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Efavirenz is associated with altered fronto-striatal function in HIV+ adolescents

Stéfan Du Plessis[1] · Alexander Perez[2] · Jean-Paul Fouche[3] · Nicole Phillips[3] · John A. Joska[3] · Matthijs Vink[4] · Landon Myer[2,5] · Heather J. Zar[6,7] · Dan J. Stein[3,8] · Jacqueline Hoare[3]

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Abstract

Neurotoxicity associated with the antiretroviral efavirenz (EFV) has been documented in HIV-infected adults, but there are no data on the impact of EFV on brain function in adolescents. We investigated potential alterations in fronto-striatal function associated with EFV use in adolescents. A total of 86 adolescents underwent a Stop Signal Anticipation Task (SSAT) during functional MRI (fMRI), 39 HIV+ adolescents receiving EFV, 27 HIV+ adolescents on antiretroviral therapy without EFV (matched on age, gender, education, CD4 cell count and HIV viral load) and 20 HIV− matched controls (matched on age and gender). The task required participants to give timed GO responses with occasional STOP signals at fixed probabilities. Reactive inhibition was modelled as a correct STOP response and proactive inhibition was modelled after response slowing as the STOP probability increases. A priori mask-based regions associated with reactive and proactive inhibition were entered into two respective multivariate ANOVAs. The EFV treatment group showed significantly blunted proactive inhibitory behavioural responses compared to HIV+ adolescents not receiving EFV. There was no difference in reactive inhibition between treatment groups. We also demonstrated a significant effect of EFV treatment on BOLD signal in proactive inhibition regions. There was no difference in regions involved in reactive inhibition. We found no differences between adolescents not receiving EFV and HIV− controls, showing that functional and behavioural differences were unique to the EFV group. Here, we demonstrate for the first time a potential adverse impact of EFV on higher cortical function in young HIV+ adolescents.

Keywords HIV · Adolescence · Efavirenz · fMRI

Introduction

Despite the early initiation of antiretroviral therapy (ART), HIV-infected children continue to be at risk of neurocognitive impairment secondary to HIV (Hoare et al. 2016). Efavirenz, a non-nucleoside reverse transcriptase inhibitor, is widely used in combination with other antiretroviral agents as a first-line treatment for human immunodeficiency virus (HIV) infection (Decloedt and Maartens 2013). Currently, the continued use of efavirenz (EFV) has raised concerns around neurotoxicity in children and adults (Frange et al. 2018). A growing body of evidence reports that efavirenz (EFV) potentially has an adverse impact on the CNS, and therefore its use in developing children/adolescents should be investigated (Ciccarelli et al. 2011). EFV has neuropsychiatric side effects with transient sleep disturbances being the most commonly reported

✉ Stéfan Du Plessis
stefandup@sun.ac.za

[1] Department of Psychiatry, Faculty of Heath Sciences, Stellenbosch University, Francie van Zijl Avenue, Tygerberg, Cape Town, South Africa

[2] Division of Epidemiology and Biostatistics, School of Public Health & Family Medicine, University of Cape Town, Cape Town, South Africa

[3] Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

[4] Departments of Experimental and Developmental Psychology, Utrecht University, Utrecht, The Netherlands

[5] Centre for Infectious Disease Epidemiology and Research, School of Public Health & Family Medicine, University of Cape Town, Cape Town, South Africa

[6] Department of Pediatrics & Child Health, Red Cross Children's Hospital, UCT, Cape Town, South Africa

[7] SA Medical Research Council Unit on Child & Adolescent Health, Cape Town, South Africa

[8] SA Medical Research Council Unit on Risk & Resilience in Mental Disorders, Cape Town, South Africa

(Molina et al. 2000). Additionally, some studies reported an increased rate of HAND in EFV-treated adults (Ciccarelli et al. 2011). Potential EFV neurotoxicity has also been reported by several animal and in vitro studies (Tovar-y-Romo et al. 2012; Funes et al. 2014; Arend et al. 2016). These findings remain inconsistent, as a recent study found little evidence to support that EFV withdrawal resulted in significant improvement of neurocognitive function in adult participants (Payne et al. 2017).

Although ART in general has been associated with increased signal and poorer performance on functional visual attention tasks in adults (Chang et al. 2007), the potential impact of such therapies on brain function in adolescents have not been investigated thus far (Hakkers et al. 2016). Our aim therefore is to investigate potential brain functional changes associated with current EFV use. As functional MRI (fMRI) is able to detect early changes even in the absence of neurocognitive impairment, it is thought to be a more sensitive measure of the impact of ART on the brain than neurocognitive testing alone (Plessis et al. 2015). The Stop Signal Anticipation Task (SSAT) is an fMRI event-related task that involves important aspects of day-to-day executive functioning (see Fig. 1). It reliably activates well-described regions of the frontal-striatal system known to be involved in HIV infection (Zandbelt and Vink 2010). The task requires participants to give timed GO responses with occasional STOP signals at fixed probabilities indicated to the participants via cues. Simple inhibition is modelled as subject responses during a correct vs. incorrect STOP response (i.e. reactive inhibition). Higher order motor inhibition is modelled as the parametric effect of the expected response slowing as the STOP probability increases (i.e. proactive inhibition) (Zandbelt and Vink 2010). Using this task, we previously demonstrated decreased subcortical activation with higher cortical functions being largely intact in early untreated HIV infection in adults (Plessis et al. 2015).

We hypothesised that alterations in proactive inhibition would be associated with an altered Blood Oxygen Level Dependant (BOLD) signal in regions well known to be active during the anticipation of a STOP signal such as the inferior frontal gyrus, supplementary motor region and the temporo-parietal junction (Zandbelt and Vink 2010). Alterations in reactive inhibition would be associated with differential activation in striatal and associated regions (Zandbelt and Vink 2010). We expected a relative increase in fronto-striatal signal for the ON_{EFV} relative to the OFF_{EFV} group, given previous findings in task-based visual attention in adults (Chang et al. 2007).

Methods

Here, 39 HIV+ adolescents ($M_{Age} = 11$) currently on EFV treatment (ON_{EFV}) as part of their ARV regimen completed the SSAT along with 27 age, education and gender frequency matched HIV+ participants on an ARV regimen not currently including EFV treatment (OFF_{EFV}). Also included were similarly matched 20 HIV− controls as a reference group. Participants included in the present study were from a larger cohort, the Cape Town Adolescent Antiretroviral Cohort (CTAAC), a prospective study of perinatally infected adolescents. All participants were recruited from the public community health care service. Parent or guardian consent as well as participant assent were received from all participants. Ethical approval was obtained from the University of Cape Town's Faculty of Health Sciences research ethics committee (HREC REF 051/2013).

Participants were included if they were aged 9–11 years, perinatally infected and had been on ART for at least 6 months. HIV+ participants consists of a randomly sampled treatment as usual cohort, treated according to the national consolidated guidelines set by the National Department of Health, South Africa (South African Department of Health 2014). All youth screened for the control cohort underwent rapid HIV testing (which included pre- and post-test counselling) prior to enrolment to confirm negative status. Participants were excluded at recruitment phase should they have had an uncontrolled medical condition, such as poorly controlled diabetes mellitus, epilepsy or active tuberculosis requiring admission; an identified CNS condition (other than HIV), such as past or current TB meningitis or bacterial meningitis, documented cerebrovascular accident, lymphoma; a history of head injury with of loss of consciousness greater than 5 min, or any radiological evidence of skull fracture; a history of perinatal complications such as hypoxic ischemic encephalopathy or neonatal jaundice requiring exchange transfusion or neurodevelopmental

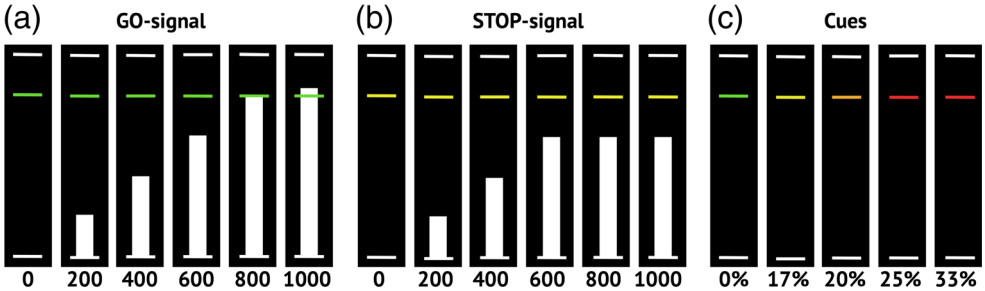

Fig. 1 The Stop Signal Anticipation Task. There are two types of trials: **a** GO signal trials interspersed with **b** occasional STOP signal trials. **c** A changing colour cue indicates the stop-signal probability, which varies from trial to trial

disorder not attributed to HIV. All participants were on standardized ART regime for at least 6 months consisting either of abacavir (ABC), lamivudine (3TC) and EFV or ABC, 3TC and lopinavir/ritonavir (LPV/r). Participants were treated ON_{EFV} or OFF_{EFV} based on weight and age of presentation as per standard national South African clinical guidelines (South African Department of Health 2014). It is not standard practice to treat patients with suspected cognitive decline on a specific treatment regime. Participants not following a standard regime were excluded from the study. A small number of participants in the OFF_{EFV} received EFV at presentation but were switched to ON_{EFV} following standard clinical guidelines (South African Department of Health 2014). HIV+ participants included in the study were randomly sampled from seven public sector health facilities in Cape Town and were intended to be representative of the local population of HIV+ adolescents in care. Recent CD4 cell count, HIV viral load results, ART regimen and date of initiation of ART were extracted from routine health care records.

Image acquisition

During MRI image acquisition, 622 whole-brain two-dimensional-echo planar imaging images (repetition time (TR) = 1600 ms; echo time (TE) = 23 ms; flip angle, 72.5°; field of view (FOV) 256 . 256; 30 slices; 4 mm isotropic voxels) were acquired. Trial-by-trial variability was accounted for by setting the total task length to 17 min. Excess scans were discarded. For image registration, a multi-echo Magnetization Prepared Rapid Acquisition Gradient Echo (ME-MPRAGE) T1-weighted image was acquired with the following parameters: FOV = 256 × 256 mm, TR = 2530 ms, TE = 1.53/3.21/4.89/6.57 ms, TI = 1100 ms, flip angle = 7°, 144 slices, in-plane resolution = 1.3 × 1.0 mm^2 and slice thickness of 1.0 mm.

Functional imaging

During the functional MRI (fMRI) experiment, participants performed the STOP signal anticipation task (Zandbelt and Vink 2010). For more details on the SSAT, see Zandbelt and Vink (Zandbelt and Vink 2010). Participants were trained on the task in their home language prior to the scan session. The experiment was performed using the Presentation® software (version 14.6, www.neurobs.com). The task is based on original work by Logan et al. (1984) who proposed a horse-race model, suggesting that a response, either GO or STOP, is a result from a race between the GO process and the STOP process. The response is stopped when the STOP process finished before the GO process reaches execution threshold (Logan and Cowan 1984). The task and experimental procedures were identical to those described before as performed by adults (Zandbelt and Vink 2010) and adolescents (Vink et al. 2016).

During task performance, participants were presented with three background lines. In each trial, a bar moved at a constant speed from the bottom line to the top line, passing the middle line within 800 ms. On GO trials, participants were required to stop the bar with a button press using their right index finger, as close to the middle/coloured line as possible. Should the bar reach the top line after 1000 ms, the GO trial was considered a failure. The inter-trial interval was kept at 1000 ms. On STOP signal trials, the bar stopped on its own, indicating a STOP signal. During STOP trials, the participant was required to withhold the button press (reactive response inhibition).

To measure proactive response inhibition, the probability of the stop-signal was explicitly indicated to the participant by the colour of the middle line. This allowed a participant to proactively anticipate a STOP signal in each STOP trial, by taking the stop-signal probability into account. There were five stop-signal probability levels, 0% (green), 17% (yellow), 20% (amber), 25% (orange) and 33% (red). In total, 414 go trials (0%, $n = 234$; 17%, $n = 30$; 20%, $n = 48$; 25%, $n = 54$; 33%, $n = 48$) and 60 STOP trials (17%, $n = 6$; 20%, $n = 12$; 25%, $n = 18$; 33%, $n = 24$) were presented in a single run in pseudorandom order.

The STOP signal delay (SSD), the interval between start of a trial and the STOP signal, was initially 550 ms and varied for each STOP signal according to a staircase procedure. That is, should a STOP trial be successful, the trial difficulty was increased by increasing the STOP signal delay by 25 ms. Should a STOP trial be unsuccessful, trial difficulty was decreased in the same manner. This technique assured an equal amount of successful and unsuccessful STOP trials.

Analysis of task performance

Reactive inhibition was studied using the Stop Signal Reaction Time (SSRT) (Logan and Cowan 1984; Zandbelt and Vink 2010). The SSRT was calculated according to the integration method (Logan and Cowan 1984) and pooled across all STOP signal probability levels. Proactive inhibition generally involves subjects instinctively slowing down their response times as the STOP probability increases. Therefore, in keeping with previous studies (Vink et al. 2005; Zandbelt and Vink 2010; Vink et al. 2014), proactive inhibition was calculated as the effect of STOP signal probability on GO signal response time. To compare inhibition across the treatment groups, a repeated measure analysis of variance (RM-ANOVA) was undertaken with mean GO signal response times as the dependent variable, and with STOP signal probability and EFV treatment status as factors.

fMRI data analysis

Images were analysed using SPM12 (http://www.fil.ion. ucl.ac.uk/spm/software/spm12/). Pre-processing and first-level

statistical analysis was undertaken as previously described (Zandbelt and Vink 2010). In brief, pre-processing involved correction for slice timing differences, re-alignment to correct for head motion, spatial normalization to the Montreal Neurological Institute template brain and spatial smoothing to accommodate inter-individual differences in neuro-anatomy. Head motion parameters were analysed to ensure that the maximum motion did not exceed a predefined threshold (maximum motion > 2 mm) (Van Dijk et al. 2012).

Region of interest analysis

The fMRI data were modelled voxel-wise, using a general linear model, in which the following events were included as regressors: Timed GO signal trials with STOP signal probability > 0%, successful STOP signal trials and failed STOP signal trials. For GO signal trials with a STOP probability above 0%, we also included a parametric regressor modelling the STOP signal probability level as well as variations in response time. GO baseline (0% STOP probability) as well as activity during rest was explicitly modelled. The fMRI data were high-pass filtered (cut-off 128 Hz) to remove low-frequency drifts. For each participant, we computed two contrast images: (1) Activation during successful STOP signal trials versus failed STOP signal trials to assess reactive inhibition and (2) the parametric effect of STOP signal probability on GO signal activation (to assess proactive inhibition).

We assessed group activation differences in predefined a priori regions of interest (ROIs), using mask-based activation maps acquired in a previous experiment (Zandbelt and Vink 2010), in which an independent sample of healthy volunteers performed the same task. The brain mask ROIs were defined using a cluster-level threshold (cluster-defining threshold $p < 0.001$, cluster probability of $p < 0.05$, family-wise error corrected for multiple comparisons). Mean values of activation during reactive inhibition as well as proactive inhibition were calculated over their respective regions as defined by a priori masks as described above. For regions included in the activation masks, see Fig. 2.

Since differences in the various activation regions are potentially complex for both reactive and proactive inhibition and highly intercorrelated, in our primary analysis, we considered correlations between the various regions using a multivariate analysis technique (Bray and Maxwell 1982). These activation values (expressed as percent signal change) were entered into two separate Multivariate analysis of variance (MANOVA) models assessing the effect of efavirenz therapy on regions involved in proactive and reactive inhibition, respectively. Subsequent post hoc ANOVAs using each individual ROI as a dependent variable were conducted exploring potential relationships between various brain regions involved in proactive and reactive inhibition, respectively. This was done to explore potential directionally of differences found in the main analysis, as well as regional specificity of the findings. As our main aims do not necessitate differences in specific subregions involved in reactive and proactive inhibition nor were they dependant on a particular directionality per se, we regarded these post hoc analyses as exploratory and therefore did not correct for multiple comparisons. We did, however, account for the number of MANOVAs performed as part of the main analysis using the Bonferroni correction method.

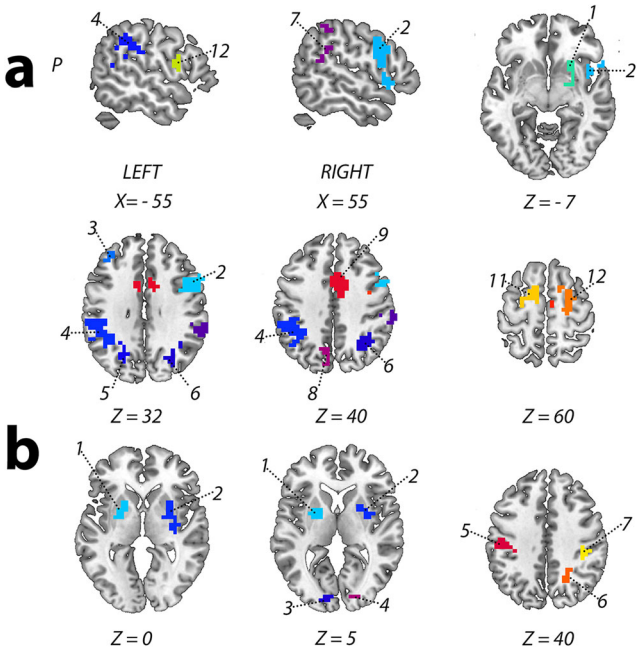

Fig. 2 A priori regions used to assess group differences in brain activation during **a** proactive and **b** reactive inhibition. All ROIs were based on a brain mask derived from a previous experiment (Zandbelt and Vink 2010) in which an independent sample of healthy volunteers performed the same task. A cluster-defining threshold of $p < 0.001$, cluster probability, $p < 0.05$ family-wise error (FWE) correction was used for both brain masks. **a** Proactive inhibition regions were the (1) right striatum; (2) right inferior frontal cortex, extending into the precentral gyrus; (3) left middle frontal gyrus; (4) left temporo-parietal junction; (5) left superior parietal gyrus, extending into the angular gyrus; (6) right superior parietal gyrus, extending into the angular gyrus; (7) right temporo-parietal junction; (8) left precuneus; (9) anterior cingulate gyrus, extending into the superior frontal gyrus; (10) right superior frontal gyrus; (11) left superior frontal gyrus; (12) left inferior frontal gyrus and (13) right anterior insula (not shown). **b** Reactive inhibition regions were the (1) left putamen, (2) right putamen, (3) left middle occipital gyrus, (4) right middle occipital gyrus, (5) left pre/postcentral gyrus, (6) right precuneus and (7) right supramarginal gyrus

As per the standard treatment guidelines, all adolescents included in the study receive either a fixed dose combination of lopinavir/ritonavir (LPV/r) or efavirenz based on their weight (i.e. > 10 kg/< 40 kg for EFV and < 10 kg for LPV/r) and age of first presentation to the local clinic (i.e. 3–10 years for EFV and < 3 years for LPV/r) (South African Department of Health 2014). This was then combined with abacavir (ABC) and lamivudine (3TC) using standard prescribed dosages for all HIV+ participants.

Results

Demographics

A total of 33 HIV+ patients and 10 controls were excluded from the study due to motion-related artefacts and quantitative signal-to-noise quality assessment (Geissler et al. 2007). Of the excluded HIV+ participants, 14 were on EFV. The number of exclusions per group did not significantly differ ($X^2 = 0.517$, $p = 0.772$). One HIV+ participant was excluded due to poor task comprehension and poor base line performance prior to analysis. The number of exclusions in the group currently on EFV treatment and the group not currently on treatment also did not differ significantly ($X^2 = 3.200$, $p = 0.202$).

After exclusions, a total of 39 HIV+ adolescents ON_{EFV} and 27 HIV+ OFF_{EFV} treatment were included in the final analysis. Twenty controls were also included in the final analysis (see Table 1).

As previously reported, HIV+ children had worse school performance ($X^2 = 6.494$, $p = 0.039$) (Hoare et al. 2018). There was no significant between-group difference in school performance between the EFV-treated and untreated groups, however ($X^2 = 2.1$, $p = 0.14$). The two ARV treatment groups were matched for age, gender, highest grade achieved, age of initiation of ART, viral load and CD4 count. HIV− controls were matched for age, gender and education. As the HIV-infected groups were matched for age, gender, highest grade achieved, age of initiation of ART, duration of ART, viral load and CD4 count, when including them in the functional and behavioural assessments, the results remained unchanged. These factors were therefore not considered in the final analysis.

Task performance

Motor responses

Participants ON_{EFV} had similar baseline GO reaction times (i.e. GO0) when no STOP signal could occur compared to HIV+ participants OFF_{EFV} ($t(64) = 0.812$, $p = 0.420$). The HIV treatment groups also did not differ in terms of GO0 response accuracy ($t(64) = 0.377$, $p = 0.708$).

Reactive inhibition

All groups responded significantly faster during incorrect STOP trials than during successful GO trials, indicating that the underlying assumptions of the SSAT task is valid for this study population ($F(1.83) = 270.185$, $p < 0.001$) (Logan et al. 1984). There was no difference between the two treatment groups in terms of stop signal reaction time ($t(64) = -.373$, $p = 0.710$) nor accuracy during reactive inhibition ($M_{ON\_EFV} = 46.4 \pm 1.2\%$; $M_{OFF\_EFV} = 50\% \pm 1.4\%$; $t(64) = 1.678$, $p = 0.098$). This task accuracy was to be expected given that we manipulated the stop signal delay according to

Table 1 Baseline demographic and clinical characteristics of the CTAAC cohort

	HIV+		Controls	Statistic	P*
	OFF EFV $N = 27$	ON EFV $N = 39$	$N = 20$		
Gender (M/F)	11/16	18/21	4/16	$X^2 = 0.532$	0.141
Age (years)	11.00 (1.04)	10.87 (0.894)	10.90 (1.02)	$F = 0.144$	0.866
Highest grade achieved	3.33 (1.78)	3.26 (0.910)	3.55 (1.432)	$F = 0.448$	0.640
Repeated grades (yes/no)	11/16	23/16	5/15	$X^2 = 6.494$	0.039*
Executive functioning	− 0.24 (0.64)	− 0.47 (0.68)	0.055 (0.58)	$F = 4.335$	0.016*
Age of initiation of ART (years)	3.29 (2.25)	4.32 (2.96)	N/A	$t = -1.530$	0.131
Duration of EFV treatment (years)	0.69 (1.23)	5.6 (2.57)	N/A	$t = -9.626$	< 0.001**
Viral load (copies/mL)	7.41 (15.83)	9.41 (16.82)	N/A	$t = -0.487$	0.628
CD4 count (cells/mm3)	1035.15 (321.70)	1003.03 (488.20)	N/A	$t = 0.299$	0.766

Demographic characteristics of the diagnostics groups. Age, viral load and CD_4 data represent mean ± SD. Annual household income brackets: 0-R5000 ZAR/5001+ ZAR. The percentage presented above represents the percentage of participants who earn up to 5000 ZAR per annum. Data represents standardized values ± SD. Adolescents on EFV received a combination of abacavir (ABC), lamivudine (3TC) and EFV according to standard treatment guidelines. Those not receiving EFV were on a standardized combination of ABC, 3TC and lopinavir/ritonavir (LPV/r)

M male, *F* female, *ART* antiretroviral therapy, *EFV* efavirenz

One-way-ANOVA across OFF_{EFV}/ON_{EFV} and Control groups:

*Results significant at a $p = 0.05$ value

**Results significant at a $p < 0.001$ value

individual performance to ensure balanced successful and unsuccessful STOP trials.

Proactive inhibition

Analysis of proactive inhibition showed a significant main effect of STOP signal probability on response times ($F(4.61) = 5.130$, $p < 0.003$). Participants generally responded slower, the more likely a response signal was to occur, demonstrating adequate task performance. There was, however, a significant treatment by STOP signal probability interaction, with the group ON_{EFV} treatment showing blunted proactive inhibitory control ($F(4.64) = 3.504$, $p = 0.021$) (see Fig. 3). To estimate the potential impact of HIV on the present results, we then repeated the same analysis for our matched controls and HIV+ participants OFF_{EFV} treatment. We found no significant effect of group, indicating poor task performance is only present in the EFV treatment group ($F(4.42) = 1.874$, $p = 0.133$), with no difference between HIV+ cases OFF_{EFV} and healthy matched controls.

fMRI results

Reactive inhibition

There was no significant main effect of group in fMRI BOLD signal on reactive inhibition on multivariate analysis ($F(7.58) = 1.721$, $p = 0.122$).

Proactive inhibition

There was a significant main effect of group on proactive inhibition task related BOLD activity on our multivariate analysis ($F(13.53) = 2.961$, $p = 0.003$) when comparing the HIV+ ON_{EFV} treatment and the OFF_{EFV} groups while correcting for multiple comparisons. Follow-up uncorrected univariate ANOVAs indicated significant decreased activity in the right striatum ($F(1.64) = 4.013$, $p = 0.049$), left temporo-parietal junction ($F(1.64) = 8.122$, $p = 0.006$), right temporo-parietal junction ($F(1.64) = 5.228$, $p = 0.026$), left precuneus ($F(1.64) = 5.588$, $p = 0.021$) and right anterior insula ($F(1.64) = 6.230$, $p = 0.015$) (see Fig. 4). Again, we found no significant group effect between HIV+ adolescents OFF_{EFV} and matched healthy controls when we repeated the same analysis as for the behaviour data. This indicated the increased activity is only present in the EFV treatment group and not associated with HIV infection per se ($F(13.32) = 0.558$, $p = 0.868$).

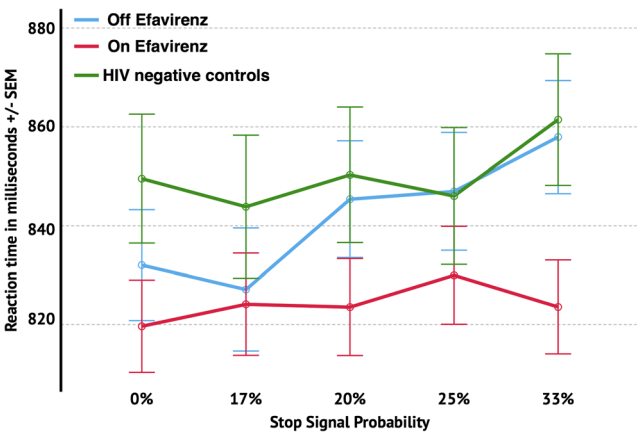

Fig. 3 Effect of stop-signal probability on GO signal response time across groups with error bars indicating the standard error of the mean. Subjects not on current EFV treatment as well as HIV− controls demonstrate normal response slowing, as the STOP probability increases. Those on current EFV treatment show no such response

Exploratory whole-brain analysis

An exploratory whole-brain analysis set at FWE error multiple comparisons correction, $p < 0.05$ with an extent threshold of 30 voxels, revealed no further additional results of note.

Discussion

In this study, we examined the potential effects of efavirenz on brain function using a SSAT task on 39 ON_{EFV} HIV+ adolescents with 27 matched HIV+ OFF_{EFV}. We also used 20 matched HIV− controls as a reference group. The EFV treatment group showed similar GO response speeds and accuracy during the GO0 control condition to the HIV+ OFF_{EFV} group. Those on ON_{EFV} treatment showed significantly blunted proactive inhibition compared to OFF_{EFV} HIV+ participants. There was no difference in reactive inhibition between the two treatment groups. We also demonstrated a significant effect of EFV treatment on BOLD signal in a priori proactive inhibition regions, with no difference in regions involved in reactive inhibition. Here, we demonstrate for the first time a potential adverse impact of EFV on higher cortical function in adolescents.

There was no difference in baseline GO0 responses between treatment groups, which indicated both groups were able to perform the basic elements of the task. There were no differences in reactive inhibition between the treatment groups, showing that basic inhibition process is unlikely to be affected by EFV use. Strikingly, proactive responses were significantly affected in the EFV treatment group. This shows that the ON_{EFV} group did not slow down their responses appropriately as the STOP signal probability increased. Reduced proactive inhibition has been linked with working memory impairment, likely linked to a general disruption in executive functioning (Zandbelt et al. 2011). As it has been shown that the ability to proactively inhibit motor responses is under

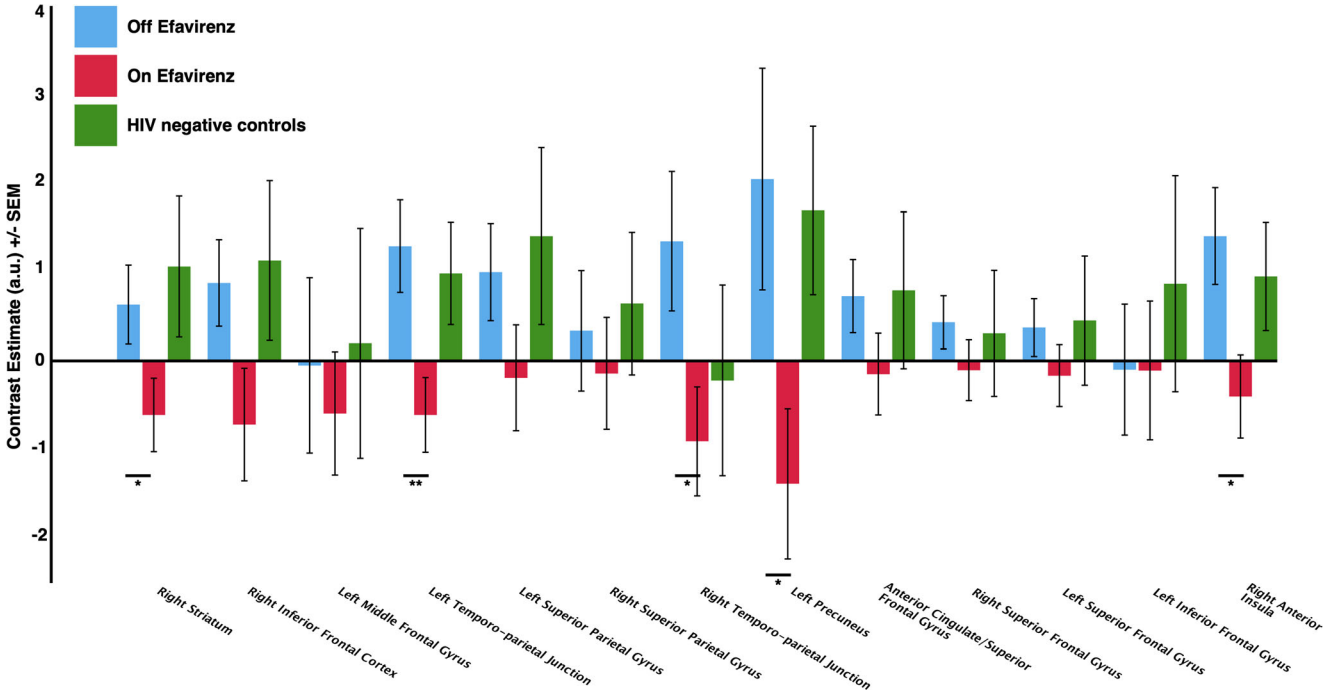

Fig. 4 Activity in a priori mask regions during proactive inhibition across groups. Error bars indicate the Standard Error of the Mean (SEM). Subjects not on current EFV treatment and healthy controls show normal activity levels, whereas subjects on EFV treatment demonstrate a significant signal decrease in several regions. *Significant at $p < 0.05$ level. **Significant at $p < 0.01$ level

active development in our participants' age group (Vink et al. 2014), children could be at increased risk for any potential neurotoxic effects. The behavioural effects found here may not only represent a specific impact of EFV on proactive response inhibition, but may also point to a negative impact on general executive functioning.

We found no differences in BOLD responses in terms of reactive inhibition. However, as predicted by the poor proactive behavioural responses seen in the EFV treated group, we demonstrated a significant difference in BOLD responses in regions involved in proactive inhibition in the EFV-treated group. Indeed, previously, it has been shown that ART is associated with differential frontal activation during visual attention in ART-treated adults (Chang et al. 2007). Given our negative findings during both behavioural measures as well as functional measures of basic inhibition, our finding of abnormal function during proactive inhibition suggests that EFV may have an impact on higher order cognitive control and potentially general cortical function. The general direction of differences seen on BOLD activation during proactive inhibition is at odds with the previous study done in HIV treated adults, which found relative increases in the ART group (Chang et al. 2007). It should be noted, however, that our post hoc exploratory test for specific regional differences were not corrected for multiple comparisons, and therefore the regional specificity as well as directionality of the functional differences should be interpreted with caution. Nevertheless, a clear trend was evident of decreased signal for all regions involved in proactive inhibition for adolescents on EFV treatment, which is in keeping with the significantly poor behavioural responses when compared to those on EFV treatment.

Our findings indicate functional as well as behavioural differences in HIV+ adolescents receiving treatment as usual. HIV+ adolescents received either LPV/r or EFV based on their age as well as weight at first presentation. There are several factors that singles EFV out as being potentially neurotoxic. Although it is known that EFV has a high CNS penetration effectiveness, it seems unlikely that the differing results in the EFV group is based on increased CSF availability of EFV alone. For instance, it has been found that the LPV/r alternative used in our present cohort has a similar CPE to that of EFV (Cusini et al. 2013). Animal studies, however, show EFV-treated rats display memory deficits and increased stress levels, which was associated with increased proinflammatory cytokines interleukin-1 beta and tumour necrosis factor-alpha levels (O'Mahony et al. 2005). Another study found creatine kinase (CK) concentrations to be significantly reduced in the brains of mice treated on EFV. Impaired energy metabolism, involving the he CK-catalysed ATP buffering system, has been considered as an important factor in neurogenerative disease (Blas-Garcia et al. 2010). EFV has also been found to cause a concentration-dependant mitochondriopathy in human hepatic cells. It is known that mitochondria disfunction impacts cell survival and has been associated with ageing and dementing processes (Swerdlow 2011). More importantly, EFV and its metabolites have shown direct toxic effects on

neuronal cultures in vitro by disrupting calcium homeostasis (O'Mahony et al. 2005).

Several limitations of the study deserve emphasis. First, as we included a relatively narrow age range, limited conclusions can be drawn regarding the potential impact of EFV on neurodevelopment. Nevertheless, a clear parallel seen in both behaviour and task function is clear cause for concern in our present cohort. The impact of EFV, however, on further neurodevelopment and potential mediating factors will need to be determined in a longitudinal design. Second, our sample was randomly sampled, receiving treatment as usual; a randomized control trial would need to be conducted to confirm the specific role of EFV on cognition and brain function. Third, CSF drug levels were not available for the present sample, but as previously stated the results of our present study are unlikely based on CPE alone. Finally, our present sample selection could potentially include a survivorship bias. That is, our results could potentially include compensatory hyperfunction in more resilient children, and the behavioural results representing ultimate decompensation. We expect very little confounding influence of clinical cognitive status on the functional and behavioural effects we found on the SSAT, as cognitive decline is not routinely an indication for EFV treatment.

Despite clear behavioural as well as functional differences seen in the EFV-treated group in keeping with an adverse impact of EFV on fronto-striatal function, the clinical relevance of these findings remains uncertain. fMRI has been shown to be highly sensitive in detecting an impact of HIV on brain function, being present even in patients with no measurable cognitive decline (Ernst et al. 2002). Nevertheless, it is concerning, as function associated with proactive inhibition continues to develop right through adolescence (Vink et al. 2014). The long-term effects of EFV on proactive inhibition and associated frontal neuro-circuitry are therefore uncertain given our present results. It is possible that children are particularly vulnerable to the potential neurotoxic effects of EFV, due to active neurodevelopment. The current findings support the ongoing evaluation of subclinical neurological effects of EFV as well as related ARVs in HIV-infected children and adolescents. Follow-up studies are needed to examine the long-term implications of EFV on neurodevelopment.

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Author's contribution SDP: Conceptualization, study design, recruitment supervision, fMRI task administration supervision, data processing, analysis, interpretation and lead author on manuscript preparation. AP: Data processing, analysis, interpretation and manuscript preparation. JPF: Conceptualization, study design, recruitment supervision, fMRI task administration supervision, data processing, analysis, interpretation and manuscript preparation. NP: Conceptualization, study design, recruitment supervision, fMRI task administration supervision and manuscript preparation. JJ: Conceptualization, study design, interpretation and manuscript preparation. MV: Task design, data processing, analysis, interpretation and manuscript preparation. LM: Conceptualization, study design, analysis, interpretation and manuscript preparation. HZ: Conceptualization, study design, interpretation and manuscript preparation. DS: Conceptualization, study design, interpretation and manuscript preparation. JH: Conceptualization, study design, interpretation and manuscript preparation.

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Conflict of interest The authors declare that they have no conflict of interest.

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