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Brain plasticity and adolescent HIV: A randomised controlled trial protocol investigating behavioural and hemodynamic responses in attention cognitive rehabilitation therapy

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ABSTRACT

Despite advances in antiretroviral pharmacology, neuroHIV in the central nervous system (CNS), causes neuronal dysregulation, which is associated with compromised neurocognition. Non-pharmaceutical interventions such as HIV cognitive rehabilitation training (HIV-CRT), have shown potential to partially reverse cognitive deficits, sequent HIV neuroinvasion. Nonetheless, no studies exist pairing cognitive outcomes with objective neuroimaging biomarkers in adolescent HIV-CRT. This longitudinal pre-post-quasi-experimental protocol examined cognitive outcomes, paired with optimal neuroimaging outcomes following customised attention training in adolescent HIV. Twenty-six adolescents living with HIV were randomly assigned to either the treatment group, which received attention CRT using ACTIVATE^{TM}, (n = 13), or to the treatment as usual group (n = 13). Cognitive outcomes were examined using the NEPSY-II, and BRIEF; whilst neuroimaging outcomes were determined by changes in oxygenated haemoglobin (HbO), as determined by functional near-infrared spectrometry (fNIRS). Functional connectivity fNIRS measures were evaluated using seed-based correlation analysis, located in the central executive network (CEN). This study serves to guide the development and identification of objective biomarkers for adolescent neuroHIV, sequent CRT amongst children living with HIV in Sub-Saharan Africa

Specifications table

Subject area	
More specific subject area:	Longitudinal changes in HIV neurocognition within an adolescent neuroHIV population
Name of your protocol:	HIV neuroplasticity: A protocol for a longitudinal follow-up cohort investigating brain plasticity and HIV neurocognition in South Africa
Reagents/tools:	A Developmental Neuropsychological Assessment, Second Edition (NEPSY-II) Behavioural Rating Inventory for Executive Functions (BRIEF) Functional Near-Infrared Spectrometry (NIRxSport2, NIRx Medical Technologies, LLC, Berlin, Germany)
Experimental design:	The study took the form of a longitudinal pre-and-post-quasi-experimental design. It was inclusive of two groups, an experimental and treatment as usual control group. Outcome measures included neuroimaging and behavioural measures to investigate the effect of an intervention.

(*continued on next page*)

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Subject area	
Trial registration:	Not Applicable
Ethics:	This study has received ethical approval from the Ethics Committee of the University of the Witwatersrand, South Africa [M211073]. Written assent and consent were obtained from all participants prior to the study.
Value of the Protocol:	• HIV crosses the blood brain barriers leading to neurocognitive deficits in children and adolescents. To this end, antiretroviral drugs have limited permeability in the central nervous system and are associated with neurotoxicity. • The current study investigated the efficacy of non-pharmaceutical interventions, namely HIV cognitive rehabilitation therapy, (HIV-CRT) to ameliorate attention function in adolescent neuroHIV. • The study took the joint investigation of behavioural and neuroimaging outcomes, sequent brain training, an approach yet to be pursued in the cognitive rehabilitation of children living with HIV in Sub-Saharan African populations.

Background

The Human Immunodeficiency Virus (HIV) has a detrimental effect on the body's immune and central nervous system (CNS). Markedly, with reference to the CNS, once HIV infiltrates the CNS, it is indicated to permeate the blood-brain barrier (BBB), and enter the cerebral cortex, where it leads to the differentiation of monocytes into macrophages, promptly resulting in pathogenetic neuroinflammation, [1] which is associated with aberrant neuronal transmission [2], white matter loss [3], neuronal apoptosis [4], and catecholaminergic dysregulation [5]. Collectively, the cognitive and motors deficits associated with HIV neuroinvasion are referred to as HIV associated neurocognitive disorder (HAND), which is observed in both adult [6] and adolescent [7] populations. Concerning adolescents, the focus of the current protocol, cognitive fallouts are characterized by deficits in working memory [8], attention [9], executive functions [10], and response inhibition [11].

Despite the development of highly active antiretroviral drugs (ARVs), HAND persists, namely due to the limited permeability of ARVs once in the CNS [12] and their associated neurotoxicity [13]. Given these limitations, there has been an urgent 'call to action' [14], for experimental studies, investigating the efficacy of non-pharmaceutical interventions, such as HIV cognitive rehabilitation (CRT)[1] to remediate cognition, sequent HIV neuroinvasion. With reference to adolescent HIV, there has been a steady rise of experimental studies, to remediate attention [15], working memory [8], and executive functions [16]. Notwithstanding these, no studies have paired behavioural outcomes sequent HIV-CRT, with objectives biomarker data, such as neuroimaging techniques, to investigate brain plasticity in adolescent neuroHIV, a major limitation of the current HIV-CRT literature [17,18].

Due to the exorbitant costs associated with technologies such as fMRI and transcranial Doppler (TCD), [19,20], there is a need for experimental studies, describing and implementing cheaper, portable technologies investigating brain outcomes in Sub-Saharan Africa contexts [21]. With reference to neuroHIV, given the dearth of studies pairing behavioural and objective neuroimaging biomarkers, to investigate HIV-CRT, the present protocol details how functional near- infrared spectrometry (fNIRS) technology may be paired with behavioural measures from the NEPSY-II, to investigate attention training, in the context of adolescent HIV. The protocol further presents details of how seed-based correlation analysis can be pursued to investigate functional connectivity in this domain of study. The protocol, specifically details the use of fNIRS, specifically when paired to investigate oxygenated hemodynamic responses, in the central executive network (CEN), a critical cortical network, implicated in neuroHIV [22,23].

Research questions

(1) Compared to controls, do HIV+ participants receiving attention brain training indicate improved cognitive outcomes on behavioural measures, at post intervention?
(2) Compared to controls, do HIV+ participants receiving attention brain training indicate greater functional connectivity in the CEN, post intervention, as indicated by seed-based functional connectivity analysis?

Description of protocol

Methods details

Ethical approval

The study was conducted in line with recommendations from the Declaration of Helsinki and received ethical approval from the Ethics Committee of the University of the Witwatersrand, South Africa [M211073].

Study sample and participant recruitment

Purposive sampling was used to recruit children living with HIV, residing at three shelters,[2] caring for orphaned and abandoned children in South Africa. At study conception, G power analysis estimated using Fraser and Cockcroft [8] ($n = 63$), indicated that to

[1] The terms cognitive rehabilitation therapy and brain training are used interchangeably in the literature, and in the protocol.
[2] The shelters (or Homes) were located in 'townships' located in Johannesburg and Makhanda, South Africa. Townships refer to dwellings previously designated for non-white residents during the apartheid era.

provide 80 % power, and detect a medium to high effect size ($d = 0.649$) at alpha 0.05, using independent samples analysis, a projected sample size of $n = 42$ was required (Experimental = 26; Control = 26) (GPower 3.1: Faul et al., [24]). Forty-three participants were initially recruited for the study (Experimental, $n = 22$; Control, $n = 21$), with 15 participants in the experimental and 15 participants in the control group, completing all pre-assessments. Sequent baseline assessment: one participant in the experimental group withdrew citing a lack of interest. Six participants in the control group dropped out, citing clashes of the study time with their school timetable ($n = 2$), a lack of interest ($n = 2$), no longer residing at the care shelter ($n = 1$), or illness ($n = 1$). At post brain-training and after all post-training assessments were obtained, data was deleted from a further three participants for the final analysis. Data deletions occurred either, due to low fNIRS signal data ($n = 1$), or corrupted imaging data due to internet loss ($n = 2$).

The final study sample consisted of 26 participants, 13 HIV+ participants in the brain training group and 13 HIV+ participants in the active control group. Participants constituted indigenous Africans, coloured and white participants, aged between 14 and 18 years of age ($M = 17.28$, $SD = 1.94$). All participants were on a course of cART and were either attending primary or secondary schooling at the time of the study. Participants were excluded if they presented with (a) TBI, (b) CNS-related ailments (e.g., cerebral palsy, meningitis), or (c) learning difficulties. Written informed consent was obtained from the Directors of the shelters and, where possible, from guardians of the children. Assent was obtained from all participants aged 14 and older. Once assent and consent was determined, participants were randomly assigned to either the experimental or control group using the Research Randomizer Software [25].

Study protocol

Experimental design

The study took the form of a longitudinal pre-and-post-quasi-experimental design. This design enabled us to collect behavioural and fNIRS neuroimaging data, at pre- and post-treatment to allow for the investigation of the HIV-CRT, on outcomes measures, detailed below.

Behavioural outcomes pre and post attention training

Demographic questionnaire assessed participants' age, sex, level of education, general medical history, and medication.

A Developmental Neuropsychological Assessment: Second Edition (NEPSY-II) [26], was administered to determine, near and far transfer gains, emanating from the attention training. The NEPSY is a standardized neuropsychological battery developed for children (3–16 years) that assesses cognitive function across six domains, namely, executive function, and attention, memory and learning, language, visuospatial processing, and social perception [26]. The study used selected subtests from the NEPSY-II, as detailed in Table S1.

Behaviour Rating Inventory for Executive Function (BRIEF): The school-age version of the BRIEF (6–18 years; 86 items) [27], was administered to evaluate, behavioural and regulation outcomes emanating from the brain training. The behavioural regulation index (BRI), and metacognitive index (MI) were administered, respectively. Higher scores on the BRI and MI indicate behavioural and executive challenges.

Imaging outcomes pre and post brain training

Neuroimaging data was collected using fNIRS optical neuroimaging techniques. We specifically designed an fNIRS-Stroop Colour Word Test (SCWT), adapted from Schroeter et al. (2002). Participants completed a computerised version of the SCWT at pre - and post-assessment. The SCWT was built using PsychoPy [28]. Before completing the computerised version of the SCWT, participants completed a pencil and paper version of the SCWT and received feedback on their performance (Supplementary Material Table S2). The SCWT took the form of an fNIRS block design as opposed to the event related design. The former has been indicated to show stronger statistical power and elucidate greater hemodynamic responses.

In the classical SCWT, a colour word, such as blue, is written in an ink colour, which may or may not be the same as the colour word. First, the participant must name the colour of the word while ignoring the actual word. Then, the participant must read the word and ignore the colour [29]. The Stroop interference effect occurs when reading the word interferes with naming the colour (incongruent condition). Generally, the interference effect requires greater attentional capacity. Responses on the incongruent task have been associated with slower responses, less accuracy, and greater cortical activation in the central executive network [30,31].

For the SCWT, participants answered the following question: "*Does the colour ink of the top word match the meaning of the bottom word*?". As indicated in Fig. 1, two conditions were implemented to answer this question, namely, Condition 1, which was a Congruent Block (the colour of the top word was the same as the meaning of the bottom word), and Condition 2, which was an Incongruent Block (colour of the top word differed from the meaning of the bottom word).

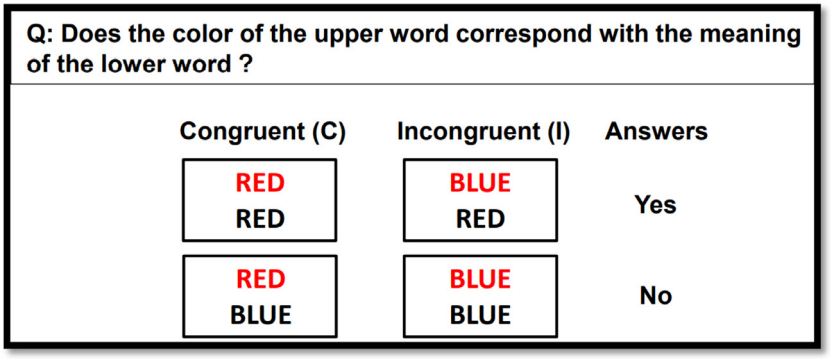


Fig. 1. The Stroop Colour Word Test (SCWT) was adapted from Schroeter et al. (2002).

As indicated in Fig. 2, blocks (Congruent, Incongruent) were randomly assigned, with each block presented for 10 s, interspaced with 15 s of rest, where participants had to stare at a '+' sign before responding to the block condition. Each block condition was presented five times for a maximum of ten blocks, during which participants were required to press *q* on the computer keyboard in response to congruent stimuli and *p* in response to incongruent stimuli. All event markers (triggers) were programmed on PsychoPy and sent to the Aurora Acquisition Software (NIRx, Medical Technologies, LLC, Berlin, Germany), via lab streaming layer (LSL).

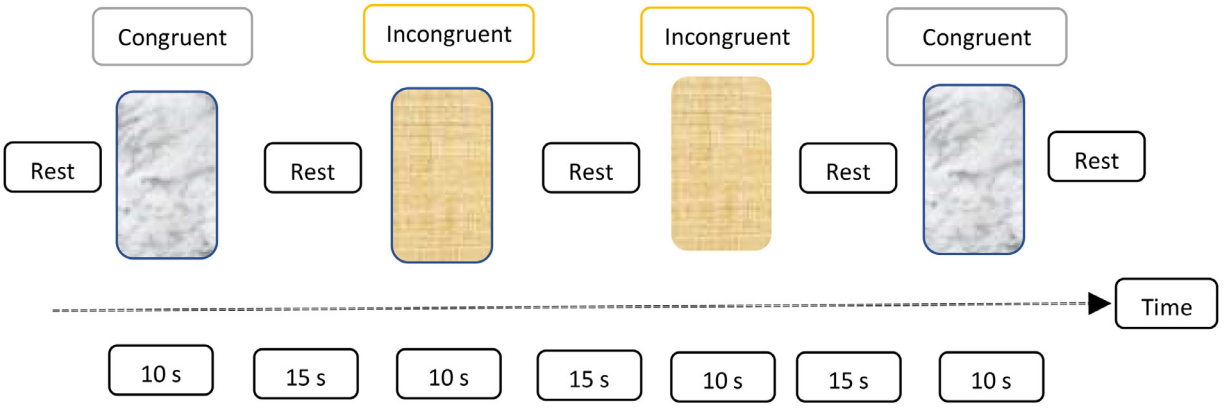


Fig. 2. The SCWT Block Design. In total, 10 blocks (five congruent and five incongruent) lasting ten seconds each were interspaced with 15 s of rest.

Functional near-infrared spectrometry

Data acquisition and montage

We measured cerebral activity based on concentration changes in oxygenated (HbO) and deoxygenated haemoglobin (Hb). Data were collected using the NIRxSport2 (NIRx, Medical Technologies, LLC, Berlin, Germany), a portable continuous wave fNIRS device, while participants completed the SCWT. As indicated in Fig. 3, we used eight LED emitters (sources), paired with seven photodiode detectors, covering the prefrontal cortex. The optodes were placed according to the 10–20 system [32], using a standardized prefrontal fNIRS Headband (EasyCap, NIRx, Medical Technologies, LLC, Berlin, Germany).

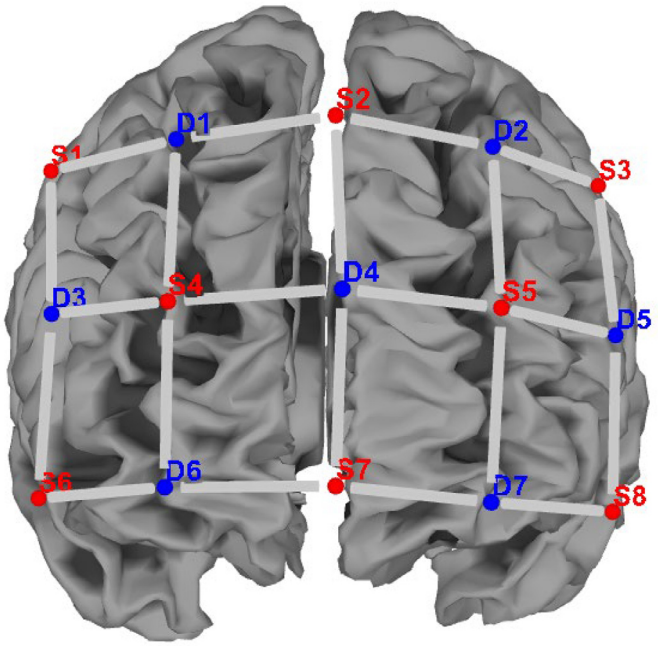


Fig. 3. Optode placement montage. Regions of interest were concentrated on regions in the CEN. Optode placements followed the 10–20 system. Red = sources. Blue = detectors.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Probe placement (sources and detectors) to identify the most sensitive placement for each optode location was determined using the 'fNIRS Optodes Location Decider' (fOLD) software [33] (Fig. 4). These locations were paired with relevant Montreal Neurological

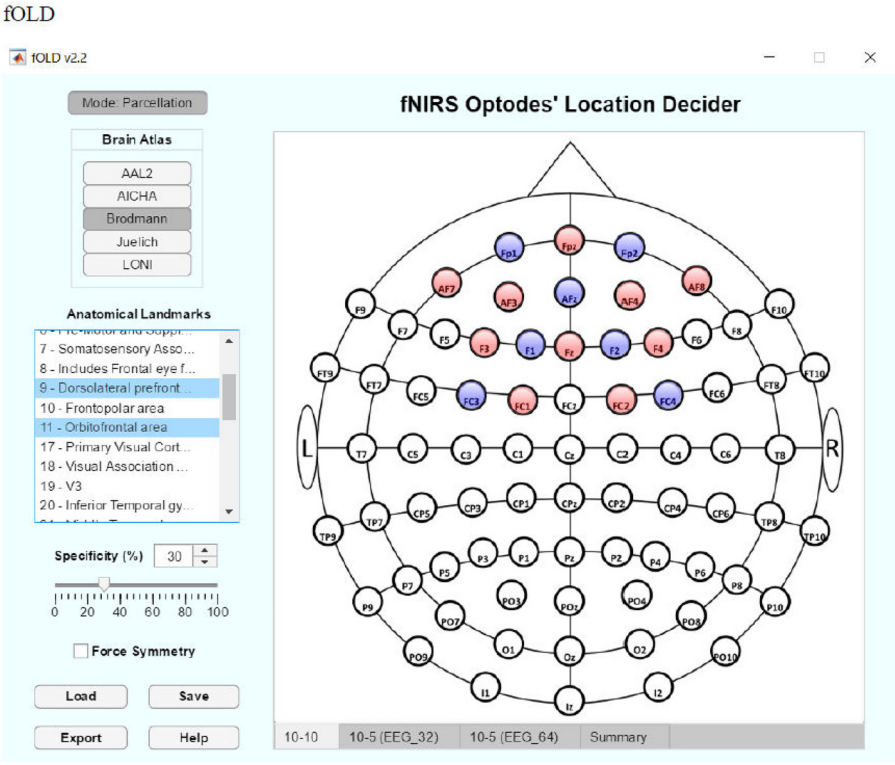


Fig. 4. Screen shot of the fOLD software. The software enabled the location for optode placement for regions within the central executive network (DLPF, Orbitofrontal).

Table 1
fNIRS Optodes and corresponding brain regions.

Channel	Optode name	MNI Position			BA	Anatomical Location (Specificity %)
		x	*y*	*z*		
CH 1	S1-D1	30	40	41	9	Right dorsolateral prefrontal cortex (69)
					46	Right dorsolateral prefrontal cortex (22)
CH 2	S1-D3	46	38	24	45	Right pars triangularis Broca's Area (71)
					46	Right dorsolateral prefrontal cortex (24)
CH 3	S2-D1	10	41	50	9	Right dorsolateral prefrontal cortex (68)
					8	Right includes frontal eye fields (29)
CH 4	S2-D2	−9	41	50	9	Left dorsolateral prefrontal cortex (63)
					8	Left includes frontal eye fields (35)
CH 5	S2-D4	2	50	39	9	Medial dorsolateral prefrontal cortex (62)
					10	Medial Frontopolar Area (20)
CH 6	S3-D2	−31	39	41	9	Left dorsolateral prefrontal cortex (67)
					46	Left dorsolateral prefrontal cortex (25)
CH 7	S3-D5	−46	39	26	45	Left pars triangularis Broca's Area (73)
					46	Left dorsolateral prefrontal cortex (22)
CH 8	S4-D3	40	50	16	46	Right dorsolateral prefrontal cortex (46)
					45	Right pars triangularis Broca's Area (30)
					10	Right frontopolar area (19)
CH 9	S4-D4	13	61	24	10	Right frontopolar area (72)
					11	Right orbitofrontal area 17)
CH 10	S4-D6	22	52	33	9	Right dorsolateral prefrontal cortex (50)
					46	Right dorsolateral prefrontal cortex (26)
CH 11	S6-D5	−39	50	17	46	Left dorsolateral prefrontal cortex (48)
					45	Left pars triangularis Broca's area (32)
CH 12	S5-D4	−12	62	23	10	Left frontopolar area (76)
					9	Left dorsolateral prefrontal cortex (15)
CH 13	S5-D7	−24	63	9	10	Left frontopolar Area (70)
					11	Left orbitofrontal area (20)
					46	Left dorsolateral (9)
CH 14	S6-D3	48	46	5	45	Right pars triangularis Broca's Area (44)
					46	Right dorsolateral prefrontal cortex (43)
CH 15	S6-D6	25	63	9	10	Right frontopolar area (69)
					11	Right orbitofrontal area (22)
CH 16	S7-D4	1	64	14	10	Medial frontopolar area (88)
CH 17	S7-D6	13	67	0	10	Right frontopolar area (52)
					11	Right orbitofrontal area (45)
CH 18	S7-D7	−12	67	0	10	Left Frontopolar area (53)
					11	Left orbitofrontal area (45)
CH 19	S8-D5	−47	46	6	45	Left pars triangularis Broca's area (48)
					46	Left dorsolateral prefrontal cortex (43)
CH 20	S8-D7	−23	62	23	9	Left dorsolateral prefrontal cortex (47)
					46	Left dorsolateral prefrontal cortex (32)
					10	Left Frontopolar Area (17)

Institute (MNI), coordinates (Table 1). Please note that the optode placement, and reporting of MNI coordinates used in the current protocol differ from our feasibility study [34]. Importantly, placement of sources and detectors corresponded with cortical regions implicated in attention, and working memory within the central executive network (CEN), inclusive of the frontopolar, orbitofrontal, and dorsolateral prefrontal cortices [35–37]. In total, signals were captured from twenty-two channels covering the prefrontal cortex. The distance between sources and detectors was set at 2.5 cm, following guidelines for data acquisition with paediatric and adolescent samples [38]. Data were recorded at a sampling frequency rate of 10.2 Hz, based on two wavelengths, 760 and 850 nm.

Imaging procedures

All participants were tested individually in a quiet room, fitted with a desk, computer, and chair, at the children's shelter. Upon arrival, participants were first seated at a desk equipped with a computer (screen diameter: 22 cm; height: 33.2 cm) and were requested to verify their demographic data, collected during study recruitment. Participants then read and signed the Study Information Sheet and completed the fNIRS protocol as detailed below.

fNIRS protocol

Step 1. Placement of Optodes by Fiducial Points

➢ Detection optodes (Table 1), were placed 25–30 mm, above the midpoint of the eyebrow of participants, in accordance with the 10–20 electrode system.

➢ To ensure consistency of optode placement, a red marker was used to indicate positions, 'Fpz', 'Oz', (Fig. 5), 'T3', and 'T4' (Fig. 6). The establishment of these fiducial positions helped establish cap placement, for each participant.

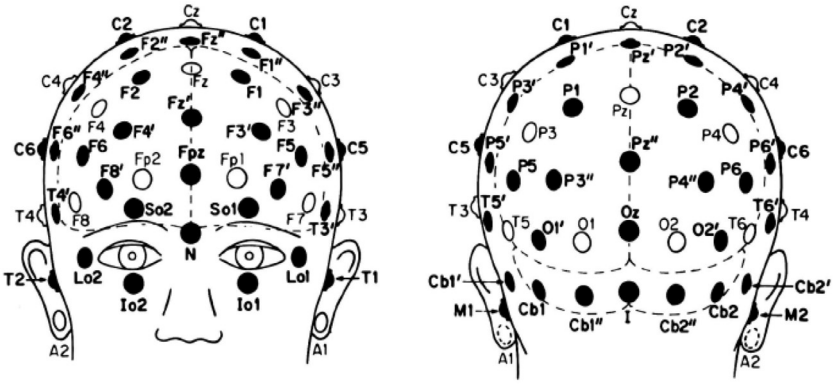


Fig. 5. The auxiliary markers, 'Fpz' and 'Oz', were identified by a red marker, to ensure correct optode placement. Markers were identified using the 10/20 system [39].

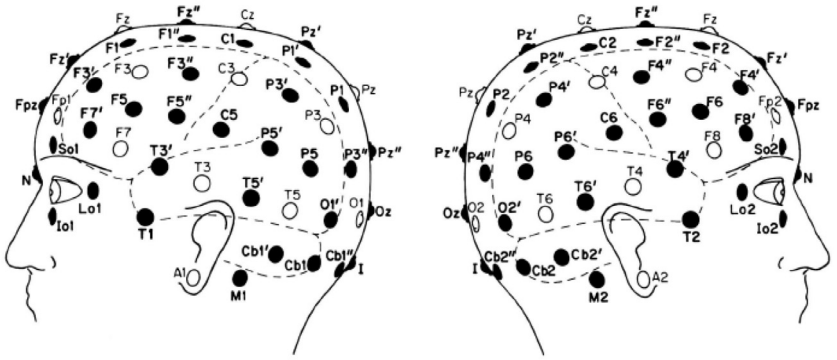


Fig. 6. The auxiliary markers, 'T3' and 'T4', were identified by a red marker, to ensure correct optode placement. Markers were identified using the 10/20 system [39]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Step 2. Placement of fNIRS Cap

➢ Once fiducial points were established, participants were fitted the fNIRS cap (see Fig. 7), tightly fixed with a dark shower overlay.

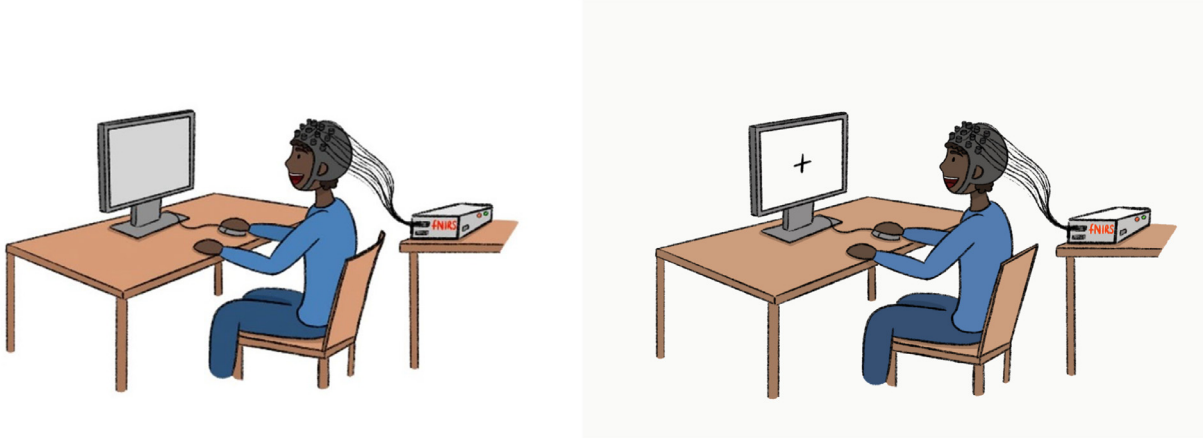


Fig. 7. Participant with fNIRS cap in seating position the completing the SCWT. Copyright: Laura Bell & Sizwe Zondo.

➢ To prevent external light affecting the fNIRS signal, room lights were deemed or turned off.
➢ fNIRS recording then commenced using Aurora Data Acquisition Software (NIRx, Germany), as indicated in Steps 3 to Step 11.

Step 3. Aurora fNIRS Data Acquisition

➢ Open the Aurora Software and go to Configurations (blue arrow) (Fig. 8).
➢ Choose the Headband 8 × 8 montage (orange arrow). The configuration for the Headband_8 × 8 headband, is inbuilt within Aurora, and should reflect eight sources, and eight detectors, as indicated in the bottom righthand of the caption.
➢ Please note the above configuration can be used with seven detectors as indicated in the 'Configuration details', in Fig. 8.

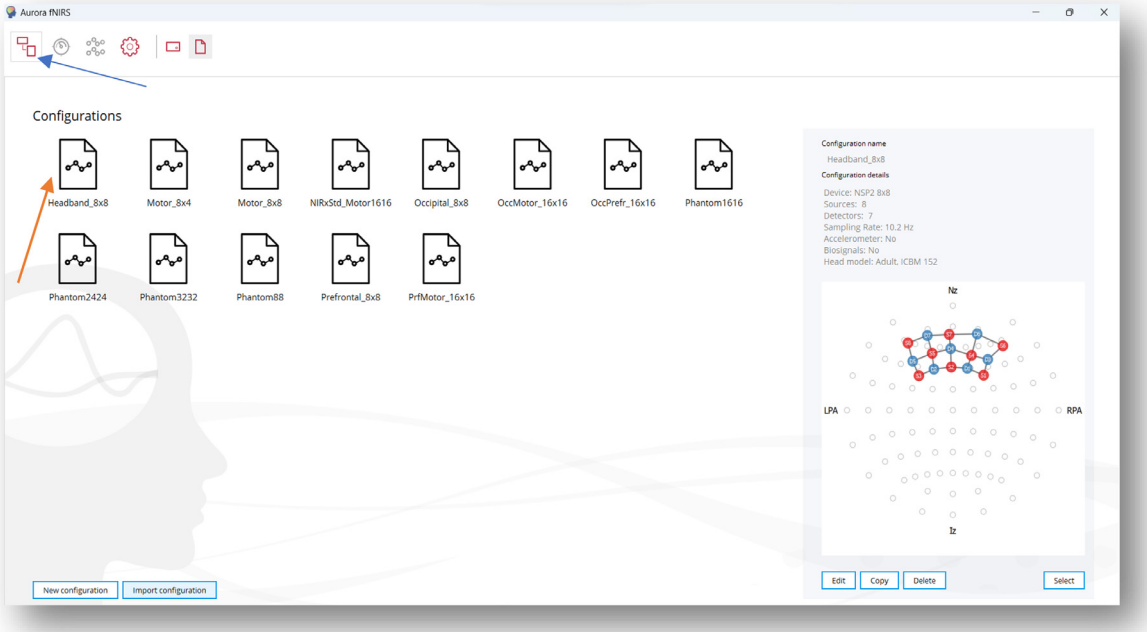


Fig. 8. The Aurora montage used for the study protocol.

Step 4. Connect Aurora Acquisition Software to NIRxSport2

➢ Insert Wi-Fi password which can be found on the NIRXSport2 (if conducting research in places with limited internet, it is recommended you choose Option 2 below).
➢ Connect Aurora to the NIRxSport2 using a cable linked to your laptop (Option 2; Fig. 9).

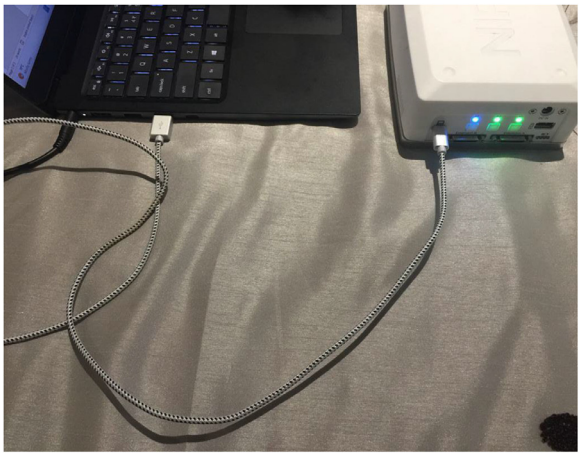


Fig. 9. Cable Link of Aurora to the NIRxSport2.

Step 5. Connect Aurora to NIRxSport2

➢ The below caption indicates a successful connection between Aurora and the NIRxSport2 fNIRS device (Fig. 10).

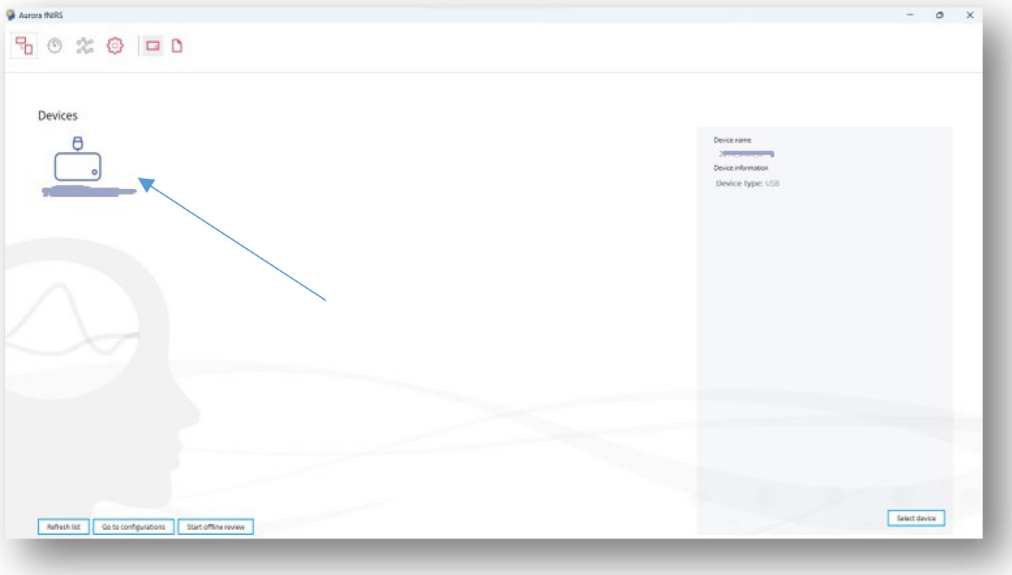


Fig. 10. An example of a successful linkage.

Step 6. Sources and Detector on NIRxSport2

➢ Detectors [8] are marked in blue and must be fitted on the left side of the NIRxSport2 fNIRS device (Fig. 11)
➢ Sources [8] are marked in red and must be fitted on the right side of the NIRxSport2 fNIRS device.

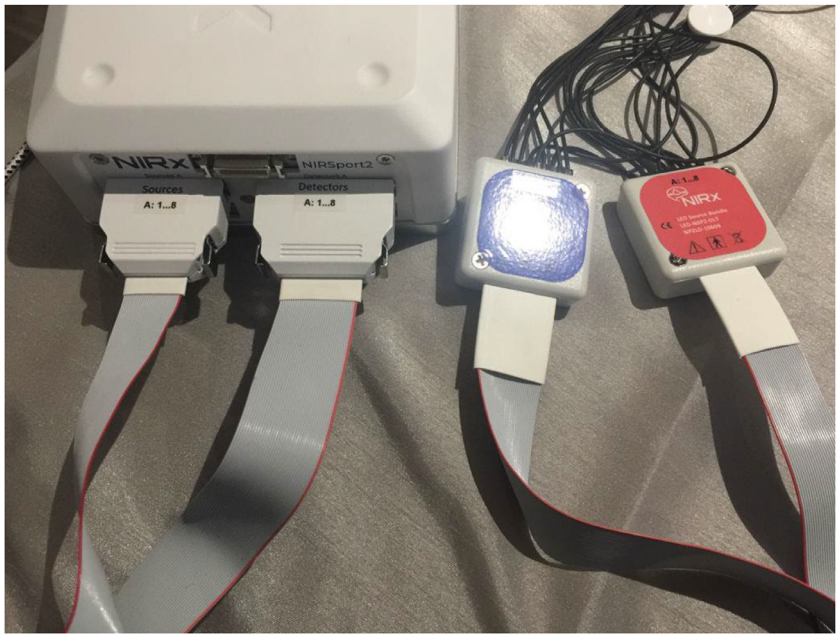


Fig. 11. NIRxSport2 correct source (red) and detector (blue) placement. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Step 7. Aurora Signal Optimization

➢ Run signal optimizations until 'excellent' green signals are obtained to ensure no signal saturation (Figs. 12).
➢ To limit saturation, dim all lights in the experimental room, and ensure that a headcap (overlay) is worn over the optodes.

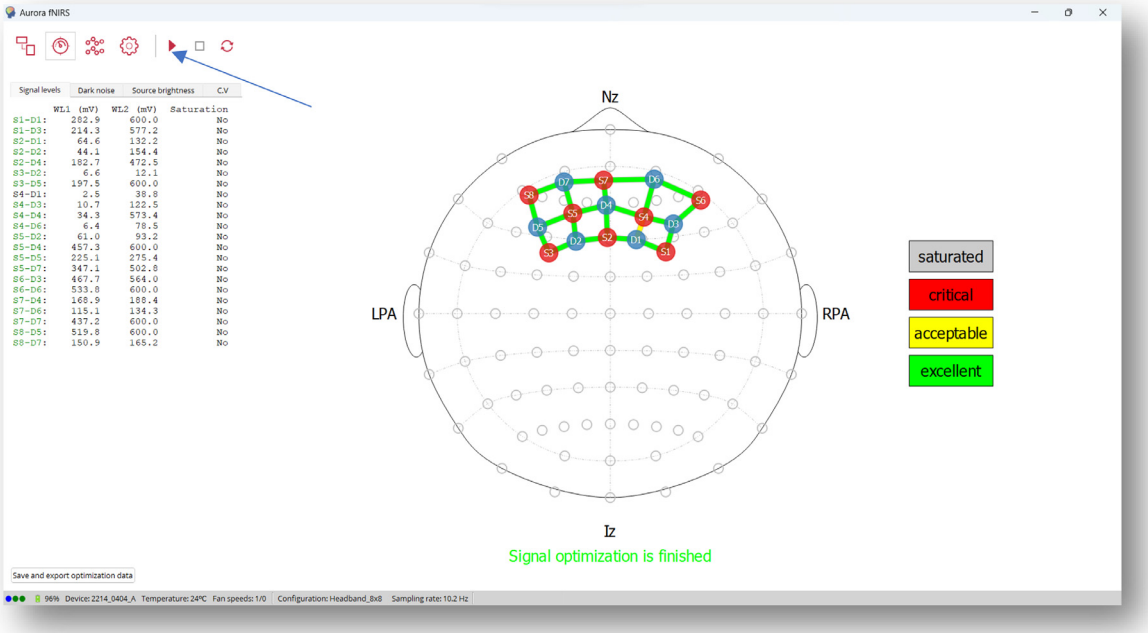


Fig. 12. Screen shot of Aurora signal optimization.

Step 8. Connect PsychoPy SCWT to NIRxSport2

➢ The SCWT (created on PsychoPy), must now be connected to Aurora, using Lab Stream Layering (LSL) as requested by the below PsychoPy caption (Fig. 13).



Fig. 13. An example of an LSL connection request on PsychoPy.

Step 9. Enabling LSL on PsychoPy for the fNIRS SCWT

➢ Under the Custom Tab (yellow arrow) (Fig. 14), insert the below code, within the Begin Experiment tab when prompted.

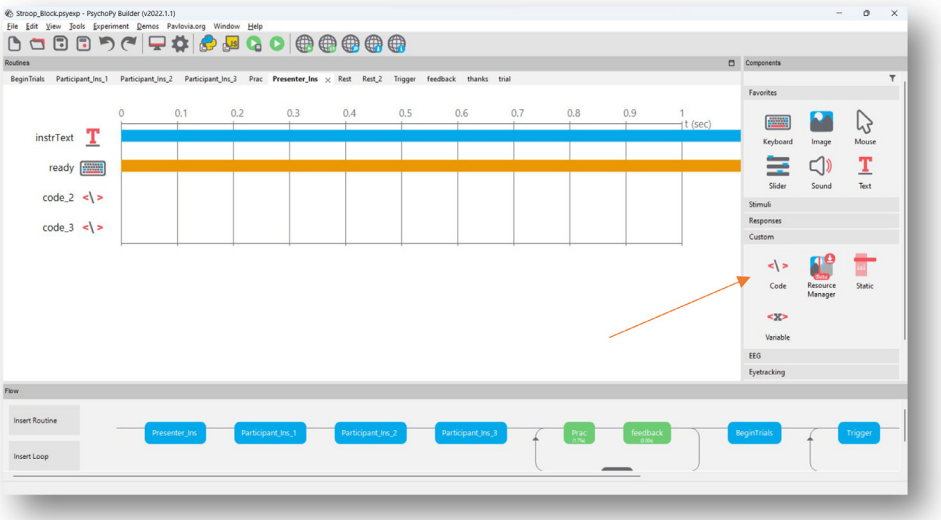


Fig. 14. Code optimization on PsychoPy for the SCWT.

```
from pylsl import StreamInfo, StreamOutlet # import required classes
info = StreamInfo(name='Trigger', type='Markers', channel_count=1, channel_format='int32', source_id='Example') # sets variables for object info
outlet = StreamOutlet(info) # initialize stream.
outlet.push_sample(x=[marker]) outlet.push_sample(x=[congruent])
```

Step 10. Commence recording of fNIRS signal (Fig. 15).

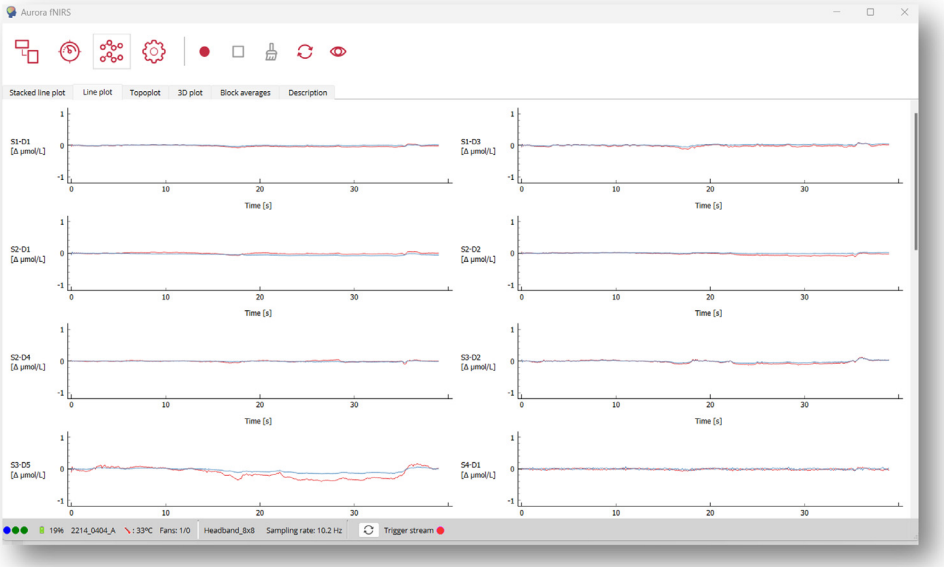


Fig. 15. Screen shot of an fNIRS signal recording (line plot) on Aurora.

Data file and data preprocessing

Once fNIRS data was collected, the analysis of changes in hemodynamic responses, was executed on Satori fNIRS [40] using oxygenated haemoglobin data (HbO). Supplementary Fig. S1 provides an example of a Satori fNIRS analysis file, indicating the channels, and properties of the nirs file used for the analysis. The pre-processing steps are detailed in Fig. S2. Channel rejections were applied using the Scalp Coupling Index (SCI) = 0.75 [41]. Motion artefacts, including head movement, were corrected by applying spike removal parameters based on monotonic interpolations [42]. Spike removal corrections were followed by temporal derivative distribution repair (TDDR) to remove baseline shifts and spike artefacts in the data [43]. Low-frequency band-pass filtering was applied to eliminate baseline drift on the data. Physiological fluctuations related to blood pressure fluctuations (1–1.5 Hz) and respiration (0.2–0.5 Hz) were removed using low-pass (LP) and high-pass Butterworth filtering method. Summarily, the LP filter (0.1–0.2 Hz) enabled further removal of high-frequency noise within the data that was not accounted for by brain activity [44]. The high pass filter (0.01 Hz) was applied to attenuate low-frequency signals by removing baseline drift that may have affected the hemodynamic signal. Once data was preprocessed, changes in light intensity were converted into concentration changes in HbO, using the Modified Beer-Lambert Law (MBLL).

Intervention

HIV-CRT attention training was conducted using ACTIVATE^{TM} [45]. The computerized brain training program consists of six brain training exercises focusing on sustained attention, working memory, inhibitory control, and cognitive flexibility. The program was installed on computers at the children's shelters, and participants completed the cognitive tasks during after school hours. The cognitive tasks included various attention exercises such as memorizing sequences of stimuli presentations, completing pattern designs, task-switching, and categorizing objects. All training activities were implemented to enhance 'top-down cognitive processes', which foster cognitive control, and maintaining vigilance, thus enhancing task readiness and attention skills [46]. The program automatically scheduled cognitive tasks based on varying levels of complexity levels, with every new level of difficulty, dynamically customised for each individual participant based on feedback mechanisms incorporated within ACTIVATE^{TM} [46]. In total, participants underwent three cognitive training sessions weekly for a period of 25–30 sessions. Each session lasted approximately 30 min, and the intervention was carried out over a 12-week period, spanning six months. Additional information about ACTIVATE^{TM} can be found on the C8 sciences website (http://www.c8sciences.com/about/games/).

Statistical analysis

Statistical analysis was performed using JASP (Version 0.18). Pre and post differences on cognitive and fNIRS performance were calculated to compare estimated mean differences between the groups. Planned comparison differences were executed by controlling for pretest scores and group interaction, using analysis of covariance (ANCOVA). All assumptions, including independence of the covariate (*pretest scores*), and homogeneity of regression slopes were undertaken for the analysis. Further statistical imputations were conducted to investigate fNIRS seed-based correlation functional connectivity as detailed below.

Seed-based correlation functional connectivity (FC) analysis

Seed-based correlation FC was executed within Satori fNIRS. Based on priori evidence, we selected the left dorsolateral prefrontal cortex (L-DLPF), as the seed region for the cross-correlation. The L-DLPF is pliable to neuronal plasticity, following HIV brain training [46], and following deep brain stimulation, to improve HIV neurocognition [47,48]. With reference to our study, this seed represented Channel 6 (Seed S3-D2), with MNI coordinates, x = - 31, y = 39, z = 41 (Table 1). This seed indicated a specificity index of 92 %, as determined by fOLD. Seed-based correlation FC analyses were performed by computing the temporal correlations between the seed (S3-D2), and *each of the channels* indicated in Table 1, for all participants. Once correlations were computed, these were transposed to create an average correlation, for each of the channels (See Supplementary Fig. S3 for an example).

Similar to [49], to increase the normality of the distribution of the individual correlation values, Fisher's r-to-z transformations, were applied to each correlation coefficient. Summarily, Fisher's transformed bivariate correlation coefficients, were calculated between the seed hemodynamic time series, and each of the individual channel time series [50], which enabled Fisher's r-to-z normalizations, to be transformed to correlation maps (r). These maps were generated on Satori, at the individual and group level. The above transformation enabled planned comparisons between the groups to be computed controlling for pretest scores (average correlations), and group interaction, using Analysis of Covariance (ANCOVAs).

Hemispheric interaction and threshold survival

Since average correlations were derived as described above, we were able to derive data to compare the average hemodynamic response effect, between the left and right hemisphere, (HbO), between the groups (please see Supplementary Fig. S4 for an example). To further explore FC, and to identify regions (channels), with the greatest connectivity with the seed region (S3-D2), at post training, (in the treatment group), we computed threshold 'survival' analysis. This additional analysis enabled us to examine the percentage of 'surviving' channels in each hemisphere, with increased correlation thresholding. Surviving channels / voxels would be indicative of

the strongest correlations with the priori seed. Importantly, there is a lack of consensus on optimal threshold parameters for functional connectivity analysis. Garrison et al. [51], for example, applied threshold surviving parameters based on increments ranging from 5 % to 95 %, while, Jia et al. [52] employed increments of 20 % to 70 % to study link density survival edges in adult HIV brain training. Our study employed threshold increments of $r = 0.2$ to $r = 0.8$, investigate 'surviving' channels with the largest correlation to the seed (S3-D2).

Discussion

South Africa, were the protocol was implemented, has the highest rates of HIV infection in pediatric and adolescent populations [53]. The protocol answers an urgent 'call to action' [14] for the implementation of longitudinal studies to investigate non-pharmaceutical measures to reverse cognitive decline in neuroHIV, especially in African populations. By undertaking the study, and pairing behavioural gains (or lack thereof), emanating from the brain training, with neuroimaging data, the study provides invaluable insights into the nature of neuroplasticity and adolescent neuroHIV. Importantly, the protocol addresses a social justice issue, to apportion mental health services to the least served regions, in South Africa, including 'townships', and rural areas [54]. Notwithstanding the above, it is important to note that the execution of neuroimaging research in African contexts, is attendant to logistical challenges. For example, execution of neuroimaging commands, particularly Lab-Streaming Layer (LSL) requires constant electricity, and internet connectivity, which may be in short supply in our context. Moreover, due to the nature of the population (children living with HIV), copious levels of clinical research are undertaken with the population which may lead to participants experiencing research fatigue, resulting in increased attrition rates, as experienced in our study. We thus recommend future researchers adapting the present protocol, to consider pursuing Single Case Experimental Designs (SCED), were possible.

Study progress

The data collection for the study has since been completed, and data analysis is underway. Given ongoing analysis, no research outputs exist from the protocol. This protocol study has a distinct scientific contribution in detailing the step-by-step fNIRS neuroimaging procedures and the behavioural assessments used to investigate neuroHIV cognition in adolescent populations, especially in the domain of attention. As the first study detailing the joint investigation of hemodynamic responses paired with behavioural changes, to investigate HIV-CRT, in the context of adolescent HIV, the study contributes to uncovering potential biomarkers for the cognitive rehabilitation of adolescent neuroHIV, in Sub-Saharan Africa.

Protocol validation

Data analysis for the protocol is currently underway. An example of a JASP data file for the analysis can be found under the Supplementary section. The data analysis includes ANCOVA and mixed methods models.

Limitations

For successful replication of the above study, it is important that optodes are correctly placed, using the details provided in the protocol. Moreover, it is advised that children practise the pencil and paper Colour Stroop Word Test (Table S2), before completing the fNIRS-SCWT. Lastly, it is advised that, to minimize ambient light affecting the optodes, the experimental room setting should be dimly lit, and a black cap should be placed over the optodes.

Related research article

None.

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.

CRediT authorship contribution statement

Sizwe Zondo: Conceptualization, Writing – original draft, Writing – review & editing, Data curation, Project administration, Formal analysis, Software. **Kate Cockcroft:** Supervision. **Aline Ferreira-Correia:** Supervision.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2024.102808.

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