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Neural correlates of maintenance working memory, as well as relevant structural qualities, are associated with earlier antiretroviral treatment initiation in vertically transmitted HIV

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ABSTRACT

There is evidence of HIV affecting cognitive functioning across age groups, with adult studies showing related deficits in fronto-striatal and hippocampal regional activity. Additionally, delayed initiation of antiretroviral treatment (ART) has been associated with poorer cognitive outcomes in HIV infected youth. Little is known, however, of the neural correlates underlying such cognitive deficits in youth populations. We investigated maintenance working memory related brain activity in South African HIV infected youth and controls, and the effect of ART initiation age on underlying structures. Sixty-four perinatally infected youth (ages 9-12) and 20 controls (ages 9-13) underwent functional magnetic resonance imaging (fMRI) while completing 1-back and 0-back blocks of the N-back task. At an uncorrected p value threshold of 0.001, the HIV infected group showed decreased activation in the left superior temporal gyrus, pre and postcentral gyri, insula, and putamen as well as bilateral hippocampus, and mid cingulum. The HIV patients with delayed ART initiation showed less activation during processing conditions in the mid cingulum, left inferior parietal gyrus, as well as right inferior frontal, bilateral thalamic, and superior temporal regions. When these regions were tested for structural differences, the mid cingulum, and right inferior frontal gyrus, insula, and thalamus were found to have less cortical thickness, surface area, or volume in the group with delayed ART initiation. Regional differences between HIV infected youth and controls noted in the N-back task are consistent with impairments in structures involved in maintenance working memory. These data support earlier ART initiation in perinatally infected individuals.

Keywords: Human immunodeficiency virus; maintenance working memory; Adolescents; Functional magnetic resonance imaging

1 INTRODUCTION

The human immunodeficiency virus (HIV) has been associated with impaired executive function, working memory, processing speed, and general intelligence in people of all ages living with HIV (Boivin et al. 1995; Nozyce et al. 2006; Hoare et al. 2016; Phillips et al. 2016). Most neuroimaging studies of cognitive impairment investigate adult onset HIV in Western populations, and note differences primarily in fronto-striatal regions (Chang et al. 2001; Melrose et al. 2008; Du Plessis et al. 2014) and the hippocampus (Maki et al. 2009). In HIV infected children numerous studies have revealed worse performance than controls on cognitive tasks (Hoare et al. 2012, 2018), but generally no differences are seen in children's cognition in relation to age of antiretroviral treatment (ART) initiation (Smith et al. 2008; Puthanakit et al. 2010, 2013). In some of these cases, where the median age of initiation is 9 years old, the lack of findings might be due to missing the crucial infancy stage of neurodevelopment (Puthanakit et al. 2010, 2013). When ART initiation is before the age of three months, improved neurodevelopment using the Griffiths Mental Development Scale is noted (Laughton et al. 2012). There is a small amount of research on the effects of HIV and ART initiation age, on white matter structures in perinatally infected children before the age of two years (Geng et al. 2012), but the effects on grey matter are less well studied. Overall, the effects of HIV infection and ART initiation on neurocognitive-relevant brain activity and structural development remain understudied in young South African populations, where the HIV burden is the highest in the world.

Members of our laboratory have previously used the N-back task to assess working memory using only the first two N-back levels in a study of methamphetamine users (Brooks et al. 2016), and other laboratories have also used this version of the task (Ragland et al. 2002; Rac-Lubashevsky and Kessler 2016; Hur et al. 2017; Meule 2017). The version using 0-back and 1-back levels has been described as involving maintenance of information held in working memory systems, while 2 back and higher levels involve manipulation thereof (Ragland et al. 2002; Rac-Lubashevsky and Kessler 2016). Said maintenance level of working memory task activates the inferior parietal gyrus, dorsolateral prefrontal cortex (DLPFC), right ventrolateral prefrontal cortex (VLPFC), and left lingual gyrus, while the manipulation levels result in further activation of these regions with additional activation in the DLPFC and anterior cingulate.

Here, perinatally infected youth from the Cape Town Antiretroviral Adolescents Cohort (CTAAC). underwent functional magnetic resonance imaging (fMRI) while completing the N-back task with alternating 1-back and 0-back blocks to examine the effect of HIV infection on task performance and underlying neural activity. Structural MRI was also used to examine the volume, cortical thickness, and surface area of the brain regions utilised while completing the maintenance working memory (MWM) task, in relation to ART age of initiation. The task requires participants to proactively monitor the presentation of randomised stimuli, and as such the ongoing processing of visual stimuli,

and shifts in attention, were expected. Altered brain activity and structure underlying divergent neurodevelopment in the HIV infected and control participants was also expected, dependent upon ART initiation being earlier or delayed.

2 METHODS

2.1 Participants

Sixty-four HIV infected youth (mean age 10.42, SD= 0.87; range = 9-12) and twenty demographically matched controls (mean age = 10.43; SD =1.3; range = 9-13) enrolled in CTAAC were recruited to participate in the current study. Previous HIV research has used similar patient to control sample size ratios (Maki et al. 2009). Ethical approval for human participant recruitment was granted by the Human Research Ethics Committee of the Faculty of Health Sciences at the University of Cape Town (HREC REF 051/2013), and the study was performed in accordance with the latest Declaration of Helsinki. All participants and their legal guardians provided written informed consent, and were excluded for: an uncontrolled medical condition, and identified central nervous system condition (other than HIV), past or present meningitis, cerebrovascular trauma or head injury with loss of consciousness of greater than 5 minutes, skull fracture, a history of perinatal complications, or neurodevelopmental disorder not attributed to HIV. All patients were on a personalised ART program. The fMRI task used here was part of a larger neurocognitive study in which 248 demographically similar youth were recruited. Only the 84 included here completed the version of the N-back task described below. All (n=20) of the control participants were black African, 63 of the patients were black African, and one patient was of mixed ethnicity. Each participant was accompanied by a guardian to all testing sessions and the guardian was paid 200ZAR for travel expenses.

2.2 Working memory maintenance task

Participants completed 0-back and 1-back levels of the classic N-back task (Kirchner 1958). This version of the N-back task was used, due to the young age and earlier educational abilities of the participants, and given its validity as a MWM task as described above. Participation involves watching a screen on which letters of the alphabet are flashed. For the 0-back block, participants pressed a button when they saw the letter “X”. For the 1-back block, they pressed a button when they saw two of the same letter presented consecutively. The participants completed the task while undergoing fMRI scanning. Alternating 1-back and 0-back blocks were presented six times each with a rest period between blocks. 1-back and 0-back blocks consisted of 20 letters, each letter presented separately on the screen for 1.5 seconds, with 1.5 seconds for the participant to respond using the space bar. Rest periods between the blocks were 9 seconds long during which the participant was presented with a blank screen, and so the total experimental run was approximately 14 minutes. The MWM task was presented in the scanner using Neurobehavioural Systems Presentation software (www.neurobs.com/menu_presentation) and subjects viewed the task on a mirror attached to the front

of the head coil. Subjects were requested to respond by pressing a button on an MRI-compatible button box. Independent sample *t*-tests were done in SPSS25 to assess for divergent response times in the MWM task. Based on previous publications using the same task (Brooks et al. 2016, 2017), commission errors (pressing the response button without the appropriate cue), and omission errors (not pressing the response button when presented with the appropriate cue) were recorded.

2.3 Neurocognitive impairment

Each participant completed a comprehensive neuropsychological battery that assessed cognitive functioning in the following 10 cognitive domains: general intellectual functioning, attention, working memory, verbal memory, visual memory, visual spatial ability, motor coordination, language processing speed, and executive function. The full list of tests is described in a previous study (Hoare et al. 2012). Each participant's neuropsychological profile was classified according to the youth Neurocognitive Disorders classification (Hoare et al. 2016) into one of three categories: no impairment, mild impairment, or major impairment.

2.4 fMRI scanning parameters

All scans were obtained using a 3 Tesla Magnetom Allegra Siemens MRI head scanner (Siemens Medical Systems GmBH, Erlangen, Germany) with a four-channel phased array head coil, at the Cape Universities Body Imaging Centre (CUBIC). Whole brain T2* weighted 2D- echo planar imaging (EPI) functional volumes were acquired with 36 ascending axial slices. The following parameters were used: EPI factor = 64, TR/TE:3s/25ms, FOV (anterior-posterior, inferior-superior, left-right): 64*64*36 slices, voxel size: 3.1 x 3.1 x 3.5mm. A T1-weighted high resolution structural scan was obtained once for each participant using the following parameters: TR/TE: 2.53/6.6ms, FOV 256*256*128 mm, voxel size: 1 x 1 x 1.33mm, with an acquisition time of 8 minutes and 6 seconds.

2.5 fMRI data analysis

MR scans were analysed using SPM12 (Wellcome Department of Imaging Neuroscience, London, UK). Pre-processing included; slice-time correction, motion correction of the 6 motion parameters, volume realignment to the middle volume, and AC-PC realignment to improve co-registration. Functional and structural volumes were co-registered and subsequently normalized to standard (MNI152) space using an indirect normalization procedure (Ashburner and Friston 1997) and resampled into 4mm isotropic voxels using 4th degree B-spline interpolation. Finally, all images were smoothed using an 8 mm FWHM Gaussian kernel, which addresses residual between-subjects variance.

Statistical analysis of fMRI data at the individual subject level was performed within the general linear model framework. The onsets and durations of three task conditions were included as

regressors. The conditions of the experiment were entered into the model. The six conditions were control/1-back, control/0-back, control/rest, HIV+/1-back, HIV+/0-back, HIV+/rest. Six rigid body transformation parameters obtained during realignment were also included as regressors. High pass filter cut-off was set at 1/128 Hz. For each participant, contrast maps were generated for the main effect of HIV status across sessions, and the interaction of task condition with HIV status.

Second level random effects modelling tested the null hypothesis of zero difference across participants between the drug and placebo conditions. Whole brain analyses were run using FWE corrected voxel level significance, thresholded at $p<0.05$ throughout. A three-factor full factorial 2x3 ANOVA (control/HIV+, 1-back/0-back/rest) was modelled using block onset and duration times of the task stimuli and tested for whole brain effects. After the primary analyses, covariates were included into the model to test for the possibly confounding effects. The main effect of HIV status was tested, as well as main effects of the task conditions. Contrast maps of condition interactions were also made. For visualisation of results in **Figures 1-4**, statistical parametric maps have been superimposed onto a high resolution canonical T1 scan with thresholds set at $p<0.001$. Mango (Research Imaging Institute, UTHSCSA) was used to create the figures.

Covariates were added to the model mentioned above in order to test for contributing effects to group differences, and thus were applied to the controls vs HIV, N-back vs rest interaction contrast (i.e. checking for increased activation in the control sample in the MWM conditions). All covariates were tested with a FWE corrected significance level of $p<0.05$.

In order to investigate the effects of ART initiation on the developing brain, the HIV infected youth were split into two groups: those who began ART before the age of two years (24 months; n=20), and those who began ART after the age of two years (n=42). Two subjects from the original 64 patients had to be excluded as their date of ART initiation was unknown. These tests were run in the same fashion as the HIV vs control contrasts. A two way ANOVA (ART age group X N-back condition) was modelled to check for differential activations and deactivation between the two groups in the N-back task conditions.

3 RESULTS

3.1 Demographic data

The groups were matched on age, sex, home language, and ethnicity. The HIV infected group had a higher number of repeated grades and a higher rate of mild neurocognitive impairment. None of the included subjects had major neurocognitive impairment. More details on the group demographics are in **Table 1.**

Table 1 Demographic and clinical characteristics

Variable	Controls N=20	HIV infected N=64	T or Chi-square score	P value
Age in years: Mean(SD)	10.43(1.30)	10.42(0.87)	0.24	0.98
Gender: Male/Female	8/12	28/36	0.09	0.77
Ethnicity: Black African/Other	20/0	63/1	0.32	0.57
Home language: isiXhosa/Other	20/0	60/4	1.31	0.25
Viral load (copies/mL): Median(IQR)	n/a	0(40)	n/a	n/a
CD4 count: Mean(SD)	n/a	942(490.3)	n/a	n/a
Current school grade	4.9(1.4)	4.4(1.1)	1.79	0.076
Repeated a school grade	No=15 Yes=5	No=23 Yes=41	0.39	**0.002**
Neurocognitive impairment	None=19 Impairment=1	None=48 Impairment=16	8.96	**0.011**

Significant p-values are in bold.

3.1 Behavioural data

The N-back response times did not differ significantly according to HIV status (see **Table 2)**, although a slight trend can be seen where all response times are marginally slower, and the number of errors marginally higher in the HIV group across task conditions. When errors and response times were compared between the participants who initiated ART under the age of 2 and over the age of 2, there were no significant differences.

Table 2 N-back button press responses

Test	Mean score		P value
	Controls (N=20)	HIV+ (N=64)	
Response time (seconds)			
1-back	0.6441	0.7182	0.095
0-back	0.6333	0.6717	0.155
Combined	0.6387	0.7057	0.066
Commission errors			
1-back	4.2	5.02	0.655
0-back	2.4	3.55	0.273
Combined	6.6	8.75	0.381
Omission errors			
1-back	3	3.4	0.673
0-back	2.45	3.05	0.616
Combined	5.41	6.46	0.611

3.2 fMRI results

3.2.1 Main effects of task

Initially, both HIV infected and control groups were combined to detect overall task effects. The main effect of the N-back stimuli compared to the rest blocks resulted in robust activation the superior temporal, precentral, and lingual gyri. Analyses were run in which both N-back conditions (1-back and 0-back) were combined to form a maintenance working memory (MWM) vs rest contrast. A 1-back vs 0-back sensitivity contrast was also tested, in order to control for visual stimuli. In the MWM vs rest contrast, a whole brain analysis revealed increased activation in large bilateral clusters in the superior temporal gyri, extending into the rolandic operculum and insula, as well as large clusters bilaterally in the precentral gyri, extending into the postcentral gyri, supplemental motor area and mid cingulate, with an additional cluster in the right occipital lobe incorporating the calcarine and lingual areas (see **Table 3A** and **Figure 1**). For the same contrast, areas of deactivation were noted in the bilateral inferior and superior parietal lobules, the bilateral middle temporal gyri, the midbrain/pons, and the right middle frontal gyrus, extending into the superior frontal gyrus and including the opercularis and triangularis (see **Table 3A** and **Figure 2**). These findings were at the cluster level using a FWE corrected $p<0.05$.

When contrasting 1-back vs 0-back effects across the combined sample, as a sensitivity analysis, a whole brain analysis revealed no differences with a cluster level FWE corrected threshold, but when using a less stringent uncorrected $p<0.001$ threshold at the peak level, differences in activation -but not deactivation- were detected in the bilateral precentral gyri (including pars triangularis and pars opercularis), extending to the superior temporal gyrus on the left, and extending into the insula on the right.

Table 3 fMRI effects of N-back task

Contrast	Region	Hem.	MNI coordinates	P value	Cluster size
A. HIV and Controls combined					

N-back > Rest	Superior temporal gyrus	R	64 2 4	<0.001	558
		L	-58 2 4	<0.001	1401
	Precentral gyrus	L	-24 -26 68	<0.001	2616
		R	22 -26 70	<0.001	326
		R	56 -4 40	0.015	36
		R	44 -10 56	0.036	5
	Lingual gyrus-Cuneus border	R	12 -82 2	0.021	22
Rest > N-back	Inferior parietal lobule	R	42 -56 54	<0.001	327
	Superior parietal lobule	L	-34 -66 52	0.001	241
	Middle temporal gyrus	R	58 -40 -8	0.009	59
		L	-58 -44 -8	0.045	1
	Midbrain, pons	Medial	6 -14 -16	0.009	59
		Medial	-10 -26 -14	0.028	12
	Middle frontal gyrus	R	40 16 44	0.010	57
B. Controls > HIV					
1-back > Rest	Superior temporal gyrus	L	-56 -24 2	<0.001	320
	Insula	L	-38 -8 -6	<0.001	393
	Inferior frontal gyrus/Caudate	L	-18 24 -4	<0.001	81
	Hippocampus	L	-28 -20 -16	<0.001	66
	Lentiform nucleus/Globus pallidus	L	-12 -4 -4	<0.001	13
	Subcallosal gyrus/Superior orbitofrontal gyrus	L	-22 12 -16	0.001	10
	Middle temporal gyrus	L	-54 4 -18	0.001	3
1-back > 0-back	Lentiform nucleus/Putamen	L	-22 8 -12	<0.001	35
	Superior frontal gyrus	L	-18 12 62	0.001	8
	Mid cingulum	R	14 -14 48	0.001	7
C. HIV > Controls					
	No results				

Cluster size is described in terms of number of voxels; fMRI voxels were resampled to 4mm isotropic during the data pre-processing.
In Table 3A, significance levels were determined at the cluster-level with FWE corrected $p<0.05$.
In Tables 3B and C, significance levels were determined at the peak-level at uncorrected $p<0.001$

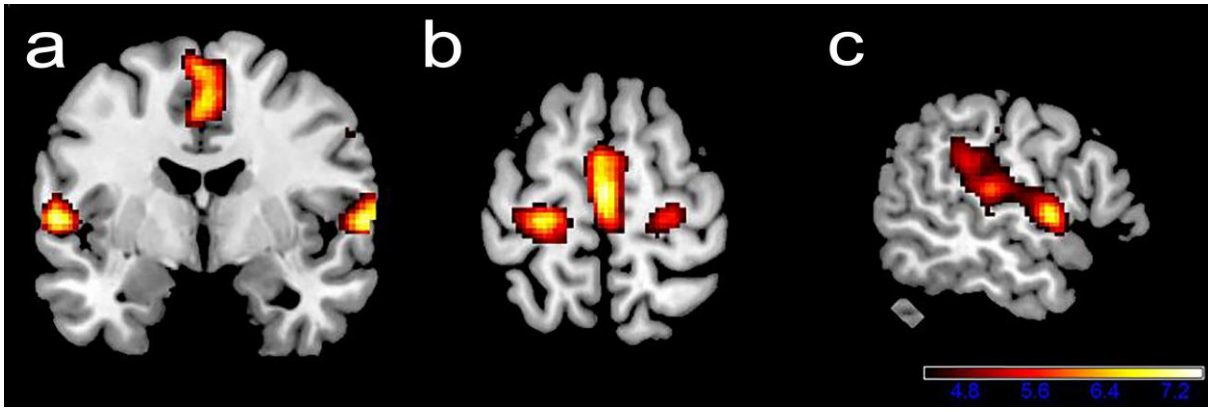

Fig1 Neuronal activations during N-back task conditions in the combined sample. Activation in the precentral gyrus (panels a and b) and left and right upper temporal/inferior parietal gyri (panels b and c). On the bottom right is a colour legend indicating T values. Panel a: y=-2; Panel b: z=64; Panel c: x=-56

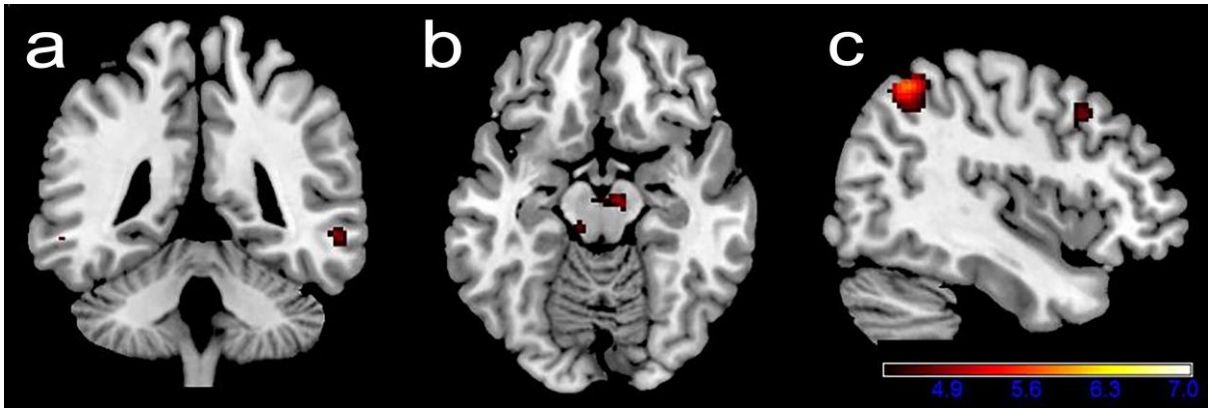

Fig2 Neuronal deactivations during N-back task conditions in the combined sample Deactivation in the bilateral temporal gyri (panel a), pons (panel b), and parietal and frontal gyri (panel c). On the bottom right is a colour legend indicating T values. Panel a: y=-43; Panel b: z=-14; Panel c: x=41

3.2.2 Group task effects

A 2x3 (HIV status X N-back condition) ANOVA was run to detect differences in BOLD activation between HIV infected youth and controls during the MWM conditions. At a FWE $p<0.05$ cluster level significance threshold, no differences were detected. However, at a less stringent threshold of $p<0.001$ (uncorrected) at the peak level some group differences in activations were noted. In the 1-back vs rest contrast these activations were seen in the control group, predominantly in the left superior temporal gyrus and insula. For full results see **Table 3B** and also **Figure 3**). When 1-back and 0-back were directly compared in a sensitivity analysis, in order to detect differences independent of visual stimuli, and for neural differences between the two levels of the task, clusters of activation were noted in the left lentiform nucleus/putamen, left superior frontal gyrus, and the right mid cingulum, with $p<0.001$ at the peak level, while no deactivations were noted. Controlling for age and neurocognitive impairment separately did not change these clusters significantly. These group task effects are listed in **Table 3B**. Overall, neural activation seen in the task conditions was contributed to more by the control group

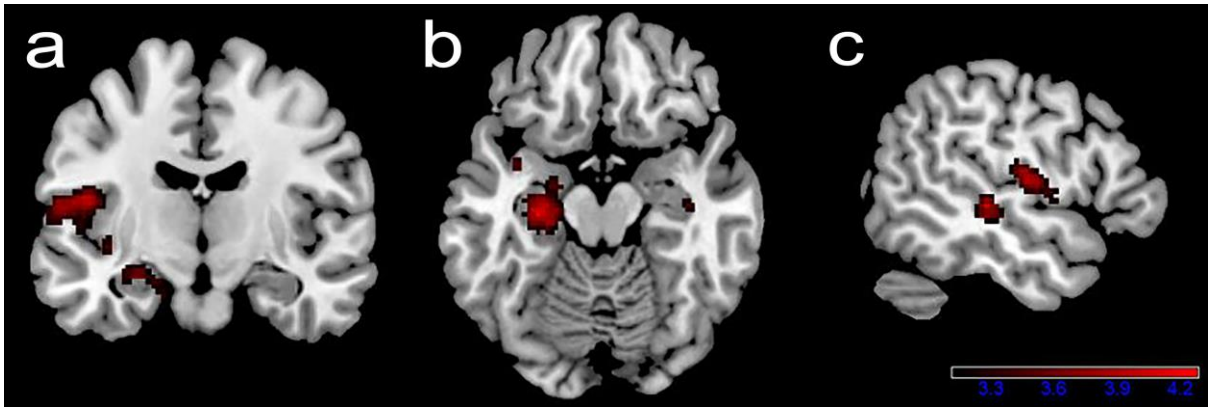

Fig3 Neuronal deactivation in the HIV patients during N-back task conditions. Clusters in the left insula (panel a), left hippocampus (panels a and b), and left superior temporal gyrus/rolandic operculum (panels a and c). On the bottom right is a colour legend indicating T values.
Panel a: y=-11; Panel b: z=-16; Panel c: x=-53

3.2.4 Group effects with covariates

The covariate 'age' (as measured in years with one decimal point) contributed significantly to activation in one small cluster in the left precentral gyrus/rolandic operculum (-58, -10, 12). The covariate 'neurocognitive impairment' contributed significantly to activation in the left amygdala, numerous clusters in the left middle and superior temporal gyrus, and a small cluster in the posterior cingulate cortex, indicating that a proposed neurocognitive deficit correlated with activation in these regions. Both covariates contributed to deactivations (neurocognitive impairment in the bilateral superior parietal lobes, and age in the left parahippocampal gyrus) but only when a less stringent threshold of uncorrected $p<0.001$ was applied. Three measures (attention, executive function, working memory) were combined and averaged to create one additional covariate. This covariate contributed to activation in the superior temporal gyrus/rolandic operculum, but only with a less stringent threshold of uncorrected $p<0.001$.

3.3.1 Functional findings based on differential age of ART initiation

A comparison of the two ART initiation age groups on primary demographic features is in **Table 4**. The two groups were matched on all relevant demographic variables. The only measures on which the two groups differ are "ART duration" and "Age of ART initiation", which is to be expected. For this analysis, the two N-back conditions were combined to form a MWM vs rest contrast. No effects were detected with a FWE corrected $p<0.05$ threshold, but an uncorrected threshold of $p<0.001$ allowed for detection of numerous activations during N-back conditions in the earlier ART initiation group. The full results for this contrast are listed in **Table 5** and visually presented in **Figure 4**.

Table 4 Demographic and clinical characteristics of HIV infected youth. Earlier ART initiators began ART by the age of 24 months of age. Late ART initiators began ART after 24 months.

Variable	Earlier ART initiators N=20	Late ART initiators N=42	T or Chi-square score	P value
Age in years: Mean(SD)	10.2 (0.9)	10.5 (0.8)	-1.21	0.23
Gender: Male/Female	11/9	16/26	1.58	0.21
Home language: isiXhosa/Other	20/0	38/4	2.04	0.15
Neurocognitive impairment	None=17 Impairment=3	None=29 Impairment=13	1.80	0.18
Repeated a school grade	No=9 Yes=11	No=13 Yes=29	1.17	0.28
CD4 count: Mean(SD)	908.1 (577.4)	964.7 (453.9)	-0.42	0.68
Viral load (copies/mL): Median(IQR)	0(140)	0(20)	n/a	n/a
ART duration	9.3 (0.9)	6.2 (2.1)	6.19	**<0.001**
ART age of initiation	1.1 (0.5)	4.6 (1.9)	-7.9	**<0.001**

Significant p-values are in bold.

Table 5 fMRI effects of N-back task in earlier ART initiation (N=20) vs late ART initiation (N=42) groups

Task condition	Region	Hem.	MNI coordinates	P value	Cluster size
N-back > Rest	Mid cingulate gyrus	L	-20 -14 42	<0.001	115
		n/a	0 0 38	<0.001	391
	Inf frontal: opercularis	R	38 4 28	<0.001	80
	Sup temp gyrus	L	-62 -24 -2	<0.001	33
	Sup temp gyrus/insula	R	42 10 -12	0.001	8
	Thalamus	L	-12 -30 4	<0.001	38
		L	-12 -26 14	<0.001	31
		R	6 -20 10	0.001	6
	Inf parietal	L	-36 -34 36	0.001	16

Cluster size is described in terms of number of voxels; fMRI voxels were resampled to 4mm isotropic during the data pre-processing.
All significant levels were determined at the peak-level with uncorrected p<0.001.

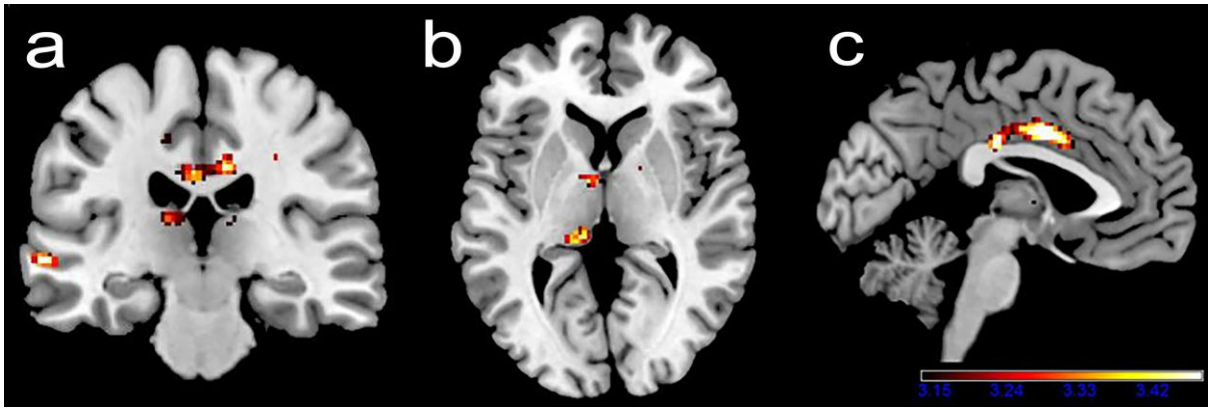

Fig4 Neuronal activation in the earlier ART initiation HIV patients during N-back task conditions. Clusters in the cingulate cortex (panels a and c), left temporal gyrus (panel a), and thalamus (panels a and b). On the bottom right is a colour legend indicating T values.
Panel a: y=-23; Panel b: z=4; Panel c: x=-3

3.3.2 Structural differences based on functional findings

Following these functional findings between the ART age groups, structural qualities (volume, cortical thickness, and surface area) of the significantly activated regions were compared. Based on Freesurfer anatomical output(Hoare et al. 2018), the two subcortical regions (thalamus, middle cingulate gyrus) were compared on volume, and the four cortical regions (inferior parietal, superior temporal, insula, inferior frontal/pars opercularis) were compared on surface area and cortical thickness. There were no structural differences between the inferior parietal, superior temporal, and left thalamus regions. However, the middle cingulate volume was significantly greater in the earlier ART initiation group (t=2.031(59); p=0.047), the right thalamus volume was almost significantly larger in the earlier ART initiation group (t=1.89(59); p=0.064), the surface area of the right insula

was greater in the earlier ART initiation group (t=2.14(59); p=0.037), and the cortical thickness of the inferior frontal/pars opercularis was almost significantly greater (t=1.78(59); p=0.08). In addition, the overall cortex volume was found to be greater in the earlier ART initiation (t=2(59); p=0.05), and the subcortical grey volume was almost significantly greater in the earlier ART initiation group (t=1.97(59); p=0.054).

4 DISCUSSION

This study attempted to detect differences in brain activity between HIV infected youth and controls during a MWM task. Within the patient group, further differences in brain activation were investigated in relation to ART age of initiation, and the brain regions showing differential activation were assessed for differences in volume, cortical thickness, and surface area.

Differences were found in neural activation dependant on HIV status, with the controls showing increased activation in the dorsal-ventral attention networks (Vossel et al. 2014), including large clusters in the superior temporal, inferior frontal, and insula regions, particularly in the left hemisphere. Smaller clusters of activation were also seen in the cingulum and hippocampus in the controls. Patients who started ART before the age of two had increased activation in similar networks, with additional activity in the bilateral thalami. These earlier ART initiators also had larger medial cingulate cortex volumes, increased overall cortex volumes, and larger surface area of the right insula, while late ART initiators had no increased volumes, thickness, or surface area. In both the HIV vs controls, and earlier vs late ART initiation groups, it is thought that the increased activation indicated improved sensorimotor recruitment, as it corresponds with lower levels of neurocognitive impairment and slightly faster response times on the N-back task in the case of the controls>HIV contrast, and with larger brain volumes in the case of the earlier ART initiators.

Our findings confirm an attentional processing network used in MWM that includes the temporoparietal junction and nearby regions such as the inferior frontal gyrus and insula. All of these regions have been linked to processing and MWM abilities, including the lateral inferior prefrontal cortex, which is additionally linked to executive functions including decision making and working memory (Oldrati et al. 2016), and the parietal lobes and thalamus (Tomasi et al. 2006). Although not considered part of the fronto-striatal network associated with altered executive function performance in HIV infected patients, reduced activation has been noted in the hippocampus in adults during verbal recall tasks (Maki et al. 2009). The decreased activation noticed in these regions in the HIV infected group may be associated to a reduced processing ability. When it is considered that previous studies have found the HIV infected brain to have microstructural damage and increased inflammation (Hoare et al. 2018), the grey matter deactivations noted here may have associated white matter limitations, thereby inhibiting grey matter activity as detectable in BOLD fMRI.

Previous studies of healthy and clinical adults have found differential effects of the dorsolateral prefrontal cortex (DLPFC) in working memory tasks (Tomasi et al. 2006; Melrose et al. 2008). The lack of DLPFC findings here may have two explanations. First, our task utilised the MWM levels of the N-back task (0-back and 1-back), which presents a lower cognitive load to participants, as compared to a 2-back or 3-back task. The DLFPC is more associated with manipulation of working memory networks that occurs with higher levels of the task (Ragland et al. 2002). Another potential reason for the relative lack of frontal effects is the young age of the subjects (Bunge et al. 2002). It is possible that incomplete myelination of associative cortical regions, as seen in youth, as compared to adults, may also mean fewer frontal differences between the tests groups (Benes 1989; Sowell et al. 1999).

The role of ART initiation age, and also adherence, is known to be important in reducing the negative HIV-related effects in cognition, among other physiological measures. Our findings provide support for the importance of earlier initiation, and therefore have implications for privately and publicly funded treatment programs in perinatally infected youth. In South Africa there is high risk of delayed treatment, due to limited resources of both patients and public health services. However, findings such as ours indicate a pressing need to enforce early detection and immediate treatment.

Limitations

The inclusion of a 2-back level in the task would have been useful to improve the scope of the task in terms of difference levels of working memory and, as such, the 2-back level has been included in a 3-year follow up study on this cohort. The group-effect results were significant only with an uncorrected p value of 0.001, likely due to low power, but were included due to the exploratory and novel nature of this research.

Conclusion

Significantly reduced activation in cognitive processing networks was noted in HIV infected subjects during an MWM task. In the patients with delayed ART initiation, further deactivations and decreased brain volumes were noted in the same networks as well as the thalamus. The findings presented here provide much needed insight into the development of cognitive systems in this population and have implications for ART programs and their initiation in young perinatally infected children. Namely, earlier ART initiation appears protective of the cognitive and structural neurodevelopment of the young HIV infected brain.

Acknowledgements and conflict of interest

There is no conflict of interest. JH has received support from Medical Research Council (MRC) of South Africa. HZ and DS are supported by the National Research Foundation (NRF) and the Medical Research Council (MRC) of South Africa. This research was supported by NICHD under grant R01HD074051.

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