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# Resting State Functional Connectivity in Children: A New Paradigm

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## Abstract

Resting state functional connectivity (rsFC) can provide a window into the neural architecture of functional networks in the brain. Functional networks measured both during task and during “resting” (task-absent) state are correlated with cognitive function, and much development of these networks occurs between infancy and adulthood. However, rsFC study in young children has been sparse, mainly due to a paucity of child-appropriate neural measures and behavioral paradigms. We present a new paradigm to measure rsFC in children, utilizing functional near-infrared spectroscopy (fNIRS) and Freeplay, a behavioral setup designed to approximate resting state in children. Results suggest this paradigm is practical and has good construct validity and test-retest reliability.

**Keywords:** resting state functional connectivity; fNIRS; early childhood; Freeplay;

## Introduction

Brain function arises from the concerted effort of multiple regions working together in what can be characterized as networks. Recent research has focused on properties of these networks and patterns of connectivity that characterize regions working together to support function (Moussa, Steen, Laurienti, & Hayasaka, 2012). These networks of brain regions, which are not necessarily spatially adjacent but exhibit correlated activity during function, are called “functionally connected”. Specifically, functional connectivity is defined as the temporal correlation between low-frequency fluctuations in activity of anatomically distinct regions (Friston, 1994). While traditionally studied in participants during task, Biswal, Yetkin, Haughton & Hyde (1995) demonstrated that functional correlations are present even in the absence of a task. Such “resting state” functional connectivities (rsFC) comprise networks, many of which overlap with or reflect known functional networks (Rosazza & Minati, 2011) and are consistent across individuals. These “resting state networks” (RSNs) -- functionally connected networks that show temporally correlated activity in subjects *not* performing any task (Fox & Raichle, 2007) -- are thought to reflect underlying functional architecture of the brain.

One question that arises is why spontaneous correlations during resting state overlap with known functional networks of regions engaged together during task. One possibility is that regions that tend to be activated together during tasks also show correlations in their spontaneous fluctuations at rest, reflecting a kind of memory trace of previous coordinated activity (Rosazza & Minati, 2011). For example, Stevens, Buckner & Schacter (2010) demonstrated that resting state fluctuations in lateral visual areas were modulated by a visual task presented prior to resting state collection, seeming to reflect experience-dependent spontaneous correlations. Although the ontology of RSNs is not fully understood, RSNs seem crucially linked to cognitive function.

Several studies have correlated strength of rsFC in RSNs with task performance or behavior. For example, Seeley et al. (2007) found that rsFC in regions of the central executive network was correlated with varying performance on the trail-making task, a test tapping into executive function. These and other studies demonstrate a link between resting state correlations and cognitive function, within known networks such as the visual, auditory, and executive control networks (Cordes et al., 2000).

This fortuitous discovery of rsFC and its link to function has widened the door to investigating mechanisms of cognitive function by providing some advantages over task-based studies: 1) whereas measuring functional connectivity during task provides us with the connectivity of only those regions specifically involved in the task, measurement during resting state can provide connectivity information simultaneously among many regions, since they are all measurable in resting state; 2) certain populations (e.g., clinical populations) or cognitive functions (e.g., certain motor functions) that are difficult to assess with tasks in the scanner, are more easily studied in resting state.

RSN research in infants and adults presents a clear developmental trend: whereas 10-20 RSNs have been identified in adults, only about 5 of these networks are found in infants, the rest likely developing throughout childhood (Jolles et al., 2011; Barkhof et al., 2014). We also know that *patterns* of connectivity change over development

-- a number of studies have shown that children display more diffuse functional connectivity patterns and increased connectivity with adjacent regions, while adults show more focal connectivity patterns and increased connectivity between distant regions (Supekar, Musen, & Menon, 2009; Fair et al., 2009). This simultaneous increase in both segregation (pruning short-range connections) and integration (strengthening long-range connections) of brain regions over development likely reflects a transition from organization around spatial proximity to organization around higher-order function (Fair et al., 2009). Also, RSN abnormalities are found in a variety of psychopathologies (for review, see Barkhof et al., 2013), suggesting that RSN development may play an integral role in healthy brain and cognitive development. Together, these findings highlight the need for studying RSN development in children.

However, study of RSNs in children has been difficult, mainly for two reasons. First, traditional neuroimaging methods are difficult to administer with awake children. Second, the standard procedure for measuring resting state connectivity -- to sit still for a period of time -- is difficult for children. Consequently, resting state studies in young children have been limited (Uddin et al., 2010). The current study uses functional near-infrared spectroscopy (fNIRS) to address the first difficulty and introduces a new paradigm called Freeplay to help address the second difficulty. By reconstructing characteristic features of resting state functional connectivity, we demonstrate the feasibility of this fNIRS-Freeplay set-up for studying resting state connectivity in preschool and early school-aged children.

**Challenges in neuroimaging methods** Traditionally studied with fMRI, PET (positron emission tomography), and EEG/MEG (magnetoencephalography), RSNs have been studied primarily in adult and sleeping infant populations, but relatively rarely in child populations, as these imaging techniques are difficult to use with awake children (Raschle et al., 2012). For example, since children have difficulty remaining still for extended periods of time, a major challenge is the sensitivity of these modalities to movement artefacts, which can systematically bias functional connectivity measures (Power et al., 2012).

fNIRS is a relatively recent light-based neuroimaging method that overcomes many of the challenges with obtaining brain activity measures in child populations. In fNIRS imaging, near-infrared light is used to obtain an estimate of changes in hemoglobin concentrations in an area. Thus, like fMRI, fNIRS gives an indirect measure of neural activity based on blood oxygenation levels. Compared to fMRI or EEG, fNIRS is robust to and unrestrictive of motion, comfortable, quick to set up, and cost effective (see Nishiyori, 2016 for comparison of techniques). These factors make it especially appropriate for use with children. The main limitation of fNIRS in the context of studying rsFC is that measurement is limited to

regions near the surface of the brain. Our study, however, as discussed below, measures from the prefrontal cortex, most of which is accessible by fNIRS.

**Challenges in behavioral methods** Traditional resting state studies record from adults instructed to remain still for a period of time (Patriat et al., 2013). Infant studies are conducted during sleep (Liu, Flax, Guise, Sukul, & Benasich, 2008). Unfortunately, neither recording situation is practical for child populations; children have difficulty remaining quietly still for an extended time, and unlike with babies, it is difficult to get a sleeping child into the scanner.

Some studies have used movie clips to help children sit still and quietly for an extended time while in the scanner (Barker, Aarabi, & Huppert, 2013; Emerson et al., 2015). Viewing such clips may engage task-specific networks, such as those involved in language or audition. In fact, movies are sometimes used in studies as stimuli or tasks, bringing into question the validity of using movies for inducing a state of "rest" (Cantlon & Li, 2013).

Our study proposes an experimental paradigm called Freeplay in an attempt to closely approximate true resting state. In this "task", participants are presented with a set of simple toys (e.g., blocks, small plastic animals) and asked to quietly play for a few minutes. The premise is that children can naturally comply much more easily with sitting still and quietly for a period of time when presented with even simple and unengaging toys. Due to the simple nature of the toys, Freeplay is expected to induce quiet boredom, a state we expect may closely approximate resting state.

**Prefrontal Cortex** The prefrontal cortex (PFC) is a major component of RSNs such as the central executive network (CEN) and default mode network (DMN). Its development, from infancy through adulthood, is most prominently linked to the development of executive function (EF) (Casey, Giedd, Kathleen & Thomas, 2000), a collective system of basic cognitive processes that includes inhibition, working memory, and cognitive flexibility, and supports higher-order processes such as planning (Diamond, 2006; Diamond, 2013). PFC's protracted development make it an important region to study over development. Also, much of the PFC is well measured by NIRS, as it is close to the surface of the skull and conveniently placed under the forehead, which lacks hair (improving NIRS signal quality). Thus, we focused data collection on the PFC in this validation study.

### **Current study**

This study aims to demonstrate feasibility of using fNIRS and Freeplay to measure rsFC in pre-school and early school-aged children. We present this paradigm as a potential means to address the gap in literature on rsFC in children, stemming from a lack of appropriate measurement and behavioral tools. This set-up is designed to place minimal restrictions on the participant, allow sufficient data

collection, and achieve relatively good signal to noise ratio (by minimizing sources of noise introduced by both the measurement tool and the participant). This study aims to establish fundamental psychometric properties of the fNIRS-Freeplay paradigm, including construct validity, test-retest reliability, and feasibility.

## Experiment 1

We first investigated whether the fNIRS-Freeplay paradigm allows us to measure rsFC -- specifically, whether the paradigm exhibits construct validity. We did this in two ways. First, we asked whether the fNIRS-Freeplay paradigm reproduces a characteristic feature of adult resting state connectivity, namely strong connectivity between homologous (bilaterally symmetric) regions (Sasai, Homae, Watanabe & Taga, 2011). Second, we studied whether rsFC in adults in Freeplay is distinguishable from that in adults in true resting state, in terms of the ability of machine learning classifiers to correctly classify instances of each.

Broadly, we hypothesize that adults “at rest” and in Freeplay will show similar connectivity patterns, suggesting Freeplay may be a good approximation of resting state. Confirming this hypothesis would begin to establish fNIRS-Freeplay as a valid paradigm for measuring rsFC in adults, and a natural next step would then be to apply this paradigm to measure rsFC in children. To begin exploring this (and also to provide a control for our second similarity measure -- classification error between adults in Freeplay and “at rest”), we additionally measured NIRS-Freeplay data in children, hypothesizing that adults and children in Freeplay will show different connectivity patterns, consistent with research suggesting significant development of rsFC from childhood into adulthood (Jolles et al., 2011).

## Methods

**Participants** Participants were 13 undergraduates (aged 18-21) from CMU and 17 children (aged 3 to 8 years,  $M_{age} = 4.8$ ) recruited from the community and the Children’s School, a CMU-affiliated laboratory school. Adults participated in both the standard resting state task and the Freeplay task within a single session. Task order was randomized. Children participated in Freeplay only.

**Standard Resting State Task** Participants sat still and quietly at a desk with eyes open for 8 minutes.

**Freeplay Set-up** Participants sat quietly and freely played with a set of toys for about 8 minutes. Toys included: lincoln logs, wooden nuts and bolts, plastic animal figurines, toy cars, and simple coloring pages (a flower, turtle, duck, or fish). Toys were chosen to be simple and minimally engaging, to help induce quiet boredom, a state that we expect may closely approximate resting state.

**fNIRS Set-up** Neural activity was recorded at 20 Hz using a

continuous wave (CW6) real-time fNIRS system (Techen, Inc., Milford, MA, USA), with 4 light sources, each containing 690-nm (12 mW) and 830-nm (8 mW) laser light, and 10 detectors, to give oxygenation measures in 12 channels on the PFC. Sensors were arranged in a layout as depicted in Figure 1. Sensors were snapped into a cap strip built from foam sheet and plastic mesh, and connected to the fNIRS system via optic cables. For each participant, the cap strip was positioned on the head, centered on position FpZ according to the international 10-20 coordinate system standard, extending over the Brodmann area 10 (anterior PFC) and area 46 (dorsolateral PFC) bilaterally. The strip was secured to the head using a neoprene scuba cap.

fNIRS data was recorded for each participant using custom data collection software that interfaced with the fNIRS system, described in Abdelnour and Huppert (2009). After fitting the fNIRS cap to the participant’s head, signal quality was checked for each source-detector channel and adjusted if needed to make sure the fNIRS fiber optics made good contact with the scalp of the participant, and that light gain was sensitive to blood oxygenation (identified by cardiac signal).

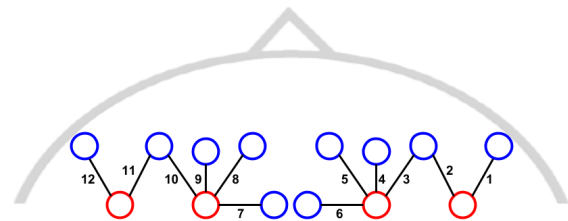


Figure 1. Probe layout for Experiment 1 -- Sources are in red and detectors are in blue. Channels are in black, labeled 1-6 on the right hemisphere and 7-12 on the left.

**fNIRS Data Processing** Raw light attenuation measures were converted to oxygenated hemoglobin concentration changes using the modified Beer-Lambert law (Huppert, 2013). We first removed long-term drifts in the data by subtracting a least-squares linear fit. We then band-pass filtered the data to remove cardiac and respiration signals (0.01-0.1 Hz, as suggested by Sasai et al., (2011)). With the resulting time series, we computed partial correlations for each channel pair (CP), given the other channels (since there were 12 channels, there were 66 (12 choose 2) distinct CPs). These 66 computed partial correlations, which are represented graphically in correlation matrices (as in Figure 2) were the main objects studied in this paper.

**Data Analysis Strategy** Our first goal was to find the homologous correlations characteristic of rsFC. To test for significant homologous connectivity, we compared functional connectivity between regions that were homologous (bilaterally symmetric) to that between non-homologous regions.

Our second goal was to test the validity of Freeplay as a task for measuring resting state. To do so, we compared the

functional connectivity in adults between the two conditions, “at rest” and Freeplay. Since it is difficult to directly measure similarity between functional connectivity patterns, we did so by estimating the accuracy of classifiers trained to distinguish different conditions (e.g., between “at rest” and Freeplay in adults). Higher accuracies suggest greater distinguishability, and hence greater dissimilarity, between classes. Given the high-dimensionality (66 CPs) of our problem, we used logistic LASSO (i.e., logistic regression with the Least Absolute Selection and Shrinkage Operator penalty), which should perform relatively well in our high-dimensional setting (Tibshirani, 1996). Accuracy was estimated using leave-one-out cross-validation (LOOCV). Within each LOOCV fold, the LASSO regularization parameter  $\lambda$  was selected by 10-fold cross validation. To reduce the chance that results were classifier-specific, we also tried a highly distinct classifier, k-nearest neighbors (kNN) classification. Accuracy was again estimated by LOOCV, with k selected within each LOOCV fold by 10-fold cross-validation (over  $k=1, \dots, 10$ , covering a range of common values (Cunningham & Delany, 2007)). To provide a baseline for comparison, we similarly compared data from adults versus children in Freeplay.

## Results

All participants (including all child participants) completed the task and provided usable data.

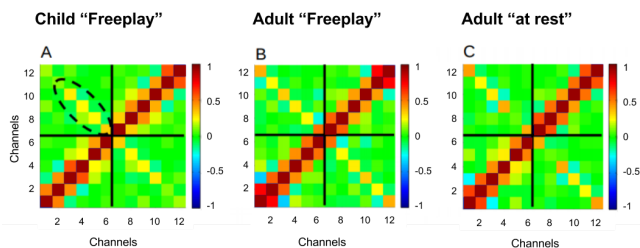


Figure 2. Group-averaged partial correlation matrices for children in Freeplay and for adult in Freeplay and at rest. Channels 1-6 were located on the right hemisphere; channels 7-12 were located on the left hemisphere. Homologous CPs are circled in the child panel.

**Homologous versus non-homologous CPs** First, we compared all homologous to all non-homologous pairs of distinct channels, to identify the strong inter-hemispheric symmetric connections. Specifically, we averaged homologous and non-homologous CPs within subjects, forming two sets of measured CP correlations ( $r$ -values; separately for adults and children). This comparison showed that homologous CPs were significantly more strongly connected than non-homologous CPs ( $p < 0.001$  for both children and adults, by a two-sample  $t$ -test of the Fisher  $z$ -transformed  $r$ -values as well as a permutation test). As can be seen in Figure 2, these homologous correlations appear stronger in adults, consistent with the trend of RSNs’

long-range connections strengthening into adulthood, although we did not formally test for this.

**Adult correlations in Freeplay versus “at rest”** Next, we compared adult correlations in Freeplay and “at rest”, to see how Freeplay compares with traditional resting state. Logistic LASSO, trained to predict Freeplay or “at rest” from CP correlations, gave a LOOCV accuracy of 45.1% (worse than chance, 50%) suggesting that the two tasks do not seem to elicit highly distinct connectivity. The kNN classification to predict Freeplay or “at rest” from CP correlations achieved a LOOCV classification accuracy of 57.7%, just over chance.

**Adult versus child correlations in Freeplay** Next, we compared correlation matrices between adults and children, both in Freeplay. Logistic LASSO, trained to predict “adult” or “child” from CP correlations, gave a LOOCV accuracy of 96.7%, suggesting that adults and children exhibit highly distinct connectivity in Freeplay.

## Experiment 2

In this experiment, we studied reliability of the NIRS-Freeplay rsFC measure in children, in terms of consistency of results across independent scans (intersession, or test-retest, reliability). To do this, we collected multiple NIRS-Freeplay scans from children on different days and used each scan to estimate a connectivity network. We then measured similarity of this estimated connectivity network both across scans within subjects and across subjects.

## Methods

**Participants** Participants were 19 children (aged 3 to 5 years,  $M_{\text{age}} = 4.49$ ) recruited from the Children’s School. Data was collected longitudinally at 2 time points (a few months apart), with 2 scans (a few days apart) at each time point. These data were collected as part of a bigger study, and the longitudinal aspect was not analyzed in this study. 17 participants completed all 4 scans; data from two participants who were not able to complete all 4 scans due to scheduling constraints was discarded.

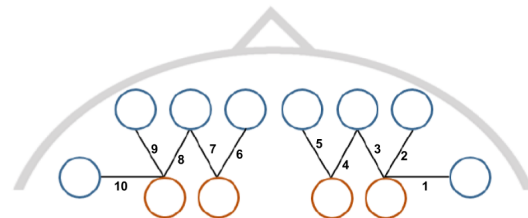


Figure 3. Probe layout for Experiment 2 -- Sources are in red and detectors are in blue. Channels are in black, labeled 1-5 on the right hemisphere and 6-10 on the left.

**Freeplay and fNIRS Set-up** The Freeplay set-up was as in Experiment 1. fNIRS set-up was almost identical to that in Experiment 1, except with a slightly different sensor layout:

4 light sources and 8 detectors, to give oxygenation measures in 10 channels on the prefrontal cortex, with sensors arranged in a layout as depicted in Figure 3.

**fNIRS Data Processing** Data was preprocessed and partial correlations computed as in Experiment 1. Since there were 10 channels in this probe design, there were 45 distinct CPs.

**Data Analysis Strategy** Because we were interested in the reliability of *patterns of connectivity* rather than the exact connectivity values, we binarized each CP by thresholding the absolute value of its partial correlation value at a “connectivity threshold”  $\theta$ ; absolute values below  $\theta$  were replaced with 0 (denoting an unconnected CP), and absolute values above  $\theta$  replaced by 1 (denoting a connected CP).

We then used two indices of inter-scan reliability: the F1 score (a.k.a., Dice coefficient), a general measure of overlap between two sets (twice the ratio of the number of CPs functionally connected in *both* scans to the sum of the numbers of functionally connected CPs over both scans) and the Matthews correlation coefficient (MCC), the correlation between the binarized pattern of 0’s and 1’s, across all 45 CPs. Accuracy (proportion of agreement) between the two scans was not used because it is extremely sensitive to the connectivity threshold; for example, using a threshold of 0 (full connectivity) or 1 (no connectivity) results in a perfect accuracy of 1. The raw (continuous, un-thresholded) correlation was also not used, as it is relatively difficult to interpret as a measure of reliability. We chose  $\theta$  to maximize (over 1000 equally spaced values between 0 and 1) each reliability index and used LOOCV to obtain an unbiased estimate of each reliability index.

## Results

The mean (across LOOCV folds) F1 score was 0.683 (95% CI: 0.050). The mean cutoff threshold chosen was  $\theta=0.547$ , corresponding to functional connectivity in an mean of 13.2% of CPs. The mean MCC was 0.483 (95% CI: 0.108), with mean cutoff threshold  $\theta=0.559$ , corresponding to connectivity in a mean of 12.7% of CPs.

We performed a permutation test comparing the F1 score or MCC to that after randomly permuting the CPs in the second scan for each participant, to test a null model where CPs are randomly identified as functionally connected, with the two scans independent. This test rejected the null for both F1 score and MCC ( $ps<0.001$ ; 1000 permutations). That is, within subjects, we find functional connectivity consistently in the same channels between scans.

Interestingly, similar tests in which second scans were randomly permuted across *participants* (i.e., for a null model of identical/non-distinct participants) were *not* significant ( $ps>0.1$ ), suggesting consistency *across* subjects in Freeplay. This is encouraging from the perspective of trying to identify a common pattern of functional connectivity across individuals. However, further work is

needed to understand the sensitivity of the paradigm to individual differences (that might correlate with other quantities of interest, such as age, or behavioral measures), for which we conjecture that the continuous (un-thresholded) CP correlations may be more informative.

## Discussion

This study explored the feasibility of using fNIRS and Freeplay to measure rsFC in children. Consistent with previous results, we were able to recover connectivity features characteristic of rsFC, helping validate our fNIRS set-up for measuring traditional rsFC. Additionally, in adults, correlation patterns in Freeplay were similar to that in traditional resting state -- trained classifiers did not perform significantly better than chance, suggesting that Freeplay may produce a state similar to that in the traditional resting state condition. Since Freeplay was designed to approximate resting state in children, who struggle with the usual resting state task, this comparison of Freeplay to the traditional task in adult participants serves as an additional check for the viability of fNIRS-Freeplay for measuring rsFC in children. Crucially, all children completed the task and provided usable data, speaking to the practical usability of the Freeplay paradigm for studying rsFC in children. Further, correlations in adults and children in Freeplay showed different patterns, from which a trained classifier was able to predict “adult” or “child” with high accuracy -- this is in line with our expectations given that we know RSNs develop significantly with age. Finally, Experiment 2 demonstrated inter-scan reliability, in that similar connectivity patterns are found between 2 independent scans of the same individual.

The current study demonstrated the practical feasibility as well as the construct validity of the Freeplay-fNIRS paradigm for studying rsFC in children by measuring correlations between pairs of regions in the PFC. As we previously discussed, the PFC is a central player in the CEN, and studying its resting state connectivity patterns over development may inform us about how the CEN develops and supports EF. Zhao et al. (2016) recently found that network properties of rsFC in the PFC, as measured by fNIRS, were correlated with varying performance in EF tasks in adults. An important next step will be to extend this investigation to children. We are especially interested in exploring, through rsFC in the PFC, the changes in neural infrastructure that parallel observed cognitive development in EF. As a practical setup for measuring rsFC in children, the fNIRS-Freeplay paradigm will allow us to investigate these questions to advance understanding of the neural mechanisms of cognitive development.

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