Portable Functional Neuroimaging as an Environmental Epidemiology Tool: A How-To Guide for the Use of fNIRS in Field Studies

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SUMMARY: The widespread application of functional neuroimaging within the field of environmental epidemiology has the potential to greatly enhance our understanding of how environmental toxicants affect brain function. Because many epidemiological studies take place in remote and frequently changing environments, it is necessary that the primary neuroimaging approach adopted by the epidemiology community be robust to many environments, easy to use, and, preferably, mobile. Here, we outline our use of functional near-infrared spectroscopy (fNIRS) to collect functional brain imaging data from Costa Rican farm workers enrolled in an epidemiological study on the health effects of chronic pesticide exposure. While couched in this perspective, we focus on the methodological considerations that are necessary to conduct a mobile fNIRS study in a diverse range of environments. Thus, this guide is intended to be generalizable to all research scenarios and projects in which fNIRS may be used to collect functional brain imaging data in epidemiological field surveys. https://doi.org/10.1289/EHP2049

Introduction

Exposure to environmental toxicants has been associated with statistically significant structural and functional brain anomalies that have been measured with magnetic resonance imaging (MRI) (Brubaker et al. 2010; Rauh et al. 2012; White et al. 2011; Yuan et al. 2006). For example, in adults, early-life exposures to lead and chlorpyrifos (a commonly used organophosphate insecticide) have been linked to gray matter volume loss and to altered myelination and axonal integrity throughout the brain (Brubaker et al. 2009; Cecil et al. 2008). In addition, developmental exposures to methylmercury and polychlorinated biphenyls have been linked to altered brain activation patterns in adolescence (White et al. 2011) and adulthood (Yuan et al. 2006).

Few epidemiological studies have used traditional neuroimaging techniques such as functional MRI (fMRI) because of their high cost and the requirement of highly specialized scan environments. Alternatively, functional near-infrared spectroscopy (fNIRS) offers a relatively inexpensive, portable, and convenient method of neuroimaging. fNIRS uses light projected into the brain to measure fluctuations in cerebral hemoglobin oxygenation that occur in response to neural activation (Boas et al. 2014, 2001). It is easy to use and amenable to many environments, and it correlates highly with fMRI cortical signals across many cognitive tasks (Cui et al. 2011). Thus, fNIRS is an optimal tool for functional neuroimaging applications in epidemiological studies that take place in remote environments. Notably, to our knowledge, fNIRS has not been used in population-based epidemiologic studies or to assess the neurotoxicity of exposure to environmental toxicants such as pesticides or heavy metals.

Given the wide range of locations and environments in which epidemiological studies take place, it is important to use caution when preparing to incorporate fNIRS into study designs. Here, we use the experience gained in a fNIRS assessment of cortical functioning in farm workers from Zarcero County, Costa Rica, as a framework for a methodological guide for employing fNIRS in epidemiological studies. Specifically, we used a portable fNIRS device (NIRSport; NIRx Medical Technologies, LLC) to assess cortical functioning in a subsample of 50 farm workers (out of 300) from 14 individual farms over the course of 11 d. Each participant completed three computer-based tests that covered the neurobehavioral domains of executive function, working memory, and response inhibition. Each scan, including set-up and completion of the task, lasted approximately one hour and was conducted on-site at each farm location.

In preparing for this study, our team recognized multiple methodological challenges—including identification of neurobehavioral domains of interest, functional (brain) regions of interest, fNIRS task selection, and testing environment—that required thoughtful planning to adequately address. Below, we detail how elements of each category were addressed in our study, and we outline a checklist that future researchers may want to follow in their own research.

Areas of Methodological Concern

Neurobehavioral Domains of Interest

The first step in any fNIRS application is the identification of the neurobehavioral function (i.e., the domain) that is hypothesized to be influenced/affected by the exposure of interest. Identification of these domains may arise from multiple sources such as behavioral neurocognitive assessments or neuroimaging outcomes reported in previous epidemiological studies or in animal studies. In our study, pesticides used by agricultural farms were the exposure of interest: some of these, including chlorpyrifos, have been shown to affect executive functioning, working memory, and...
attention and response inhibition in children and adults (Bouchard et al. 2011; Marks et al. 2010; Muñoz-Quezada et al. 2016; Rauh et al. 2011; Rohlman et al. 2014; Ross et al. 2013; van Wendel de Joode et al. 2001; Wesseling et al. 2006).

Functional Regions of Interest
After identifying the neurobehavioral domain(s) of interest, it is possible to determine the cortical regions of the brain that should be targeted with fNIRS. This step is important because the portable fNIRS devices in production at the time that this manuscript was written do not provide enough source/detector pairs to adequately cover the entire cortex. Thus, researchers should allocate the available source/detector pairs over the cortical regions that have previously been shown to be involved in each neurobehavioral domain of interest. This information will most likely be found in published neuroimaging studies. In our study, we distributed eight source/detector pairs evenly over the bilateral dorsolateral prefrontal cortex because these regions are known to underlie our domains of interest. For cases in which no overlap exists between neurobehavioral domains and functional regions of interest (i.e., source/detector locations must change to target the functional region of interest for each cognitive domain), or cases in which more regions of interest are identified than can be covered by a single optode design, the fNIRS source/detector pairs may be moved in between fNIRS scans.

Task Selection
As is common in behavioral neurocognitive assessments, it is likely that a battery of tests may already exist that effectively targets the neurobehavioral domain(s) of interest. Indeed, many test repositories [e.g., the National Institutes of Health (NIH) Toolbox® (http://www.healthmeasures.net/explore-measurement-systems/nih-toolbox)] and standardized batteries [e.g., Wechsler Intelligence Scales (Wechsler 2008)] contain a multitude of neurobehavioral tests that are commonly used in epidemiologic studies. Although the cognitive domain(s) and functional brain region(s) of interest should be considered before task selection, many other factors should also be considered when determining the tasks that are most appropriate for an fNIRS study.

Population of interest. Characteristics of the population of interest may affect the tasks that will be suitable for use with fNIRS. Namely, sociodemographic characteristics such as age and education level (e.g., literacy, computer literacy) may influence the stimuli presented in a task. For children or for highly illiterate populations, it may be more appropriate to require the participants to respond to nonlinguistic stimuli, such as pictures (Durston et al. 2002) or physical actions (Beato et al. 2007). The difficulty of a task may also require adjustment so that the population of interest can complete the task with sufficient accuracy.

Time to conduct scans. When selecting the task(s) to be implemented with fNIRS, researchers should consider the scan time required to complete each task and the scan time required for the entire fNIRS session. It is important to minimize the amount of time each participant spends in the fNIRS cap because fNIRS optodes, which are held tightly to the participant’s head, may become uncomfortable over time. In our experience, the longest an adult will tolerate the fNIRS optodes without experiencing discomfort is ∼45–60 min, whereas this time may need to be reduced to as little as 15–20 min for children or clinical populations. When preparing an fNIRS test battery, researchers should consider the number of tasks that may be accomplished within this time frame. Although the problem of discomfort due to time spent in the fNIRS cap may be ameliorated by removing the cap in between tasks, this will increase the fNIRS session length because of the added set-up time when the cap is removed. However, in cases such as our study, in which farm workers had limited available time, removing the cap between tasks may not be feasible.

Task design. Because of statistical concerns that require sufficient repeats of task trials to identify task-related hemodynamic responses, a redesign of existing behavioral neurocognitive tests is often required. That is, although many tests that target a particular domain of interest may already exist, researchers may still find it necessary to modify the task to suit fNIRS’s requirements for block- or event-related designs (Plchta et al. 2006, 2007). To address these concerns in our study, each of our tasks was reprogrammed using the Psychtoolbox extension (Brainard 1997; Kleiner et al. 2007; Pelli 1997) in Matlab (version 14b; MathWorks Inc.). This program and others (e.g., E-Prime, SuperLab, PsychoPy) provide users with the flexibility to accurately time each aspect of a task and to log timestamps for each block or event. Specifically, our programs sent trial event markers from the stimulus computer (Figure 1A) to our fNIRS data acquisition source (Figure 1D) via a StimTracker™ (Figure 1B; Cedrus Corp.) device. These markers were integrated into our fNIRS data stream, providing the basis for statistical analysis. However, this setup also required that our tasks be administered on a computer, which in turn raised methodological concerns that are addressed below. Notably, fNIRS tasks do not always require computer-based administration, but for instances in which a computer is not used, the same concerns regarding repetition, timing, and logging of events remain. Thus, researchers using noncomputer-based approaches should consider video recording all aspects of their fNIRS scan sessions so that accurate timing of blocks or events can be identified post hoc.

Movement. One possible application of fNIRS is its use during naturalistic tasks that require or cause head motion. Although motion was not a major consideration in our study, it should be carefully considered when it is a factor (e.g., task designs that require motion, when scanning clinical and/or young populations that may have trouble remaining still) (Brigadoi et al. 2014; Cui et al. 2010; Yücel et al. 2014). The primary source of head-motion-related noise in the fNIRS signal comes from mechanical “shearing” of the optodes on the scalp. Thus, one primary method of reducing such noise is to securely attach the optodes to the participant’s scalp via a tight over-cap or band (see Figure 2C). However, these methods may also influence other assessment factors, including the time a participant may be in the fNIRS cap before reporting discomfort. Although many signal processing measures have been developed to help correct fNIRS signals containing motion artifacts, such methods may not be optimal for emergent study designs that inherently involve greater degrees of head motion than are typical in laboratory-based studies (Brigadoi et al. 2014).

Testing Environment
As discussed above, our scan sessions took place at 14 individual farm locations, each of which provided a wide range of amenities (e.g., availability or nonavailability of power, shelter from rain and wind). Therefore, to collect valid data that were not influenced by the testing environment, it was necessary for our team to address the following concerns.

Power source. The NIRSport unit used in our study was battery-powered and was capable of collecting data for roughly eight hours. However, depending on the type of fNIRS unit employed and the task selection and implementation, it may be necessary to have an active power source. For instance, the StimTracker™ device used to send trial event markers for our tasks requires power to maintain communication between the stimulus computer and the data acquisition source. In the event
that power is not ensured in a testing environment, alternative power supplies such as a power generator or car batteries may be employed. If the use of a battery is necessary, we advise minimizing the number of devices connected to the battery during the scans.

**Ambient light.** Because fNIRS is an optical (i.e., light-based) neuroimaging technique, high levels of ambient light in the scan environment may reduce signal quality by increasing the signal-to-noise ratio (Chenier and Sawan 2007; Coyle et al. 2007). In our study, the ambient light conditions varied greatly. In environments with high levels of ambient light, such as outdoors, it is important to minimize the light as much as possible by covering the fNIRS optodes with dark material (e.g., a loose-fitting black shower cap) or, in our case, by using NIRx product-specific over-caps (see Figure 2C).

**Ambient noise and visual distractions.** A quiet location is preferable when collecting fNIRS data in the field. However, as is the nature of many field projects, this is not always possible. Nevertheless, in conjunction with the study-specific power source needs, it is important that these factors be considered when identifying a possible scan location. Ambient noise may be overcome by employing earplugs. Moreover, as illustrated in Figure 1, barriers may be used to isolate the participant and stimulus computer from the surrounding environment.

**fNIRS Scan Session Implementation**

Once each of the concerns raised above is addressed, it is possible to create a detailed fNIRS protocol that may be used at each scan session to help standardize administration across participants. Below, we outline the fNIRS scan protocol that we used in our study.

1. **Identify scan location.** Upon arriving at the day’s scan location, we first sought a suitable place to conduct our fNIRS scans. As discussed above, an optimal location provided covered shelter (i.e., protected from rain and wind) and isolation from noise; reduced ambient light, visual distractions, and pungent or foul odor (e.g., pesticides, manure); and had access to at least one electrical outlet. When one or more of these conditions were not met, we used additional supplies (e.g., a large plastic tarpaulin, cardboard dividers, a long extension cord) to improve the scanning conditions.

2. **Equipment setup.** We situated each participant in a comfortable, seated position, such that there was enough space around him/her for an examiner to move around and to sit or stand. For our study, this was frequently accomplished by arranging a small portable table and chair in the most suitable scan location (see Figure 1). The fNIRS equipment

![Figure 1. Participant engaged in a computer-based functional near-infrared spectroscopy (fNIRS) task in Costa Rica. Here, an equipment shed is used as the scan location. Because of activity outside of the shelter (off photo right), we chose to occlude the participant’s vision in that direction with the use of poster board. In the photo, the pertinent equipment needed to conduct a mobile computer-based fNIRS study has been labeled.](image-url)
The functional near-infrared spectroscopy (fNIRS) cap setup (e.g., gel, hair separator tools) were situated nearby (i.e., within range of the cords connecting each device) but out of the participant’s view. To orient the placement of the cap onto the participant’s head such that the optodes consistently covered our regions of interest despite changes in head size across participants, we referred to the international 10/20 system for transcranial functional brain mapping (Jasper 1958; Okamoto et al. 2004). Briefly, the 10/20 system is an internationally recognized atlas of functional brain region localization that relies on the distance between anatomical landmarks, such as the nasion (i.e., the location on the face, in between the eyes, at which the top bridge of the nose meets the face) and the inion (i.e., the lowest point on the back of the skull that is normally indicated by a prominent bump). The “10” and “20” in 10/20 refer to percentages of the nasion-to-inion distance, at which the 10/20 locations lie, respectively.

3. **Explaining the procedure to the participant.** Once the equipment was ready and the cap had been selected and prepared for the fNIRS administration, we found it helpful to give the participant a detailed explanation of what they would experience during the scan. Because many participants had little to no experience with technology (e.g., computers), we showed each participant the computer and the fNIRS device, and we explained what they would experience throughout the task.

4. **fNIRS setup on the participant.** We placed the anterior ridge of the fNIRS cap on the participant’s forehead such that the middle anteriormost point of the cap ridge laid directly over the midline frontal polar 10/20 location. Next, we pulled the lateral straps of the cap down such that the cap wrapped snugly around the participant’s head. It was important to secure the cap under the participant’s chin with Velcro® straps, making sure that the original placement of the cap ridge remained in place over the eyebrows and was situated evenly across the left and right hemispheres of the head. Before attaching the fNIRS optodes to the cap, cord management/support devices were set up. Next, we examined the condition of the hair underneath the cap; fNIRS optodes should be in direct contact with the scalp. If any hair impeded the fNIRS optodes, we used a blunt syringe and water-soluble gel to move the hair at each optode location. In our study, two people set up the optodes to reduce the setup time. After each optode was securely fastened to the participant’s head, we conducted a signal calibration and quality scan. In case of poor signal quality, we corrected the placement of the optodes in the affected channels by gently “wiggling” them in place or carefully removing them and repeating the steps outlined above.

5. **fNIRS administration.** Once the fNIRS optodes were set up and an adequate signal quality was achieved, the instructions for each task were explained. The examiner ensured that the tasks were understood by asking the participant to explain it in his/her own words. Alternatively, although we did not use such methods, researchers may elect to employ a criteria-based practice wherein the participants are asked to complete a set of practice trials until they reach a predetermined performance threshold (e.g., percent of trials correct, response time). Finally, the fNIRS scan was started immediately before the beginning of each task.

6. **Post-scan.** Once the participant completed the scan, we carefully removed the cap, making sure to avoid pulling any hair that may have been caught by the fNIRS optodes. After removing the cap, we wiped each optode with disposable isopropyl alcohol wipes and sprayed the cap with a 10% isopropyl alcohol:water solution. Next, we stored...
the fNIRS device so that it would not be struck or damaged in between scans, and we reset all materials needed to perform the next scan. Finally, we backed up each data file (i.e., fNIRS and behavior data files) to an encrypted external hard drive.

Conclusion
The overarching goal of this paper is to demonstrate the feasibility of employing functional neuroimaging in epidemiological studies regardless of the location or the conditions in which the study is taking place. Critically, this is now possible using readily available, portable fNIRS neuroimaging devices to assess neurobehavioral functioning across multiple domains. Most importantly, in our study, the addition of the fNIRS assessment did not interfere with the goals of the ongoing epidemiological study and was seamlessly integrated into daily operations.

This paper provides a set of general guidelines that epidemiologists and other researchers may follow to incorporate fNIRS neuroimaging into their own research. As such, many of the solutions we offer are not exhaustive but are instead presented to raise issues that we encountered and to provide the solutions we used for our study. We invite and encourage the reader to seek out more in-depth examinations of the issues we have raised, which include but are not limited to our cited references. As the availability and prevalence of functional neuroimaging in epidemiological studies increases, it is our hope that researchers in the field will use this paper as an introduction to functional neuroimaging as it pertains to their research.

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