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Title: Testing and Calibration of Non-invasive Optical Imaging Technology for Functional Brain Imaging

NIH Principal Investigator: Amir Gandjbakhche, PhD
National Institute for Child Health and Human Development (NICHD)
Building 49, Room 5A82
49 Convent Drive
Bethesda, MD 20892
Phone: 301-435-9235
E-mail: gandjbaa@mail.nih.gov

Précis:

Objective: to a) cross-validate our near infrared spectroscopy (NIRS) imaging system with existing functional magnetic resonance imaging (fMRI) and electroencephalogram (EEG) data, and b) to investigate any significant technical issues associated with optode placement and motion artifacts, and to explore techniques that will potentially improve the feasibility and reliability of the system according to the needs of the population for whom existing imaging systems are unsuitable.

Study population: 250 healthy volunteers

Design: The study will look for correlations between NIRS signal changes in healthy subjects when performing functional tasks, and existing fMRI data.

Outcome Measures: graded changes in blood flow and oxygen, measured with NIRS, in response to different functional tasks.

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List of Abbreviations

NIRS: near-infrared spectroscopy, fMRI: functional magnetic resonance imaging, PET: positron emission tomography

1 Introduction and Background

Near-infrared spectroscopy (NIRS) is an emerging technology for noninvasive measurement of local changes in cerebral hemodynamic activity using infrared light in the range of 700 to 1000 nm (Villringer and Chance, 1997, Yodh et al., 2003, Boes et al. 2004, Gratton et al. 2005, Huppert et al. 2009). Compared to other well-established brain imaging modalities, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), this technique has a higher temporal resolution (in order of milliseconds), and provides additional spectroscopic information about changes in the oxygenation content of cerebral blood. Since this technology measures localized hemodynamic changes associated with brain activity, it is considered a measure of functional neural activation over time and thus is interchangeably referred to as both NIRS and functional NIRS (fNIRS).

The application of NIRS technology for neuroimaging relies on the principle that active neural tissues demand and consume more oxygen. The relative increase in oxygenated hemoglobin in active brain tissues is called the hemodynamic response. Like fMRI, NIRS imaging is a non-invasive technique which measures the relative changes in the oxygenation content of hemoglobin in neuronal tissue. However, NIRS utilizes changes in the absorption optical spectrum of oxygenated versus deoxygenated hemoglobin rather than their magnetic properties. Because of this difference, NIRS imaging makes functional brain studies possible for sensitive populations who cannot safely undergo MRI, such as hemispherectomy patients, individuals with specific types of implanted medical devices, or war veterans with embedded ferromagnetic shrapnel associated with a penetrating Traumatic Brain Injury (TBI). NIR instruments are also smaller in size, relatively less restraining, and more tolerant of subject motion compared to fMRI or PET, making them appealing tools for functional brain imaging studies with infants and children (Zaramella et al, 2001, Elwell et al, 2005, Sowell et al, 2004, Nagamitsu et al, 2006, Wilcox et al, 2005). Validating the use of NIRS as a brain imaging modality is crucial for serving populations in which MRI, fMRI, or PET environments are unsuitable.

In a typical brain imaging experiment using fNIRS, an array of detectors and low power NIR light sources are placed on the head, orthogonal to the scalp. Due to the low optical absorption of biological tissue at NIR wavelengths, NIR light can penetrate the head skin and skull (approximately 1.5-2 cm) to reach the outer5–10mm of the brain tissue surface. (Fukui et al, 2003, Okada et al 2003, Huppert et al, 2009). Light entering the head at a source and exiting the head at a detector samples a diffuse volume of tissue between these source and detector positions (Huppert et al, 2009). Changes in the optical absorption of this tissue, represented as changes in the amount of detected light, can be related to alteration in the oxyhemoglobin and deoxyhemoglobin concentrations in cerebral blood. By measuring changes in the intensity of detected light at distinct wavelengths over time,

functionally evoked changes in hemodynamic activity can be captured and used for imaging brain activity in cortical areas.

Like other neuroimaging modalities, NIRS technique has its own unique limitations, including limited optimal spatial resolution (approximately 5mm) and limited effective brain imaging depth (approximately 3 cm). Specific issues concerning the use of optical technology for bioimaging and reconstruction of tomographic images, such as dealing with motion artifacts and cross talk, motivate researchers to pursue further investigation on the design and application of NIRS imaging. While the use of NIRS technology for functional brain imaging has been studied over the past three decades, its involvement in clinical research remains extremely limited. Thus, this research intends to address critical questions regarding the application of NIRS in clinical settings. Specifically, we intend to address the following questions concerning the validation and methodologically testing of NIRS neuroimaging.

First, we intend to validate the use of a basic NIR functional imaging (NIRFI) system for neuroimaging by comparing NIRFI data to that which has been obtained through fMRI and electroencephalogram (EEG) studies. Previous research has compared the study of the cerebral hemodynamic response to physiological activation using both blood-oxygen-level-dependent imaging and arterial spin labeling (ASL) MRI techniques. These studies have demonstrated that while the spatial and temporal measures are concordant, there are minor differences in latency between the onset of activation and NIRS and MRI measures (Kleinschmidt, Obrig et al. 1996, Strangman, Culver et al. 2002, Hoge, Franceschini et al. 2005, Huppert, Hoge et al. 2006). Using two types of NIRFI devices, fNIRSoft from BIOPAC Systems and NIRScout from NIRx, we will measure patterns of functional hemodynamic activity in healthy volunteers while they perform tasks which have been well-studied and reported in fMRI. Ultimately, we will compare our findings with corresponding fMRI results to form a benchmark for validation and calibration our fNIR system in assessing functional cerebral activity. As standard NIRFI devices, the light sources of both the fNIRSoft and NIRScout systems apply light intensities less than 100 mW, well below the Federal Drug Administration's (FDA) eye safety requirements for Class I devices. They are passive, non-invasive tools for the measurement of NIRFI data and do not require further FDA approval. The specifications of both devices are detailed in a subsequent section of this protocol.

Additionally, electroencephalogram (EEG) measurement of cortical neural activity may be performed for the purposes of validating NIRS measurements as well as enhancing their temporal resolution. Specifically, we will use the actiCAP (Brain Products LLC), a 32-channel electrode array, which is designed for use in combination with a variable number of NIRS source-detector arrangements. Like both the fNIR Devices and NIRScout devices, this is a safe, passive, and noninvasive method of measuring cortical brain activity which is commonly used for the functional assessment of coordinated neural activity. The specifications of this device are detailed in a subsequent section of this protocol. Utilization of these compatibly designed neuroimaging systems will be important for accomplishing the study objective to cross-validate the use of near infrared spectroscopy (NIRS) with other existing functional methodologies.

During functional task experiments, subjects will be fitted with the fNIR device including a specific array of sources and detectors. For each task, the subject will be given clear instructions before being presented with the task stimulus. As the subject completes each specified task, the NIRFI system will continuously record the light intensity data which corresponds to changes in oxyhemoglobin and deoxyhemoglobin concentrations in the brain tissues. We will utilize systems such as E-Prime to record and correspond the timing of stimulus presentation with the measured hemodynamic response. Each functional task will have its own base line task designed as a control level, lasting a duration sufficient for the resumption of resting state hemodynamic activity before the presentation of any subsequent stimuli. Examples and detailed descriptions of the functional tasks which may be used are provided in Section 3 of this protocol.

In testing, we will compare block and event related designs. We will also study the impacts of varying inter stimulus intervals and repetition rates of the stimulus. Once light intensity data is collected from the detectors, principal component analysis (PCA), and independent component analysis (ICA) techniques will be applied to remove the noise and motion artifact components and obtain the corresponding hemodynamic response data.

Second, we intend to test the effect of subject behaviors and physiology on the collection of NIRFI data. Specifically, we aim to study factors which may confound the collection of representative NIRS data, such as the motion of the subject during testing. As in other neuroimaging modalities, motion during NIRFI can generate noise in the collected data and is considered a confounding issue and limitation. While NIRS imaging techniques are more tolerant of noise than fMRI or PET, understanding how motion affects the quality of NIRFI data is critical to validation of the technique. In this study, we will evaluate the efficacy of various techniques, such as the use of arrays of interlaced electronic sensors, in maximizing the system tolerance for motion and minimizing the confound of motion-related noise in NIRFI data. This will assist in determining the standard design and requirements for a NIRFI system which can be used with populations for whom motion restriction is not possible.

The use of motion extraction techniques to improve localization of the NIR signal has been demonstrated numerically. Expanding upon this, we aim to examine how the mapping of NIRFI data to MNI space might be improved and enhanced through correlation with fMRI data. Such a study is important for addressing the question of whether NIRFI can be localized to the correct gyri, as previous studies have shown that localization to the expected functional area can be problematic due to artifact errors. This is significant in critically assessing the efficacy and validity of using NIRFI techniques in currently non-imageable populations.

2 Study Objectives

Primary Objectives: Our first primary objective is to collect functional optical imaging data from healthy volunteers, study patterns of near-surface cortical activation during performance of known tasks, and validate results with published literature. Our second

primary objective is to validate NIRFI imaging data results with fMRI data and assess the uses of NIRS techniques as a developing neuroimaging modality.

Secondary Objectives: Our secondary objective is to assess neurovascular changes, including blood volume and blood oxygenation fluctuations, during functional events and validate observed changes with published literature. In addition, we will assess any significant issues associated with NIRS optode placement and evaluate the effect of subject motion on data collection, quality, and noise.

3 Subjects

a. Description of study populations

We will recruit a sample of 250 healthy adults.

b. Inclusion criteria

Age 18 years or greater.

c. Exclusion criteria

- Healthy volunteers with any skin disease.
- Healthy volunteers with any past or present vascular disease.
- Known adverse reaction to latex.
- Any medical condition that, in the opinion of the Principal Investigator would preclude the inclusion of a patient onto this research study.
- Unable or unwilling to give informed consent.

4 Study Design and Methods

a. Study overview

This study will be conducted in a group of Healthy Volunteers, all of which will present at two outpatient visits (1 visit for screening, consenting and general health check and 2 visit for experimental imaging measurement). Each healthy volunteer will be admitted for screening, consenting and general health check and imaging studies related to functional brain activation. The subject will go through neuropsychological tests first, and then will perform the selected protocol for functional imaging studies.

The qualifications of each healthy volunteer will be discussed with the Principal Investigator, Study Chairperson, or an Associate Investigator involved with the medical conduct of the study. Once it has been determined that a healthy volunteer qualifies, has read the informed consent, has had an opportunity to discuss the protocol with Principal Investigator, Study Chairperson, or an Associate Investigator involved with the medical conduct of the study, and wishes to participate, informed consent will be obtained from the healthy volunteer, as documented by a signed statement of informed consent approved by the IRB.

This protocol is not linked to any other active or prospective protocols at the NIH, and study participants are not required to participate in any other protocols.

b. Recruitment

Adult participants will be recruited through the NIH Healthy Volunteer office. All recruitment materials and venues will be approved by the IRB.

c. Screening, consenting and general health check

After expressing interest in participating in the study via e-mail or phone call, a member of the research staff will contact the subject to verify their identity. Questions will be asked to rule potential participants in or out of participation in the study, with respect to the basic inclusion and exclusion criteria.

Prior to any research activities, participants will provide written, informed consent before study visit. After signing the consent, participants will have the first visit for general health check including: Medical History, physical exam and vital signs. Then, the participants will be asked to come back for another visit to do the experiments.

d. Study procedures

Behavioral measures:

Subjects will be asked to take a series of behavioral questionnaires. The specific selection of questionnaires administered will depend on the functional tasks performed in the experiment. Subject responses to these tests will assist in controlling for between-subject differences in behavioral measures which may be relevant to task performance capability, ultimately reducing the confounding effect these differences may have in subsequent imaging analyses. *Participants are free to refuse to answer any of the questions contained on any of the questionnaires they are given, which has been made clear on the informed consent document.* These series of behavioral tasks/ questionnaires may include the following scales:

- **Wechsler Adult Intelligence Scale (WAIS) III or IV**
  WAIS yields three IQ scores (verbal IQ (VIQ), performance IQ, (PIQ) and full scale IQ (FSIQ)) and four Index scores (verbal comprehension, perceptual organization, working memory and processing speed). Verbal comprehension index (VCI) measures general verbal skills (verbal fluency, ability to understand and use verbal reasoning, and verbal knowledge). Perceptual organization index (PRI) measures non-verbal and in-the-moment reasoning skills (ability to examine a problem, draws upon visual-motor and visual-spatial skills, organizes thoughts, create solutions and then test them). Working memory index (WMI) assesses the ability to memorize new information, hold it in short-term memory, concentrate, and manipulate that information to produce some result or reasoning processes. Processing speed index (PSI) assesses skills on focusing on attention and quickly scanning, discriminating

between and sequentially ordering visual information. It refers to the speed at which cognitive processes can be carried out. Time varies depending on which tests will be administered.

- **Kaufman Brief Intelligence Test, Second Edition (Kaufman & Kaufman, 2004)**
  The Kaufman Brief Intelligence Test a short norms-referenced intelligence test assessing verbal/crystallized and nonverbal/fluid intelligence. It is designed as an intelligence screener for individuals aged 4 to 90 years. Administration take approximately 20 minutes to complete the 3 subtests: verbal knowledge, riddles, and matrices. This test may be used as an alternative to the WAIS as a measure of intelligence.

- **Symptom Checklist 90-Revised**
  The Symptom Checklist-90-Revised instrument helps evaluate a broad range of psychological problems and symptoms of psychopathology.

- **Wisconsin Card Sort Test**
  The Wisconsin Card Sort Test measures neuropsychological functioning, assessing abstract thinking, cognitive flexibility and executive function.

- **PhenX Broad Psychopathology Screen (Appendix A)**
  The PhenX Broad Psychopathology screen provides basic information regarding participants' psychological functioning over the past two weeks. It is part of the PhenX Toolkit, a toolkit funded by the National Human Genome Research Institute (NHGRI) that consists of measures developed to standardize phenotypic measurement in biomedical research studies (https://www.phenx.org/). It is commonly used in research to ensure that participants have no current psychological symptoms (i.e., within the past two weeks). All participants who participate in this protocol are recruited through the healthy volunteer office at the NIH; however, this screen has been added to ensure that the investigators are as thorough as possible in controlling for any potential between-subject variance in our sample during data analysis.
- **Health History Questionnaire (Appendix B)**
  The health history questionnaire was created to provide a broad overview of participants' past and current health issues that could interfere with their imaging measurements or introduce variance into the data at their time of participation. This information will be used to control for any health differences in our participants' by using any relevant health variables as covariates in our analyses. Again, all participants who participate in this protocol are recruited through the healthy volunteer office at the NIH; however, this questionnaire has been added to ensure that the investigators are as thorough as possible in controlling for any potential between-subject variance in our sample during data analysis.
- **The Autism Spectrum Quotient (Appendix C)**

The Autism Spectrum Quotient (AQ) is a validated, self-report questionnaire that provides a continuous measure of social functioning (Baron-Cohen et al., 2001). This scale has been shown to reflect a continuum of social functioning ability in typically developing controls, as well as clinical populations. Since some of the imaging tasks involve social scenarios, such as the self-reflection task (see Section 3.2.2.3) it is important to take all participants' level of social functioning ability into account. Despite its name, this scale is in no way a diagnostic tool and is only intended for research purposes. It will only serve as a continuous measure of social functioning to be controlled for in subsequent analyses, as has been shown to be important in many previous studies of typically developing populations (Ruzich et al., 2015; Nummenmaa et al., 2012; Hasegawa et al., 2013; con dem Hagen et al., 2010).

- **The Cognitive Flexibility Inventory (Appendix D)**
  The Cognitive Flexibility Inventory (CFI) is a validated self-report questionnaire that measures participant's day-to-day ability to adapt to changes in their environment and cognitive stressors (Dennis et al., 2010). The scores on this scale directly pertain to participants' behavioral inhibition ability and will be important to factor into analyses considering their performance on behavioral inhibition tasks, such as the go/no-go (Section 3.2.2.4).
- **The Adult Repetitive Behavior Questionnaire, 2nd Edition (Appendix E)**
  Like the CFI, the Adult Repetitive Behavior Questionnaire, 2nd Edition (RBQ-2A) is a validated self-report behavioral scale which measures participants' daily repetitive behavior repertoire that are associated with behavioral inflexibility (Barrett et al., 2015) and was developed in neurotypical adults. It provides a continuous measure of repetitive behavior, which again is important information to consider during analyses involving tasks pertaining to behavioral flexibility, such as the Go/No-Go Task (Section 3.2.2.4)
- **The Edinburgh Handedness Inventory (Appendix F)**
  The Edinburgh Handedness Inventory (EDI; Old Field, 1971) is a commonly used, validated self-report measure of participants' handedness as assessed by which hand their use to perform everyday activities. It provides a score which indicated participants' degree of left or right handedness. It is important to take this into account in imaging analyses due to laterality differences in neural structure related to handedness (Willems et al., 2014).
- **Psychopathic Personality Inventory -Revised (PPI-R) (Appendix G)**
  The PPI-R score is a self report questionnaire to measure emotional callousness (coldheartedness), carefree non-planfulness, social influence and some other traits, which form global psychopathy traits, in adults (Lilienfeld, Widows et al. 2005). PPI-R has been designed to assess those traits in healthy people without considering any links to anti-social or criminal behaviors. It also can find a pattern in the responses to control for careless or random responses. Since some of the imaging studies on human decision making involve different factors such as social influences as well as personal traits it is important to take all participants' level of social and personal functioning ability into account. Despite its name, this score is in no way a diagnostic tool and is only intended for research purposes. It will only serve as a continuous

measure of social and personal functioning to be controlled for in subsequent analyses, and has been shown to be important in many previous studies of typically developing populations (Rilling, Glenn et al. 2007, Harenski, Kim et al. 2009).

- **Visual Aural Read/Write Kinesthetic (Appendix H**)
  VARK is a self-assessment learning style (© Copyright Version 7.8 (2014) held by VARK Learn Limited, Christchurch, New Zealand). The results of this test indicate preferences rather than strengths and can be used for assessing learning styles. This questionnaire consists of 16 multiple-choice questions regarding subjects' preferences when conducting a variety of activities. For instance, subjects will choose how they prefer to learn something for the first time or communicate with others. The multiple choice would allow subjects to select the preferred methods to deal with the given situation. The VARK questionnaire and the results focus on how people like to receive information or deliver their communication. We use the VARK score to find the correlation between learning preferences and brain activation during performance of the cognitive tasks that use different stimuli such as auditory and visual.

Biological Measures:

fNIRS Devices & Application: After completing these questionnaires, subjects will be seated on an adjustable chair. An fNIR device will be applied to the appropriate head position on the head and held in place by an adjustable band. One of two fNIRS devices will be used to assess participants in this protocol. The optode array in Device 1 (fNIRSoft) is most commonly used for assessment of hemodynamic activity in the prefrontal region, while Device 2 (NIRScout) can be applied to measure activity from other areas of the cortex. Due to this specificity, both devices have been included in the protocol. Selection of the device used will depend on the aims of the experiment and nature of the tasks performed by the subject.

Device 1 – fNIRSoft: This NIRFI device consists of three rows of sources and detectors, organized into a band. The bottom and top row of this band each contain 5 detectors, while the middle row contains 4 sources. The system is designed to cover the forehead of the subject and capture activations that occur in the prefrontal cortex region. The current version of this device being used in the protocol is the fNIR Devices 2000S imager (fNIR Devices, LLC). Specifically, This device uses light emitting diodes (LEDs) provide highly monochromatic sources with very fast time sequencing (50 msec), available at 730, 805, and 850 nm. These three LEDs are contained in a 3 mm diameter housing and are operated as central clustered sources with circumferential detectors. A power consumption of approximately 0.2 watt, time shared at 1 msec is typical. The light intensities are within FDA requirements for Class I devices (<100 mW) for eye safety, and do not require further FDA approval. Published results from tests utilizing this device were performed at Drexel University, where they were also controlled by an assigned safety engineer and reviewed by the Office

of Regulatory Research Compliance. This device has already been approved for human subject studies by the Drexel University Institutional Review Board.

Device 2 - NIRScout: The NIRx NIRScout 8x12 fNIRS imaging system is a second NIRFI device which may also be used to measure cerebral hemodynamic activity. This device includes 8 laser sources (Class 1 lasers operating at 760nm and 850nm) which shine low-intensity levels of infrared light onto the participant's scalp, and 12 detectors (Avalanche photodiode). The device also includes 8 additional small detectors of the same type used to create short source-detector separation channels. The sources and detectors will be fit into a comfortable elastic cap worn by the participant. Like the fNIR Devices system, this is a safe, passive, and noninvasive method of measuring the oxygenation content of cortical blood flow.

Device 3 – Brain Products actiCAP
In conjunction with NIRFI, electroencephalogram (EEG) measurement of cortical neural activity is important for the validation of NIRS as well as enhancing the temporal resolution of measured activity. The NIRx NIRScout system, previously discussed in detail, is designed to operate in an integrated fashion with EEG systems produced by Brain Products LLC. Specifically, the Brain Products actiCAP is a 32-channel silver chloride electrode array which can be worn in combination with a variable number of NIRS source-detector pairs integrated at key anatomical sites. Like both the fNIR Devices and NIRScout devices, this is a safe, passive, and noninvasive method of measuring cortical brain activity which is commonly used for the functional assessment of coordinated neural activity.

Physiological measures: Surface electrodes and/or a respiration monitor will also be applied to subjects to monitor various physiological signals, such are heart rate, respiration, skin conductance response, and blood flow. These signals are commonly collected in conjunction with neuroimaging data, particularly fMRI studies, as they can then be used to reduce noise in the neuroimaging signal due to physiological interference (Caballero-Gaudes & Reynolds, 2017). Physiological signals such as heart rate, respiration, skin conductance response will be collected through the BioPac MP160, a physiological data acquisition and analysis system. The BioPac MP160 can measure several physiological signals such as electrocardiogram (ECG), respiration rate, skin conductance response, through the use of surface electrodes and signal transducers (e.g., a respiratory effort transducer). The BioPac MP160 is a commercially available product from BioPac Systems, Inc (Goleta, CA). Electrodes and transducers will be applied to each subject to monitor physiological signals throughout their data collection session so that they can later be compared to their neuroimaging (fNIRS) data.

*Blood flow measurement***:** Blood flow will be measured using a Laser speckle imaging (LSI) device. LSI is a NIRS technology that is sensitive to flow. LSI

consists of a coherent non-collimated light source (typically 785 nm) and a camera that images the illuminated tissue area. When biological tissue is illuminated with coherent light, an interference pattern will be formed at the detector, known as a speckle pattern. LSI is based on the dynamic change in this backscattered light as a result of interaction with red blood cells. Blood flow speed affects the pattern captured by the camera. Characterizing these changes in the pattern allows for a measurement of blood flow in the skin microvasculature as well as cardiac parameters. For this experiment, we are going to use a figure tip LSI, which was built in the Section on Biomedical Optics, NICHD. This device consists of a light source on one side of the finger or toe, and a small CMOS camera with a pinhole aperture making direct contact with the other side of the finger or toe. Blood flow and cardiac parameters measured by LSI will be used to remove systemic physiological noise from cerebral hemodynamic responses (fNIRS). Like other fNIRS techniques, LSI uses low-intensity levels of infrared light, which is safe, passive, and noninvasive.

Eye tracking:
While completing select cognitive tasks, including the Anti-saccade, emotional Anti-saccade, and Flanker tasks, the Eyegaze Edge eyetracker system (LC Technologies) will be used to monitor participant gaze and eye movement while looking at stimuli presented on a computer screen. This is necessary for the assessment of saccadic eye movements, which are rapid, simultaneous movement of both eyes in the same direction occurring between two or more phases of visual fixation, as well as tracking smooth pursuit eye movement and attention.

The Eyegaze Edge system utilizes a binocular, double fixed-camera system to observe the movement of both eyes at a sampling rate of 120 Hertz. Video cameras are positioned below a computer monitor, where a small, low power, infrared light emitting diode (LED) located at the center of the camera lens illuminates the eye. Gaze detection is measured using the pupil-center corneal reflection (PCCR) method, a noninvasive technique in which near-infrared light is shone towards the participant's pupils, causing reflections in both the pupil and the cornea. The vector between the corneal reflection and the pupil center are detected and tracked by an infrared camera. The use of both infrared light sources and detectors is critical for the accuracy of detecting corneal reflection, as infrared light provides much higher levels of contrast. Importantly, LED illumination of the eyes is safe, as the illumination is approximately 20 percent of the HEW maximum permissible exposure at a range of 15 inches (LaMarre, 1977).

The Eyegaze Edge system and PCCR method is a well-used, standard eye tracking method which is passive, noninvasive, and has a negligible effect on the user. During eye tracking, participants will be seated in a comfortable position with the stimulus presentation screen fixed at eye level. The Eyegaze Edge system works with both glasses and contact lenses, as its automatic calibration procedure accommodates the reflective properties of lenses. It also tolerant of minor head

movements and accommodates a wide range of variation in the eye image, including shape, brightness, and other features.

Tasks: We propose to conduct a wide battery of experiments using NIRFI to assess our ability to map functional events in the near-surface of the cortex. Each task will be based on no-risk models from existing fMRI literature for comparative purposes. Here we provide references of various example studies using executive activations. We will study CBF in the prefrontal cortex while the subject will judge event complexity task (Krueger et al., 2009), judge emotional stimulus related to the self (Johnson et al., 2002), and perform multitask (Koechlin et al, 1999), as well as a variety of cognitive tasks based on those previously used in the fMRI literature

The task details are outlined below:

Event Complexity task
Subjects will be asked to make a dichotomous decision regarding the complexity of daily life activities. In particular, participants are asked to rate each activity in terms of the number of events involved in the activity. Each activity may consist of few events (e.g. 'stirring a cup of coffee') or may consist of many events (e.g. 'planning a wedding').

Multi-task
Subjects will perform a letter-matching task in four conditions including a control, a delay, a dual-task and a branching condition. The subject will respond to visually presented letter (500ms duration) by pressing response buttons with their right or left hand.

Self Reflection
Subjects will be asked to make decision about themselves on specific statement requiring self-evaluation in the domains of mood, social interactions, cognitive and physical abilities. In the control condition, participants will make decisions about statements of factual knowledge.

Go/No-Go Task
Subjects will be asked to complete go/no-go tasks on the computer. The go/no-go task is a commonly used cognitive task to assess behavioral inhibition abilities in neuroimaging studies. During the task, subjects will respond to visual stimuli presented on a computer by either pressing a button or inhibiting the button press response.

Cerebral Autoregulation Task:

- ***Sit-to-Stand Procedure***

Subjects will undergo a passive orthostatic test to quantify cerebral autoregulation through an active sit-to-stand procedure. Cerebral autoregulation and cerebral blood perfusion have been studied through fMRI using arterial spin

labeling; however, conducting such a measurement through fNIRS is less time consuming and more economical. Monitoring of fNIRS and physiological signals will be performed throughout, as previously described. In the sit-to-stand procedure, subjects will stand in an upright posture for a short, standardized time period (< 1 minute). They will then be instructed to transition from the standing to seated position, where they will hold an upright seated posture. This task will be repeated for multiple trials, with a short rest period between each trial (Lee at al., 2014). This is an active although noninvasive and nonstrenuous procedure which is both a safe and standard method for the evaluation of orthostatic tolerance. Moreover, physiological measures (such as heart rate) will be recorded throughout the procedure to monitor exertion levels.

A limitation of a sit-to-stand task is that measured cerebral activities using fNIRS and EEG could be contaminated by movement artifacts associated with the procedure because this experiment used wire-devices/equipment. An alternative cerebral autoregulation task which does not require body movement is a cold pressor test.

- Cold pressor test

Cold pressor test (CPT) is a long-standing, simple, and validated physiological tool to evoke temporary increases in the arterial blood pressure with an elevation in the sympathetic nerve activity (an autonomic neural control of the cerebral circulation) (Hines et al., 1936; Wood et al. 1984; Menkes et al. 1989; Wirch et al. 2006; Mourot et sl. 2009; Washio et al., 2020). During this test, in which the subject immerses one hand or foot into an ice bath for a short time (e.g.1-3 minutes), a vascular sympathetic response occurs that results in an increase in blood pressure. Due to its effects on blood pressure, CPT has been used frequently for predicting hypertension (Wood et al. 1984) and simulating cerebral vascular change (Washio et al. 2020). Previous studies have indicated a change in cerebrovascular response using fMRI (Hendriks-Balk et al. 2020) and neural activities using EEG (Chang et al. 2002). In this protocol, we will monitor hemodynamic responses using fNIRS and neural activities using EEG during a CPT, in which participants will put their limb in an ice bath (temperature ~ 33.8^{o}F to 41^{o}F) for as long as they can with a maximum time of 3 minutes. Temperature of the cold bath will be monitored with a standard thermometer. Participant will be allowed to remove their limb at any time and the test will be ended to prevent severe pain. Physiological signals will be monitored throughout the test to ensure a safe condition. Though it is not anticipated to happen, but in case any physiological parameter changes to a critical level, the test will be ended immediately. After the test, we will correlate our measured signals with literature fMRI and EEG data obtained during the task. If a high correlation is found, fNIRS can then be used to replace a high-cost fMRI system.

Computerized Cognitive Tasks:
Subjects will be asked to complete one or more cognitive tasks, similar to the type that appear in the NIH Toolbox Cognition Battery. Tasks will be created and administered using EPrime Psychology Software Tools and will be based on

established tasks that test various aspects of cognition and executive function (e.g., the Flanker test, the Dimensional Change Card Sort test, etc). All of the tasks are administered on a computer and consist of the subject being given a set of task instructions, then viewing a series of stimuli (consisting of different words, images, symbols, numbers, and/or sounds) and responding to these stimuli based on the task instructions through a variety of potential behavioral responses (such as a button press, mouse click, eye gaze, etc). Computerized cognitive tests such as these are common in the fMRI literature and thus comparing fNIRS data. Elaborated examples of two potential tasks are described below.

Antisaccade Task
The antisaccade task, commonly used as an assessment of frontal lobe functioning, tests a subject's inhibition of reflexive eye movement towards a presented stimulus. Subjects will be asked to fixate their gaze on a non-moving target, such as a small dot or cross, presented on a computer screen. A stimulus will be rapidly presented without warning in the periphery of the screen, on varying sides of the fixation point. The subject will be instructed to make a saccade (a rapid shift in gaze) in the direction away from the stimulus.

Flanker Task
Subjects will be asked to complete a series of Eriksen Flanker Tasks. During the task, the subject will be presented with a target stimulus surrounded by congruent, incongruent, or neutral flankers. Subjects will be instructed to make directional responses associated with the target stimulus by pressing right or left arrow buttons on a keyboard. In the congruent condition, flankers elicit the same response as the target stimulus. In the incongruent condition, flankers elicit the opposite response to the target stimulus. In the neutral condition, flankers have no relationship to the directional response.

Finger tapping task
The finger tapping task is a neuropsychological task which assesses motor functioning. Subjects will be asked to perform real, imagined, and imitated finger tapping. All finger tapping tasks will be done on the right hand (right-handed subject preference). The subject will look at a screen which will display prompts to physically tap or imagine tapping their finger. During the imitated finger tapping, the subject will watch a video or live demonstration of another person tapping his or her finger and be instructed to imitate the same action.

e. End of participation
After completion of the task, a summary of the task and main purpose of the experiment will be given to participants.

5 Management of Data and Samples

a. Storage

Electronic data records will be stored in a secured database located at NIH and paper records will be stored in a locked office in a locked filing cabinet. The data will only be accessible to investigators listed on the study protocol. At the completion of the study, all data will be stored by the Principal Investigator for 3 years

b. Data and sample sharing plan

Data may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated databases if consent for sharing was obtained. Repositories receiving data from this protocol may be open-access or restricted access.

Data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators but will remain at NIH. Data may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases will be submitted for prospective IRB approval.
Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6 Additional Considerations

a. Research with investigational drugs or devices

The system being used to collect fNIRS data is either the fNIR Devices 2000 or the NIRx NIRScout Imager. Laser speckle imaging (LSI) device. LSI is a NIRS technology and like other fNIRS techniques, LSI uses low-intensity levels of infrared light, which is safe, passive, and noninvasive. ActiCap for the measurement of cortical electrical activity will be used conjunction with NIRS measurement. ActiCAP is a 32-channel electrode array designed to be worn in combination the NIRx NIRFI devices. This is a safe, passive, and noninvasive method of measuring cortical brain activity which is commonly used for the functional assessment of coordinated neural activity. All of these are commercially available, non-invasive devices that are being used for their intended purpose (i.e, to measure neural activity). For this reason, the devices are not investigational and the study is IDE exempt.

b. Gene therapy

Not Applicable

7 Risks and Discomforts

There are no known risks associated with the procedures described above, which are widely used in cognitive neuroscience studies. Members of the research team will ensure that participants remain comfortable throughout the course of the experiment.

The cold pressor test may provoke pain and discomfort at the limb, which is emerged in the cold bath. However, we will emphasize to the participants that their participation is completely voluntary and that they can remove their limb from the cold bath at any time and we will end the test immediately.

Optical imaging – NIRS, LSI: There are no known risks associated with the procedures described above, which are extensively used in different biomedical optic fields. Near-Infrared Spectroscopy (NIRS) is a noninvasive technique used widely in cognitive neuroscience studies.

The silicon cushioning material is used to cover all electronic parts as well as to provide comfort to subjects. However, it is conceivable (although never observed during experiment to date) that individuals with latex allergies or other abnormal skin conditions may experience adverse reactions. This will be made known at every major point of contact between the subject and the investigators up to and including the time of informed consent. The silver-silver chloride (Ag-AgCl) disposable surface electrodes used to record physiological signals are peel-and-stick, applied through an adhesive backing, like a band-aid. There may be mild redness due to removal of these surface electrodes at the end of the data collection session, similar to what one would expect when removing a band-aid. The respiratory effort transducer is an elastic band worn over the clothes, across the chest that is tightened to a snug fit. The snug fit may be mildly irritating to some subjects. If they do not tolerate either the electrodes of the respiration band, they are free to refuse to wear either or both at any point in the data collection session.

To minimize discomfort, the experimenter will ask subjects about their comfort and adjust the procedure as needed.

8 Subject Safety Monitoring

All subjects will be monitored during their data collection session by a member of the research team. All study personnel who are in contact with subjects will be First Aid and CPR-certified. In the case of a medical emergency, emergency services will be immediately contacted, and the participant will be attended to by one of the study personnel for proper acute management until emergency services arrive on scene. Any adverse events that may occur during the study procedures will be monitored by the Principal Investigator. While any risks incurred by participating in the study are minimal, any discomfort while wearing the fNIRS cap will be monitored during subjects' data collection sessions. Since the research being conducted is behavioral research, we will not use any toxicity tables for safety monitoring. Participation in the present study is voluntary. Healthy adult volunteer subjects are free to withdraw from the study at any time without penalty.

9 Outcome Measures

The main outcome measure of this study will be the neuroimaging measure. Specifically, graded changes in blood flow and oxygen, measured with NIRS, in response to different functional tasks.

10 Statistical Analysis

a. Analysis of data/ study outcomes

The collated data for each protocol will be compared to the published results from pre-existing fMRI studies for both inter and intra patient studies. This will be done by comparing the Atlased locations of FNIR data to the Atlased locations of the equivalent fMRI study in the literature. We will determine the mean coordinates of activation along with the deviation in the population in the same manner as the original studies. The populations can then be tested using t-tests and ANOVA tests to determine if the population data are from the same or different populations.

11 Human Subjects Protection

a. Subject selection

No selection will be based on race, ethnicity, or gender. Exclusion for healthy volunteers under 18 years is based on several issues related to the design of the current subject interface. This interface was designed for use on subjects with a fully adult sized head. Whilst it is conceivable that a smaller subject would be viable it introduces potential confounding factors into the data analysis.

b. Justification for inclusion of children

No children will be included in this study.

c. Justification for inclusion of other vulnerable subjects

This study allows the participation of NIH employees, who are considered a vulnerable population as per policy 404: *Research Involving NIH Staff as Subjects*. It is a minimal risk study and there are no known risks associated with the use of fNIRS with this population. No other vulnerable subjects will be included.

d. Justification for sensitive procedures

Not Applicable

e. Safeguards for NIH Staff or family members of study team members

NIH staff and family members of study team members may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH. Every effort will be made to protect participant information, but such information may be available in medical records and may be available to

authorized users outside of the study team in both an identifiable an unidentifiable manner.
The NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research will be made available. Please see section 13 for consent of NIH Staff

12 Anticipated Benefit

The potential benefit is that the data collected will assist us in developing a functional brain imaging system which will be useable on a much broader patient base than fMRI.

13 Consent Documents and Process

a. Designation of those obtaining consent

Study investigators designated as able to obtain consent will obtain informed consent from healthy adult volunteers.

b. Consent procedures

Consent will be obtained for all adult participants. Participants will have an opportunity to have their questions answered before being asked to sign the consent form.

c. Consent documents

The consent form contains all the elements required. The consent forms that have been included are as follows: a healthy adult volunteer consent form.

d. Considerations for Consent of NIH staff, or family members of study team members

Consent for NIH staff will be obtained as detailed above with following additional protections:
Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

14 Data and Safety Monitoring

Data and safety monitor

Because this study involves minimal risk to participants, data and safety will be monitored by the Principal Investigator, who will be responsible for ensuring validity of data collection methods as well as adherence to the protocol.

IRB and DSMC Documentation

- All IRB documentation can be found in iRIS. Principal Investigator, is responsible for maintaining IRB documentation, including records of all reviews of the study and submissions to the IRB.
- Clinical Trial Studies (non IND/IDE) will have random audits performed by the NICHD Office of the Clinical Director (policy: https://science.nichd.nih.gov/confluence/display/ocd/Protocol+Navigation).

Study Completion

Principal Investigator will maintain subjects' records for at least three years after completion of the study.

Criteria for stopping the study or suspending enrollment or procedures

If enrollment or procedures for the study are suspended for any reason, the Principal and Associate Investigators will conduct a review to determine whether the research can resume.

15 Quality Assurance

a. Quality assurance monitor

Quality assurance will be monitored by the Principal Investigator and Associate Investigators, as well as the NICHD Quality Assurance Program, as the study is behavioral in nature and does not involve any intervention or drugs.

b. Quality assurance plan

Oversight of monitoring will be performed by the NICHD Quality Assurance (QA) Program. The NICHD Quality Assurance Program performs annual audits randomly on 10% of actively accruing NICHD protocols.

(https://science.nichd.nih.gov/confluence/display/ocd/Protocol+Navigation).

16 NIH Reporting Requirements

16.1 Reportable events definitions

Please refer to definitions provided in Policy 801: Reporting Research Events.

16.2 Expedited reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events.

16.3 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events.

16.4 Reporting to Clinical Director (CD)

Non-compliance, major deviations, unanticipated problems, new information, and suspension or termination of research activities will be reported to CD as soon as possible and in writing not more than 7 days after the PI first learns of the event. Death will be reported within 24 hours after an investigator becomes aware.

17 Alternatives to Participation

Subjects do not receive any treatment in this study, nor do they forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

18 Privacy

All research activities will be conducted in as private a setting as possible. All research activities will be conducted by researchers who have been trained in the study protocol.

19 Confidentiality

a. For research data and investigator medical records

All data obtained from the research subjects is considered confidential. The data will be coded to protect subject confidentiality; electronic records will be stored in a secured database located at NIH and paper records will be stored in a locked office in a locked filing cabinet. Only study investigators will have access to data.

b. For stored samples

Not Applicable

c. Special precautions

Results will be published as group data without identifying characteristics. All subjects' information will be kept private. However, if, during the course of the study, there is any concern that a subject is at risk of being harmed, study staff will report it to the appropriate authorities, as obligated by law.

20 Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts-of-interest to report.

c. Role of a commercial company or sponsor

Not Applicable

21 Technology Transfer

Not Applicable

22 Research and Travel Compensation

Travel expenses for each participant and an accompanying parent(s) will be reimbursed according to standard NIH travel reimbursement policy. Subjects will be compensated $30 for the first hour and $20 for every hour thereafter if required.

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24 Attachments/ Appendices

- PhenX Broad Psychopathology Screen (Appendix A)
- Health History Questionnaire (Appendix B)
- The Autism Spectrum Quotient (Appendix C)
- The Cognitive Flexibility Inventory (Appendix D)
- The Adult Repetitive Behavior Questionnaire, 2nd Edition (Appendix E)
- The Edinburgh Handedness Inventory (Appendix F)
- Psychopathic Personality Inventory -Revised (PPI-R) (Appendix G)
- Visual Aural Read/Write Kinesthetic (Appendix H)