

# Prefrontal cortex intrinsic functional connectivity and executive function in early childhood and early adulthood using fNIRS

Cassandra M. Eng<sup>a,b,\*</sup>, Roberto J. Vargas<sup>b</sup>, Howard L. Fung<sup>a,c</sup>, Selena R. Niemi<sup>a,d</sup>,  
Melissa Pocsai<sup>e</sup>, Anna V. Fisher<sup>b</sup>, Erik D. Thiessen<sup>b,\*\*</sup>

<sup>a</sup> Department of Psychiatry & Behavioral Sciences, Stanford University, Center for Interdisciplinary Brain Sciences Research, 1520 Page Mill Road, Stanford, CA 94304, USA

<sup>b</sup> Department of Psychology, Carnegie Mellon University, 5000 Forbes Ave, Pittsburgh, PA 15213, USA

<sup>c</sup> Department of Psychology, Trinity College, 300 Summit Street, Hartford, CT 06106, USA

<sup>d</sup> Department of Human Biology, Stanford University, 450 Jane Stanford Way, Building 20, Stanford, CA 94305, USA

<sup>e</sup> Department of Psychology, The Graduate Center & Queens College, City University of New York, 365 5th Ave, New York, NY 10016, USA

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## ABSTRACT

Executive function (EF) is crucial for goal-directed behavior and predicts overall wellbeing, academic and interpersonal success. Intrinsic (i.e., non-evoked) resting state functional connectivity (rsFC) during naturalistic paradigms offers insight into neural mechanisms underlying EF. However, few studies have explored EF-rsFC associations using functional near-infrared spectroscopy (fNIRS) across age groups. This cross-sectional study validates a naturalistic viewing paradigm (*Inscapes*) using fNIRS and examines the link between rsFC in the prefrontal cortex (PFC) and EF in children ages 4–5 and in young adults ages 18–22. Adults were presented with two rsFC paradigms in a counterbalanced within-subjects design: a traditional static crosshair and *Inscapes*. Representational similarity analysis revealed robustly similar rsFC patterns between the crosshair and *Inscapes* conditions, and both were associated with EF (Stroop performance). Children were presented with *Inscapes* to assess rsFC, and exhibited high compliance using fNIRS. Importantly, rsFC assessed with *Inscapes* in children was associated with EF (Stroop-like Day-Night Task performance). Age-related differences showed intrinsic functional connections within the PFC strengthening over development. This study uses child-friendly, noninvasive optical neuroimaging and a publicly available rsFC paradigm to elucidate the role of the PFC in EF development, illuminating practical methodological approaches to study the developmental trajectory and neural underpinnings of EF.

## 1. Introduction

In the past two decades, the use of resting state functional connectivity (rsFC) has gained popularity in cognitive and developmental neuroscience to help understand brain network organization and the change in patterns of connectivity during development (Kardan et al., 2022; Marek et al., 2019; Van Essen et al., 2013). Unlike task-based neuroimaging methods, rsFC methods leverage simultaneous neural activation in spatially-distributed brain regions that occur in the absence of external tasks or stimuli that would elicit goal-directed behavior (i.e., when the brain is “at rest”) (Damoiseaux et al., 2006; Greicius et al.,

2003). This approach relies on measurement of both between and within brain region neural co-activation, revealing intrinsic functional architecture of the brain (for review see Power et al., 2011; Vogel et al., 2010; Uddin et al., 2024). In many cases, task-based assessments that are appropriate for adults may be too difficult for use with children, and must be modified so extensively for use with children that the resulting data cannot be easily compared across ages (e.g., Fisher and Kloos, 2016; Eng et al., 2024), whereas rsFC methods do not suffer from these limitations to nearly the same extent.

There has been much research relating group and individual differences in rsFC in adults to several cognitive processes, including

\* Corresponding author at: Department of Psychiatry and Behavioral Sciences, Stanford University, Center for Interdisciplinary Brain Sciences Research, 1520 Page Mill, Office 160, Stanford, CA 94304, USA.

\*\* Corresponding author.

E-mail addresses: [cassie24@stanford.edu](mailto:cassie24@stanford.edu), [cassonde@alummi.cmu.edu](mailto:cassonde@alummi.cmu.edu) (C.M. Eng), [thiessen@andrew.cmu.edu](mailto:thiessen@andrew.cmu.edu) (E.D. Thiessen).

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executive function (EF; Cole et al., 2016; Reineberg et al., 2018; Sato et al., 2016; Sherman et al., 2014; Tan et al., 2024). EF encompasses complex, higher-order cognitive processes that facilitate goal-oriented behavior (Best and Miller, 2010; Miller and Cohen, 2001; Werchan and Amso, 2017). Although there is no single standard definition of EF, it is widely accepted that EF is characterized by carrying out goal-relevant responses while counteracting irrelevant stimuli (Zhou et al., 2012). The most commonly studied EF processes include working memory, cognitive flexibility, and inhibitory control (Davidson et al., 2006; Diamond, 2013; Lerner and Lonigan, 2014; Miyake, 2000). These processes play a crucial role in learning, problem-solving, decision-making, emotional regulation, and successful life transitions (Cowan, 2014; Friedman and Robbins, 2022; Kupis and Uddin, 2023). Improvements in EF have also been shown to support academic achievement, interpersonal and occupational success, and overall well-being by developing crucial skills such as planning, reasoning, and problem-solving (Diamond, 2011; Liu et al., 2020).

As childhood is a time of rapid EF development, neuroimaging data from participants in this age group are tremendously informative (Best and Miller, 2010; Davidson et al., 2006; Diamond, 2020; Eng et al., 2022; Kharitonova et al., 2013; Kraybill et al., 2019; Mehnert et al., 2013; Perone et al., 2018a; Werchan and Amso, 2017). However, several challenges are associated with applying traditional rsFC research methods to child participants. For instance, rsFC methodological approaches typically involve asking participants to look at a static crosshair or keep their eyes open or closed for five or more minutes (Dong et al., 2015; Keles et al., 2016). This technique is impractical for preschool-aged children with limited attention spans and low tolerance for boredom. Young children find being asked to sit still while looking at a static crosshair for a period of time to be an aversive and difficult task, thus making conventional rsFC techniques impractical for this developmental population. Additionally, functional magnetic resonance imaging (fMRI)—the most common modality of rsFC research—can be effortful and inefficient for use with children. fMRI requires participants to remain confined and immobile for prolonged periods in a loud and unfamiliar environment during experimentation. These requirements may impact participant recruitment, procedure practicality, and the quality of data collected. Importantly, these requirements result in high exclusion rates and oversampling for both typically developing preschool children and children with neurodevelopmental disorders (Fishburn et al., 2014; Yerys et al., 2009). There have been innovative methods developed to optimize testing procedures and equipment to address these shortcomings (Frew et al., 2022; Greene et al., 2018; Horien et al., 2020; Raschle et al., 2009), but the use of fMRI for rsFC research with young children remains challenging and costly.

Fortunately, advances in functional near-infrared spectroscopy (fNIRS) brain imaging, alongside recent advances in rsFC methodological practices, made it more practical to study rsFC across development. Functional NIRS is a neuroimaging technology that provides the opportunity to noninvasively monitor hemodynamic activity within the human brain (Pinti et al., 2020). The technology uses diffuse optical measurements in the red to near-infrared range to measure changes in cerebral blood oxygenation within the first few millimeters of the cerebral tissue. Changes in these biomarkers indicate cerebral blood flow, which is used as a proxy for neural activity in the cortex, similar to fMRI (Santosa et al., 2018). Compared to traditional neuroimaging methods, fNIRS is more practical for studies with children because it is unobtrusive, relatively inexpensive, does not require participants to remain immobile, and has been applied to a growing number of populations and experimental studies over the last four decades (Li et al., 2023; Yücel et al., 2021). fNIRS offers unique advantages for studying brain function in child populations compared to other neuroimaging modalities. While fMRI provides higher spatial resolution than fNIRS, fMRI is costly and requires participants to remain immobile in a confined and loud environment. Electroencephalogram (EEG) has higher temporal resolution than fNIRS but is more sensitive to motion artifacts and unsuitable for

activities involving extensive or unpredictable movements. Due to these advantages, fNIRS neuroimaging experiments have become increasingly valuable for investigating brain-behavior associations, permitting the measurement of brain cortical areas associated with EF development in children.

EF is supported by a network of brain regions, with the prefrontal cortex (PFC) in particular serving as a key hub in this network due to its high-level, top-down regulation of signals originating from other brain regions (Friedman and Robbins, 2022; Menon and D'Esposito, 2022; Zink et al., 2021). As such, PFC function is highly correlated with EF (Fuster and Bressler, 2015; Moriguchi and Hiraki, 2011). Because resting state is task-free by definition, this correlation does not arise from any specific goal-relevant behavior. Instead, it is thought to reflect an extensive history of co-activation that arises from the exercise of coordinative aspects of EF. More extensive co-activation, associated with more effective use of EF, leads to a greater degree of resting state connectivity among coordinated brain regions (Boon et al., 2020; Roye et al., 2020; Reineberg and Banich, 2016). These effects have typically been documented using fMRI. For example, Reineberg et al. (2015) showed that higher common EF, the umbrella term encompassing all features of EF tasks, is associated with increased rsFC within the PFC, the parietal and the occipital lobes, as well as between these regions in adults. Similarly, another fMRI study found that participants who showed higher rsFC within the PFC, and between the PFC and the parietal cortex, scored higher on the Stroop Task, a widely utilized EF assessment with adults (Dong et al., 2015). These findings are consistent with previous literature that found decreased rsFC in the PFC predicted EF dysfunction in those diagnosed with psychiatric conditions, such as Autism Spectrum Disorder (ASD; Padmanabhan et al., 2013), Major Depressive Disorder (Murrough et al., 2016), Huntington's Disease (Thiruvady et al., 2007), and Schizophrenia (Chen et al., 2017). The current landscape of the literature suggests PFC rsFC to be strongly associated with EF in adulthood (Cole et al., 2016; Keles et al., 2016; Rosazza and Minati, 2011; Sasai et al., 2012; Shine, et al., 2019; Smith et al., 2009; Smitha et al., 2017; Van Den Heuvel and Hulshoff Pol, 2010); however, literature mirroring these findings in children is scarce using fNIRS.

Decades of rsFC research using fMRI reveal distinct developmental trajectories of different functional networks in the brain. Although large-scale functional networks are present after the third trimester of gestation and in newborn infants (Zhang et al., 2019), initial data from infants and children suggest that early-life functional connectivity is likely restricted to proximal brain regions (Fair et al., 2009; Gao et al., 2015). Myelin formation and synaptic pruning throughout development is believed to produce more efficient neural pathways, with a consistent synchronization during the first two years of life, where the organization of functional networks develops from a local anatomical or primary networks emphasis in children to a more distributed higher-order architecture in young adults (Oldham and Fornito, 2019). The development of such infrastructure facilitates greater communication between regions in which communication was previously substantially less efficient. The developmental shift from an initial state of largely proximal connections to a network involving longer-distance connections may support the efficiency in information processing. Given that EF requires coordination across multiple disparate brain regions, a bias toward local connectivity may be one reason why EF is minimally present at birth and undergoes prolonged development. Following evidence suggesting that the PFC acts as a central coordinative hub in multiple EF-related networks, the developmental trajectory of PFC connectivity may be especially relevant to development in the EF domain. While this possibility is intriguing to explore with fNIRS, it cannot be firmly supported on the basis of current limited data, largely owing to the absence of a sufficient sample of rsFC data in children.

The application of fNIRS has facilitated advances in linking EF task-evoked activity and PFC development (Mehnert et al., 2013; Moriguchi and Hiraki, 2013; Negoro et al., 2010; Perlman et al., 2016; Perone et al.,

2018b). However, there is a gap in the literature regarding EF and rsFC across development using fNIRS (Pinti et al., 2020). One study using fNIRS with elementary school-aged children (7–8 years old) and early adolescents (11–12 years old) found changes in the topological organization of rsFC networks across two developmental stages from early childhood to early adolescence (Cai et al., 2018). This work illustrates the maturation of network integration and cognitive capacity over time. The current study examines the associations between EF and PFC rsFC with a younger population of preschool-aged children (4–5 years old) and young adult participants (18–22 years old) with fNIRS.

Vanderwal and colleagues (2015) developed *Inscapes*: a publicly accessible, naturalistic viewing paradigm designed specifically to measure intrinsic (i.e., non-evoked) functional connectivity in child populations with fMRI. Compared to most commercial media, *Inscapes* is nonsocial, nonverbal, and utilizes abstract but nature-related shapes, images, and computer-generated animations without any scene-cuts or camera-based perspective changes (Vanderwal et al., 2018). Similar to eyes-open and eyes-closed rest, *Inscapes* provides a context to study the state of the brain without specific goal-directed cognitive input. Compared to eyes-open rest or commercial media, *Inscapes* yields decreased head movement, reduced fatigue, and increased compliance with adults and children (Vanderwal et al., 2015). Furthermore, neural patterns obtained using *Inscapes* more closely resembled those of eyes-open rest compared to a commercial movie (Vanderwal et al., 2015). In a related study, Finn and Bandetinni (2021) found that rsFC measured during naturalistic viewing with adults using fMRI exhibited more accurate predictions of cognitive processes compared to viewing a traditional fixation cross and is a reliable measure of individual differences in behaviorally relevant brain networks.

*Inscapes* has been used as a measure of non-evoked, intrinsic functional connectivity and is considered a reliable alternative to conventional rsFC measures in fMRI with adults (Kirsch et al., 2023), in adolescents diagnosed with ASD (Prillinger et al., 2021), and with children (Geng et al., 2019). *Inscapes* has been used to measure rsFC with other neuroimaging modalities such as magnetoencephalography (MEG; Vandewouw et al., 2021) and EEG (Espenhahn et al., 2020). Sato et al. (2021) employed *Inscapes* using fNIRS with 3- to 4-year-olds diagnosed with microcephaly caused by congenital Zika virus infection and reported the optimal fNIRS preparation and desensitization procedures for clinical populations with hypersensitivity and found *Inscapes* to be particularly suitable. However, this study lacked a healthy control group for comparison. While *Inscapes* is typically described as a naturalistic viewing paradigm that captures intrinsic, non-evoked functional connectivity, for the purposes of this paper—and in line with the definition of rsFC as neural activation in spatially distributed brain regions occurring in the absence of external tasks that elicit goal-directed behavior—we refer to *Inscapes* as a measure of rsFC for conceptual clarity and consistency with the literature, with the important caveat that rsFC remains a nuanced and evolving construct to measure.

While the prior results are encouraging, research investigating the feasibility and criterion validity of the *Inscapes* measure using fNIRS with typically developing children is quite scarce. Not only is such work helpful for comparisons with atypical development, but it can also provide unique insights into typical developmental trajectories. The aims of this cross-sectional study are to 1) investigate the robustness of the naturalistic viewing paradigm, *Inscapes* using fNIRS in young adults (construct validity), 2) report the feasibility and practicality of employing the aforementioned paradigm using fNIRS in young children, 3) examine the association between PFC rsFC and EF in both children and adult populations (criterion validity), and 4) identify age-related patterns in rsFC and EF using fNIRS with children aged 4–5 years and young adults aged 18–22 years.

## 2. Experiment 1 executive function and functional connectivity using fNIRS in a young adult sample

The goal of this within-subjects counterbalanced experiment was to conceptually replicate and investigate whether functional connectivity patterns observed during a naturalistic viewing paradigm resemble those obtained from a traditional rest measure (i.e., eyes-open fixation on a static crosshair). While this experiment aims for a conceptual replication of prior research, it also extends that research in novel directions. The advances of this replication from prior work are 1) the utilization of fNIRS; this can inform us about the utility of this tool for investigation of cognitive development 2) primarily focusing on functional connectivity in the PFC with healthy young adults; this allows us to assess the generalizability of prior results from different participant groups and 3) examining the association of PFC rsFC and behavioral performance on an EF task; while there is much evidence that the data derived from fMRI is indicative of cognitive outcomes, there is much less evidence to support this claim for fNIRS.

### 2.1. Materials and methods

Research aims and analyses were preregistered on the Open Science Framework, where the data reported in the study and the scripts used to analyze these data are available (Eng and Vargas, 2020): <https://doi.org/10.17605/OSF.IO/NXC2B>.

### 2.2. Participants

Thirty right-handed young adults ages 18–22 ( $M = 20.04$ ,  $SD = 1.13$  years; 22 Females, 8 Males) with no history of serious medical, neurological, or psychiatric illnesses were recruited through the Carnegie Mellon University Psychology Subject Pool in Pittsburgh, PA in the United States. Participants were 46.7 % Asian, 43.3 % White, and 10 % Black or African American. 16.7 % were Hispanic or Latino. The experimental protocol was approved by the Carnegie Mellon University Institutional Review Board (#IRB00000352). Signed consent was obtained from all participants, who were compensated with course credit for participation in the study. Participants were randomly assigned to one of two orders: Crosshair First ( $n = 15$ ) or *Inscapes* First ( $n = 15$ ). Resting-state scans were recorded for five minutes—the minimum time needed for rsFC correlation coefficient data from functional connectivity matrices to stabilize for accurate signal acquisition using fMRI (Whitlow et al., 2011). This duration is also sufficient for producing stable networks with fNIRS (Geng et al., 2017; Lester et al., 2024), particularly for achieving local efficiency stability (Wang et al., 2017). However, Wu et al. (2022) demonstrated that two-minute rsFC fNIRS scans were sufficient in differentiating children with and without ASD—a methodological adaptation accounting for motion constraints in clinical child populations. Consequently, the optimal fNIRS scan duration can vary depending on the research question (e.g., neuroatypical developing populations, local vs. global rsFC). A five-minute duration was chosen in this study to align with evidence from both fNIRS and fMRI studies, ensuring comparability across methodologies and a duration sufficient for producing stable and accurate signal acquisition with fNIRS for rsFC correlation coefficient data. Then, participants were administered a behavioral EF assessment, as described below. Resting-state and EF assessments were presented on a 13.3-inch diagonal Retina display MacBook Pro with a resolution of  $2560 \times 1600$ .

### 2.3. Adult behavioral executive function assessment

The Stroop Task (Stroop, 1935; MacLeod, 1991; Hirsh and Inzlicht, 2010) is one of the most commonly used assessments of EF for older populations. In the most common version of the task, participants see a word written in a color that either matches its meaning (congruent trial, e.g., the word 'green' written in green) or conflicts with its meaning



(incongruent trial, e.g., the word 'green' written in red). When asked to identify the font color, participants respond more slowly and make more errors when the color conflicts with the word's meaning than when it matches. This phenomenon is known as the 'Stroop Effect' or a 'Conflict Effect'. A smaller Conflict Effect is taken as a sign of more effective EF (Kane and Engle, 2003; Engle and Kane, 2004; Duell et al., 2018). Consistent with this claim, performance on the Stroop Task has been shown to predict educational outcomes and psychopathology, and the Conflict Effect is larger in many clinical populations (Laurenson et al., 2015; Khan et al., 2022).

In the present study, participants were instructed to press one of four colored buttons on a response box corresponding to the font color of the stimulus word (red, green, blue, or yellow). Each word appeared for 200 ms, with a maximum response window of 800 ms and an inter-trial interval of 1000 ms. A session of 24 practice trials (16 congruent, 8 incongruent) preceded five blocks of 48 trials each (32 congruent, 16 incongruent), for a total of 240 test trials. For both congruent and incongruent trials, accuracy was calculated as the percent of correct trials out of total trials, and reaction time (RT) was calculated as the mean response time across each trial type. Mean RT on the incongruent trials was preregistered as the primary dependent variable of interest.

#### 2.4. Functional connectivity measures using functional near-infrared spectroscopy

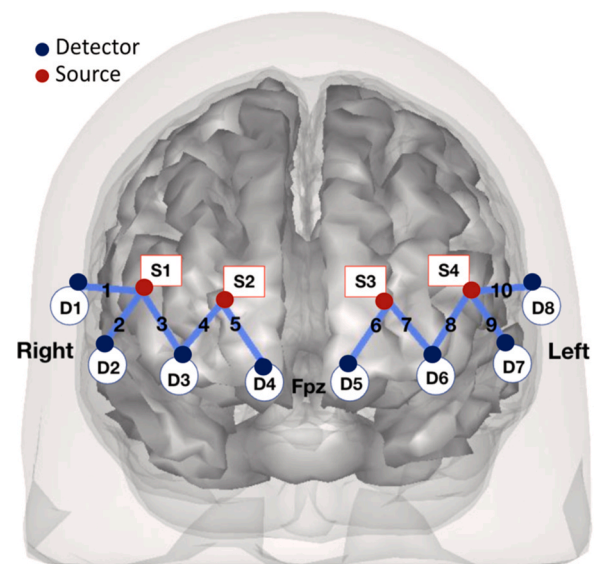
Two resting paradigms were presented to the young adult population. Each scan was administered for five minutes, also maintaining the experimenter, lighting, and computer screen attributes in the same testing room. Each participant experienced both a crosshair and an *Inscapes* resting scan. During **Crosshair** sessions, participants were instructed to relax and remain still while fixing their gaze on a central crosshair on the screen for the task's duration, to blink naturally, to avoid unnecessary movements, and were told the scan would last for five minutes. During *Inscapes* (Vanderwal et al., 2015) sessions, participants were shown the first five minutes of the paradigm and associated audio, instructed to relax and remain still while fixing their gaze at *Inscapes* on the screen for the duration of the task, to blink naturally, to avoid unnecessary movements, and were told the scan would last for five minutes. The only difference between the two conditions was the paradigm presented.

#### 2.5. Functional near-infrared spectroscopy data acquisition

Data were recorded using a TechEn CW6 (TechEn Inc., Massachusetts) continuous wave NIRS system with optical fiber sources and detectors at a sampling rate of 20 Hz for two wavelengths (690-nm and 830-nm laser light). The optode array consisted of ten channels from four sources and eight detectors across the PFC (see Fig. 1), for a total of 45 unique channel-pairs. Sources and detectors were arranged at an inter-optode distance of 30 mm, required for adequate cortex depth sensitivity. Optodes were positioned on a probe strip in reference to the 10–20 EEG international coordinate system covering areas Fp1–F8 on the PFC and secured with a neoprene thermal cap fitted to the head circumference of participants. In addition to the neoprene cap, the room lights were dimmed during the rsFC data acquisition scans to reduce signal contamination from ambient light. First, the quality of the raw signal for each channel was checked in real-time to ensure that fNIRS signals were not contaminated by external light sources or hair, exhibiting poor signal-to-noise ratio, or in out-of-range amplitude ( $< 1.5$  uV or  $10.0 < \text{uV}$ ) prior to starting data acquisition.

#### 2.6. Functional near infrared spectroscopy data preprocessing and analysis

The publicly available NIRS Brain AnalyzIR Toolbox (Santosa et al., 2018) in MATLAB (Mathworks, version 2023b) was utilized for data



**Fig. 1.** Anterior view of functional near-infrared spectroscopy montage and optode placement: four sources (S1–S4 represented by red circles) with 690 and 830 nanometer laser light and eight detectors (D1–D8 represented by dark blue circles). Light blue lines represent ten channels. Optodes were positioned in reference to the 10–20 EEG international coordinate system, covering the prefrontal cortex region with Fp1 referenced to Detector 5 and Fp2 referenced to Detector 4.

visualization, preprocessing, and analysis (Santosa et al., 2018). Raw fNIRS intensity signals were first converted to changes in optical density. Using the modified Beer-Lambert law, signals were transformed to oxygenated hemoglobin concentrations using a differential pathlength factor of six (Cope and Delpy, 1988). This study specifically focused on oxygenated hemoglobin (HbO) because changes in HbO have been shown to elicit a higher signal-to-noise ratio compared to the deoxygenated (HbR) signal (Strangman et al., 2002). The data were then corrected for motion artifacts using the Temporal Derivative Distribution Repair (TDDR) method, a motion correction procedure based on robust regression that effectively reduces the magnitude of large fluctuations (i.e., motion) in the signal, while leaving small fluctuations (i.e., hemodynamics) intact to prevent physiological artifacts from biasing the results (Fishburn et al., 2019). Most fNIRS preprocessing approaches account for noise by employing the general linear model (GLM). Simplified, the GLM utilizes predictors to describe the largest sources of variability within the fNIRS data, but fNIRS data violate core GLM assumptions in that any noise (e.g., motion artifacts and systematic physiological) is not serially correlated and independent (Yucel et al., 2021). To solve these GLM violations, an autoregressive iteratively-reweighted least squares approach was used to estimate the coefficients to account for serial correlations in the data (Barker et al., 2013; Huppert, 2016). The estimated coefficients were submitted to a robust weighted mixed effects model, with condition (Crosshair, Inscapes) modeled as a fixed effect and subject as a random effect. Using this iterative reweighting approach, it is possible to produce a set of weights which shrinks the excessively large fluctuations most commonly associated with head motion and is an optimal method to remove confounding noise in fNIRS data compared to other motion correcting techniques without the need for any user-supplied parameters (Fishburn et al., 2019).

#### 2.7. Data analytic approach

As per preregistered analyses, first, to measure whether adults exhibited similar patterns of rsFC between Crosshair and Inscapes paradigms, a representational similarity analysis (RSA) was computed

(Kriegeskorte et al., 2008) for Study 1. RSA is a modern multivariate approach for measuring the (dis)similarity of distributed activation across multiple regions of interest (ROIs) using fMRI. This study extends the application of this method by innovatively adapting the analysis to analyze fNIRS data. For each participant, functional connectivity matrices, consisting of interchannel correlations, were converted to distances ( $1-r$ ) within each condition. Functional connectivity distance matrices were then correlated between conditions for each participant separately. The resulting inter-condition correlation was statistically evaluated against a null distribution using a 10,000-iteration permutation test by randomizing the channel-pair labels. If the correlation coefficient falls on the tail of this distribution ( $p < 0.05$ ), then the rsFC during Crosshair and *Inscapes* has significantly similar patterns of relationships between channels.

Second, after subject-level rsFC correlation coefficients were extracted from all 45 channel-pairs, a Fisher's  $r$ -to- $Z$  transformation was then applied to normalize the variance of the correlation values—the standard for addressing the non-linear nature of correlation coefficients, particularly when averaging across correlation values. Z-scores were averaged for each participant, resulting in a single mean PFC rsFC value for Crosshair and *Inscapes*. We then conducted a correlation analysis to further investigate the naturalistic paradigm's construct validity and examine the association between mean PFC rsFC for *Inscapes* and mean PFC rsFC for the Crosshair. To investigate the construct validity of the naturalistic paradigm, we ran a correlation analysis to examine the associations between both resting-state paradigms and executive function

behavior (Stroop Task performance).

Alpha was set at .05 for all tests. 95 % confidence intervals (CIs) and Cohen's  $d$  for effect size estimates were reported for analyses. 95 % CIs for Pearson Correlation Coefficients were calculated utilizing the Pearson product-moment correlation coefficient observed within the sample and the number of paired observations of the sample.

### 3. Results

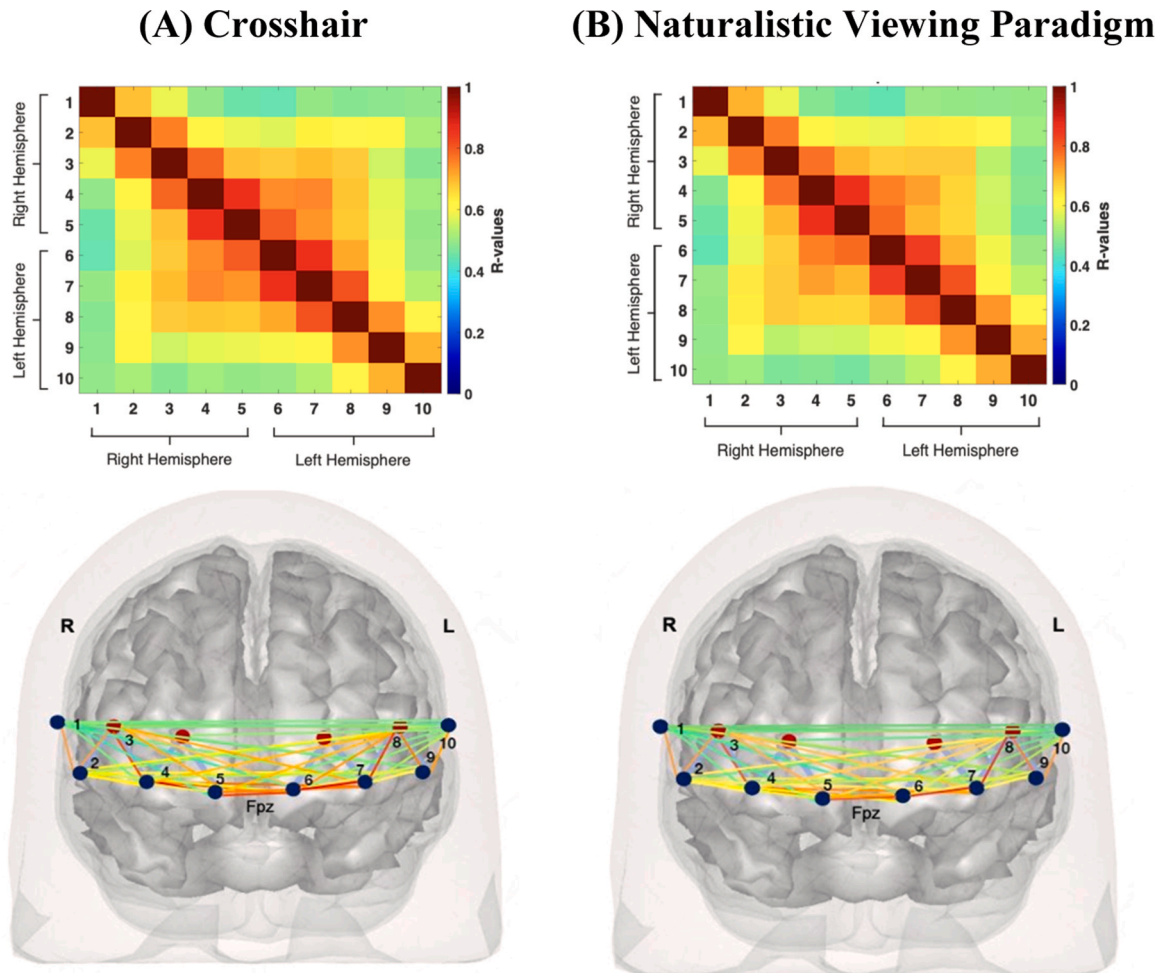
After preprocessing, rsFC was quantified by concatenating the individual block time courses, and the Pearson correlation coefficients were computed between all 45 channel-pairs for each resting-state condition (see Fig. 2).

#### 3.1. Association between functional connectivity of crosshair and *Inscapes*

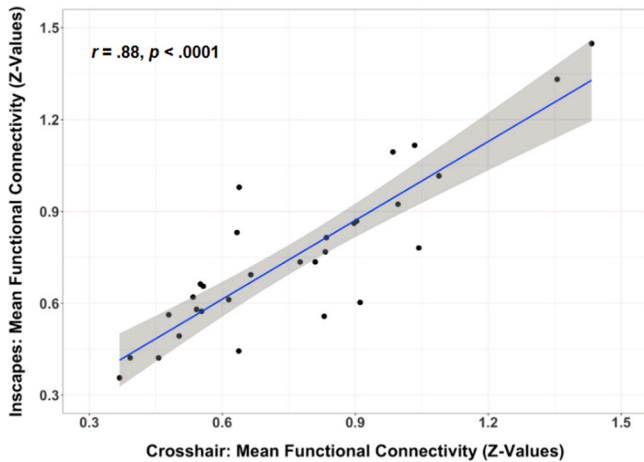
Correlation analysis showed a positive association between mean PFC rsFC for *Inscapes* and mean PFC rsFC for the traditional Crosshair,  $r = .88$ , 95 %CI [.75,.94],  $p < .0001$ , suggesting the naturalistic viewing paradigm exhibits high construct validity (see Fig. 3).

#### 3.2. Validation of naturalistic viewing paradigm within adult participants

The RSA and 10,000-iteration permutation test showed that the correlation between the Crosshair and *Inscapes* Conditions ranged from



**Fig. 2.** Adult Group-level Functional Connectivity of the Prefrontal Cortex for (A) Crosshair and (B) *Inscapes*. Pearson correlation coefficients were calculated for all 45 channel-pairs, resulting in a symmetrical functional connectivity matrix for each condition. The matrix was then mapped onto a 3D Brain Model to visualize connections between channel-pairs. Warmer colors denote greater connectivity strength.



**Fig. 3.** Scatterplot of the positive association between mean prefrontal cortex functional connectivity for young adults viewing a crosshair and the naturalistic viewing paradigm for the same scan duration. Subject-level correlation coefficients were extracted from all channel-pairs across the prefrontal cortex, normalized with Fisher's  $r$ -to- $Z$  transformations, and then averaged together to result in a single mean value for Crosshair and for *Inscapes*. Shaded regions represent the 95 % confidence interval of the prediction line.

$r = 0.64$  to  $r = 0.97$  across the 30 adult participants (all estimates were significant at  $p < 0.001$ ). Mean  $r$  statistics were computed by taking the mean of the Fisher's  $r$ -to- $z$  transformed coefficients and transforming the resulting mean  $z$  back to  $r$ , mean  $r_z = 0.90$  (Corey et al., 1998). These results indicate that the Crosshair and *Inscapes* paradigms evoked robustly similar functional connectivity patterns within adults.

### 3.3. Resting-state functional connectivity using fNIRS and executive function

**Manipulation Check.** Mean Stroop RT on congruent trials ( $M = 393.21$  ms,  $SD = 64.82$ ) was faster compared to mean RT on incongruent trials ( $M = 520.17$  ms,  $SD = 72.01$ ), paired-sample  $t(29) = 18.22$ ,  $SE = 6.97$ , 95 %CI [112.72, 141.21],  $p < .001$ , Cohen's  $d = 3.33$ . Mean accuracy on congruent trials ( $M = 93.93$  %,  $SD = 4.76$ ) was higher

compared to mean accuracy on incongruent trials ( $M = 78.29$  %,  $SD = 13.17$ ), paired-sample  $t(29) = 8.43$ ,  $SE = 1.85$ , 95 %CI [11.84, 19.43],  $p < .001$ , Cohen's  $d = 1.54$ . These findings reproduce the well-known Conflict Effect of more accurate and faster responses on congruent compared to incongruent trials.

EF performance–mean RT on the incongruent trials–was significantly associated with mean PFC rsFC for *Inscapes*,  $r = -.448$ , 95 %CI [-.11, -.70],  $p = .013$ , and with mean PFC rsFC for the traditional Crosshair,  $r = -.435$ , 95 %CI [-.09, -.69],  $p = .016$  (see Fig. 4). Consistent with prior literature, adults who exhibited better EF performance (faster RT on incongruent trials) tended to have higher rsFC in the PFC. Furthermore, overall faster RTs were not due to a speed-accuracy tradeoff. As participants' mean accuracy on incongruent trials increased, mean RT decreased ( $r = -.531$ , 95 %CI [-.21, -.75],  $p = .003$ ). This result indicates that faster responses during the Stroop Task did not lead to a decrease in accuracy.

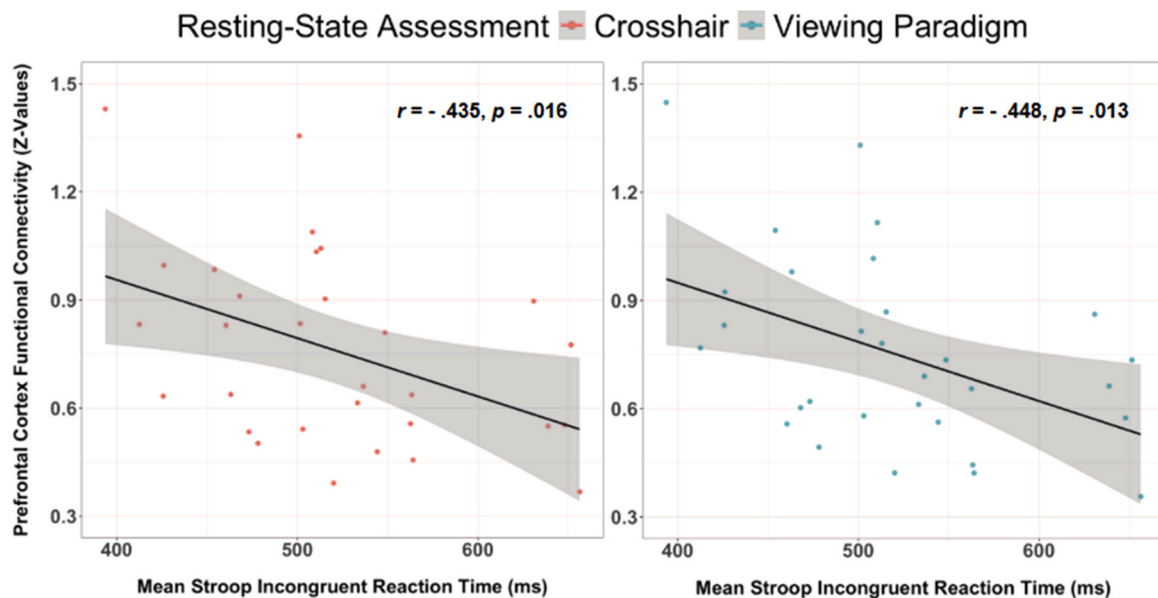
## 4. Experiment 1 discussion

Results from Experiment 1 indicate that the functional connectivity patterns in the PFC for the naturalistic viewing paradigm closely resemble the functional connectivity patterns for a traditional crosshair. The RSA and permutation testing revealed that participants exhibited robustly similar patterns of relationships among channels, comparing Crosshair and *Inscapes*. It was also found that mean PFC rsFC using fNIRS for both resting-state conditions was associated with behavioral EF performance. It is an open question of how PFC rsFC patterns using fNIRS compare from young adulthood to young childhood and if the association between PFC rsFC and EF generalizes across development. Experiment 2 addresses this question by employing the naturalistic viewing paradigm with children ages 4–5 years and examines the association between rsFC and a developmentally appropriate EF task.

## 5. Experiment 2

### 5.1. Executive function and functional connectivity using fNIRS with young children

The goal of this experiment was to investigate the feasibility and practicality of *Inscapes* as a rsFC measure with young children using



**Fig. 4.** Scatterplots of the association between executive function performance (faster response time) and higher mean prefrontal cortex functional connectivity for the static crosshair and the naturalistic viewing paradigm with adults. Shaded regions represent the 95 % confidence intervals of the prediction lines. Note: ms = Milliseconds.



fNIRS, the association between behavioral EF performance and PFC rsFC, and how rsFC patterns in the PFC change developmentally from young childhood to young adulthood. Research using fMRI has shown that the PFC undergoes notable changes in rsFC from childhood through adulthood, typically characterized by increased rsFC PFC strength and integration over development, reflecting the maturation of cognitive processes like EF (Fair et al., 2008, 2009; Menon, 2013). The intrinsic brain networks typically observed in adults—such as the default mode, salience, and executive control networks—undergo significant nonlinear maturation throughout childhood and adolescence (Sanders et al., 2023). Based on this literature, we hypothesized that mean rsFC in the PFC would be positively associated with EF in preschool-aged children and that adults would exhibit higher average PFC rsFC compared to children.

## 5.2. Methods and materials

Procedure, fNIRS technology, preprocessing, and data analytic approach were nearly identical to those described in Experiment 1 in Sections 2.4–2.7. The only difference was that children were not presented with a static Crosshair and were only exposed to the naturalistic viewing paradigm. Additionally, the children participated in a developmentally appropriate Stroop-like EF assessment, rather than the Stroop Task designed for adults. As in Experiment 1, children were fitted with caps based on head circumference, presented with *Inscapes* for five minutes using fNIRS, followed by administration of the behavioral EF task. Identical to the analysis with adults, we ran a correlation analysis to examine the association between mean PFC rsFC and executive function behavior (Day Night Task performance). Then, we report the mean PFC rsFC of the naturalistic paradigm for children and adults with side-by-side 3D Brain Visuals.

## 5.3. Participants

55 children between the ages of 4–5 ( $M = 4.83$ ,  $SD = 5$  months; 35 Males, 20 Females) were recruited from a pre-primary school in Pittsburgh, PA, in the United States. The school population reflects local racial and economic diversity, with children being 59 % White, 24 % Asian or Pacific Islander, 5 % Black or African American, and 12 % Middle-Eastern. 5 % were Hispanic or Latino. Additionally, 28 % of the children received scholarship financial aid. Participants of this study are part of a larger ongoing intervention study examining the short- and long-term effects of an EF training paradigm with preschool-aged children. The focus of the current study was the neurological and behavioral EF data acquired during the first baseline visit at 4–5 years of age. The experimental protocol was approved by the Institutional Review Board (#IRB00000018). Signed consent was obtained from the parents of participants, and verbal assent was secured from all children. Participants were given stickers at the conclusion of the study session.

## 5.4. Early childhood stroop-like executive function assessment

The Stroop Task used in Study 1, designed for adults, has limited external validity in preschool children, as many of them lack the reading proficiency required for the task. One widely utilized variation of the Stroop Task adapted for use with children is the Day-Night Task (Gerstadt et al., 1994; Montgomery and Koeltzow, 2010). In this task, children are first presented images of the sun and moon and asked to respond with “day” and “night,” respectively (congruent trials), then they are asked to respond instead with “night” when shown an image of the sun and “day” when shown the image of the moon (incongruent trials). The Day-Night Task (Gerstadt et al., 1994) mirrors Stroop by requiring children to maintain current task goals, follow rules, switch response sets, and inhibit practiced responses—fundamental yet burgeoning EF skills. To verify that the Day-Night Task instructions were understood during the study, the children completed two practice trials in the congruent condition, followed by 16 congruent test trials.

Similarly, the children completed two practice trials in the incongruent condition, followed by 16 incongruent test trials. For each condition, practice trials were repeated until the child demonstrated sufficient understanding of task demands as ascertained by the examiner. During the practice trials in each condition, children were encouraged to respond as quickly and accurately as possible, but no encouragement or correction was provided during the 16 test trials. Mean accuracy on the incongruent trials was preregistered as the primary dependent variable of interest, calculated as a percentage of total correct responses on incongruent trials out of the 16 total incongruent trials.

## 6. Results

Alpha was set at .05 for all tests. 95 % CIs and Cohen's  $d$  for effect size estimates were reported for analyses.

### 6.1. Practicality and feasibility of *Inscapes*

Out of 55 children, neuroimaging data were not collected for two children because they opted out of wearing the cap, resulting in low attrition (3.63 %) and high compliance (96.36 %) rates.

### 6.2. Developmental patterns of prefrontal cortex functional connectivity

After subject-level rsFC correlation coefficients were extracted from all 45 channel-pairs, a Fisher's  $r$ -to- $Z$  transformation was then applied to normalize the variance of the correlation values.  $Z$ -scores were averaged for each participant, resulting in a single mean PFC rsFC value for the naturalistic viewing paradigm. Children exhibited mean rsFC values of 0.57 ( $SD = 0.16$ ; Range = 0.18–0.87) during *Inscapes* (Fig. 5A), compared to young adults who exhibited mean rsFC values of 0.75 ( $SD = 0.26$ ; Range = 0.36–1.45) during *Inscapes* (see Fig. 5B).

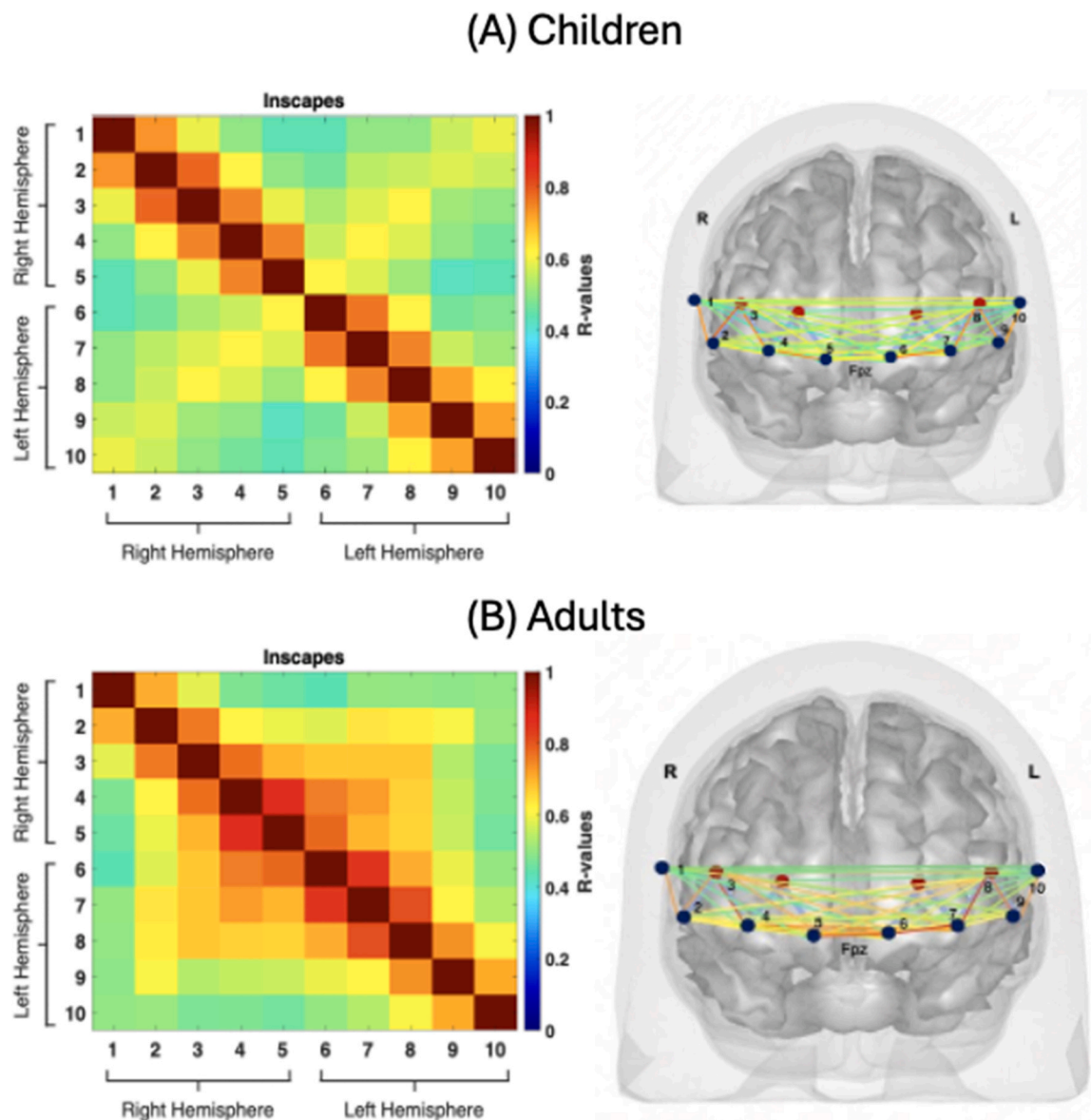
### 6.3. Association between resting-state functional connectivity and executive function

**Manipulation Check.** Mirroring the ‘Conflict Effect’ with adults, children's mean RT was significantly slower for incongruent trials ( $M=2065.09$   $SD=466.09$  ms) compared to congruent trials ( $M=1813.27$   $SD=610.29$  ms), paired-sample  $t(54) = 4.38$ ,  $p < .0001$ ; Cohen's  $d = 0.59$ , 95 %CI [0.30, 0.88]. Children's mean accuracy was significantly higher for Congruent trials ( $M=90.80$  %,  $SD=7.96$  %) compared to Incongruent trials ( $M=59.32$  %,  $SD=17.63$  %), paired-sample  $t(54) = 14.12$ ,  $p < .0001$ ; Cohen's  $d = 1.90$ , 95 %CI [1.46, 2.35]. The mean accuracy values are consistent with prior studies with 4- to 5-year-old children and reproduce findings of children exhibiting higher accuracy when responding with well-established associations between words and images on the congruent trials, compared to incongruent trials in which children must override a prepotent response and substitute a conflicting one (Baker et al., 2010; Gerstadt et al., 1994).

EF performance—mean accuracy on the incongruent trials—was significantly associated with mean PFC rsFC,  $r = .698$ , 95 %CI [.527, .814],  $p < .001$  (see Fig. 6). These results mirror the findings with young adults in that higher EF is associated with increased functional connectivity in the PFC at rest.

## 7. Study 2 discussion

Results from Study 2 indicate that individual differences in rsFC in PFC—as measured via the *Inscapes* paradigm—are associated with individual differences in EF. Notably, this result was obtained in a developmental population using different EF measures than the Stroop Task employed in Study 1. This suggests that the measurement of rsFC as an index of EF ability is robust to variations in cognitive testing paradigms and age groups. Using fNIRS proved highly feasible and practical with



**Fig. 5.** Group-level Functional Connectivity of the Prefrontal Cortex for *Inscapes* for (A) Children and (B) Adults. Pearson correlation coefficients were calculated for all 45 channel-pairs, resulting in a symmetrical functional connectivity matrix. The matrix was then mapped onto a 3D Brain Model to visualize connections between channel-pairs. Warmer colors denote greater connectivity strength.

preschool-aged children, demonstrating a low attrition rate of 3.63 % and high compliance at 96.36 %. Children also exhibited less PFC functional connectivity strength overall than young adults, consistent with prior results derived from fMRI (Fair et al., 2008, 2009; Menon, 2013).

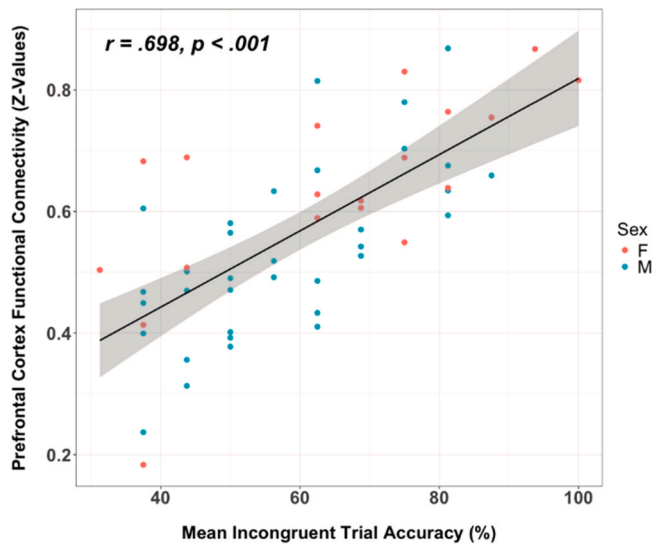
## 8. General discussion

In recent years, rsFC has become an important tool in the arsenal of developmental neuroscientists. One reason for the excitement over this tool is that the “task-free” nature of procedures that collect rsFC data also offers tremendous potential for developmental researchers to compare neural patterns across age groups. This potential has not yet been fully realized, partly due to the limitations of rsFC neuroimaging methods using fMRI, which are not easily applied to young children. Additionally, rsFC requires a minimum of five minutes with the participant either keeping their eyes open, closed, or fixating on a static crosshair, which is so difficult for young children that trying to assess resting state connectivity in a comparable task between children and

adults has proven, in many cases, to be an intractable challenge. Our goal in this study was first to validate the robustness of a naturalistic viewing paradigm as a rsFC methodological approach in developmental research, and to investigate the association between PFC rsFC and EF using fNIRS.

With respect to the first goal, we found that in healthy young adults, rsFC patterns in the PFC were robustly similar whether measured while participants gazed at a crosshair (the typical paradigm used with adults in fMRI) or watched the naturalistic paradigm. As *Inscapes* affords more practicality in use with children, we believe it is promising that the naturalistic viewing paradigm yielded similar rsFC to more traditional resting-state methodologies. Research using fMRI has shown the PFC undergoing notable changes in rsFC from childhood through adulthood, typically characterized by increased PFC rsFC strength and integration over development (Fair et al., 2008, 2009; Menon, 2013), and reflecting the maturation of cognitive processes, such as EF. The developmental differences we found in PFC rsFC using fNIRS are mainly consistent with results from the fMRI literature, further reinforcing the possibility that the rsFC measured here is tapping into the same underlying brain





**Fig. 6.** Scatterplot of the association between executive function performance (percentage of correct responses on the incongruent Day Night Task trials) and mean prefrontal cortex functional connectivity for the naturalistic viewing paradigm with children. Data points are colored by sex. Shaded region represents the 95 % confidence intervals of the prediction lines.

networks as fMRI measures.

Furthermore, we found that rsFC measured by fNIRS viewing a naturalistic paradigm is an informative measure of individual differences in both adults and children. In adults, the correlation with EF and rsFC is equally strong whether rsFC is measured with a traditional resting state paradigm or with *Inscapes*, suggesting convergent validity between these measures. We did not administer the traditional crosshair method of measuring rsFC with children because in piloting this procedure with preschoolers we observed lack of compliance (i.e., children repeatedly looked at the experimenter and asked when they would be done, despite reminders to look at the screen) and fidgeting behaviors (e.g., pressing computer buttons or kicking legs). Although we did not use the traditional measure with children, the significant correlations between rsFC measured using *Inscapes* and EF assessments suggest that the rsFC patterns elicited by this paradigm are associated with EF processes in both children and adults, while also proving to be a feasible and practical approach. Our findings are consistent with the general finding that rsFC in the PFC is associated with individual differences in EF and demonstrate that this association can be observed with fNIRS. These results align with findings from rsFC measured during naturalistic viewing in adults using fMRI, which show accurate predictions of cognitive processes and serve as a reliable measure of individual differences in behaviorally relevant brain networks (Finn and Bandettini, 2021). Altogether, these results indicate that fNIRS may be a promising avenue to investigate the neurocognitive development of EF and potentially other skills developing in early and middle childhood and other populations for whom fMRI poses obstacles.

It is important to acknowledge several limitations of the present findings. The sample in the present study is small and non-representative of the U.S. population. Additionally, there are inherent limitations to the fNIRS technology in addition to the limitations of our sample. First, fNIRS is limited to imaging the first few millimeters of the cerebral tissue and cannot capture the spatial resolution into subcortical areas that fMRI affords.

Second, while the RSA yielded robust results in demonstrating near-identical rsFC patterns between the traditional crosshair and the naturalistic viewing paradigm, these findings specifically reflect our targeted investigation of rsFC within the PFC, which is particularly relevant for examining associations with EF. While these initial results with adults support the hypothesis that PFC rsFC yields similar patterns for a

traditional crosshair and *Inscapes* paradigm, we cannot make claims about rsFC within and between occipital, parietal, and temporal regions. Future fNIRS research with full head coverage or simultaneous fNIRS-fMRI studies are needed to make claims about rsFC networks using this naturalistic viewing paradigm. A less costly and promising future direction to address the spatial specificity limitation of this study is the inclusion of a 3D Digitizer, which permits precise measurement of the head surface points in real-world coordinates and enables projection of fNIRS channels onto the cortical surface using MNI coordinates and an automatic anatomical labeling method commonly used in fMRI methodology (Singh et al., 2005). This approach could delineate more specific regions encompassed by the fNIRS channels and facilitate translation from fNIRS to methods capable of distinguishing between different PFC subregions.

Another limitation of this study is that we did not use short-separation channels (inter-optode distance of ~8 mm) to account for blood flow in the extracerebral tissue layer, which can remove noise from the signal (Santosa et al., 2020). However, younger populations are less susceptible to interference from extracerebral tissues due to smaller scalps and thinner skulls (Yücel et al., 2021). Additionally, the robust weighted mixed-effects autoregressive model with TDDR motion correction used for fNIRS signal processing effectively reduces the influence of extracerebral tissues even when short-separation channels are unavailable (Santosa et al., 2020). Furthermore, physiological noise (e.g., cardiac, respiratory, and blood pressure fluctuations) that could contaminate the signal was minimized compared to task-evoked paradigms involving body movement because participants were at rest. Future work could also benefit from incorporating additional behavioral monitoring tools (e.g., eye-tracking, facial affect coding, mobile sensors) to capture subtle movements or behaviors during naturalistic viewing paradigms. This would provide an empirical basis to test how these factors may influence the fNIRS results.

Due to the cross-sectional nature of our research design, we cannot infer the causal direction of the observed relationship between rsFC and EF abilities. While replicating these findings in different age groups is essential for generalizability, it remains insufficient to establish causality. To address whether rsFC predicts EF, or vice versa, future studies should adopt longitudinal designs that assess changes in rsFC and EF at multiple time points. This approach would allow for a more nuanced analysis of temporal dynamics and directional relationships, providing critical insights into the developmental interplay between these constructs. Although our sample was underpowered for this analysis, given the developmental significance of sex-based differences in cognitive processes and functional connectivity (Fenske et al., 2023; Solé-Padullés et al., 2016), future studies should explore these effects using appropriately powered designs.

Despite these limitations, the current results help open exciting avenues for future research. As discussed previously, the measurement of EF is fundamental during periods of rapid development, and fNIRS can provide us with new insight about the neural changes that occur over developmental time. Further, because fNIRS is more easily accessible than fMRI for various populations and reasons, novel translational neuroscience applications may be possible. For example, several rsFC studies have shown atypical connectivity patterns as potential biomarkers of psychiatric disorders using fMRI (Greicius et al., 2007). Due to the expense associated with fMRI, it is not currently feasible to scan for this biomarker widely, especially in young children, for whom intervention might be most impactful. It is also the case that the use of *Inscapes* makes resting-state procedures more tolerable for children; this may especially be the case for children familiar with screens and coming from environments that provide lots of screen time, potentially since infancy. Although screen time is prevalent across developmental stages in the modern digital era, the extent to which this finding generalizes across contexts and populations remains unclear. Nevertheless, we think it is possible that the relative ease of use, low cost, and minimal task demands of *Inscapes* and fNIRS may make rsFC methodological

approaches accessible to a broader array of clinical practitioners and developmental neuroscience researchers. We believe that the current results represent an important step, but only one of many steps necessary, to validate the use of fNIRS as a versatile neuroimaging modality appropriate for use with a wide range of developmental and clinical populations.

## CRediT authorship contribution statement

**Fisher Anna V.:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Thiessen Erik D.:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition. **Eng Cassandra M.:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Vargas Roberto J.:** Writing – review & editing, Validation, Formal analysis, Data curation. **Fung Howard:** Writing – review & editing, Writing – original draft. **Niemi Selenia:** Writing – review & editing, Writing – original draft. **Melissa Pocsai:** Project administration, Investigation.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

All code and data will be made publicly available in the preregistered link for this study in the open science framework.

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