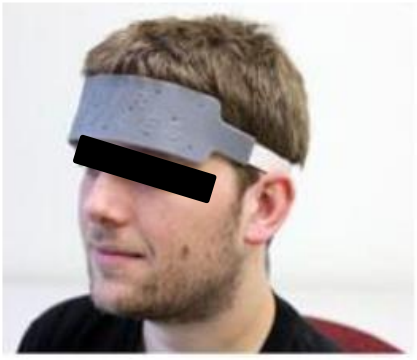
Title of research project: Developmental changes of the default mode network in the prefrontal cortex on children with and without autism using fNIRS

Who does the research study? Ling-Yin Liang (Lynn) and Dr. Nancy Getchell from the University of Delaware

We would like you to help us learn about how your brain works while you are resting. Ling-Yin (Lynn) Liang is in charge of this work. We want to see what your brain does when you are resting. We want you to be in the study because you are between 8-18 years old. We want to use what we learn to help us understand more about children like you. Your parents or legal guardians know about this and have agreed to let you join us, but the final choice is yours. If you agree to participate, you will be one of 60 participants in this research study.

What will you need to do?

You will come to our lab one time. You will rest on a chair for 10 minutes then we will clean your forehead with an alcohol swab. A pad will be placed on your forehead like you are wearing a headband. The pad will project light on your forehead and let us see your brain. All you need to do is sitting on the chair quietly for 6 minutes, 3 minutes with your eyes closed and 3 minutes with your eyes open. And then play a Hanoi tower game and a tapping game on iPad. The Hanoi Tower has three rods and different numbers of discs of different sizes depending on the difficulty. It requires moving the discs from the left rod to the right rod following specific rules. We will tell you when to open or close your eyes. We will do that twice with 30 minutes apart. You might be asked to come back if we need your help again.



Initials: _____

Are there good things and bad things about the study?

There are no expected benefits.
When we place the pad on your forehead, we will clean your forehead using alcohol swabs. You might feel a cold sensation on your forehead. But the cold sensation should not last too long.

Do you have to be in the study?

It is your choice. You can say "yes" now and then change your mind later. No one will be mad or upset with you if you do not want to be in this study or if you start the study now and change your mind later. There is no penalty for stopping. You will not be graded. All you have to do is to tell us you want to stop.

Who will know that you are in the study?

We will not write your name anywhere. The data we collect from you will be stored in password protected computers in our lab. This form will also be stored in our locked cabinet here at the University of Delaware. Only the people who do the research will have a key to the cabinet. We might write an article about this research study, but we will not use your name or the name of your school. No one who reads the article will know that you were involved in it.

Do you have any questions?

If you feel uncomfortable or uncertain about being a part of this study, please talk to your parents. You can also talk to Lynn with any questions about the study at any time. You can ask now or you can ask later. You can talk to us or you can talk to someone else at any time during the study. Here are the telephone numbers to reach us.

Ling-Yin Liang (Lynn) at 302-831-8211 or Dr. Nancy Getchell at 302-831-6682.

CONSENT SIGNATURES:

I have read this form. My parents or the people who will do the study answered my questions. A copy of this form has been given to me.

IF YOU AGREE TO BE IN THIS STUDY, SIGN YOUR NAME ON THE LINE BELOW

Print your name here: _____

Sign your name here: _____

Witness' Signature: _____ Date: _____

Principal Investigator's Signature: _____ Date: _____

Initials: _____