Test-retest Reliability of Neurovascular Coupling and Impairment in Epilepsy Measured by fNIRS and EEG

Quinn Smith,
Intouch (August) Limvaree,
Jesse Farrand,
Summer Edwards
Stephenson School of Biomedical Engineering line 1: 2nd Given
University of Oklahoma
Norman, OK

Tressie Stephens,
Ian F. Dunn,
Andrew Conner
Department of Neurosurgery
University of Oklahoma Health Sciences Center
Oklahoma City, OK, USA

Lei Ding, Han Yuan*
Stephenson School of Biomedical Engineering
Institute for Biomedical Engineering, Science and Technology
University of Oklahoma
Norman, OK, USA

*hanyuan@ou.edu

***Abstract*—Simultaneous fNIRS-EEG is a cost efficient, noninvasive, and adaptable multimodal imaging method that measures both the hemodynamics and electrical activity of the brain. Neurovascular coupling (NVC) is an important mechanism in the brain in which increased neuronal activity propels an increase in oxygenated hemoglobin as well as decrease in deoxygenated hemoglobin in the local cerebral blood flow to that specific area. Previous literature has suggested that NVC may be impaired in individuals with epilepsy. The unique approach to measure and quantify NVC via multimodal fNIRS-EEG imaging is an intriguing method to probe the NVC in clinical settings. Therefore, the purpose of the present study is to determine the reliability of NVC in a group of healthy subjects and investigate NVC in a pilot sample of epileptic patients to compare with healthy through a series of auditory tasks using simultaneous fNIRS-EEG. Results showed excellent test-retest reliability agreement in the fNIRS responses and NVC for the healthy control group. Meanwhile, abnormally lower fNIRS and NVC responses were observed in the epileptic patient group. These results provide important data for the reliability of fNIRS-EEG-based NVC testing and show promise that simultaneous fNIRS-EEG imaging can detect impaired NVC in epileptic individuals.**

Keywords—fNIRS, EEG, reliability, epilepsy, NVC

I. Introduction

Neurovascular coupling (NVC) is a mechanism of the brain in which neuronal activity in an area of the brain is followed by an increase in oxygenated cerebral blood flow to that area to ensure neurons have sufficient oxygenation to maintain function [1]. NVC is the basis for functional brain imaging modalities such as PET, SPECT, fMRI, and fNIRS, so an intact NVC is crucial for these blood flow based imaging modalities to assess neuronal functions [1]. However, impaired NVC has been implicated in epilepsy and can lead to abnormal hemodynamics and furthermore incorrect imaging outcomes [2-4]. Thus, being able to assess whether a patient has impaired NVC is desirable.

Simultaneous electroencephalography (EEG) and functional near-infrared spectroscopy (fNIRS) is an established multimodal imaging method that can be used to assess NVC [5-8]. EEG measures neuronal activity by recording changes in the electrical potential of populations of neurons acting together. fNIRS measures the hemodynamic response of the brain to neuronal activity by recording relative changes in concentration of oxygenated and deoxygenated hemoglobin. NVC can then be assessed by determining what ratio neuronal activity is associated with an increase in blood oxygenation. Since both EEG and fNIRS are portable, noninvasive and can be accessed in a clinical setting, assessing NVC could be a useful test adjuvant to fMRI as part of the presurgical workflow.

In this study, we aimed to determine if simultaneous fNIRS-EEG provides reliable NVC measures and can be used to identify impaired NVC in epileptic patients. As temporal lobe epilepsy is the most common form of focal epilepsy, this study recruited patients of this type and investigated neuronal activity originating from the temporal lobe. Since the auditory cortex is located in the temporal lobe, subjects in this study were presented with auditory stimuli. Past studies used concurrent EEG-fMRI to detect impaired NVC in those with epilepsy [2-4]; therefore, we expect to see abnormal NVC in the epileptic subjects of this study while using simultaneous fNIRS-EEG.

II. Materials and Methods

A. *Experimental Setup and Data Acquistion*

Prior to the experiment, informed consent was obtained from all participants within this study. A healthy control group and epileptic patient group were recruited. Healthy subjects had no signs of neurological disorders, normal hearing, good or corrected vision, good use of both hands, and were fluent in written and spoken English. For the epileptic group, patients were diagnosed with epilepsy with intentions to undergo the Wada test before surgery. In addition, they had normal hearing, good or corrected vision, good use of both hands, and were fluent in written and spoken English. All study protocols were approved by the Institutional Review Board at The University of Oklahoma Health Sciences Center.

<u>Experiment 1:</u> Ten healthy subjects (four females) aged 19-23 years participated in a test-retest study, spaced 2-7 days apart. Participants performed two different tasks including two resting and two auditory sessions. Each resting session consisted of the subject sitting at complete rest for six minutes while focusing on

This work was supported by NSF RII Track-4 2132182, NIH NIGMS 1P20GM135009, NIH P20GM103447-24S1, University of Oklahoma IBEST-OUHSC Grant, Undergraduate Research Opportunities Program and Undergraduate Research and Creative Activity (UReCA) Summer Fellowship.

a cross at the center of the computer screen. For auditory sessions, subjects were at rest and underwent seven blocks of 20 s auditory noise stimulus followed by a 30 s resting period. To ensure minimal jaw movement, a bite bar made of dental putty was utilized for all subjects [6]. This experimental procedure was followed for the test and retest visits.

Data Acquisition for Experiment 1: A NIRScout system (NIRX, New York, United States) collected fNIRS data at a sampling rate of 3.91 Hz, while an ActiChamp system (Brain Vision, North Carolina, United States) acquired EEG data. Sixteen fNIRS light sources, 26 fNIRS light detectors, 16 short-separation light detectors, and a 32 channel EEG were placed on an integrated fNIRS-EEG cap surrounding the left and right auditory and motor regions of the brain. For correct and accurate placement of the cap, multiple head measurements were taken including, head circumference, nasion to inion, and left tragus to right tragus.

Experiment 2: Seven epileptic patients (three females) aged 14-50 years participated. Patients performed the same two tasks as the healthy group, two resting and two auditory sessions. To ensure minimal jaw movement, a bite bar made of dental putty was utilized for all patients. fMRI at resting state was also collected but not used in the current study. Two epileptic subjects' data were thrown out due to incomplete procedure or excessive motion, resulting the analyzed sample to be five.

Data Acquisition for Experiment 2: A NIRScout system (NIRX, New York, United States) collected fNIRS data at a sampling rate of 3.91 Hz while an ActiCHamp system (Brain Vision, North Carolina, United States) collected EEG data. Sixteen fNIRS light sources, 22 fNIRS light detectors, 16 short-separation light detectors, and a 32 channel EEG was placed on an integrated fNIRS-EEG cap with emphasis surrounding the left and right auditory regions of the brain. For correct and accurate placement of the cap, multiple head measurements were taken including head circumference, nasion to inion, and left tragus to right tragus.

B. fNIRS Data Preprocessing

Two processing pipelines were used to quantify the fNIRS response, a minimum processing and principal component analysis general linear model (PCA-GLM) [7]. Minimum processing was done using NirsLAB_v201 (NIRX, New York, United States), including bad channel rejection, bandpass filtering from 0.008-0.2 Hz and computing HbO and HbR states. Block averages of fNIRS were then calculated by averaging across all task blocks with reference to a baseline from -5 to 0 s.

In parallel with minimal preprocessing, we also evaluated fNIRS and NVC using a denoising pipeline, PCA-GLM [7], which removes physiological noises of superficial tissue absorption, respiration, cardiac pulsation and head motion. Briefly, the procedure performs PCA on long-separation channels (LS) to find the component most representative of superficial skin responses, PC-LS. Then PCA of short-separation (SS) channels was also performed. The time course of the SS component with the highest temporal correlation to PC-LS was used as a regressor in the GLM. Other nuisance regressors included in the GLM design matrix are acceleration, respiration, and cardiac pulsation from auxiliary EEG measurements; a third-order polynomial drift; and hemodynamic response function regressor to model the task-related effect. Due to missing auxiliary data, one healthy and two epileptic subjects were removed from this portion of analysis.

C. EEG Data Preprocessing

EEG data was preprocessed using the EEGlab toolbox (https://sccn.ucsd.edu/eeglab/) and codes in MATLAB®. Key steps include bandpass filtering of 0.1 and 70 Hz with a notch centered at 60 Hz, epochs extraction regarding the auditory stimulus onset, and ICA to remove ocular, muscular artifacts etc. Preprocessed data was then re-referenced to the common average reference and epochs with residual artifacts were rejected from averaging, which yielded the event related potential (ERP) as auditory response.

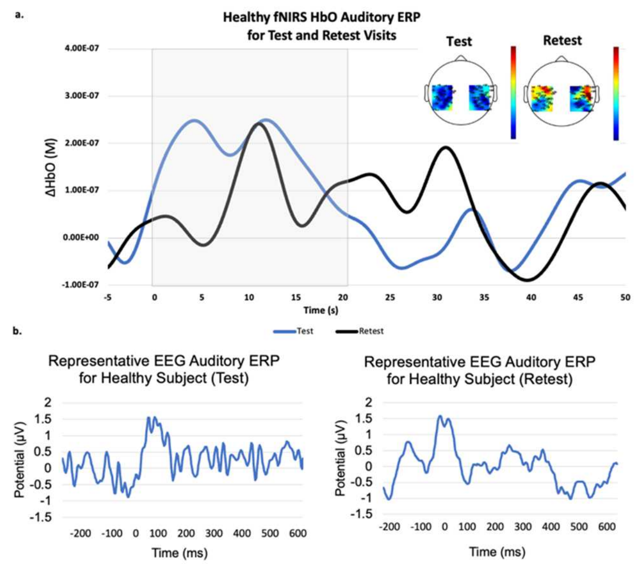

Figure 1. a) Representative fNIRS HbO responses from a healthy subject at test (visit 1) and retest (visit 2) visits. Shaded area indicates the block stimuli from 0-20 s. Inserts are the topography of beta values for test (left) and retest visits (right). b) Representative auditory ERPs for test (left) and retest session (right).

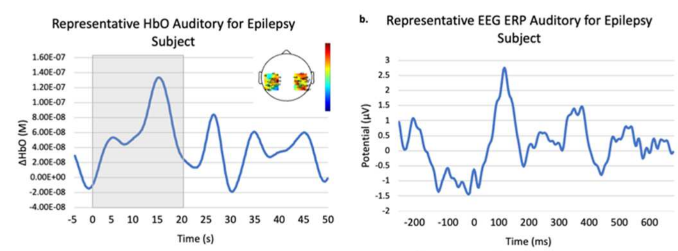

Figure 2. a) Representative fNIRS and ERP in epileptic subject. Shaded area indicates the block of stimuli from 0 to 20 s. Insert is the topography of beta values for this subject. **b)** Representative EEG Auditory ERP for epileptic subject. Channel shown (TP9) is located over the left auditory cortex.

D. Test-rest Reliability Assessment

Prior to studying NVC in epileptic patients, reliability was assessed in healthy subjects with two visits. We evaluated EEG, fNIRS and NVC for HbO and HbR conditions, using minimal processing. fNIRS and NVC were also evaluated for both conditions using PCA-GLM.

EEG: The peak EEG amplitude of the ERP averaged over two selected channels were evaluated. fNIRS: fNIRS ROI consisted of three channels over the left and right auditory cortices, chosen to best represent the hemodynamic responses to the sound stimuli. Quantities of HbO and HbR were calculated by averaging the six-channel ROI over the peak time window of 5-20 s. NVC: The power of ERP was used instead of voltage. Specifically, ERP time courses were squared, summed from 0 to 300 ms and averaged over two representative channels, yielding a quantity reflecting total power of neural response to stimuli. NVC was calculated by dividing fNIRS (average of HbO/HbR relative changes) over EEG (average of the ERP power).

After calculations, a two-tailed, paired t-test assuming unequal variances was applied to the test (visit 1) and retest (visit 2) in the healthy. To identify which quantity was more reliable, a one-way random-effect intraclass correlation coefficient (ICC) was calculated [9]. For these measurements, the primary components are the between-subject variance that uses between-subjects mean squares (MS_B) and the between-test variance that uses within-subject mean squares (MS_W) [9]. The following equation was used to quantify the reliability:

$$ICC = \frac{MS_B - MS_W}{MS_B}$$

E. *Comprison Between Epileptic and Healthy Individuals*

To explore if NVC is impaired in epileptic individuals, we compared epileptic and healthy subjects using minimal processing and PCA-GLM pipelines. For healthy, values were averaged across both visits, while epileptic group values were averaged across the single visit.

III. Results

A. *Reliability Assessment Among Healthy Subjects*

Representative time courses of EEG and fNIRS in a single healthy subject from test and retest visits are shown in **Fig. 1**. fNIRS show an increase of HbO and decrease of HbR accompanying the block of sound stimuli, peaking from about 5 to 20 s with regard to the beginning of blocks. The EEG ERP show activation too, peaking at a much faster time scale, about 100 ms. Since NVC is defined as the ratio between fNIRS and EEG responses, we evaluated the reliability of EEG and fNIRS separately and assessed the NVC. **Figure 3** displays the quantified metrics for EEG, fNIRS and NVC for HbO and HbR. In all metrics, there was no difference between test and retest visits ($p > 0.1$ for all). These results suggest the measures in the healthy control group to be reliable.

Further assessments were made by calculating the ICC for each condition. Reliability was determined using a 0.0 to 1.0 scale that ranged from poor (ICC < 0.40), fair (0.40 < ICC < 0.59), good (0.60 < ICC < 0.74) and excellent (0.75 < ICC < 1.00) [8]. fNIRS and NVC HbO metrics, shown in **Table 1**, resulted in the greatest reliability. PCA-GLM processing ICC values were slightly lower; however, demonstrated greater reliability in NVC than standalone fNIRS and EEG values.

Table 1. Reliability ICC Values for EEG, fNIRS and NVC.

EEG	fNIRS HbO	HbO NVC	fNIRS HbR	HbR NVC
0.46	0.92	0.97	0.34	0.26

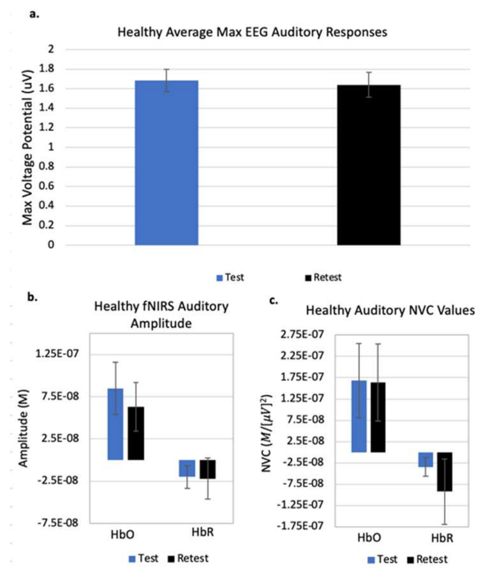

Figure 3. Group level comparison of healthy subjects for test (blue) and retest (black) visits. a) Peak EEG amplitude, b) fNIRS HbO and HbR responses, c) NVC HbO and HbR. Error bars indicate standard error across all subjects.

B. *Epilepsy and Healthy Comparison Across fNIRS, EEG, and NVC Responses*

Representative time courses of fNIRS and ERP response from an epileptic patient are shown in **Fig. 2**. The group level data is plotted in **Fig. 4**. EEG peak amplitude did not differ between healthy and epileptic subjects ($p = 0.36$), shown in **Fig. 4a**. For HbO and HbR (**Fig. 4b**), epileptic subjects consistently had lower responses than healthy. For HbO, the average max amplitude response in epileptic subjects resulted in a significantly lower amplitude compared to the healthy group ($p = 0.04$). In HbR, while the healthy group maintained a visual mean difference, the significance value was less than that of the HbO condition ($p = 0.70$). Regarding NVC, the healthy group had a notable difference compared to the epilepsy group, approaching significance ($p = 0.09$ for NVC HbO, $p = 0.29$ for NVC HbR).

Applying the PCA-GLM method to fNIRS and NVC datasets resulted in significant differences among the epileptic and healthy subject groups, suggesting PCA-GLM enhances the significance by reducing insignificant noise. In **Fig. 5,** the epileptic fNIRS HbO average demonstrated consistent negative responses compared to the healthy group, resulting in a remarkable difference ($p = 0.0005$). The fNIRS HbR response (**Fig. 5**) resulted in similar trends; however, not as significant ($p = 0.17$). For NVC, in HbO and HbR, similar trends were seen which resulted in significance for both conditions ($p = 0.02$ for NVC HbO, $p = 0.03$ for NVC HbR).

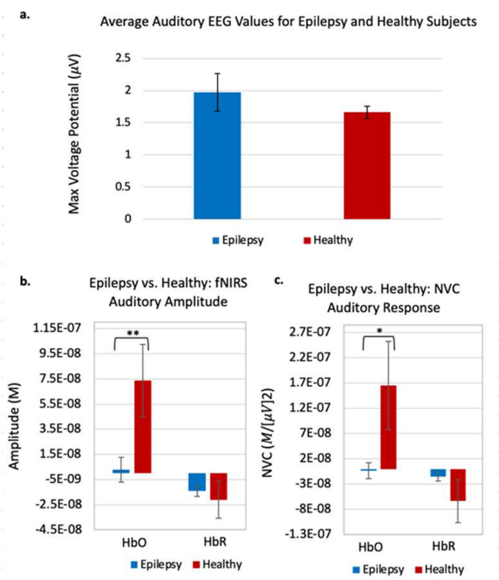

Figure 4. Compare epileptic and healthy subjects, in a) Peak EEG amplitude, b) fNIRS HbO and HbR responses. c) NVC of HbO and HbR. Error bars indicate the standard error. ** = $p < 0.05$ *= $p<0.1$.

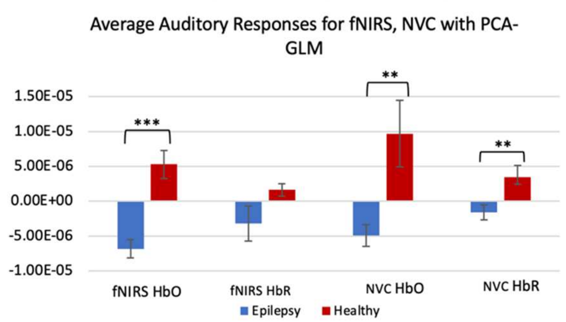

Figure 5. Compare epileptic and healthy subjects with applying PCA-GLM fNIRS HbO and HbR responses. And NVC of HbO and HbR. Error bars indicate the standard error. ***=$p<0.001$ **= $p<0.05$.

IV. Discussion

The reliability assessment performed among the healthy subjects demonstrated the consistency and reliability of the simultaneous fNIRS-EEG instruments used in these experiments. All measures of fNIRS, EEG, and NVC showed consistent data between the test and retest visits. Further, fNIRS and NVC HbO with minimal processing, showed excellent reliability [8]. From this, these results were used as a baseline when evaluating the healthy control against the epileptic patients.

The quantitative assessment between the healthy and epileptic group illustrated the simultaneous fNIRS-EEG setup used in this study was able to identify significant differences in the epilepsy and healthy control group. More specifically, when applying PCA-GLM, our results further indicate the impaired NVC in epileptic individuals. Compared with healthy, epileptic subjects showed abnormally lower hemodynamic responses (HbO) despite normal neuronal responses (ERP). The presentation of abnormal NVC is consistent with their underlying condition of temporal lobe epilepsy.

V. Conclusion

Three primary conlcusions were obtained from this study. 1) Based off several paired t-tests, no signfiicant differences were found in the Test and Retest visits. 2) Auditory responses among healthy subjects resulted in high reliability agreement, specifically within fNIRS and NVC HbO conditions. 3) Quantitative assessments between the epilepsy and healthy subject group demonstrated that simulataneous fNIRS-EEG is a feasible method for detecting impaired NVC in epileptic subjects. Previous fMRI-EEG litearture point to the idea of a dynamic HRF, in which the HRF may not be seen across the same window of time in all individuals, a possible cause for the lower hemodynamics seen in the epileptic group [2],[10]. Therefore, future work includes investigating NVC on an individualized basis to compare with the static measures taken in this study. Additionally, the signficant response difference for fNIRS HbO and HbR for healthy should further be looked into. While ROIs were chosen upon initial activation seen in the HbO condition, it should not signficantly impact either condition. Applying these implementations and increasing recruitment of subjects should lead to more conclusive results that will determne the efficacy of simultaneous fNIRS-EEG.

References

[1] G. C. Teskey and C. H. Tran, "Neurovascular coupling in seizures," *Neuroglia*, vol. 2, no. 1, pp. 36–47, 2021. doi:10.3390/neuroglia2010005

[2] R. Rathakrishnan, F. Moeller, P. Levan, F. Dubeau, and J. Gotman, "BOLD signal changes preceding negative responses in eeg-fmri in patients with focal epilepsy," *Epilepsia*, vol. 51, no. 9, pp. 1837–1845, 2010. doi:10.1111/j.1528-1167.2010.02643.x

[3] M. Zhao *et al.*, "Preictal and ictal neurovascular and metabolic coupling surrounding a seizure focus," *The Journal of Neuroscience*, vol. 31, no. 37, pp. 13292–13300, 2011. doi:10.1523/jneurosci.2597-11.2011

[4] T. H. Schwartz, "Neurovascular coupling and epilepsy: Hemodynamic markers for localizing and predicting seizure onset," *Epilepsy Currents*, vol. 7, no. 4, pp. 91–94, 2007. doi:10.1111/j.1535-7511.2007.00183.x

[5] Chen Y, Tang J, Chen Y, Farrand J, Craft MA, Carlson BW, Yuan H. Amplitude of fNIRS Resting-State Global Signal Is Related to EEG Vigilance Measures: A Simultaneous fNIRS and EEG Study. Front Neurosci. 2020;14:560878. doi: 10.3389/fnins.2020.560878. eCollection 2020.

[6] Zhang F, Reid A, Schroeder A, Ding L, Yuan H. Controlling jaw-related motion artifacts in functional near-infrared spectroscopy. J Neurosci Methods. 2023 Feb 2;388:109810.

[7] Zhang F, Cheong D, Khan AF, Chen Y, Ding L, Yuan H. Correcting physiological noise in whole-head functional near-infrared spectroscopy. J Neurosci Methods. 2021 Aug 1;360:109262. doi: 10.1016/j.jneumeth.2021.109262. Epub 2021 Jun 17. PMID: 34146592.

[8] Zhang F, Khan AF, Ding L, Yuan H. Network organization of resting-state cerebral hemodynamics and their aliasing contributions measured by functional near-infrared spectroscopy. J Neural Eng. 2023 Jan 18;20(1):016012. doi: 10.1088/1741-2552/acaccb.

[9] L. Li, L. Zeng, Z.-J. Lin, M. Cazzell, and H. Liu, "Tutorial on use of intraclass correlation coefficients for assessing intertest reliability and its application in functional near-infrared spectroscopy–based brain imaging," *Journal of Biomedical Optics*, vol. 20, no. 5, p. 050801, 2015. doi:10.1117/1.jbo.20.5.050801

[10] Y. Lu *et al.*, "Using voxel-specific hemodynamic response function in EEG-fmri data analysis," *NeuroImage*, vol. 32, no. 1, pp. 238–247, Aug. 2006. doi:10.1016/j.neuroimage.2005.11.