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Neuroimaging field methods using functional Near Infrared Spectroscopy (fNIRS) Neuroimaging to Study Global Child Development: Rural sub-Saharan Africa --Manuscript Draft--

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Abstract:	Portable neuroimaging approaches provide new advances to the study of brain function and brain development with previously inaccessible populations and in remote locations. This paper shows the development of field functional Near Infrared Spectroscopy imaging to the study of child language, reading, and cognitive development in a rural village setting of Côte d'Ivoire. Innovation in methods and the development of culturally appropriate neuroimaging protocols allows a first-time look into the brain's development and children's learning outcomes in understudied environments. This paper demonstrates protocols for transporting and setting up a mobile laboratory, discuss considerations for field versus laboratory neuroimaging, and present a guide for developing neuroimaging consent procedures and building meaningful long-term collaborations with local government and science partners. Portable neuroimaging methods can be used to study complex child development contexts, including the impact of significant poverty and adversity on brain development. The protocol presented here has been developed for use in Côte d'Ivoire, the world's primary source of cocoa, and where reports of child labor in the cocoa sector are common. Yet, little is known about the impact of child labor on brain development and learning. Field neuroimaging methods have the potential to yield new insights into such urgent issues, and the development of children globally.
Author Comments:	Re: Using functional Near Infrared Spectroscopy (fNIRS) Neuroimaging to Study Global Child Development: Imaging Field Methods in Rural sub-Saharan Africa Kaja K. Jasinska* and Sosthène Guei *Corresponding Author Dear Dr. Vineeta Bajaj: We sincerely thank you for considering our manuscript for publication in Journal of Visualized Experiments and especially for the opportunity to improve this manuscript

as per the specific suggestions provided by the reviewers. We greatly appreciate the time and effort that went into their thoughtful reviews. We are delighted to report that the manuscript is now improved following from their helpful suggestions. Every suggestion identified by the reviewers is now addressed. Please see our detailed replies to each reviewer's critiques below. The overall expansion of our descriptions reflect our deep gratitude and extraordinary respect for the reviewers' invaluable input. We thus sincerely hope you will now find our manuscript suitable for publication in the Journal of Visualized Experiments.

With my sincere best wishes,

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Editorial comments

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version. Response: All corrections have been made.

1. Please abbreviate all journal titles. Response: All corrections have been made.
2. Please define all abbreviations before use. Response: All corrections have been made.
3. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Response: A new table has been included.
4. Please add commas to separate affiliation numbers for the authors. Response: All corrections have been made.
5. Please provide an email address for each author. Response: All corrections have been made.
6. Please revise the title to better reflect the technique shown.
Response: Our title has been revised to: "Neuroimaging field methods using portable functional Near Infrared Spectroscopy (fNIRS) Neuroimaging to Study Global Child Development: Rural sub-Saharan Africa."
7. Please rephrase the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to ...". The current short abstract is above 50 words.
Response: Our abstract has been revised as follows: "Portable neuroimaging approaches (functional Near Infrared Spectroscopy) provide advances to the study of the brain in previously inaccessible regions; here, rural Côte d'Ivoire. Innovation in methods and development of culturally-appropriate neuroimaging protocols permits

novel study of the brain's development and children's learning outcomes in environments with significant poverty and adversity."

8. Unfortunately, there are a few sections in the manuscript that show overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please see lines: 82-86

Response: We have corrected this text.

Please see lines 81-94: "fNIRS neuroimaging is well-suited for field research. Similar to functional magnetic resonance imaging (fMRI), fNIRS measures the brain's hemodynamic response⁶. However, fNIRS uses a series of light emitting optodes and light detectors rather than generate electromagnetic fields. There are no restrictions on metal in or near the testing area, and no electric shielding is necessary, as in the case for electroencephalography (EEG). A key advantage of fNIRS is its portability (i.e. some systems may fit in a suitcase) and ease of use. fNIRS is also easy to use with children; the child is comfortably seated in a chair during the experiment and the fNIRS system tolerates movement well compared to fMRI. Compared with fMRI, fNIRS also provides separate measures of deoxygenated and oxygenated hemoglobin during recording, compared to fMRI which yields a combined blood oxygen level density (BOLD) measure. fNIRS has superior temporal resolution to fMRI: sampling rates can vary between ~7-15 Hz. fNIRS has good spatial resolution: fNIRS' depth of recording in the human cortex is less than fMRI, measuring about 3 to 4 cm in depth, this is well-suited for studying cortical functions, especially with infants and children who have thinner skulls than adults 3,7-10."

9. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: Shimadzu LightNIRS, etc.

Response: All references to Shimadzu LightNIRS have been removed.

10. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). Response: All corrections have been made.

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures, etc. Response: All corrections have been made.

12. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Response: We have adjusted the protocol and added explanatory "notes" to clarify specific protocol steps. We have also moved text that corresponds to discussion into the Discussion section.

13. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion. For e.g. 2.1.1, 2.2, 3.2.1, 4.2.2, 5.1.1, 7.1, etc. Response: See note above.

14. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Response: We have added additional details to the protocol section.

Please add section 3.5 along with the ethics statement. Response: We have added details of the IRB procedure in country and the ethics statement in section 3.4.

4.2.1: Please provide the specific scalp locations so this manuscript can stand alone. Response: Scalp locations are noted in the international 10-20 scalp location standard.

4.3.2: Please explain how to arrange the 3D digitizer in detail. Where is the second sensor placed? Please be more specific.

Please see revision 4.3.2: "Arrange 3D digitizer equipment. One sensor should be placed on the participant's head at Cz and affixed securely (i.e. using elastic or hair accessory), and the second block sensor should be placed behind the participant. For example, the participant can be seated in a chair with their back to a table. The second sensor can be placed on the table directly behind the participant's head. Neither sensor can move during the course of obtaining the 3D digitizer measurement."

4.4.3: Please explain how to select the probe arrangement. What is the basis of the experimental design?

Please see revision 4.4.2: "Using the fNIRS built-in software, select the probe arrangement that corresponds to the experiment design. fNIRS probes can be placed to cover the entire head (i.e. full head coverage), or alternatively, an array can be

placed over general regions of interest. For example, this protocol used a 10 x 3 probe array (30 probes arranged in 3 rows of 10 probes each). This probe arrangement was placed to maximally overlay left hemisphere language areas and their right hemisphere homologues, as well as the frontal lobe. See Figure 2."

4.4.4: Please describe the optode map. Where does it come from? Please see revision on 4.4.4: "Using the optode map in the fNIRS inbuilt software as a guide, place each optode in the appropriate optode opening on the cap. The optode map indicates the location of each optode in the array (e.g. 10 x 3)"

5.3: How are the experimental tasks presented? Is there a rest period? How long is each task presented?

How are the speech and non-speech sounds presented? How are the rhyming and non-rhyming words selected?

How are the images presented on the screen and how are they selected?

5.8: Is there a specific reason to measure the given tasks? Are there any other tasks which can be measured?

Response (to above comments re: task): We have revised the session describing the task. In fact, any researcher following this protocol will customize the experimental task to suit their research aims. Our tasks are only an example. In the first version of the manuscript, our protocol gave the incorrect sense that these are required steps. We have noted our error and as such, we have limited our description and clearly state that Please explain how to analyze the data in more details. Response: We've added additional details as per the reviewers' (particularly reviewer 1) incredibly helpful and insightful comments.

7.4.1: Corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s). Response: This is now corrected.

7.5.1: Citation? Response: This is now corrected.

16. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

17. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Response: Relevant sections are highlighted.

18. Please note that only steps 4, 5, and 6 can be filmed. The other steps are not appropriate for the video. Response: This is noted. We do also have a lot of footage we accumulated in Cote d'Ivoire that we would be happy to potentially share with the production team if deemed relevant.

19. Please explain the figures in more detail in the Representative Results. Response: We have added additional details to our figures, particularly following Reviewer 1's suggestions as well.

20. Please do not have one sentence paragraphs. Response: This is now corrected.

21. Please revise discussion to also include the significance with respect to existing methods and any future applications of the technique. Response: We have added a section into our discussion for existing methods and future applications.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This article demonstrated a protocol of using a portable neuroimaging technique fNIRS to study child labor on brain development in rural Africa area. The practical protocol including fNIRS equipment shipping and set up, fNIRS data analysis, and finding local research team, can be very useful to researchers in the field.

Response: We sincerely appreciate the reviewer's careful reading of our manuscript. Particularly the reviewer's detailed attention to analysis issues that were lacking or unclear in our previous version of the manuscript, including nirs preprocessing steps. This is a very critical point in the fNIRS field right now, particularly because robust

analysis methods are not always utilized. As such, we are very grateful for the opportunity to make the important improvement to our manuscript, which now expresses a higher standard for nirs data preprocessing and statistical inference.

Major Concerns:

1. It is suggested to provide a picture of the NIRS device set up as well as the mobile lab, so that readers can see the size of the NIRS system and the mobile lab.

Response: We thank the reviewer for this wonderful suggestion. We have added a new figure with a schematic of our laboratory set up and photos. See Figure 1. We have also accumulated video footage of laboratory setup, in addition to photos, to share with JoVE team for potential production.

2. Is there any data backup plan, since online data storage service is usually not available on site?

Response: This is a very important concern, and in fact, in the field, we did not have any access to online data storage. As such, data was transferred to multiple to external portable hard drives during field data collection dates. We note this data backup plan in section 7. See Line 340:

"7.Backup data

7.1. Ensure that data is exported and backed up to multiple portable hard drives, as internet access for online data storage is unlikely to be available. Data should be transferred to online data storage as sufficient internet connectivity is available."

Minor Concerns:

3. protocol 1.2.1, more details about how temperature affect fNIRS functionality are expected. e.g. what temperature range does fNIRS equipment require, and within the range, will the signal quality change along the temperature variation.

Response: Electronics operating in high humidity conditions, generally above 60%, are more susceptible to corrosion as excessive moisture can settle on parts and react with metal. Humidity levels in an indoor lab (e.g. inside a university building) are generally between 30-50%. Humidity in southern Côte d'Ivoire ranges between 80 and 90%. We have revised the text, see section 1.2.2.: "1.2.1. Prepare for climate conditions in the field. Note: Temperature and humidity conditions in the field can vary significantly from laboratory setting and may affect equipment function and longevity, as well as participant comfort during experimentation. Electronics operating in high humidity conditions, generally above 60%, are more susceptible to corrosion as excessive moisture can settle on parts and react with metal components. Humidity levels in an indoor lab (e.g. inside a university building) are generally between 30-50%. Humidity in southern Côte d'Ivoire can be 80-95%. Set up a portable air conditioning unit with low wattage demands."

4. protocol 4.1.2, the authors should describe how to use the measured head size information, e.g. how to use the information when placing the cap on participants, the description in 4.2.1 is vague.

Response: We appreciate the reviewer bringing this important detail to our attention. We have revised the text to improve clarity. Please see section 4.2.1.: "4.2.1. Place the fNIRS optode holder cap onto the participant's head, aligning the cap to the international 10-20 system for scalp locations¹⁶. Cap position should be identical for all participants. Align points on the cap (e.g. probe holder) with scalp positions. For example, center the front of the cap on the head to frontopolar (FP) position. This position corresponds to 10% of the nasion-inion over top distance dorsal to nasion position."

5. protocol 4.3.2, if a sensor of the 3D digitizer is placed at Cz, it can be very easily moved during the localization data collection, please provide details of how the sensor is secured.

Response: Please see revision in 4.3.2.: "Arrange 3D digitizer equipment. One sensor should be placed on the participant's head at Cz and affixed securely (i.e. using elastic or hair accessory), and the second block sensor should be placed behind the participant. Neither sensor can move during the course of obtaining the 3D digitizer measurement."

6. Protocol 7.5.1, please provide more details about the time derivatives, the authors should describe the reason of including the time derivatives in the analysis model.

Response: We are grateful to the reviewer for raising this important analysis issue, which indeed should be further clarified. Please see our revisions in section 8.5.1.: "The hemodynamic response function can have greater variability in higher cortical regions and across participants. These types of variability can be accommodated in analysis models by expanding the HRF to include temporal derivatives. A temporal derivative can model differences in the time to peak hemodynamic response (Friston et al., 1998)."

7. Since 7.4.1 and 7.4.2 was optional preprocessing steps, please discuss the performance of the low pass filter and Wavelet artifact correction method. It is also helpful to introduce more methods for data preprocessing so that readers can make their selection.

Response: Indeed, many rigorous analysis approaches exist, including outlined by Huppert et al (2009). We now stress in the manuscript that fNIRS researchers have to make a selection that should be guided by careful investigation of methods that are best suited to their research projects, and we indicate other approaches to consider.

Please see revisions in section 8.5: "Data preprocessing. Multiple options exist for preprocessing fNIRS data. For example, Huppert et al. (2009) propose very rigorous methods for different sources of noise²⁰. These include eigenvector-based reduction of motion artifacts, bandpass filtering techniques and eigenvector-based reduction of spatial covariance for physiological interference in data (e.g. respiration, blood pressure, heart rate). Huppert also shares a thorough commentary on sources of noise in fNIRS research and implications for statistical analysis ²¹. The fNIRS researcher must investigate preprocessing applications that are most appropriate for a given study. Below, an analysis approach modeled after Worsely and Friston (1995)²⁴ and Jang et al. (2009)²³ is presented."

8. Figure 1 panel C, why not also provide a coronal view of the digitization results?

Response: We have now added a coronal view.

9. Figure 4, the axis labels need revision, is the signal normalized?

Response: Thank you for bringing this to our attention. We have revised the figure and figure caption. A - raw data, not normalized, B - filtered data after wavelet detrending. Please see Figure 4.

10. Figure 5 and Figure 6, the authors should provide statistical results, e.g. labelling the color bar as t-value, also please provide the p-value associated with the results, especially for Figure 5 panel b.

Response: We have updated our figures. Figure 5 panel b presents a visualization of oxy and deoxy responses with standard error (shaded areas). This visualization is obtained from Cui Xi's plotAverage.m code (<http://www.alivelearn.net/?p=1191>)

Reviewer #2:

Manuscript Summary:

This is an excellent manuscript that addresses and situates the scientific and social benefits of an inclusive, collaborative, and culturally appropriate research protocol for rural contexts. As the authors write, "Methods that advance our field's capacity to study development in a fuller range of human experience can dramatically advance our understanding of the complex relation between brain development and the life experiences that shape it." This manuscript lays out the practical means to achieve this important objective for our field.

The scientific and social benefits (as well as the risks of not following this paradigm) are made clear as they relate to multiple parties (the researcher, the participants' community, and the research scientists based in that region.) This manuscript is also timely given the increasing interest in fNIRS systems and will serve well both new and established fNIRS users that may be seeking to extend their research into studying populations that have been historically underrepresented in research studies.

The fNIRS protocols for setting up the equipment for participants, spatial registration, data collection, and data analyses are all appropriate and are within published cognitive neuroscience standards.

Response: We are very grateful for the reviewer's positive comments about our manuscript -- particularly noting the importance of "scientific and social benefits of an

inclusive, collaborative, and culturally appropriate research protocol for rural contexts". We have addressed all the very valuable issues the reviewer raised with respect to culturally-appropriate engagement. We are deeply appreciative of the reviewer raising important issues related to culture and science capacity building that pushed us to improve this new version of the manuscript.

Major Concerns:

§6.4: Given the emphasis provided on culturally appropriate engagement with rural communities and the importance of establishing appropriate channels for approval, the manuscript abruptly ceases to consider this after the cap is removed from the participant's head. This important consideration extends beyond obtaining consent to collect data. At the very minimum, section 6.4 should at least provide some discussion about how the participant is released from the experiment. Do they accept payment for time/participation? What is the appropriate method of acknowledging and thanking the participant? It would be more ideal if there was some mention of efforts to return the knowledge back to the community. This is partially alluded to in earlier sections that discuss the importance of building research capacity in rural regions. The manuscript would benefit well from a more explicit discussion on this matter.

Response: We thank the reviewer for this pertinent observation. We have addressed the question related to the benefits and compensation for participants and other stakeholders in the Discussion Section.

Please see revisions in Discussion:

Local Science Engagement and Opportunities for Capacity Building

One of the main challenges that local, particularly junior, researchers in Côte d'Ivoire are faced with when they complete their studies is the lack of opportunity for hands-on research experience with experienced researcher mentors and/or international collaborators. For this purpose, researcher should make all efforts to establish robust collaboration with local researchers from relevant disciplinary backgrounds, and include trainees at all levels (undergraduate, graduate, postdoctoral). Trainees can leverage the insight gained from this experience to work independently and further research. This experience can also be a stepping stone to build their capacities as researchers and develop their competitiveness at the international level in writing research proposals, papers and applying for grants. A research team excluding local researchers may have a reduced chance of success as local researchers will best know the local social and cultural values and systems, the local languages spoken in addition to the geographic knowledge of the area. Their contribution is therefore extremely important in understanding the local realities and designing culturally-appropriate protocols for the successful research projects.

Culturally-Appropriate Research Methods

The development of informed consent protocols to conduct research in rural settings specifically in Cote d'Ivoire is critical and failure to adopt the appropriate approach can inhibit the successful achievement of the research even though well-intentioned and scientifically robust 28-32. Generally, in rural settings in Côte d'Ivoire, asking a villager to read a consent form and sign it can break any trust building between the researcher and the participant. In fact, the perceived formality of this procedure may create a psychological distance and a feeling of insecurity in the participant's mind. This may result in a clear or unexpressed unwillingness to collaborate. This attitude can be explained by many factors including a long history of oral tradition whereby communication is more oral than written and high rates of illiteracy that may be found in target communities. Communities in rural settings trust their chief and rely on his decision-making power. Therefore, the protocol presented incorporates the consent of the chief of the village at the community level. This is arguably more culturally important than individual consent. Additionally, participants and community members may have had limited or no exposure to neuroimaging technology or computers before. Therefore, researchers need to take into consideration that the informed consent procedure, and instructions, may be misunderstood. The function of the fNIRS system should be communicated in lay terms and appropriate language easily understandable by child participants and community members who may have had very limited exposure to technology. These considerations can strongly influence the comfort and confidence of all community members involved in a field neuroimaging research project.

The protocol presented here also highlighted the importance of sharing research findings with community members and government partners. Partnerships built on continued dialogue aid in the eventual translation of research findings into policy. It is imperative to arrange post data collection field visits to disseminate research findings and deliver reports and, possibly, share any tools that resulted from the study (e.g. assessments in local languages). Participating communities in rural settings may never otherwise receive information about study completion and findings given lack of internet service and/or computers. Likewise, researchers in country may have limited access to academic journal subscriptions and poor internet connectivity at regional universities. Published results should be shared in a regional forum, and made available in an accessible language.

Minor Concerns:

Line 81 on page 1: "the fNIRS system tolerates movement well compared to both fMRI and EEG." NIRS systems are not any more tolerant of motion than EEG systems, but they are certainly much more tolerant of motion than fMRI systems (and even PET or MEG systems, which can tolerate small minor movement.) This sentence should be corrected.

Response: We have made this correction, thank you for bringing this important detail to our attention.

§7.1: It is correct that NIRS-SPM & Homer2 are widely used, but the fNIRS Toolbox by Huppert is quickly gaining considerable attention and use. To reflect current trends, this toolbox should also be cited.

Response: We are grateful for this important note. Indeed, we are excited about the development of the fNIRS toolbox and plan to incorporate the advance into our future research program. We did note that on the fNIRS Toolbox website (<https://bitbucket.org/huppert/nirs-toolbox/wiki/Home>) there is currently an omission for the direct citation of the toolbox.

Please see our revision: "Multiple data analysis packages exist for fNIRS: Statistical Parametric Mapping for Near-Infrared Spectroscopy (NIRS-SPM)19, Homer220 (widely used), and the fNIRS toolbox 21,22 (new and gaining popularity) are used for fNIRS data analysis. This protocol reviews data analysis methods using NIRS-SPM, but it is to the discretion of the researcher to select preferred method of analysis.

Typos:

§7.4.1: Citation for Jang is not formatted correctly.

Figure 3: 2 instances of "Trail" as opposed to "trial"

Response: We have made these corrections. Thank you for noting this.

Reviewer #3:

Manuscript Summary:

The protocol described using portable functional near infrared spectroscopy (fNIRS) in studying children's reading development and ability in rural sub-Saharan Africa, especially in regions with a high risk of illiteracy. The study is performed in Côte d'Ivoire where an estimated of 1.3 millions of child labor in the coca agricultural sector. It is an important topic because little has been studied about the impact of child labor on brain development and learning. The manuscript is well-written and easy to understand. The protocol can help researcher understand fNIRS is portable, low-cost and easy to operate in field research, especially in the pediatric populations. Although similar setup of fNIRS field studies in low-resource countries have been published (Bergus et al, 2016, PMID: 26782222; Papademetriou et al, 2014), the protocol provides useful information, including preparations for traveling with laboratory devices in low-resource contexts and set up, to researcher who interested in setting up field fNIRS studies.

Response: We would like to thank the reviewer for their thoughtful comments on our manuscript. Particularly referencing the growing group of fNIRS research in low-resource contexts. We have added an additional section to our discussion to review some of the existing fNIRS neuroimaging studies in similarly low-resourced contexts. We agree with the reviewer that is important to note the extent to which portable

	<p>NIRS technology is already revolutionizing cognitive neuroscience.</p> <p>Major Concerns: Are the tasks used in the fNIRS measurements culturally appropriate or adapted to children who can not read or/and write? Please address the considerations of the potential impact on the test results.</p> <p>Response: The fNIRS tasks and measurements are adapted to children in a village setting who have very low/beginning reading ability. Indeed, most of the participants and community members have not have exposure to neuroimaging technology or computers before, but efforts are made by the experimenters to explain the instructions and the function of the fNIRS system in lay terms and appropriate language easily understandable by child participants. Experimenters also ensure that the participants feel confident and relaxed all along the experiment. From our experience, children are keen to participate in the experiment whatever their literacy level as they consider the research activities like a game. All these factors strongly influence their comfort with participating and have a positive impact on the quality of data collected.</p> <p>Please see 5.4.2.1.: "Note: Tasks should be created in a manner suitable for the participant. In the study reference here, researchers were investigating language, cognitive, and reading development in children who were very poor readers. The reading neuroimaging task developed with words that would be appropriate for a child with minimal literacy skills. As well, children were selected for the neuroimaging paradigm based on scores obtained on a reading assessment."</p> <p>Minor Concerns: The author stated the protocol has been approved by the Institutional Review Board of the University of Delaware. Please confirm the study is also approved by local IRB in Côte d'Ivoire. If not, please explain why.</p> <p>Response: We sincerely appreciate the reviewer raising this issue. IRB approval was obtained from the University of Delaware (and now noted in the manuscript). At the time of writing this manuscript, Cote d'Ivoire did not have any formal IRB procedures, albeit there has been mention that a branch of government was working on developing a comprehensive policy and procedure. Date of completion and policy implementation are not known. In lieu of this, we obtained approval directly from the Ministry of Education.</p> <p>Please see revision in 3.5: "All research activities need to be approved by local authorities, for example, the Ivorian Ministry of Education or the Ministry of Higher Education and Research. The country the project will take place in may not have a formal procedure for ethical approval through an institutional review board (IRB). Check regulation to ensure you are following the correct protocols for obtaining ethical approval for research. Note: at time of obtaining approval, Cote d'Ivoire did not have a formal IRB review process. In lieu of this, the research team proceeded by preparing documentation akin to a IRB application to submit to the Ministry of Education. Multiple meetings were arranged with Ministry of Education and Ministry of Higher Education and Research officials. At these meetings, the research team presented the proposed research plan to all officials followed by group discussion and question and answer sessions. Ethical approval was obtained directly from the Ministry of Education in the form of a signed document granting authorization to conduct research with children at specific schools.</p>
Additional Information:	
Question	Response
If this article needs to be "in-press" by a certain date, please indicate the date	

below and explain in your cover letter.

TITLE:

Neuroimaging Field Methods Using Functional Near Infrared Spectroscopy (fNIRS) Neuroimaging to Study Global Child Development: Rural Sub-Saharan Africa

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KEYWORDS:

fNIRS, field methods, neuroimaging, cognition, neuroscience, child development, global literacy, language, reading, Ivory Coast, sub-Saharan Africa

SHORT ABSTRACT:

Portable neuroimaging approaches (functional Near Infrared Spectroscopy) provide advances to the study of the brain in previously inaccessible regions; here, rural Côte d'Ivoire. Innovation in methods and development of culturally-appropriate neuroimaging protocols permits novel study of the brain's development and children's learning outcomes in environments with significant poverty and adversity.

LONG ABSTRACT:

Portable neuroimaging approaches provide new advances to the study of brain function and brain development with previously inaccessible populations and in remote locations. This paper shows the development of field functional Near Infrared Spectroscopy (fNIRS) imaging to the study of child language, reading, and cognitive development in a rural village setting of Côte d'Ivoire. Innovation in methods and the development of culturally appropriate neuroimaging protocols allows a first-time look into the brain's development and children's learning outcomes in understudied environments. This paper demonstrates protocols for transporting and setting up a mobile laboratory, discusses considerations for field versus laboratory neuroimaging, and presents a guide for developing neuroimaging consent procedures and building meaningful long-term collaborations with local government and science partners. Portable neuroimaging methods can be used to study complex child development contexts, including the impact of significant poverty and adversity on brain development. The protocol presented here has been developed for use in Côte d'Ivoire, the world's primary source of cocoa, and where reports of child labor in the cocoa sector are common. Yet, little is known about the impact of child labor

on brain development and learning. Field neuroimaging methods have the potential to yield new insights into such urgent issues, and the development of children globally.

INTRODUCTION:

Portable fNIRS imaging provides the ability to study brain function and development outside the laboratory, in previously inaccessible settings or with understudied populations. Much of the knowledge in the domain of cognitive neuroscience comes from imaging studies conducted in university or hospital laboratory settings, in predominantly Western countries. By design, this contributes to a seldom-spoken-of problem in research: much of what is known about the brain is based on studies with participants for whom laboratory settings in (mostly) Western countries are accessible. That is, most neuroimaging research involves participants who live in reasonable proximity to a neuroimaging laboratory and have both the time and resources necessary to participate in a study. As a discipline, cognitive neuroscience aims to understand the brain and the factors that shape its development—including the powerful effects of a child's environment and their early-life experiences¹⁻³. Methods that advance the field's capacity to study development in a fuller range of human experience can dramatically advance the understanding of the complex relation between brain development and the life experiences that shape it.

This paper presents a protocol for field neuroimaging, which was developed for use in rural sub-Saharan Africa, specifically southern Côte d'Ivoire. The aim of this field neuroimaging research program was to understand children's reading development in an environment with a high-risk of illiteracy. Côte d'Ivoire's youth (15–24 years) literacy rate is 53%, despite 93% primary school enrollment rates⁴. Côte d'Ivoire is the world's primary source of cocoa, and there are an estimated 1.3 million child laborers in the cocoa agricultural sector⁵. Yet, little is known about the impact of child labor on brain development and learning, specifically learning to read. Applying the latest tools of cognitive neuroscience, *i.e.*, portable neuroimaging methods, yields impacts on children's learning outcomes. insights into how the developing brain responds to this high-risk environment. For example, field neuroimaging with fNIRS can allow the identification of neurodevelopmental periods during which targeted educational programs or interventions may have maximal impacts on children's learning outcomes.

fNIRS neuroimaging is well-suited for field research. Similar to functional magnetic resonance imaging (fMRI), fNIRS measures the brain's hemodynamic response⁶. However, fNIRS uses a series of light emitting optodes and light detectors rather than generating electromagnetic fields. There are no restrictions on metal in or near the testing area, and no electric shielding is necessary, as in the case for electroencephalography (EEG). A key advantage of fNIRS is its portability (*i.e.*, some systems may fit in a suitcase) and ease of use. fNIRS is also easy to use with children; the child is comfortably seated in a chair during the experiment and the fNIRS system tolerates movement well compared to fMRI. Compared with fMRI, fNIRS also provides separate measures of deoxygenated (HbR) and oxygenated hemoglobin (HbO) during recording, compared to fMRI which yields a combined blood oxygen level density (BOLD) measure. fNIRS has superior temporal resolution to fMRI: sampling rates can vary between ~ 7–15 Hz. fNIRS has good spatial resolution: the fNIRS' depth of recording in the human cortex is less than fMRI, measuring about

3 to 4 cm in depth, which is well-suited for studying cortical functions, especially with infants and children who have thinner skulls than adults^{3,7-10}.

This field neuroimaging protocol outlines considerations for traveling with and setting up a portable neuroimaging laboratory in low-resource contexts. The protocol also highlights the essential nature of meaningful, long-term collaborations with local science partners and ways by which this approach serves to build local science capacity. The neuroimaging protocol for collecting and analyzing fNIRS brain data from a battery of language, reading, and cognitive tasks, is demonstrated including recommendations for creating culturally appropriate informed consent procedures for imaging research. While this protocol is designed for cognitive development research with primary school aged children in rural Côte d'Ivoire, the protocol is highly relevant for any field neuroimaging study in challenging, low-resource environments, and can be adapted for novel contexts.

PROTOCOL:

All methods described here have been approved by the Institutional Review Board (IRB) of the University of Delaware.

1. Mobile Laboratory Transport and Setup

1.1. Traveling with the fNIRS equipment

1.1.1. Transport fNIRS equipment.

Note: fNIRS equipment can be transported as checked-luggage on a major international airline, but it is imperative to confirm with the given airline. Equipment restrictions may vary by origin or destination country. Alternatively, fNIRS equipment can be shipped.

1.1.2. Know the procedures for importing or traveling with fNIRS equipment for the destination country, and if applicable, obtain appropriate import approval documentation.

1.1.3. Prepare for inspections.

Note: Authorities (*e.g.*, Transportation Security Administration) reserve the right to inspect checked-baggage. Fragile fNIRS fiber optics may be damaged during inspections. Arrange for appropriate documentation to accompany all equipment.

1.2. Essential laboratory equipment in the field

1.2.1. Prepare for climate conditions in the field.

Note: Temperature and humidity conditions in the field can vary significantly from laboratory setting and may affect equipment function and longevity, as well as participant comfort during

experimentation. Electronics operating in high humidity conditions, generally above 60%, are more susceptible to corrosion as excessive moisture can settle on parts and react with metal components. Humidity levels in an indoor lab (*e.g.*, inside a university building) are generally between 30–50%. Humidity in southern Côte d'Ivoire can be 80–95%. Set up a portable air conditioning unit with low wattage demands.

1.2.2. Ensure adequate electrical supply. Since electrical supply may not be available in rural settings, or may function only intermittently or with insufficient wattage, use portable solar generators to power small to medium sized electronics. Make available a diesel generator as backup power. Employ a local electrician who is familiar with electrical supply challenges in rural contexts.

1.2.3. Prepare a suitable laboratory structure with minimal setup time such as a large customized tent with opaque and waterproof roof and walls.

Note: Facilities (*e.g.*, classroom at local school) are unlikely to be available, or provide waterproof and quiet testing space.

1.3. Setting up the portable laboratory (Figure 1)

1.3.1. Assemble the mobile laboratory (*e.g.*, customized tent). Ensure that the laboratory is large enough to accommodate seating for the participant at a desk, seating for two experimenters, stimulus presentation computer, fNIRS data collection computer, fNIRS portable unit, three-dimensional (3D) digitizer, and portable air conditioner.

[insert Figure 1]

2. Local Research Teams and Science Partners

2.1. Invest into formation of scientific collaborations and provide opportunities within the research framework to local researchers.

2.2. Establish partnership with local scientific institutions for the purpose of inclusivity. Gaining recognition from peers at the local level is important to communicating eventual research finding in the region.

2.3. Consult relevant local authorities before any research activities to receive authorization and license to operate. Familiarize with ethical review procedures in the target country, and make appropriate accommodations if no formal scientific review procedure is in place.

Note: For example, direct communication with and approval from representatives in the Ministry of Higher Education and Research (or comparable government body) may be done in lieu of an ethical review procedure.

3. Informed Consent and Child Assent

3.1. Develop a consent procedure that is culturally appropriate and ensures that participants, their families, and communities are informed about research and their decision to participate in the study.

3.2. Review local customs and history in typical protocol development and include members of the group with whom research needs to be conducted.

3.2.1. Make sure to obtain a clear consent from local leader(s) (*e.g.*, village chief, community elders, *etc.*) before proceeding with research.

Note: This may be expressed by an ancestral benediction or by any other means typical to the community. After consent from the village chief, cultural customs may include the pouring of wine onto the earth and asking the ancestors to approve and bless the research activities.

3.3. At a more formal level, seek the consent of parent and educator groups which are responsible for making decision concerning children's participation in school approved activities. For example, the parent-teacher group ('Comité de Gestion d'Ecoles – COGES' in Cote d'Ivoire) is a key stakeholder in the national primary educational system consisting of members appointed by student's parents to defend their interests in decision making and in all other aspects related to the education of their children.

3.4. Approve all research activities by local authorities, for example, the Ivorian Ministry of Education or the Ministry of Higher Education and Research. The country that the project will take place in may not have a formal procedure for ethical approval through an IRB. Check regulations to ensure that the correct protocols for obtaining ethical approval for research are being followed.

Note: At the time of obtaining approval, Côte d'Ivoire did not have a formal IRB review process. In lieu of this, the research team proceeded by preparing documentation akin to an IRB application to submit to the Ministry of Education. Multiple meetings were arranged with the Ministry of Education, Ministry of Higher Education, and research officials where the research team presented the proposed research plan to all officials, followed by group discussion and question and answer sessions. Ethical approval was obtained directly from the Ministry of Education in the form of a signed document granting authorization to conduct research with children at specific schools. This study received ethical approval from the University of Delaware IRB.

3.5. Explain the research purpose in simple words to participating children in a child assent procedure. The local community may highly value a child's obedience, in which case a child may assent to participate or continue participating in a study despite their unwillingness to do so because of cultural expectations. Ensure that the assent procedure carefully communicates voluntary participation in the research.

3.6. Clearly define how the research will benefit the participants and if they will receive compensation for their participation in the research. Ensure the compensation is appropriate both culturally and for the participants.

3.7. Conduct all consent and assent procedures in the local or preferred language of the participant by a trained member of the research team who is also a member of the language and cultural group.

4. fNIRS Scalp Placement and Measurement

4.1. Collecting head measurements

4.1.1. Direct the participant to sit on a chair, and explain the process to be expected during head measurement.

4.1.2. Using a standard tape measure, measure the distances between: (1) the nasion and inion around the head, (2) the nasion and inion over top of the head through the midline central (Cz)¹¹, and (3) the distance between the left and right ear tragus over the top of the head through Cz.

4.2. Placement of the fNIRS cap and optodes on the participant's head^{3,8,9,12}

4.2.1. Place the fNIRS optode holder cap onto the participant's head, aligning the cap to the international 10-20 system for scalp locations¹¹. Ensure that the cap position is identical for all participants. Align points on the cap (*e.g.*, probe holder) with scalp positions.

Note: For example, center the front of the cap on the head to the frontopolar (FP) position. This position corresponds to 10% of the nasion-inion over top distance dorsal to nasion position.

4.2.2. Secure the cap with a strap and ensure the participant is comfortable.

4.3. 3D digitizer measurement

4.3.1. Once the cap is in position, instruct the participant to sit still in the position for obtaining a 3D digitizer measurement of the key 10-20 system scalp positions¹¹ and each optode place holder.

4.3.2. Arrange the 3D digitizer equipment. Place one sensor on the participant's head at Cz and affixed securely (*i.e.*, using an elastic or hair accessory), and place the second block sensor behind the participant. Let the participant be seated in a chair with their back to a table. Place the second sensor on the table directly behind the participant's head. Ensure that neither sensor move during the course of obtaining the 3D digitizer measurement.

4.3.3. Open the Brainstorm software¹³ on the data collection computer. Make sure the 3D digitizer system is in communication with the Brainstorm software through the appropriate COM port.

4.3.4. Move the 3D digitizer stylus to each probe location and across the key 10-20 system positions (nasion, inion, left ear, right ear, Cz). At each location, obtain position data through the Brainstorm function on the data collection computer.

4.4. Placing light emitting optodes and detectors on the scalp

4.4.1. After the 3D digitizer data are collected, direct the participant to be seated comfortably in front of the stimulus presentation computer.

4.4.2. Using the fNIRS built-in software, select the probe arrangement that corresponds to the experiment design. fNIRS probes can be placed to cover the entire head (*i.e.*, full head coverage), or alternatively, an array can be placed over general regions of interest. For example, this protocol used a 10 x 3 probe array (30 probes arranged in 3 rows of 10 probes each). This probe arrangement was placed to maximally overlay left hemisphere language areas and their right hemisphere homologues, as well as the frontal lobe (**Figure 2**).

4.4.3. Ensure that each probe (emitter and detector) is numbered and the numbering system corresponds to the probe arrangement map.

4.4.4. Using the optode map in the fNIRS inbuilt software as a guide, place each optode in the appropriate optode opening on the cap. The optode map indicates the location of each optode in the array (*e.g.*, 10 x 3).

4.4.5. Move any hair out of the way to ensure direct contact between the tip of the optode and participant's scalp.

4.4.6. After all, optodes are in position, check for signal quality using the fNIRS system built-in software.

4.4.7. Adjust individual probes as necessary until sufficient signal quality is achieved. Once all optodes' signal quality checks have passed, proceed with experimental tasks.

5. Experimental Tasks

5.1. Design each neuroimaging task with the appropriate number of trials and conditions in line with the research goals. Understand that the neuroimaging tasks will vary depending on the research aims. For example, three tasks were used in this protocol: (1) a language processing and reading task, (2) a rhyme judgment task, and (3) a cognitive flexibility task.

Note: The procedure (and representative results) of the rhyme judgment task are highlighted.

5.2. Place noise-cancelling headphones on the participant's head, being mindful not to interfere with fNIRS probe placement. Ensure that the headphones will deliver auditory speech stimuli to the participant, as well as block any ambient noise.

Note: Laboratory testing typically takes place in a sound attenuated room. Field laboratory testing does not provide the same degree of noise control, and noise-cancelling headphones can ensure quiet testing conditions for all participants.

5.3. Instruct the participant to face the computer monitor and fixate on the cross in the middle of the screen, and to remain still during the experiment. Present all experimental tasks on a computer screen.

5.4. Rhyme judgment task

5.4.1. Instruct the participant to listen to the word pairs presented aurally through headphones. Ask the participant to indicate whether word pairs rhymed or not (e.g., 'cat'-'hat' or 'cat'-'log') with a button press on the keyboard.

5.4.2. In this example, use an event related design. Let participants complete 12 non-rhyming and 12 rhyming trials separated by jittered inter-stimulus intervals of between 8 and 17 s.

Note: Tasks should be created in a manner suitable for the participant. In the study referenced here, researchers were investigating language, cognitive, and reading development in children who were very poor readers. The reading neuroimaging task developed with words that would be appropriate for a child with minimal literacy skills. As well, children were selected for the neuroimaging paradigm based on scores obtained on a reading assessment.

5.5. Dim lights and begin recording the participant on the built-in video camera.

5.6. Begin fNIRS data recording on the fNIRS command computer, and commence tasks on the stimulus presentation computer.

5.7. Monitor participant performance throughout all tasks. Provide breaks between tasks and runs.

5.8. Ensure that triggering from the experimental stimuli presentation computer is received by the fNIRS command computer.

5.9. At the end of all tasks, stop collecting video and fNIRS data.

6. Post-experimental Task Measurements

6.1. Remove each optode from the optode holder cap.

6.2. Without disrupting the position of the optode holder cap on the participant's head, direct the participant to sit in a position to obtain a second 3D digitizer measurement.

6.3. Repeat the 3D digitizer measurement as in the fNIRS Scalp Placement and Measurement Section 4 to ensure that any disruptions to the scalp probe position during the experiment can be detected by comparing the two position files.

6.4. Remove the optode holder cap from the participant's head.

6.5. At the end of the experiment, provide participants with a small gift (*e.g.*, books and school supplies) and the acknowledgements of the research team for their participation.

7. Plan for Disseminating Data

7.1. Share the research findings with community members and relevant local authorities for their eventual translation into policy addressing the investigated issue.

Note: Participants may not benefit directly from the experiment.

7.2. Make plans for follow-up visits to the participating communities. Prepare reports and tools that local educators can use. For example, any assessments created in local languages should be made available to school officials in the region. Prepare the members of the research team who speak the local language to meet with community leaders to communicate study findings.

7.3. Make plans to publish study findings in regional academic journals and in the language of the region, if applicable. For example, study findings should be disseminated in French if research was conducted in Francophone countries.

7.4. Make plans to meet with and deliver reports of the study findings to the government branch that granted approval for the research program.

8. Backup Data

8.1. Ensure that data are exported and backed up to multiple portable hard drives, as internet access for online data storage is unlikely to be available. Transfer the data to online data storage as sufficient internet connectivity is available.

9. Data Analysis

Note: Multiple data analysis packages exist for fNIRS¹⁴. Statistical Parametric Mapping for Near-Infrared Spectroscopy (NIRS-SPM)¹⁵, Homer2¹⁶ (widely used), and the fNIRS toolbox^{17,18} (new and gaining popularity) are used for fNIRS data analysis. This protocol reviews data analysis

methods using NIRS-SPM, but it is to the discretion of the researcher to select preferred method of analysis.

9.1. Analyze data from the fNIRS system using NIRS-SPM, Version 4^{15,19}. This toolbox for the neuroimaging suite SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>) analyzes NIRS data with a general linear model based analysis approach and allows for the creation of activation maps with super-resolution localization.

9.2. Data conversion to HbO and HbR

9.2.1. Use the modified Beer-Lambert equation (with NIRS-SPM) to convert optical density values into concentration changes in HbO and HbR response.

9.3. Data preprocessing

9.3.1. Use one of the multiple options which exist for preprocessing fNIRS data.

Note: Huppert *et al.*¹⁷ propose very rigorous methods for different sources of noise¹⁶. These include eigenvector-based reduction of motion artifacts, bandpass filtering techniques, and eigenvector-based reduction of spatial covariance for physiological interference in data (*e.g.*, respiration, blood pressure, heart rate). They also share a thorough commentary on sources of noise in fNIRS research and implications for statistical analysis. The fNIRS researcher must investigate preprocessing applications that are most appropriate for a given study. Below, an analysis approach modeled after Worsely and Friston²⁰ and Jang *et al.*¹⁹ is presented.

9.3.2. Decompose changes in HbO and HbR concentrations using a Wavelet-Minimum Description Length (MDL) detrending algorithm in order to remove global trends resulting from breathing, blood pressure variation, vasomotion, or participant movement artifacts and to improve the signal-to-noise ratio¹⁹.

9.3.3. Apply a low-pass filter with the shape of the hemodynamic response function to the data and use the Worsely and Friston²⁰ precoloring method to remove temporal correlations.

9.4. Model generation and statistical analysis

9.4.1. Generate models for HbO and HbR containing experimental regressors convolved with the corresponding hemodynamic response function with time derivatives²¹.

Note: The hemodynamic response function can have greater variability in higher cortical regions and across participants. These types of variability can be accommodated in analysis models by expanding the HRF to include temporal derivatives. Use a temporal derivative to model differences in the time to peak hemodynamic response²¹.

9.4.2. Use NIRS-SPM to create the models for HbO and HbR with opposing polarity so that a significant model fit for HbO indicates increased concentration and for HbR decreased concentration^{5 18}.

9.4.3. Set up experimentally relevant *t*-test or *F*-test contrasts to test for the effect of one (or several) regressors (given the design matrix) on modulation of the fNIRS time series data.

9.5. Visualizing results

9.5.1. Perform spatial registration of NIRS channels to the Montreal Neurological Institute (MNI) space using data from a 3D digitizer.

9.5.2. Use registered fNIRS data to create activation maps of HbO, HbR, and total hemoglobin (THb) based on the general linear model and Sun's tube formula correction^{22,23}.

9.5.3. Load activation maps onto an appropriate brain template. For example, the recent Haskins Pediatric Brain Atlas provides a standardized template for children between 6–12 years of age²⁴.

REPRESENTATIVE RESULTS:

Probe position data obtained by the 3D digitizer (**Figure 2**) can be visualized on a standard brain template. Register fNIRS channels to MNI space using NIRS-SPM's stand-alone registration function²⁵. The spatial registration function generates MNI coordinates, anatomical labels, and Brodmann areas maximally represented by each channel.

[insert Figure 2]

Probe position data can also be visualized over cortex surface template or anatomical MRI template using Brainstorm software (**Figure 3**).

[insert Figure 3]

Here, representative data from the rhyme judgment task are shown (**Figure 4**). Participants completed two identically-structured runs of this task. Each run contained 13 trials; rhyming and non-rhyming trials were randomly ordered.

[insert Figure 4]

The 3D position data and experimental design data were combined with fNIRS time-series data (**Figure 5**) for analysis in order to map experiment-related significant neural activation patterns on a standard brain template (**Figure 6**). Representative single subject data and results are shown in **Figure 5** and **Figure 6**.

[insert Figure 5]

This subject showed greater activation in the left hemisphere Superior Temporal Gyrus (STG) during rhyming trials compared with rest (baseline fixation cross). Averaged HbO and HbR responses for rhyming trials show a canonical hemodynamic response: increasing HbO concentrations and corresponding decreasing HbR concentrations following stimulus presentation.

[insert Figure 6]

Single-subject results differed between participants (see **Figure 7**). This individual variation may reflect underlying functional differences or developmental differences in the organization of specific brain networks. For example, subject 1 showed greater activation in the left inferior frontal gyrus region during non-rhyming versus rhyming trials; whereas subject 2 showed greater activation in the left STG region during the same experimental contrast (non-rhyming versus rhyming trials).

[insert Figure 7]

FIGURE AND TABLE LEGENDS:

Figure 1. Schematics. (A) Schematic of laboratory setup. (B) Preparing the participant for data collection.

Figure 2. Data collection. (A) Placement of the fNIRS cap on the participant's head and collection of position data using the 3D digitizer. (B) International 10-20 system used to guide placement of the cap on the participant's head. (C) Spatial localization algorithm plotting x, y, z coordinate data on the MNI brain template. The image generated during stand-alone NIRS registration using 3D digitizer data in NIRS-SPM^{15,19,25}.

Figure 3. fNIRS probes. fNIRS probes visualized on (A) the surface of the cortex and (B) the MNI anatomical MRI template. Left, dorsal, and right views are presented. Images generated using Brainstorm software¹³.

Figure 4. Task design. The rhyme judgment task scheme is shown. Participants continuously viewed a fixation cross while periodically listening to French rhyming or non-rhyming word pairs. The task was completed in two runs, each comprised of 13 trials. 13 rhyming and 13 non-rhyming trials were randomly presented. Each trial lasted 3 s; 1 s per word with a 1 s ISI. Jittered presentation of rest periods between trials, which lasted 8–17 s.

Figure 5. Representative time-series data from one fNIRS channel. (A) Raw time-series data corresponding to entire task length (rhyme judgment task; first run), not normalized. (B) Filtered time-series data using wavelet detrending. Rhyming and non-rhyming trials are indicated by solid and dashed box-car, respectively.

Figure 6. Representative single-subject results. (A) Greater activation is observed for rhyming trials versus baseline (fixation cross) in the left hemisphere, overlaying the posterior portion of the superior temporal gyrus (STG). Image generated during NIRS results steps using NIRS-SPM^{15,19,25}. (B) Averaged event-related waveforms for HbO (red) and HbR (blue) during rhyming trials (rhyming word pair stimuli). Image generated using Xu Cui's plot average function²⁶.

Figure 7. Representative single-subject results from two different participants for identical contrast. Greater activation for non-rhyming versus rhyming trials in the left hemisphere is shown in both A and B. (A) Subject 1 showed greater activation in the left inferior frontal gyrus. (B) Subject 2 showed greater activation the left superior temporal gyrus.

DISCUSSION:

This paper presented a field neuroimaging protocol suitable for low-resource contexts in remote locations. The key advance of this field neuroimaging protocol is the first-time ability to study brain function and its development in understudied (or never-before studied) contexts. Critical steps in this protocol include traveling with and setting up a mobile laboratory suitable for quality data collection in tropical climates without electricity or available facilities. This protocol provides a general guide to forming strong partnerships with local scientific, educational, and government institutions, and we highlight the reciprocal knowledge transfer that occurs when successful long-term partnerships are formed between local and visiting scientists. Guidelines for the development of culturally-appropriate informed consent procedures and testing protocols are discussed with the aim of incorporating multiple cultural perspectives in research methods. Finally, this protocol provides detailed steps for field data collection and data analysis.

Local Science Engagement and Opportunities for Capacity Building:

One of the main challenges that local, particularly junior, researchers in Côte d'Ivoire are faced with when they complete their studies is the lack of opportunity for hands-on research experience with experienced researcher mentors and/or international collaborators. For this purpose, researcher should make all efforts to establish robust collaboration with local researchers from relevant disciplinary backgrounds, and include trainees at all levels (undergraduate, graduate, postdoctoral). Trainees can leverage the insight gained from this experience to work independently and further research. This experience can also be a stepping stone to build their capacities as researchers and develop their competitiveness at the international level in writing research proposals and papers and applying for grants. A research team excluding local researchers may have a reduced chance of success as local researchers will best know the local social and cultural values and systems, the local languages spoken in addition to the geographic knowledge of the area. Their contribution is therefore extremely important in understanding the local realities and designing culturally-appropriate protocols for the successful research projects.

Culturally-appropriate Research Methods:

The development of informed consent protocols to conduct research in rural settings specifically in Cote d'Ivoire is critical and failure to adopt the appropriate approach can inhibit the successful achievement of the research even though well-intentioned and scientifically robust²⁷⁻³¹. Generally, in rural settings in Côte d'Ivoire, asking a villager to read a consent form and sign it can break any trust building between the researcher and the participant. In fact, the perceived formality of this procedure may create a psychological distance and a feeling of insecurity in the participant's mind. This may result in a clear or unexpressed unwillingness to collaborate. This attitude can be explained by many factors including a long history of oral tradition whereby communication is more oral than written and high rates of illiteracy that may be found in target communities. Communities in rural settings trust their chief and rely on his decision-making power. Therefore, the protocol presented incorporates the consent of the chief of the village at the community level. This is arguably more culturally important than individual consent. Additionally, participants and community members may have had limited or no exposure to neuroimaging technology or computers. Therefore, researchers need to take into consideration that the informed consent procedure, and instructions, may be misunderstood. The function of the fNIRS system should be communicated in lay terms and appropriate language easily understandable by child participants and community members who may have had very limited exposure to technology. These considerations can strongly influence the comfort and confidence of all community members involved in a field neuroimaging research project.

The protocol presented here also highlighted the importance of sharing research findings with community members and government partners. Partnerships built on continued dialogue aid in the eventual translation of research findings into policy. It is imperative to arrange post data collection field visits to disseminate research findings and deliver reports and, possibly, share any tools that resulted from the study (e.g., assessments in local languages). Participating communities in rural settings may never otherwise receive information about study completion and findings given lack of internet service and/or computers. Likewise, researchers in the country may have limited access to academic journal subscriptions and poor internet connectivity at regional universities. Published results should be shared in a regional forum, and made available in an accessible language.

Limitations and Potential Challenges:

This field neuroimaging protocol should be modified to suit the planned data collection sites. The protocol presented here has been developed for research with primary school aged children in rural Cote d'Ivoire. However, the methods outlined here may not be suitable, specifically with respect to informed consent procedures, in other countries or even other regions of Cote d'Ivoire. Researchers who aim to conduct field neuroimaging must first carefully research local customs and incorporate local perspectives into study design. Therefore, a research team working on study designs must include members from the local cultural groups.

Field neuroimaging has limitation in comparison with laboratory methods. Importantly, control of the testing environment is considerably reduced in the field. Field neuroimaging researchers should plan extended data collection trips. Tropical rains, risk of contracting tropical diseases,

civil strikes, and political unrest may significantly impact research plans. Researchers need to ensure security levels in the region are sufficient and monitor for updates to any situations that may affect security levels. Continuous communication between team member, specifically with respect to security levels, may mitigate potential risks.

Future Applications and Relevance to Existing Methods:

The use of this field neuroimaging method can be applied to evaluating the impact of early risk on infant and child development in global health settings. Researchers have begun using this approach to study child development in rural Gambia and an urban slum in Bangladesh³². In an urban slum in Dhaka, researches are using fNIRS to examine how factors such as nutrition and sanitation contribute to growth and brain development³³. In rural Gambia, researchers have used fNIRS to study cognitive function of infants, and have demonstrated that fNIRS is a viable imaging tool in resource-poor settings^{34 35}. Such work promises to reveal new insights into the development of children in the developing world, who are disproportionately affected by malnutrition and poor sanitation. Portable neuroimaging technologies continue to become more accessible and applicable for research in low-resource environments, thus highlighting the need for rigorous methods for field neuroimaging.

Conclusion:

Portable neuroimaging systems with the capability to function on battery-supplied power have recently become available. As these techniques are relatively new, advances to battery systems will provide ongoing improvements. Diverse communities of international scientists developing research programs using these tools will undoubtedly optimize mobile laboratory settings to provide increased control of the testing environment. Meaningful engagement between international and local scientists and local communities can ensure that members of study populations have active roles in the development of research programs and represent the interests of their communities. Only such collaborative and integrated research teams would be well-positioned to apply field neuroimaging methods to study all human brain development, and reveal both theoretically- and practically-relevant information aimed at understanding the most urgent child development issues.

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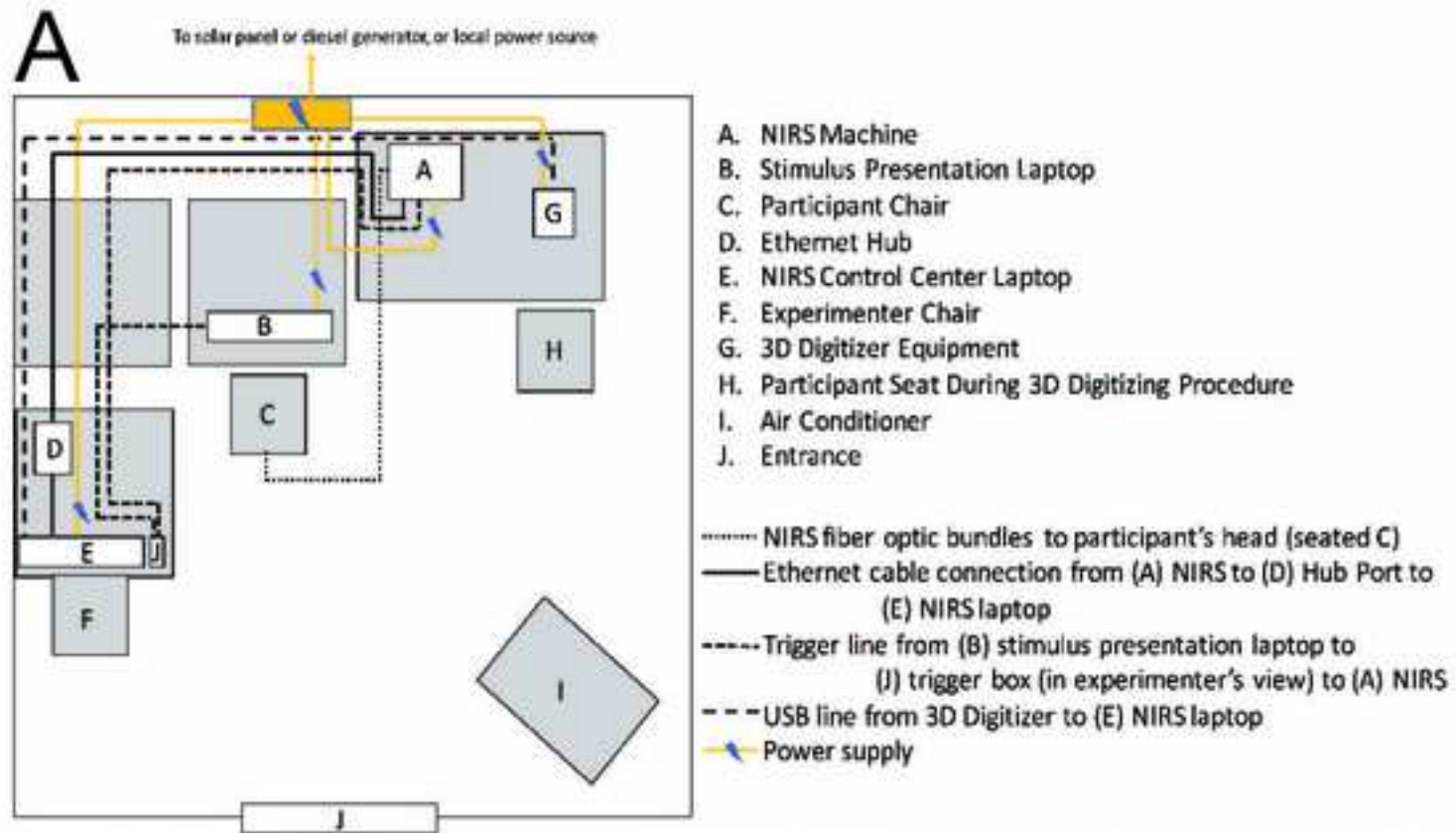
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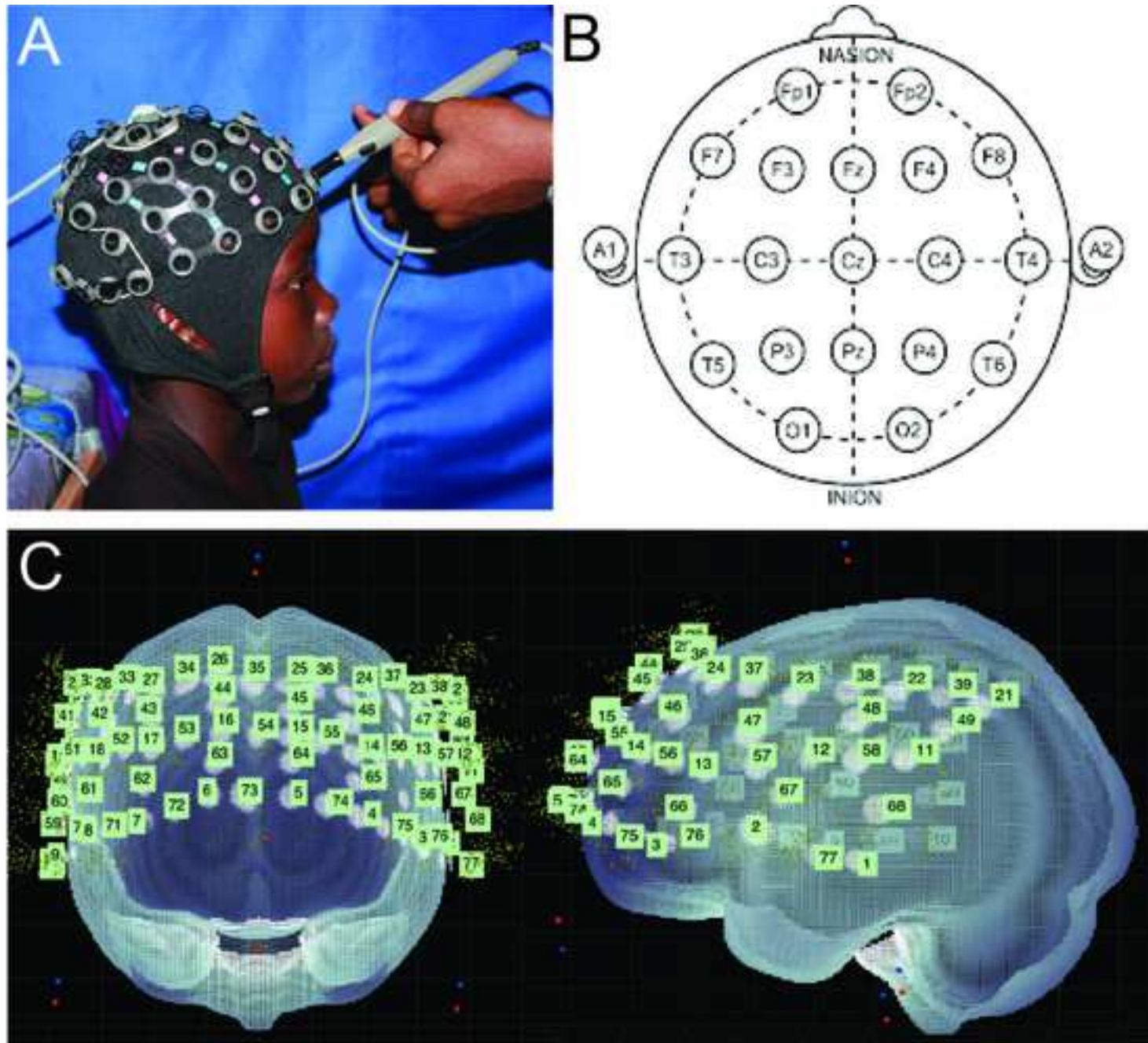
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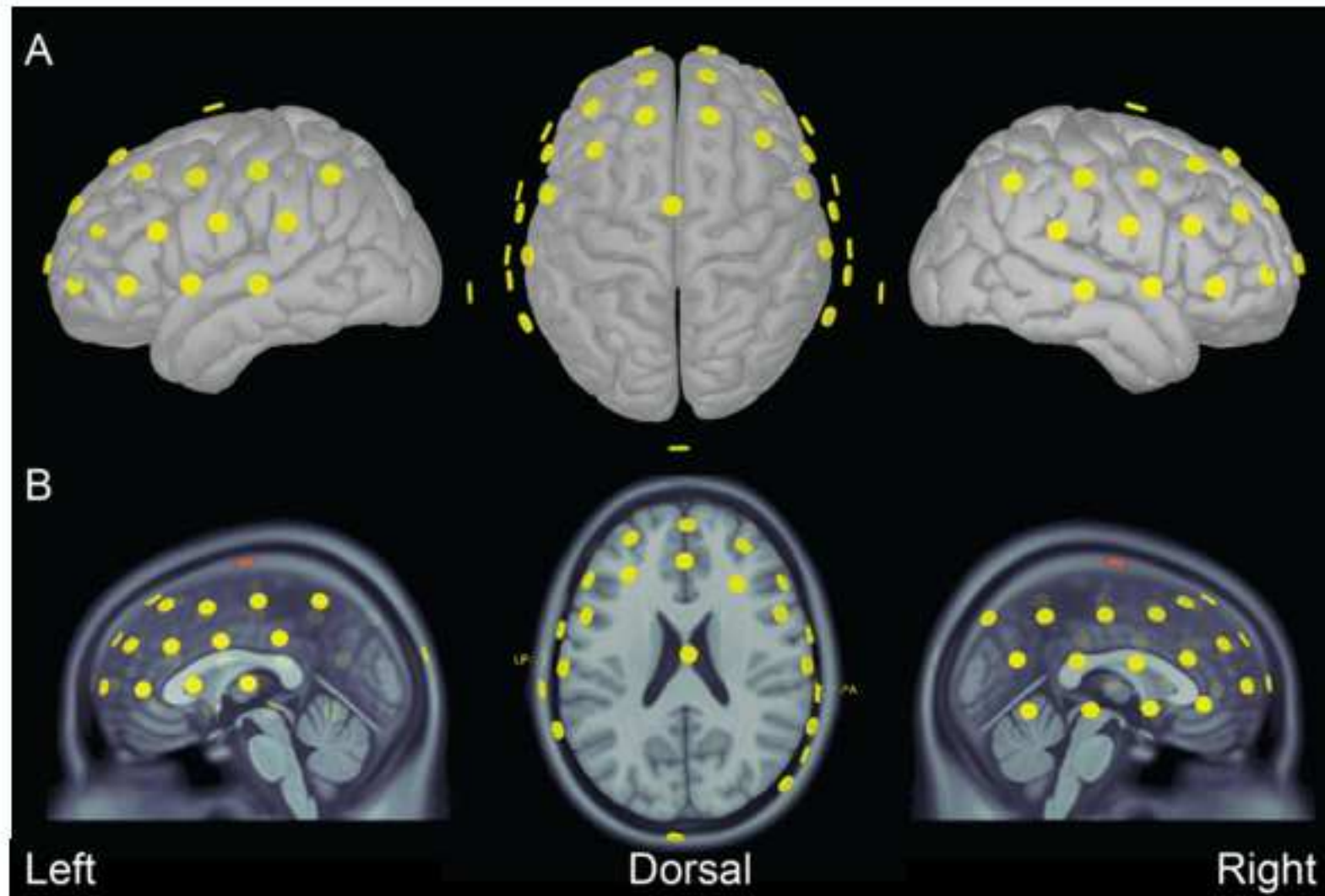
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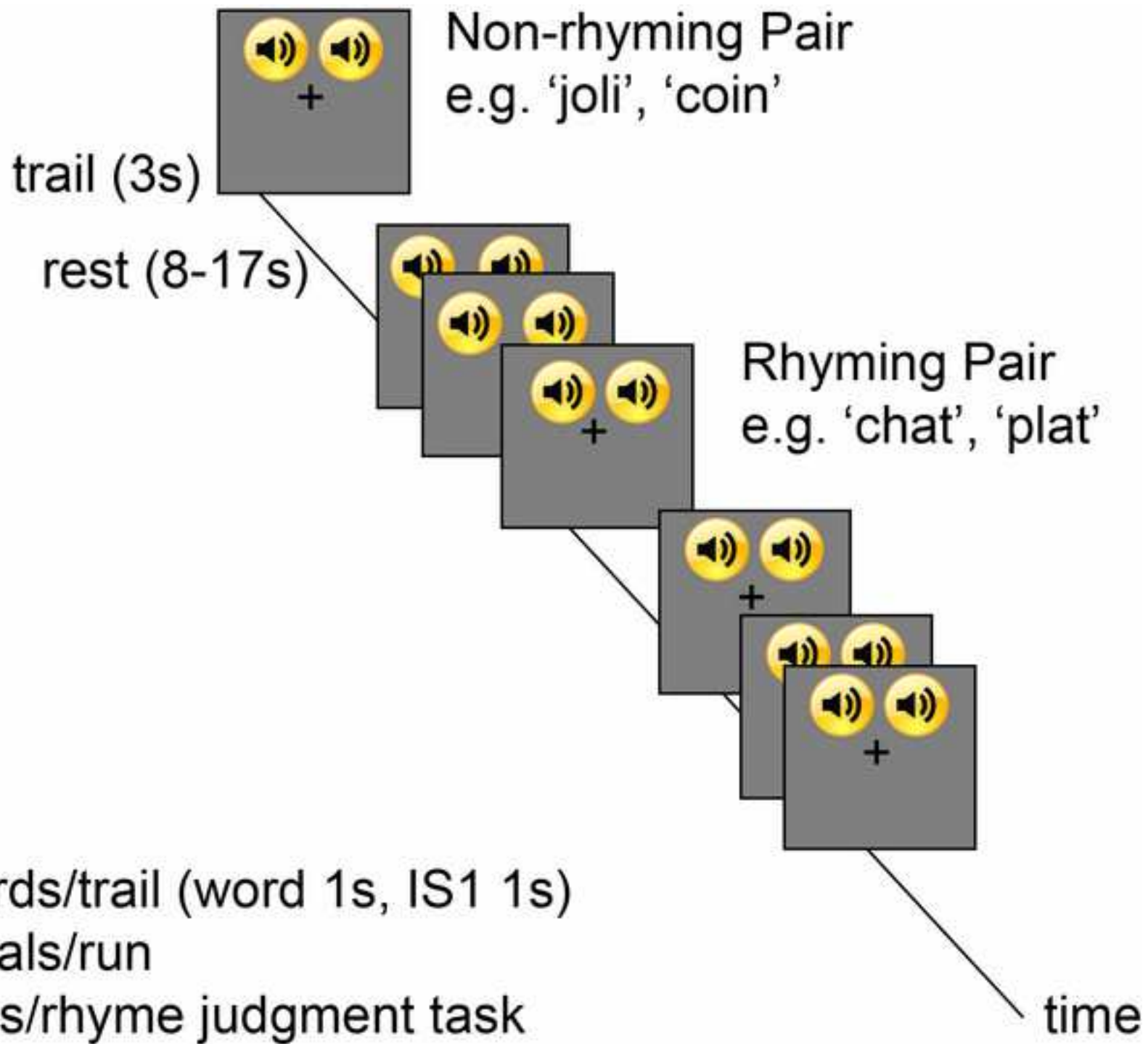
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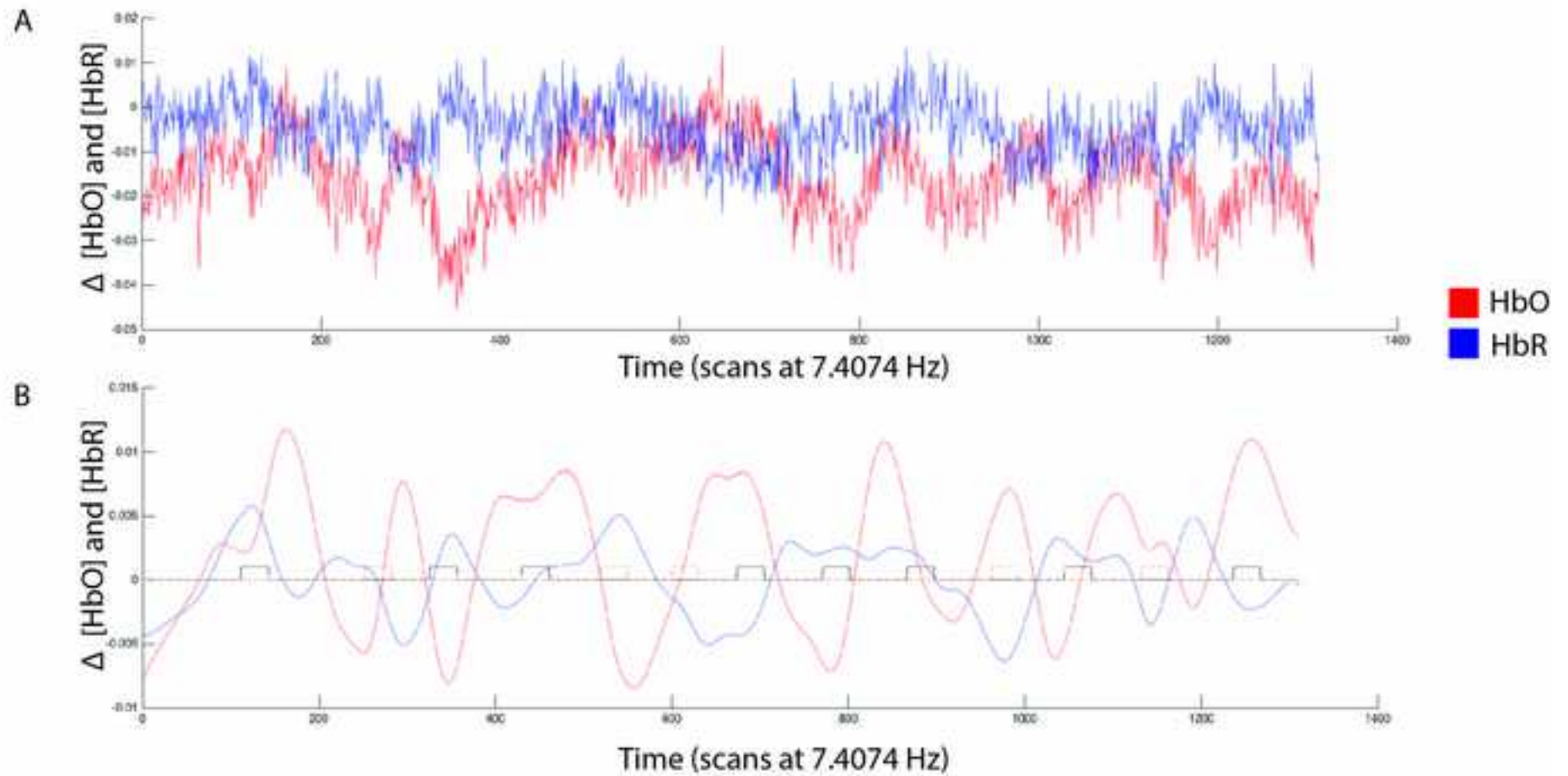


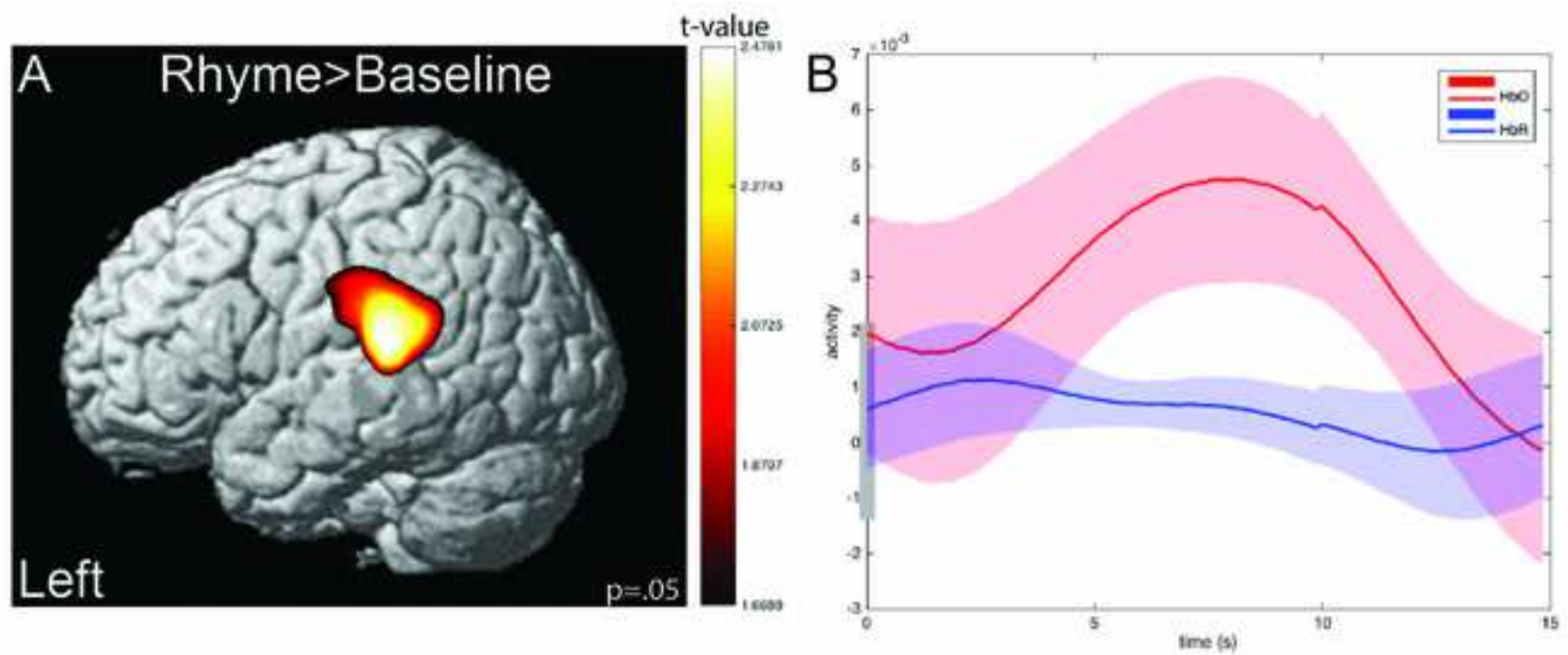


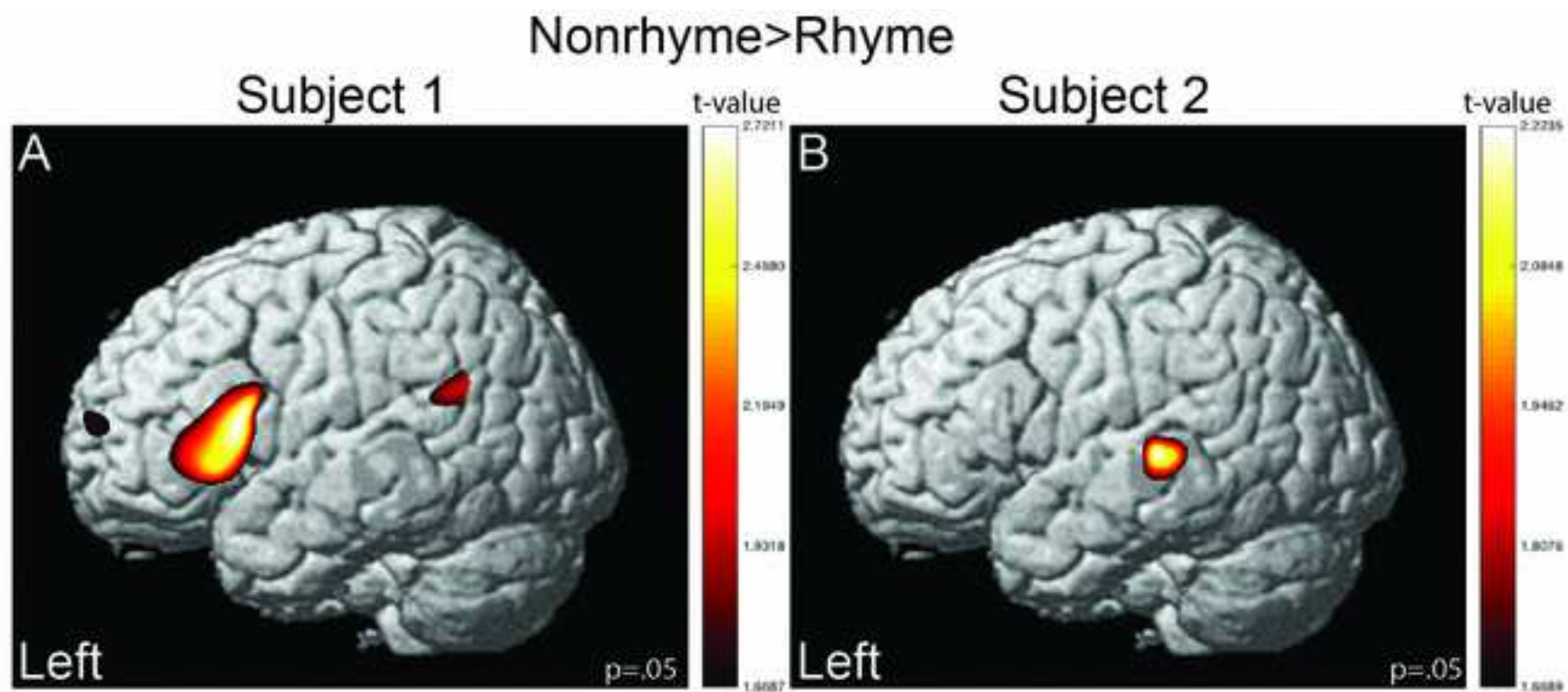




Time-Series Data from Representative Channel







Name of Material/ Equipment	Company
LIGHTNIRS Main Unit Pack 120V	Shimadzu
HOLDER ASSY, ALL- CAP	Shimadzu
LIGHTNIRS connection cable	Shimadzu
Fiber set for LIGHTNIRS, 1m (8 sets)	Shimadzu
Dell Latitude Laptop	Shimadzu (from Dell)
PATRIOT SEU (System Electronics Unit)	POLHEMUS
Power Supply	POLHEMUS
Power Supply cord	POLHEMUS
RS-232 null modem cable	POLHEMUS
USB cable	POLHEMUS
RX2 Sensor 10' cable	POLHEMUS
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Catalog Number	Comments/Description
292-34000-42	Component of the fNIRS system
594-07618-01	Component of the fNIRS system
567-10976-11	fNIRS system component
567-11350-01	fNIRS system component
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1A0453-001	PATRIOT System component
2C0809	PATRIOT System component
17500B-BLK	PATRIOT System component
1C0288	PATRIOT System component
1C0289	PATRIOT System component
4A0492-20	PATRIOT System component
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Author(s): Kaja K. Jasińska, Sosthène Guei

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Re: Using functional Near Infrared Spectroscopy (fNIRS) Neuroimaging to Study Global
Child Development: Imaging Field Methods in Rural sub-Saharan Africa
Kaja K. Jasinska* and Sosthène Guei
*Corresponding Author

Dear Dr. Vineeta Bajaj:

We sincerely thank you for considering our manuscript for publication in *Journal of Visualized Experiments* and especially for the opportunity to improve this manuscript as per the specific suggestions provided by the reviewers. We greatly appreciate the time and effort that went into their thoughtful reviews. We are delighted to report that the manuscript is now improved following from their helpful suggestions.

Every suggestion identified by the reviewers is now addressed. Please see our detailed replies to each reviewer's critiques below. The overall expansion of our descriptions reflect our deep gratitude and extraordinary respect for the reviewers' invaluable input. We thus sincerely hope you will now find our manuscript suitable for publication in your esteemed *Journal of Visualized Experiments*.

Sincerely,

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Editorial comments

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Response: All corrections have been made.

1. Please abbreviate all journal titles. Response: All corrections have been made.
2. Please define all abbreviations before use. Response: All corrections have been made.
3. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Response: A new table has been included.
4. Please add commas to separate affiliation numbers for the authors. Response: All corrections have been made.
5. Please provide an email address for each author. Response: All corrections have been made.
6. Please revise the title to better reflect the technique shown.
Response: Our title has been revised to: "Neuroimaging field methods using portable functional Near Infrared Spectroscopy (fNIRS) Neuroimaging to Study Global Child Development: Rural sub-Saharan Africa."
7. Please rephrase the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Here, we present a protocol to ...". The current short abstract is above 50 words.

Response: Our abstract has been revised as follows: "Portable neuroimaging approaches (functional Near Infrared Spectroscopy) provide advances to the study of the brain in previously inaccessible regions; here, rural Côte d'Ivoire. Innovation in methods and development of culturally-appropriate neuroimaging protocols permits novel study of the brain's development and children's learning outcomes in environments with significant poverty and adversity."

8. Unfortunately, there are a few sections in the manuscript that show overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please see lines: 82-86

Response: We have corrected this text.

Please see lines 81-94: "fNIRS neuroimaging is well-suited for field research. Similar to functional magnetic resonance imaging (fMRI), fNIRS measures the brain's hemodynamic response⁶. However, fNIRS uses a series of light emitting optodes and light detectors rather than generate electromagnetic fields. There are no restrictions on metal in or near the testing area, and no electric shielding is necessary, as in the case for electroencephalography (EEG). A key advantage of fNIRS is its portability (i.e. some systems may fit in a suitcase) and ease of use. fNIRS is also easy to use with children; the child is comfortably seated in a chair during the experiment and the fNIRS system tolerates movement well compared to fMRI. Compared with fMRI, fNIRS also provides separate measures of deoxygenated and oxygenated hemoglobin during recording, compared to fMRI which yields a combined blood oxygen level density (BOLD) measure. fNIRS has superior temporal resolution to fMRI: sampling rates can vary between ~7-15 Hz. fNIRS has good spatial resolution: fNIRS' depth of recording in the

human cortex is less than fMRI, measuring about 3 to 4 cm in depth, this is well-suited for studying cortical functions, especially with infants and children who have thinner skulls than adults^{3,7-10}.”

9. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: Shimadzu LightNIRS, etc.

Response: All references to Shimadzu LightNIRS have been removed.

10. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.). **Response:** All corrections have been made.

11. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures, etc.

Response: All corrections have been made.

12. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. **Response:** We have adjusted the protocol and added explanatory "notes" to clarify specific protocol steps. We have also moved text that corresponds to discussion into the Discussion section.

13. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion. For e.g. 2.1.1, 2.2, 3.2.1, 4.2.2, 5.1.1, 7.1, etc. **Response:** See note above.

14. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. **Response:** We have added additional details to the protocol section.

Please add section 3.5 along with the ethics statement. **Response:** We have added details of the IRB procedure in country and the ethics statement in section 3.4.

4.2.1: Please provide the specific scalp locations so this manuscript can stand alone.

Response: Scalp locations are noted in the international 10-20 scalp location standard.

4.3.2: Please explain how to arrange the 3D digitizer in detail. Where is the second sensor placed? Please be more specific.

Response: Please see revision 4.3.2: "Arrange 3D digitizer equipment. One sensor should be placed on the participant's head at Cz and affixed securely (i.e. using elastic or hair accessory), and the second block sensor should be placed behind the participant. For example, the participant can be seated in a chair with their back to a table. The second sensor can be placed on the table directly behind the participant's head. Neither sensor can move during the course of obtaining the 3D digitizer measurement."

4.4.3: Please explain how to select the probe arrangement. What is the basis of the experimental design?

Response: Please see revision 4.4.2: "Using the fNIRS built-in software, select the probe arrangement that corresponds to the experiment design. fNIRS probes can be placed to cover the entire head (i.e. full head coverage), or alternatively, an array can be placed over general regions of interest. For example, this protocol used a 10 x 3 probe array (30 probes arranged in 3 rows of 10 probes each). This probe arrangement was placed

to maximally overlay left hemisphere language areas and their right hemisphere homologues, as well as the frontal lobe. See Figure 2.”

4.4.4: Please describe the optode map. Where does it come from? [Please see revision on 4.4.4: “Using the optode map in the fNIRS inbuilt software as a guide, place each optode in the appropriate optode opening on the cap. The optode map indicates the location of each optode in the array \(e.g. 10 x 3\)”](#)

5.3: How are the experimental tasks presented? Is there a rest period? How long is each task presented?

How are the speech and non-speech sounds presented? How are the rhyming and non-rhyming words selected?

How are the images presented on the screen and how are they selected?

5.8: Is there a specific reason to measure the given tasks? Are there any other tasks which can be measured?

[Response \(to above comments re: task\): We have revised the session describing the task. In fact, any researcher following this protocol will customize the experimental task to suit their research aims. Our tasks are only an example. In the first version of the manuscript, our protocol gave the incorrect sense that these are required steps. We have noted our error and as such, we have limited our description and clearly state that Please explain how to analyze the data in more details. Response: We’ve added additional details as per the reviewers’ \(particularly reviewer 1\) incredibly helpful and insightful comments.](#)

7.4.1: Corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s). [Response: This is now corrected.](#)

7.5.1: Citation? [Response: This is now corrected.](#)

16. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

17. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. [Response: Relevant sections are highlighted.](#)

18. Please note that only steps 4, 5, and 6 can be filmed. The other steps are not appropriate for the video. [Response: This is noted. We do also have a lot of footage we accumulated in Cote d’Ivoire that we would be happy to potentially share with the production team if deemed relevant.](#)

19. Please explain the figures in more detail in the Representative Results. [Response: We have added additional details to our figures, particularly following Reviewer 1’s suggestions as well.](#)

20. Please do not have one sentence paragraphs. [Response: This is now corrected.](#)

21. Please revise discussion to also include the significance with respect to existing methods and any future applications of the technique. [Response: We have added a section into our discussion for existing methods and future applications.](#)

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This article demonstrated a protocol of using a portable neuroimaging technique fNIRS to study child labor on brain development in rural Africa area. The practical protocol including fNIRS equipment shipping and set up, fNIRS data analysis, and finding local research team, can be very useful to researchers in the field.

Response: We sincerely appreciate the reviewer's careful reading of our manuscript. Particularly the reviewer's detailed attention to analysis issues that were lacking or unclear in our previous version of the manuscript, including nirs preprocessing steps. This is a very critical point in the fNIRS field right now, particularly because robust analysis methods are not always utilized. As such, we are very grateful for the opportunity to make the important improvement to our manuscript, which now expresses a higher standard for nirs data preprocessing and statistical inference.

Major Concerns:

1. It is suggested to provide a picture of the NIRS device set up as well as the mobile lab, so that readers can see the size of the NIRS system and the mobile lab.

Response: We thank the reviewer for this wonderful suggestion. We have added a new figure with a schematic of our laboratory set up and photos. See Figure 1. We have also accumulated video footage of laboratory setup, in addition to photos, to share with JoVE team for potential production.

2. Is there any data backup plan, since online data storage service is usually not available on site?

Response: This is a very important concern, and in fact, in the field, we did not have any access to online data storage. As such, data was transferred to multiple to external portable hard drives during field data collection dates. We note this data backup plan in section 7. See Line 340:

"7.Backup data

7.1. Ensure that data is exported and backed up to multiple portable hard drives, as internet access for online data storage is unlikely to be available. Data should be transferred to online data storage as sufficient internet connectivity is available."

Minor Concerns:

3. protocol 1.2.1, more details about how temperature affect fNIRS functionality are expected. e.g. what temperature range does fNIRS equipment require, and within the range, will the signal quality change along the temperature variation.

Response: Electronics operating in high humidity conditions, generally above 60%, are more susceptible to corrosion as excessive moisture can settle on parts and react with metal. Humidity levels in an indoor lab (e.g. inside a university building) are generally between 30-50%. Humidity in southern Côte d'Ivoire ranges between 80 and 90%. We have revised the text, see section 1.2.2.: "1.2.1. Prepare for climate conditions in the field. Note: Temperature and humidity conditions in the field can vary significantly from laboratory setting and may affect equipment function and longevity, as well as

participant comfort during experimentation. Electronics operating in high humidity conditions, generally above 60%, are more susceptible to corrosion as excessive moisture can settle on parts and react with metal components. Humidity levels in an indoor lab (e.g. inside a university building) are generally between 30-50%. Humidity in southern Côte d'Ivoire can be 80-95%. Set up a portable air conditioning unit with low wattage demands.”

4. protocol 4.1.2, the authors should describe how to use the measured head size information, e.g. how to use the information when placing the cap on participants, the description in 4.2.1 is vague.

Response: We appreciate the reviewer bringing this important detail to our attention. We have revised the text to improve clarity. Please see section 4.2.1.: “4.2.1. Place the fNIRS optode holder cap onto the participant’s head, aligning the cap to the international 10-20 system for scalp locations¹⁶. Cap position should be identical for all participants. Align points on the cap (e.g. probe holder) with scalp positions. For example, center the front of the cap on the head to frontopolar (FP) position. This position corresponds to 10% of the nasion-inion over top distance dorsal to nasion position.”

5. protocol 4.3.2, if a sensor of the 3D digitizer is placed at Cz, it can be very easily moved during the localization data collection, please provide details of how the sensor is secured.

Response: Please see revision in 4.3.2.: “Arrange 3D digitizer equipment. One sensor should be placed on the participant’s head at Cz and affixed securely (i.e. using elastic or hair accessory), and the second block sensor should be placed behind the participant. Neither sensor can move during the course of obtaining the 3D digitizer measurement.”

6. Protocol 7.5.1, please provide more details about the time derivatives, the authors should describe the reason of including the time derivatives in the analysis model.

Response: We are grateful to the reviewer for raising this important analysis issue, which indeed should be further clarified. Please see our revisions in section 8.5.1.: “The hemodynamic response function can have greater variability in higher cortical regions and across participants. These types of variability can be accommodated in analysis models by expanding the HRF to include temporal derivatives. A temporal derivative can model differences in the time to peak hemodynamic response (Friston et al., 1998).”

7. Since 7.4.1 and 7.4.2 was optional preprocessing steps, please discuss the performance of the low pass filter and Wavelet artifact correction method. It is also helpful to introduce more methods for data preprocessing so that readers can make their selection.

Response: Indeed, many rigorous analysis approaches exist, including outlined by Huppert et al (2009). We now stress in the manuscript that fNIRS researchers have to make a selection that should be guided by careful investigation of methods that are best suited to their research projects, and we indicate other approaches to consider.

Please see revisions in section 8.5: "Data preprocessing. Multiple options exist for preprocessing fNIRS data. For example, Huppert et al. (2009) propose very rigorous methods for different sources of noise²⁰. These include eigenvector-based reduction of motion artifacts, bandpass filtering techniques and eigenvector-based reduction of spatial covariance for physiological interference in data (e.g. respiration, blood pressure, heart rate). Huppert also shares a thorough commentary on sources of noise in fNIRS research and implications for statistical analysis²¹. The fNIRS researcher must investigate preprocessing applications that are most appropriate for a given study. Below, an analysis approach modeled after Worsely and Friston (1995)²⁴ and Jang et al. (2009)²³ is presented."

8. Figure 1 panel C, why not also provide a coronal view of the digitization results?

Response: We have now added a coronal view.

9. Figure 4, the axis labels need revision, is the signal normalized?

Response: Thank you for bringing this to our attention. We have revised the figure and figure caption. A - raw data, not normalized, B - filtered data after wavelet detrending. Please see Figure 4.

10. Figure 5 and Figure 6, the authors should provide statistical results, e.g. labelling the color bar as t-value, also please provide the p-value associated with the results, especially for Figure 5 panel b.

Response: We have updated our figures. Figure 5 panel b presents a visualization of oxy and deoxy responses with standard error (shaded areas). This visualization is obtained from Cui Xi's plotAverage.m code (<http://www.alivelearn.net/?p=1191>)

Reviewer #2:

Manuscript Summary:

This is an excellent manuscript that addresses and situates the scientific and social benefits of an inclusive, collaborative, and culturally appropriate research protocol for rural contexts. As the authors write, "Methods that advance our field's capacity to study development in a fuller range of human experience can dramatically advance our understanding of the complex relation between brain development and the life experiences that shape it." This manuscript lays out the practical means to achieve this important objective for our field.

The scientific and social benefits (as well as the risks of not following this paradigm) are made clear as they relate to multiple parties (the researcher, the participants' community, and the research scientists based in that region.) This manuscript is also timely given the increasing interest in fNIRS systems and will serve well both new and established fNIRS users that may be seeking to extend their research into studying populations that have been historically underrepresented in research studies.

The fNIRS protocols for setting up the equipment for participants, spatial registration, data collection, and data analyses are all appropriate and are within published cognitive neuroscience standards.

Response: We are very grateful for the reviewer's positive comments about our manuscript -- particularly noting the importance of "scientific and social benefits of an inclusive, collaborative, and culturally appropriate research protocol for rural contexts". We have addressed all the very valuable issues the reviewer raised with respect to culturally-appropriate engagement. We are deeply appreciative of the reviewer raising important issues related to culture and science capacity building that pushed us to improve this new version of the manuscript.

Major Concerns:

§6.4: Given the emphasis provided on culturally appropriate engagement with rural communities and the importance of establishing appropriate channels for approval, the manuscript abruptly ceases to consider this after the cap is removed from the participant's head. This important consideration extends beyond obtaining consent to collect data. At the very minimum, section 6.4 should at least provide some discussion about how the participant is released from the experiment. Do they accept payment for time/participation? What is the appropriate method of acknowledging and thanking the participant? It would be more ideal if there was some mention of efforts to return the knowledge back to the community. This is partially alluded to in earlier sections that discuss the importance of building research capacity in rural regions. The manuscript would benefit well from a more explicit discussion on this matter.

Response: We thank the reviewer for this pertinent observation. We have addressed the question related to the benefits and compensation for participants and other stakeholders in the Discussion Section.

Please see revisions in Discussion:

Local Science Engagement and Opportunities for Capacity Building

One of the main challenges that local, particularly junior, researchers in Côte d'Ivoire are faced with when they complete their studies is the lack of opportunity for hands-on research experience with experienced researcher mentors and/or international collaborators. For this purpose, researcher should make all efforts to establish robust collaboration with local researchers from relevant disciplinary backgrounds, and include trainees at all levels (undergraduate, graduate, postdoctoral). Trainees can leverage the insight gained from this experience to work independently and further research. This experience can also be a stepping stone to build their capacities as researchers and develop their competitiveness at the international level in writing research proposals, papers and applying for grants. A research team excluding local researchers may have a reduced chance of success as local researchers will best know the local social and cultural values and systems, the local languages spoken in addition to the geographic knowledge of the area. Their contribution is therefore extremely important in understanding the local realities and designing culturally-appropriate protocols for the successful research projects.

Culturally-Appropriate Research Methods

The development of informed consent protocols to conduct research in rural settings specifically in Cote d'Ivoire is critical and failure to adopt the appropriate approach can

inhibit the successful achievement of the research even though well-intentioned and scientifically robust 28-32. Generally, in rural settings in Côte d'Ivoire, asking a villager to read a consent form and sign it can break any trust building between the researcher and the participant. In fact, the perceived formality of this procedure may create a psychological distance and a feeling of insecurity in the participant's mind. This may result in a clear or unexpressed unwillingness to collaborate. This attitude can be explained by many factors including a long history of oral tradition whereby communication is more oral than written and high rates of illiteracy that may be found in target communities. Communities in rural settings trust their chief and rely on his decision-making power. Therefore, the protocol presented incorporates the consent of the chief of the village at the community level. This is arguably more culturally important than individual consent. Additionally, participants and community members may have had limited or no exposure to neuroimaging technology or computers before. Therefore, researchers need to take into consideration that the informed consent procedure, and instructions, may be misunderstood. The function of the fNIRS system should be communicated in lay terms and appropriate language easily understandable by child participants and community members who may have had very limited exposure to technology. These considerations can strongly influence the comfort and confidence of all community members involved in a field neuroimaging research project.

The protocol presented here also highlighted the importance of sharing research findings with community members and government partners. Partnerships built on continued dialogue aid in the eventual translation of research findings into policy. It is imperative to arrange post data collection field visits to disseminate research findings and deliver reports and, possibly, share any tools that resulted from the study (e.g. assessments in local languages). Participating communities in rural settings may never otherwise receive information about study completion and findings given lack of internet service and/or computers. Likewise, researchers in country may have limited access to academic journal subscriptions and poor internet connectivity at regional universities. Published results should be shared in a regional forum, and made available in an accessible language.

Minor Concerns:

Line 81 on page 1: "the fNIRS system tolerates movement well compared to both fMRI and EEG." NIRS systems are not any more tolerant of motion than EEG systems, but they are certainly much more tolerant of motion than fMRI systems (and even PET or MEG systems, which can tolerate small minor movement.) This sentence should be corrected.

Response: We have made this correction, thank you for bringing this important detail to our attention.

§7.1: It is correct that NIRS-SPM & Homer2 are widely used, but the fNIRS Toolbox by Huppert is quickly gaining considerable attention and use. To reflect current trends, this toolbox should also be cited.

Response: We are grateful for this important note. Indeed, we are excited about the development of the fNIRS toolbox and plan to incorporate the advance into our future

research program. We did note that on the fNIRS Toolbox website (<https://bitbucket.org/huppertt/nirs-toolbox/wiki/Home>) there is currently an omission for the direct citation of the toolbox.

Please see our revision: "Multiple data analysis packages exist for fNIRS18. Statistical Parametric Mapping for Near-Infrared Spectroscopy (NIRS-SPM)19, Homer220 (widely used), and the fNIRS toolbox 21,22 (new and gaining popularity) are used for fNIRS data analysis. This protocol reviews data analysis methods using NIRS-SPM, but it is to the discretion of the researcher to select preferred method of analysis.

Typos:

§7.4.1: Citation for Jang is not formatted correctly.

Figure 3: 2 instances of "Trail" as opposed to "trial"

Response: We have made these corrections. Thank you for noting this.

Reviewer #3:

Manuscript Summary:

The protocol described using portable functional near infrared spectroscopy (fNIRS) in studying children's reading development and ability in rural sub-Saharan Africa, especially in regions with a high risk of illiteracy. The study is performed in Côte d'Ivoire where an estimated of 1.3 millions of child labor in the coca agricultural sector. It is an important topic because little has been studied about the impact of child labor on brain development and learning. The manuscript is well-written and easy to understand. The protocol can help researcher understand fNIRS is portable, low-cost and easy to operate in field research, especially in the pediatric populations. Although similar setup of fNIRS field studies in low-resource countries have been published (Begus et al, 2016, PMID: 26782222; Papademetriou et al, 2014), the protocol provides useful information, including preparations for traveling with laboratory devices in low-resource contexts and set up, to researcher who interested in setting up field fNIRS studies.

Response: We would like to thank the reviewer for their thoughtful comments on our manuscript. Particularly referencing the growing group of fNIRS research in low-resource contexts. We have added an additional section to our discussion to review some of the existing fNIRS neuroimaging studies in similarly low-resourced contexts. We agree with the reviewer that is important to note the extent to which portable NIRS technology is already revolutionizing cognitive neuroscience.

Major Concerns:

Are the tasks used in the fNIRS measurements culturally appropriate or adapted to children who can not read or/and write? Please address the considerations of the potential impact on the test results.

Response: The fNIRS tasks and measurements are adapted to children in a village setting who have very low/beginning reading ability. Indeed, most of the participants and community members have not have exposure to neuroimaging technology or computers before, but efforts are made by the experimenters to explain the instructions and the

function of the fNIRS system in lay terms and appropriate language easily understandable by child participants. Experimenters also ensure that the participants feel confident and relaxed all along the experiment. From our experience, children are keen to participate in the experiment whatever their literacy level as they consider the research activities like a game. All these factors strongly influence their comfort with participating and have a positive impact on the quality of data collected.

Please see 5.4.2.1.: “Note: Tasks should be created in a manner suitable for the participant. In the study reference here, researchers were investigating language, cognitive, and reading development in children who were very poor readers. The reading neuroimaging task developed with words that would be appropriate for a child with minimal literacy skills. As well, children were selected for the neuroimaging paradigm based on scores obtained on a reading assessment.”

Minor Concerns:

The author stated the protocol has been approved by the Institutional Review Board of the University of Delaware. Please confirm the study is also approved by local IRB in Côte d'Ivoire. If not, please explain why.

Response: We sincerely appreciate the reviewer raising this issue. IRB approval was obtained from the University of Delaware (and now noted in the manuscript). At the time of writing this manuscript, Cote d'Ivoire did not have any formal IRB procedures, albeit there has been mention that a branch of government was working on developing a comprehensive policy and procedure. Date of completion and policy implementation are not known. In lieu of this, we obtained approval directly from the Ministry of Education.

Please see revision in 3.5: “All research activities need to be approved by local authorities, for example, the Ivorian Ministry of Education or the Ministry of Higher Education and Research. The country the project will take place in may not have a formal procedure for ethical approval through an institutional review board (IRB). Check regulation to ensure you are following the correct protocols for obtaining ethical approval for research. Note: at time of obtaining approval, Cote d'Ivoire did not have a formal IRB review process. In lieu of this, the research team proceeded by preparing documentation akin to a IRB application to submit to the Ministry of Education. Multiple meetings were arranged with Ministry of Education and Ministry of Higher Education and Research officials. At these meetings, the research team presented the proposed research plan to all officials followed by group discussion and question and answer sessions. Ethical approval was obtained directly from the Ministry of Education in the form of a signed document granting authorization to conduct research with children at specific schools.