SLEEPJ, 2021, 1–9

doi: 10.1093/sleep/zsab058
Advance Access Publication Date: 30 March 2021
Original Article

ORIGINAL ARTICLE

HIV increases sleep-based brain age despite antiretroviral therapy

Michael J. Leone[1,*], Haoqi Sun[1,*], Christine L. Boutros[1], Lin Liu[2,4], Elissa Ye[1], Lee Sullivan[1], Robert J. Thomas[3], Gregory K. Robbins[1], Shibani S. Mukerji[1] and M. Brandon Westover[1,†]

[1]Department of Neurology, Massachusetts General Hospital, Boston, MA [2]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA [3]Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA [4]Present address: Institute of Natural Sciences, MOE-LSC, School of Mathematical Sciences and SJTU-Yale Joint Center for Biostatistics and Data Science, Shanghai Jiao Tong University, Shanghai, China

*These authors contributed equally to this work.
†Corresponding author. M. Brandon Westover, Department of Neurology, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114. Email: mwestover@mgh.harvard.edu.

Abstract

Study Objectives: Age-related comorbidities and immune activation raise concern for advanced brain aging in people living with HIV (PLWH). The brain age index (BAI) is a machine learning model that quantifies deviations in brain activity during sleep relative to healthy individuals of the same age. High BAI was previously found to be associated with neurological, psychiatric, cardiometabolic diseases, and reduced life expectancy among people without HIV. Here, we estimated the effect of HIV infection on BAI by comparing PLWH and HIV– controls.

Methods: Clinical data and sleep EEGs from 43 PLWH on antiretroviral therapy (HIV+) and 3,155 controls (HIV–) were collected from Massachusetts General Hospital. The effect of HIV infection on BAI, and on individual EEG features, was estimated using causal inference.

Results: The average effect of HIV on BAI was estimated to be +3.35 years ($p < 0.01$, 95% CI = [0.67, 5.92]) using doubly robust estimation. Compared to HIV– controls, HIV+ participants exhibited a reduction in delta band power during deep sleep and rapid eye movement sleep.

Conclusion: We provide causal evidence that HIV contributes to advanced brain aging reflected in sleep EEG. A better understanding is greatly needed of potential therapeutic targets to mitigate the effect of HIV on brain health, potentially including sleep disorders and cardiovascular disease

Statement of Significance

There is concern that HIV causes advanced brain aging despite antiretroviral therapy, and biomarkers are greatly needed to identify this effect in people living with HIV (PLWH). Using a machine learning model of brain aging based on sleep EEG, we found that HIV increases brain age after adjusting for potential confounders. We also found that slow waves were markedly attenuated during deep sleep among PLWH. Our study shows that sleep EEG can be used to measure brain age in PLWH, which can serve as an inexpensive and easily deployable biomarker.

Key words: HIV; sleep; EEG; brain age; machine learning

Submitted: 18 June, 2020; **Revised:** 6 January, 2021
© Sleep Research Society 2021. Published by Oxford University Press on behalf of the Sleep Research Society.
All rights reserved. For permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022

Introduction

People living with HIV (PLWH) in the current antiretroviral therapy (ART) era are at increased risk for age-related comorbidities including cardiovascular diseases [1–3], metabolic disorders [4, 5], osteoporosis, frailty [6], and HIV-associated neurocognitive disorders (HAND) [7, 8]. Recent studies based on magnetic resonance imaging (MRI) show that brains of PLWH have structural changes characteristic of older individuals [9, 10], suggesting advanced brain aging.

One critical factor not addressed in the current ART era is the relationship between brain aging and sleep. Sleep changes predictably with age [11–13]. For example, slow wave activity (SWA, EEG oscillations of <4 Hz) during deep sleep is known to attenuate gradually beyond puberty [14]. Notably, reductions in SWA are also seen in disease states such as alcohol use disorder, insomnia, and dementia. Descriptions prior to effective ART described HIV-mediated changes in slow wave power during non-REM sleep early during infection [15], and later on diminished sleep spindles [16] and increased sleep fragmentation [17]. Recent studies show high rates of insomnia and other sleep disturbances [18, 19], but more comprehensive analyses are needed.

We previously developed a machine learning algorithm that predicts sleep-EEG-based brain age using a dataset of 2,532 healthy HIV– participants [20]. Our prior work showed that neuropsychiatric diseases, hypertension, and diabetes are associated with increased brain age [20], and excess brain age predicts higher mortality [21]. Recently we showed that brain age monotonically increases from cases of non-dementia to mild cognitive impairment to dementia [22]. Due to advances in home-based EEG, sleep EEGs are inexpensive, participant-friendly [20], and more accessible for use in low–middle-income countries [23–25]. Here, we investigate the impact of HIV on brain aging measured by the brain age index (BAI), the difference between brain-predicted age and chronological age. We first estimated the effect of HIV infection on BAI. Then we estimated the effect on individual sleep EEG features in HIV+ compared to HIV– controls. In preliminary analysis, we additionally found that cardiovascular and sleep-related diseases may potentially mediate the effects of HIV on BAI. Overall, sleep EEG is found to be a potential new biomarker of brain aging in PLWH.

Methods

Standard protocol approvals, registrations, and patient consents

The study was conducted under a protocol approved by the Partners Institutional Review Board, with waiver of written consent.

HIV+ and HIV– cohorts

We conducted a retrospective cohort study at Massachusetts General Hospital. Using the Partners Research Patient Data Registry (RPDR), a large database of historical electronic health records, we retrospectively searched for HIV+ patients (Figure 1). We identified all patients who had undergone a full night diagnostic sleep study between 2008 and 2018, who had received an International Classification of Diseases, Ninth or Tenth Revision, Clinical Modification (ICD-9-CM or ICD-10-CM, respectively) billing code of B20 (ICD-10) or 042 (ICD-9), which indicates HIV infection. HIV billing codes are highly sensitive for patients with a diagnosis of HIV [26]; however, there are cases where an HIV billing code is assigned for other reasons such HIV testing, even if the test is negative. Therefore, for each of these patients, we performed manual chart review to confirm HIV infection and that the HIV diagnosis occurred before the date of the sleep study. HIV is the exposure of interest in this study. HIV– controls were drawn from the Massachusetts General Hospital sleep lab dataset described in [20] that had the same sleep study type as the HIV+ participants (full-night, diagnostic studies). No HIV+ participants were of Asian or Middle Eastern descent, so we excluded controls of those racial and ethnic backgrounds.

Sleep EEG-based brain age: outcomes

We used the same preprocessing and brain age computation used in [20]. Sleep EEG features from six channels (F3-M2, F4-M1, C3-M2, C4-M1, O1-M2, O2-M1) were computed and averaged across all 30-s epochs according to sleep stage (Wake, REM, N1, N2, N3). We log-transformed and standardized (z-score) the features before using them as inputs to the brain age prediction model. There were 480 features, 96 for each of the five sleep stages. See [20] for details on all features. Sleep EEG frequency bands which a portion of the features were based on are defined as follows: delta band (δ, 1–4 Hz), theta band (θ, 4–8 Hz), alpha band (α, 8–12 Hz), and spindle or sigma band (σ, 11–15 Hz). For each participant, the model outputs a brain age (BA), which was compared to chronological age (CA) to obtain the BAI: BAI = BA – CA. We treated BAI as the primary outcome of interest. The log-normalized, unstandardized version of each individual EEG feature was treated as a secondary outcome of interest.

Clinical data acquisition: HIV-related

We performed manual chart review to determine AIDS history, viral load, ART adherence and HIV medication history, as well as a history of secondary brain infection, a brain tumor, or HIV

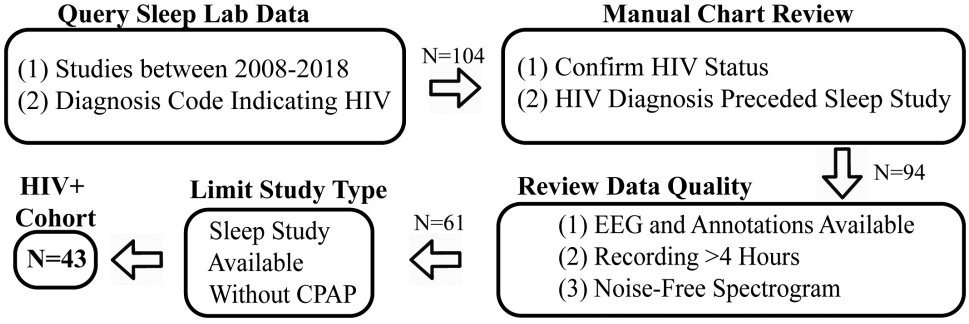

Figure 1. Flowchart of HIV+ participants inclusion and exclusion. N is the number of HIV+ participants eligible following each evaluation step. 43 HIV+ participants with diagnostic studies of sufficient quality were ultimately selected for analysis. CPAP, continuous positive airway pressure.

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022

encephalitis. AIDS was defined as a documented CD4 count below 200, or confirmation of AIDS or an opportunistic infection preceding the sleep study in a physician's medical note. We found the viral load measure nearest to the sleep study within 1 year to determine viral suppression near sleep testing. Viral loads labeled as undetected or below 200 copies/mL were considered undetectable in this study. Adherence to ART was determined by evidence of continuously prescribed medications and maintenance of CD4+ cell levels. We gathered data on efavirenz, a commonly prescribed non-nucleoside reverse transcriptase inhibitor, due to its known neuropsychiatric effects and effects on sleep [27, 28]. Additionally, we gathered usage of integrase strand transfer inhibitors (INSTIs; dolutegravir, raltegravir, bictegravir, elvitegravir), because of their potential neuropsychiatric effects [29].

Clinical data acquisition: covariates

We gathered the following covariates (collectively denoted as C): age, sex (male/female), race (Black/Hispanic/White/Other), and history of tobacco use disorder or alcohol use disorder. Substance use disorder was not included among covariates because we determined that this categorization was too ambiguous and potentially unreliable, as discussed in more detail in Supplementary Material: Results. The other covariates mentioned above were treated as potential confounders; that is, they could affect both the prevalence of the exposure, HIV, and the primary outcome, BAI, or the secondary outcomes, the features used to compute BAI. We used Phenorm [30] to reduce the risk of bias in gathering data. Phenorm is a validated machine learning tool to infer the presence of medical conditions based on automated analysis of clinical notes and ICD codes.

We collected data on additional covariates which we determined were unlikely to act as confounders, but rather could be influenced by HIV infection and act as mediators of HIV's effects on brain aging. These include co-morbidities that are more common in the setting of HIV and may affect brain health (presence determined by Phenorm). We also treated multiple categories of medications that can affect sleep as potential mediators because HIV can potentially increase the risk of neuropsychiatric disorders and the need to be treated with these medications. All data and results of mediation analysis are presented in the Supplemental Material.

Causal inference analysis

The total effect (TE) of HIV infection on BAI is the difference in average BAI between HIV+ and HIV– participants after adjusting for potential confounders. The TE can be estimated via a randomized controlled trial, or in cases where such a trial is not possible, by adjusting for confounding computationally. We therefore estimated TE from retrospective observational data through the potential outcome framework. In this framework, two potential outcomes are estimated for each participant through statistical modeling: the potential BAIs if assigned either to the HIV+ group or to the HIV– group. Each potential outcome is then averaged across the participants. The TE is the difference between the sample-averaged BAI in the HIV+ assignment group and the sample-averaged BAI in the HIV– assignment group. Although we show these sample-averaged potential outcomes, the TE is of greatest interest. Note that the TE is also referred to as the Average Causal Effect (ACE). See Supplemental Material: Methods: Causal Inference Assumptions for the precise definition of the ACE/TE and for details on assumptions made in causal statistical inference.

We used doubly robust estimation (DRE) to estimate the TE due to its robustness against model misspecification bias. DRE requires fitting two models: (1) the outcome model, a model of how HIV status and covariates predict BAI and (2) the propensity model, a model of how covariates influence HIV status. We fit these models with nested fivefold cross validation. See Supplemental Material: Methods: Doubly Robust Estimation for more details on DRE, and Supplemental Material: Methods: Cross-Validation for further details on the cross-validation procedure. We also performed matching as an additional nonparametric analysis to estimate the TE of HIV on BAI. Methods and results are described in the Supplemental Material.

Statistical analysis

In comparison of BAI between the HIV+ and HIV– cohorts, we calculated a p-value using the two-sided student's t-test. For the outcomes in analyses (BAI and BAI features), we calculated p-values, the standard error of the mean (*SEM*), and 95% confidence intervals. For p-values, we report the actual value, except where $p < 0.01$. For other continuous variables, p-values are calculated via the rank-sum test, and for categorical variables, by the chi-squared test. To adjust for multiple comparisons when evaluating the statistical significance of BAI features, we determined the appropriate significance threshold for a maximum false discovery rate (FDR) of 0.1 [31]. We estimated confidence intervals using bootstrapping, repeatedly sampling with replacement such that each bootstrapped sample size was equal to the sample size of the original dataset. For each bootstrapped dataset, the TE of HIV infection on BAI was estimated. We performed bootstrap resampling 1,000 times. We obtained 95% confidence interval from the 2.5% and 97.5% percentiles of the bootstrap distribution.

Sensitivity analysis

We performed sensitivity analysis using the E-value, defined as the minimum effect that an unmeasured confounder would need to have with both the prevalence of HIV infection and BAI to reverse our findings [32]. *E*-value is computed as $E = \mathrm{RR} + \sqrt{\mathrm{RR}\,(\mathrm{RR}\text{-}1)}$, where RR is the risk ratio of the effect of HIV on BAI. Since BAI is a continuous outcome, we assume it follows a Gaussian distribution with mean from the causal inference and standard deviation from bootstrapping and compute the cumulative probability of BAI greater than 0. Therefore, RR = P(BAI>0 | HIV+ among all participants) / P(BAI>0 | HIV– among all participants).

Data availability statement

De-identified, derived data supporting the findings of this study are available from the corresponding author on request.

Results

BAI and covariates characteristics in HIV+ and HIV–

We identified 3,155 HIV– control patients, and 43 HIV+ patients (Figure 1) with comparable diagnostic sleep studies. Among

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022

HIV+ compared to HIV– patients (Table 1), there were more men (79% HIV+ vs. 51% HIV–, $p < 0.01$), greater percentages of individuals with tobacco use (46% vs. 21%, $p < 0.01$) and alcohol use (37% vs. 8%, $p < 0.01$). Age (50 vs. 49 years old, $p = 0.77$) and the proportion of white patients between HIV+ and HIV– cohorts (79% vs. 70%, $p = 0.16$) were similar. Of 41 HIV+ participants with viral load data available, 38 had suppressed viral loads at the time of the sleep study. There were no cases of past secondary brain infection or a brain tumor, and one case of HIV encephalitis thought to be due to immune reconstitution inflammatory syndrome (neuro-IRIS), from which the patient recovered. Their BAI was –2.50. A total of 9 (44%) HIV+ participants had a history of AIDS. Four participants were prescribed efavirenz. A total of 13 were prescribed an integrase strand transfer inhibitor (INSTI) at the time of their sleep study. We compared the distribution of BAIs of the INSTI group versus the group that was not on INSTIs. The average BAI of the INSTI group was 1.33 while the average BAI of the non-INSTI group was 7.34 (Mann–Whitney *U*-test, $p = 0.017$). All HIV+ participants were determined by chart review (M.L., C.B., G.R.) to be adhering to ART at the time of the sleep study.

HIV– participants had a mean BAI of –0.18 years (*SD* = 10.24) and 1,540/3,155 (49%) of participants had a positive BAI, ie. a brain age predicted to be higher than chronological age (Figure 2). In contrast, HIV+ participants had a mean BAI of 4.4 years (*SD* = 8.77), with 28/43 (65%) of participants having a positive BAI. In sub-analyses restricted to the HIV+ cohort, we did not find a significant association of BAI with AIDS history (Supplementary Figure S1).

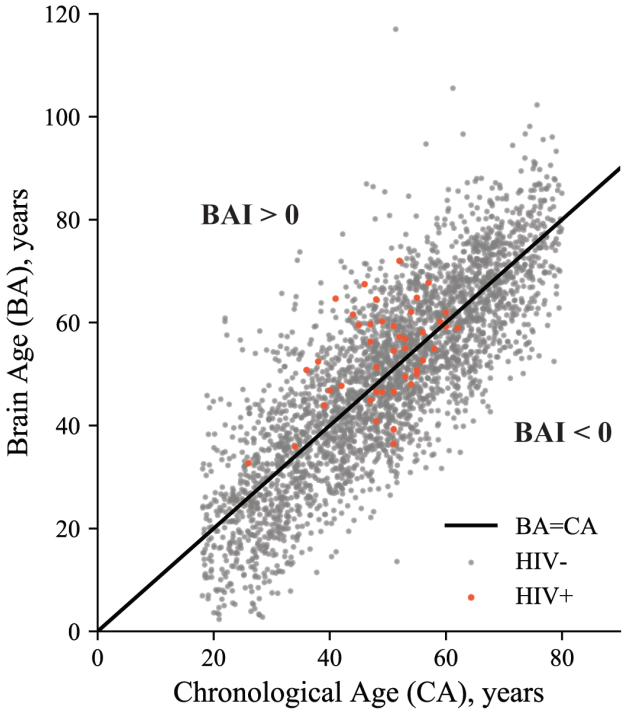

Figure 2. Brain ages among HIV+ and HIV– participants. Scatter plot showing each participant's Chronological Age (CA), the age at the time of the sleep study, versus the Brain Age (BA), the sleep EEG-predicted age. The solid line represents BA = CA, or BAI = 0. Above and below the line are indicated as the BAI > 0 and BAI < 0 regions, respectively.

HIV increases BAI after adjusting for confounders

To estimate the TE of HIV infection on BAI, we defined a causal diagram (Figure 3, A) based on our clinical knowledge depicting the potential relationships among BAI, HIV, and the set of potential confounders C: age, sex, race, tobacco use disorder, and alcohol use disorder. Using this model, we computed the average potential outcome difference of BAI between HIV– versus HIV+ participants. Average BAI for the HIV– group was estimated to be –0.16 years (*SEM* = 0.18 years), while average BAI for the HIV+ group was estimated to be 3.19 years (*SEM* = 1.43 years). TE, which is the difference in the average potential outcomes of BAI between HIV statuses, was therefore 3.35 years ($p < 0.01$, 95% CI = [0.67, 5.92]) (Figure 3, B). We found a similar and statistically significant effect using matching which we report in Supplementary Table S1, Supplementary Figure S2. With both methods, BAI was significantly elevated in the setting of HIV infection.

Table 1. Dataset demographics, potential mediators, and HIV-related variables

	HIV+	HIV–	*p*-value
Number	43	3,155	
Demographics (*n*, %)			
Age (median, IQR)	49 (46, 54)	50 (38, 62)	0.77
Male (*n*, %)	34 (79%)	1,594 (51%)	< 0.01
White (*n*, %)	30 (70%)	2,481 (79%)	0.16
Tobacco use disorder	20 (46%)	686 (21%)	< 0.01
Alcoholism	16 (37%)	267 (8%)	< 0.01
HIV-related (*n*, %)			
AIDS history	19 (44%)	NA	
Virally suppressed at sleep study	38/41 (93%)		
INSTIs taken at sleep study	13 (30%)	NA	
Efavirenz taken at sleep study	4 (9%)	NA	

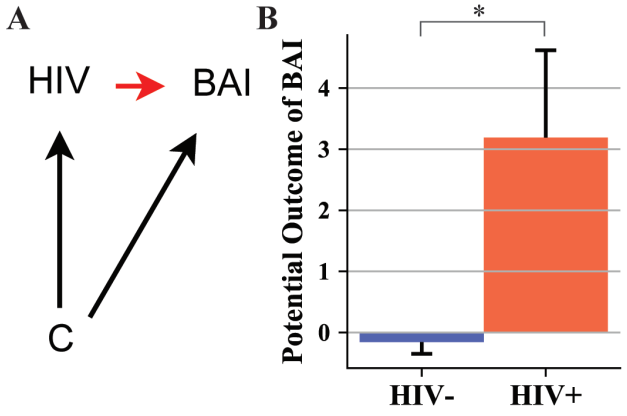

Figure 3. BAI is elevated by HIV after adjusting for potential confounders. (A) Causal diagram of the variables. An arrow from a variable X to another variable Y indicates our assumption that X causally affects Y. C is the set of covariates. HIV represents the presence or absence of the exposure to HIV infection. BAI represents the outcome variable, the BAI. The red arrow represents the effect of interest, which is measured as the difference in the expected potential outcomes of BAI in the presence and absence of HIV. (B). Bar chart showing the expected potential outcome of BAI in the absence (HIV–) and presence (HIV+) of HIV. Error bars depict the standard error of the mean (SEM; HIV– = 0.18 years, HIV+ = 1.43 years). The difference in expected potential outcomes of BAI is significant ($p < 0.05$), indicated by asterisks.

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022

Sensitivity analysis for unmeasured confounding

The risk ratio (RR), defined as P(BAI>0 | HIV+ among all participants) / P(BAI>0 | HIV– among all participants) was 2.3. Therefore, sensitivity analysis yielded an *E*-value of 4.0, which means, to explain away the effect of HIV on BAI, an unmeasured confounder should have at least a risk ratio of 4.0 for both HIV infection and BAI.

EEG features underlying BAI are altered by HIV

We also estimated the effect of HIV on the specific EEG features used to compute BAI. In the causal diagram (Figure 3, A), for each analysis we performed the same method of estimating the TE, but replaced the primary outcome BAI with an individual sleep EEG feature as a secondary outcome. With a pre-determined maximum FDR of 0.1, we identified 34 EEG features statistically significantly altered by HIV infection (Figure 4). There were no statistically significant changes in features in the Wake state (Supplementary Figure S3). In REM, three features were altered by HIV, each associated with reduced delta band power (Figure 4, A and B). In stage N1 (Figure 4, C and D), five significant feature changes were identified, all reflecting increased line length (a measure of signal complexity). In Stage N2 (Figure 4, E and F), there were 10 significant feature changes; 9 were related to reduction in delta power, and 1 to an increase in the theta-to-alpha power ratio. In stage N3 (Figure 4, G and H), there were 12 significant features, all corresponding to a relative reduction in HIV of delta band power.

Representative sleep spectrograms and hypnograms of HIV+ and HIV– participants are shown in Figure 5. Compared to that of the HIV– participant, the spectrogram of the HIV+ participant visibly reflects low-frequency delta power (1–4 Hz) markedly reduced throughout the night, most notably during N2 sleep.

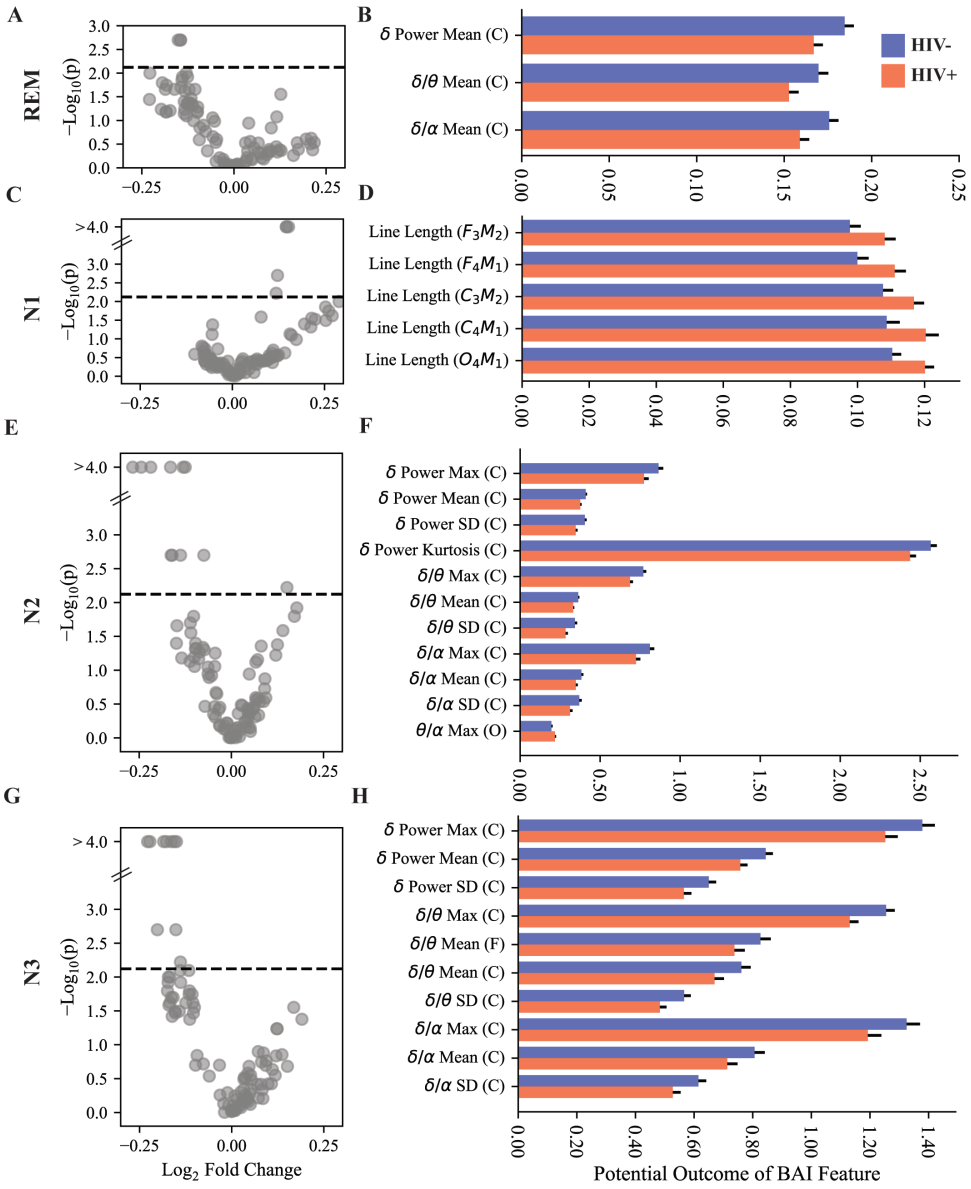

Figure 4. Individual EEG features underlying brain age are altered by HIV. Rows show features by sleep stage: (A, B) REM. (C, D) N1. (E, F) N2. (G, H) N3. (A,C,E,G) Volcano plots of significance level of changes in potential outcome of BAI features versus $\log_2$ fold change. Dotted lines represent the significance threshold for a FDR of 0.1. (B, D, F, H) Bar charts comparing potential outcomes of specific BAI features in the presence (HIV+) and absence (HIV–) of HIV. Only the significant features are shown. Solid horizontal black lines show SEM.

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022

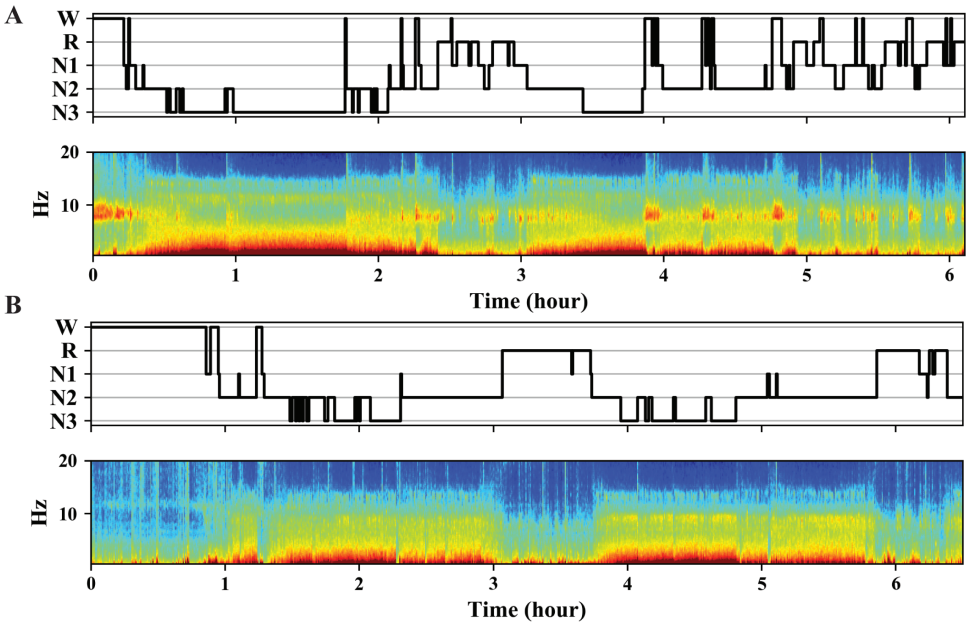

Figure 5. Hypnograms and spectrograms of representative HIV– and HIV+ participants. (A) HIV–. (B) HIV+. The x-axis is time since the sleep EEG recording in hours. The upper panel in each subplot shows the trajectory of sleep stages; the lower panel shows the spectrogram with y-axis being frequency in Hz.

Discussion

In this study, we found that HIV independently increases apparent brain age as measured by the BAI, with an average increase of 3.35 years (95% CI [0.67, 5.92], DRE). HIV+ subjects not on integrase inhibitors (INSTIs) during their sleep study had a higher average BAI. One HIV+ subject had a past history of HIV encephalitis, but given that their BAI was –2.50, their inclusion in the study cannot explain the average increase in BAI observed in the HIV+ group. Otherwise, HIV+ subjects had no history of secondary brain infection, encephalitis, or a brain tumor. We identified 34 sleep EEG features that were significantly altered by HIV infection. The most dominant EEG change in HIV is a reduction in delta band power (1–4 Hz), also referred to as SWA, during NREM sleep (N2, N3). Finally, we found that comorbidities may act as mediators and account for a portion of HIV's effect on BAI (26%); however, these results did not reach statistical significance possibly due to limited sample size.

Prior work has established that PLWH are at increased risk of advanced aging, [33] with previously discovered biomarkers such as somatic mitochondrial DNA mutations [34, 35] and markers of T-cell senescence [36], and decreased HLA methylation levels [37], telomere length [38, 39], and MRI. Advanced brain aging, vascular cognitive impairment, and Alzheimer's disease (AD) or AD-related dementia are higher risk among PLWH due to cerebrovascular disease [2, 40, 41], immune activation [42, 43], deposition of amyloid plaques and other neurodegenerative-associated proteins [44–46], persistence of HIV-infected cells in sanctuary sites [47], and higher rates of smoking [48]. Our study provides several new insights about advanced brain aging in PLWH and offers a new biomarker for brain aging. Our results show that sleep EEG identifies increased brain aging among PLWH, after adjusting for demographic and lifestyle factors, similar to prior work on MRI-based brain age. Sleep EEG is more cost-effective and easier to deploy than MRI, and thus shows promise as a biomarker for tracking brain aging in the HIV+ population, including in the outpatient and home setting. Additionally, our lab recently found that BAI increases with mild cognitive impairment and dementia [22], providing new evidence that BAI tracks clinically meaningful cognitive characteristics of the aging brain. We did not find an association of BAI with AIDS history or efavirenz, which we consider an inconclusive finding given the limited sample size. HIV+ subjects not on INSTIs had a higher average BAI than HIV+ subjects on INSTIs; however, we have no clear hypothesis for why this would occur in general, and the numbers of subjects in each group are quite small, thus we consider this a finding of uncertain significance and validity. We also identified specific sleep features that are altered in HIV. About 76% (26/34) of features with a statistically significant change were associated with a reduction in delta (1–4 Hz) band power; this was identified in multiple EEG channels and across REM, N2, and N3. SWA is a predominant feature of N2 and N3, and the presence of SWA corresponds to greater sleep depth [14]. The amplitude and incidence of SWA is reduced in normal aging, in sleep disorders such as insomnia and obstructive sleep apnea, and reduced SWA is also seen secondary to fibromyalgia, ADHD, and dementia. While the exact functional effect of SWA on health remains unclear, SWA is associated with increased glymphatic flow, [49] which clears metabolic waste products from the interstitial fluid of the parenchyma, including β-amyloid [50],and supports memory consolidation [51, 52]. Recently, a potential causal mechanism linking low-frequency oscillations to glymphatic clearance was discovered [53], providing new evidence for the hypothesis that reduced low-frequency oscillations during non-REM sleep is a risk factor for Alzheimer's dementia [54].

Our study has several methodological strengths. First, we were able to use a large dataset containing thousands of control cases, which facilitates estimation of the effects of covariates on BAI and reduces uncertainty about the average HIV– BAI. Second, our use of DRE, and replication of our results by matching, demonstrates that our findings are qualitatively robust to different choices of estimators. Third, we controlled the

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022

FDR when analyzing the effect of HIV on EEG features, such that only relatively large effect sizes were found to be statistically significant. Reduction in delta band power in particular was also consistent across multiple sleep stages and EEG channels.

As a retrospective cohort study, our study has several limitations. First, by only including participants from the sleep laboratory, our participants suffer disproportionately from sleep disorders relative to the general population. Second, we identified few women with HIV, and no HIV+ participants of Asian or Middle Eastern descent, and this limits the external validity of our findings. Third, our HIV+ cohort of 43 participants is relatively small, although as described above a large control cohort improves the statistical power. Fourth, to allow a causal interpretation of our results, we imposed standard causal identification assumptions (see Supplemental Material: Methods: Causal Inference Assumptions) [55]; violations of these assumptions would lead to biases in our results. Notably, there may be unmeasured confounders such as income and education, as well as trauma and post-traumatic stress disorder. To address this concern, we performed sensitivity analysis to estimate the effect an unmeasured confounder would need to have to explain away our findings. In Supplemental Material: Discussion, we discuss why, based on the *E*-value we calculated, and the prior literature, our results appear to be robust to unmeasured confounding. Additionally, we made informed assumptions about which clinical covariates are confounders and which are mediators. A fifth limitations is that we represented clinical conditions in our data as present versus absent, without information about disease severity. Six, HIV-specific data collection through chart review has limitations: the actual time of HIV infection and ART initiation is often unknown or undocumented in medical records, as is the case in our study. We considered AIDS history the best indicator of HIV immunosuppression [56], which was reasonably apparent in notes. Seventh, since we did not conduct our own HIV testing of participants, it is possible that some controls may have HIV; however, we expect that <1% of controls could be living with undiagnosed HIV and this would not significantly affect our findings. Eighth, our brain age algorithm also has limitations, discussed in [20]. Finally, this was a single-site study.

As a future step, tracking and predicting future cognitive decline among HIV+ participants is of particular clinical importance. Further research is needed to establish whether sleep EEG can be useful to track the progress of HIV+ individuals, and specifically their risk of cognitive decline. Based on our preliminary mediation findings, a prospective clinical study may be warranted to determine whether intervention on comorbidities mitigate risk of cognitive decline in PLWH. A sufficiently powered mediation analysis could help establish if interventions such as cardiac risk reduction and obstructive sleep apnea screening are likely to benefit adults with HIV specifically with respect to brain aging. Such a study would also allow one to better take into account potential confounders. In addition to documenting information such as education and socioeconomic background, one could assess for post-traumatic stress disorder and conduct urine drug screening for active drug use.

In conclusion, we studied brain aging in PLWH using overnight sleep EEG recordings, and provided evidence that adults with HIV on ART experience advanced brain aging. PLWH have reduced slow wave power during non-REM sleep, an EEG feature implicated in cognitive decline among people without HIV infection. Our results suggest that sleep EEG is a potentially useful biomarker for HIV-associated brain aging, and provides preliminary evidence of the specific sleep EEG features that are altered in the setting of treated HIV infection. These results should be replicated in a more representative sample of the population. Further work is needed to establish the effects of comorbidities on brain aging in HIV, and to what extent treating these co-morbidities can mitigate accelerated brain aging.

Supplementary material

Supplementary material is available at *SLEEP* online.

Funding

H.S. and M.L. were supported by a Developmental Award from the Harvard University Center for AIDS Research (HU CFAR NIH/NIAID fund 5P30AI060354-16). M.B.W. was supported by the Glenn Foundation for Medical Research and the American Federation for Aging Research through a Breakthroughs in Gerontology Grant; through the American Academy of Sleep Medicine through an AASM Foundation Strategic Research Award; from the Department of Defense through a subcontract from Moberg ICU Solutions, Inc, and by grants from the NIH (1R01NS102190, 1R01NS102574, 1R01NS107291, 1RF1AG064312). S.M. was supported by the NIH (K23MH115812-03) and Harvard University Center for AIDS Research (HU CFAR NIH/NIAID fund 5P30AI060354-16). L.L. was supported by Shanghai Pujiang Program Research grant (20PJ1408900), Shanghai Municipal Science and Technology Major Project (2021SHZDZX0102) and Major Program of National Natural Science Foundation of China (12090024).

Conflict of interest statement

Financial disclosure: None.

Nonfinancial disclosure: None.

References

1. Triant VA. HIV infection and coronary heart disease: an intersection of epidemics. *J Infect Dis*. 2012;**205**(suppl_3):S355–S361.
2. Losina E, *et al*. Projecting 10-year, 20-year, and lifetime risks of cardiovascular disease in persons living with human immunodeficiency virus in the United States. *Clin Infect Dis*. 2017;**65**(8):1266–1271.
3. Feinstein MJ, *et al*. Mortality following myocardial infarction among HIV-infected persons: the Center for AIDS Research Network Of Integrated Clinical Systems (CNICS). *BMC Med*. 2019;**17**(1):149.
4. Mulligan K, *et al*. Mixed patterns of changes in central and peripheral fat following initiation of antiretroviral therapy in a randomized trial. *J Acquir Immune Defic Syndr*. 2006;**41**(5):590–597.
5. Gandhi RT, *et al*. Metabolic and cardiovascular complications in HIV-infected patients: new challenges for a new age. *J Infect Dis*. 2012;**205**(suppl_3):S353–S354.
6. Kooij KW, *et al*. HIV infection is independently associated with frailty in middle-aged HIV type 1-infected individuals compared with similar but uninfected controls. *AIDS*. 2016;**30**(2):241–250.

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022

7. De Francesco D, *et al*. Depression, lifestyle factors and cognitive function in people living with HIV and comparable HIV-negative controls. *HIV Med*. 2019;**20**(4):274–285.
8. Métral M, *et al*. The Neurocognitive Assessment in the Metabolic and Aging Cohort (NAMACO) study: baseline participant profile. *HIV Med*. 2020;**21**(1):30–42.
9. Cysique LA, *et al*. HIV, vascular and aging injuries in the brain of clinically stable HIV-infected adults: a (1)H MRS study. *PLoS One*. 2013;**8**(4):e61738.
10. Cole JH, *et al*. Increased brain-predicted aging in treated HIV disease. *Neurology*. 2017;**88**(14):1349–1357.
11. Ohayon MM, *et al*. Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. *Sleep*. 2004;**27**(7):1255–1273.
12. Luca G, *et al*. Age and gender variations of sleep in subjects without sleep disorders. *Ann Med*. 2015;**47**(6):482–491.
13. Gorgoni M, *et al*. Sleep electroencephalography and brain maturation: developmental trajectories and the relation with cognitive functioning. *Sleep Med*. 2020;**66**:33–50.
14. Léger D, *et al*. Slow-wave sleep: from the cell to the clinic. *Sleep Med Rev*. 2018;**41**:113–132.
15. Ferini-Strambi L, *et al*. Slow wave sleep and cyclic alternating pattern (CAP) in HIV-infected asymptomatic men. *Sleep*. 1995;**18**(6):446–450.
16. Terstegge K, *et al*. Spectral power and coherence analysis of sleep EEG in AIDS patients: decrease in interhemispheric coherence. *Sleep*. 1993;**16**(2):137–145.
17. Wiegand M, *et al*. Nocturnal sleep EEG in patients with HIV infection. *Eur Arch Psychiatry Clin Neurosci*. 1991; **240**(3):153–158.
18. Low Y, *et al*. Comparison of polysomnographic data in age-, sex- and axis I psychiatric diagnosis matched HIV-seropositive and HIV-seronegative insomnia patients. *Clin Neurophysiol*. 2012;**123**(12):2402–2405.
19. Gamaldo CE, *et al*. Sleep, function and HIV: a multi-method assessment. *AIDS Behav*. 2013;**17**(8):2808–2815.
20. Sun H, *et al*. Brain age from the electroencephalogram of sleep. *Neurobiol Aging*. 2019;**74**:112–120.
21. Paixao L, *et al*. Excess brain age in the sleep electroencephalogram predicts reduced life expectancy. *Neurobiol Aging*. 2020;**88**:150–155.
22. Ye E, *et al*. Association of sleep electroencephalography-based brain age index with dementia. *JAMA Netw Open*. 2020;**3**(9):e2017357.
23. Myllymaa S, *et al*. Assessment of the suitability of using a forehead EEG electrode set and chin EMG electrodes for sleep staging in polysomnography. *J Sleep Res*. 2016;**25**(6):636–645.
24. Younes M, *et al*. Accuracy of automatic polysomnography scoring using frontal electrodes. *J Clin Sleep Med*. 2016;**12**(05):735–746.
25. Younes M, *et al*. Performance of a new portable wireless sleep monitor. *J Clin Sleep Med*. 2017;**13**(2):245–258.
26. Antoniou T, *et al*. Validation of case-finding algorithms derived from administrative data for identifying adults living with human immunodeficiency virus infection. *PLoS One*. 2011;**6**(6):e21748.
27. Gallego L, *et al*. Analyzing sleep abnormalities in HIV-infected patients treated with Efavirenz. *Clin Infect Dis*. 2004;**38**(3):430–432.
28. Clifford DB, *et al*. Impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals. *Ann Intern Med*. 2005;**143**(10):714–721.
29. Fettiplace A, *et al*. Psychiatric symptoms in patients receiving dolutegravir. *J Acquir Immune Defic Syndr*. 2017;**74**(4):423–431.
30. Yu S, *et al*. Enabling phenotypic big data with PheNorm. *J Am Med Inform Assoc*. 2018;**25**(1):54–60.
31. Benjamini Y, *et al*. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc*. 1995;**57**(1):289–300.
32. VanderWeele TJ, *et al*. Sensitivity analysis in observational research: introducing the e-value. *Ann Intern Med*. 2017;**167**(4):268–274.
33. De Francesco D, *et al*. Do people living with HIV experience greater age advancement than their HIV-negative counterparts? *AIDS*. 2019;**33**(2):259–268.
34. Payne BA, *et al*. Mitochondrial aging is accelerated by antiretroviral therapy through the clonal expansion of mtDNA mutations. *Nat Genet*. 2011;**43**(8):806–810.
35. Var SR, *et al*. Mitochondrial injury and cognitive function in HIV infection and methamphetamine use. *AIDS*. 2016;**30**(6):839–848.
36. Papagno L, *et al*. Immune activation and CD8+ T-cell differentiation towards senescence in HIV-1 infection. *PLoS Biol*. 2004;**2**(2):E20.
37. Gross AM, *et al*. Methylome-wide analysis of chronic HIV infection reveals five-year increase in biological age and epigenetic targeting of HLA. *Mol Cell*. 2016;**62**(2): 157–168.
38. Srinivasa S, *et al*. Soluble CD163 is associated with shortened telomere length in HIV-infected patients. *J Acquir Immune Defic Syndr*. 2014;**67**(4):414–418.
39. Zanet DL, *et al*. Association between short leukocyte telomere length and HIV infection in a cohort study: no evidence of a relationship with antiretroviral therapy. *Clin Infect Dis*. 2014;**58**(9):1322–1332.
40. Sico JJ, *et al*. HIV status and the risk of ischemic stroke among men. *Neurology*. 2015;**84**(19):1933–1940.
41. Chow FC, *et al*. Elevated ischemic stroke risk among women living with HIV infection. *AIDS*. 2018;**32**(1):59–67.
42. Lyons JL, *et al*. Plasma sCD14 is a biomarker associated with impaired neurocognitive test performance in attention and learning domains in HIV infection. *J Acquir Immune Defic Syndr*. 2011;**57**(5):371–379.
43. Guha D, *et al*. Proteomic analysis of cerebrospinal fluid extracellular vesicles reveals synaptic injury, inflammation, and stress response markers in HIV patients with cognitive impairment. *J Neuroinflammation*. 2019;**16**(1):254.
44. Chai Q, *et al*. HIV-1 counteracts an innate restriction by amyloid precursor protein resulting in neurodegeneration. *Nat Commun*. 2017;**8**(1):1522.
45. Solomon IH, *et al*. Brain and liver pathology, amyloid deposition, and interferon responses among older HIV-positive patients in the late HAART era. *BMC Infect Dis*. 2017;**17**(1):151.
46. Zeinolabediny Y, *et al*. HIV-1 matrix protein p17 misfolding forms toxic amyloidogenic assemblies that induce neurocognitive disorders. *Sci Rep*. 2017; **7**(1):10313.
47. Spudich S, *et al*. HIV persistence in the central nervous system during antiretroviral therapy: evidence and implications. *AIDS*. 2019;**33**:S103–S106.
48. Mdodo R, *et al*. Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: cross-sectional surveys. *Ann Intern Med*. 2015;**162**(5):335–344.

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022

49. Hablitz LM, *et al*. Increased glymphatic influx is correlated with high EEG delta power and low heart rate in mice under anesthesia. *Sci Adv*. 2019;**5**(2): eaav5447.
50. Xie L, *et al*. Sleep drives metabolite clearance from the adult brain. *Science*. 2013;**342**(6156):373–377.
51. Marshall L, *et al*. Boosting slow oscillations during sleep potentiates memory. *Nature*. 2006;**444**(7119): 610–613.
52. Diekelmann S, *et al*. The memory function of sleep. *Nat Rev Neurosci*. 2010;**11**(2):114–126.
53. Fultz NE, *et al*. Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. *Science*. 2019;**366**(6465):628–631.
54. Irwin MR, *et al*. Implications of sleep disturbance and inflammation for Alzheimer's disease dementia. *Lancet Neurol*. 2019;**18**(3):296–306.
55. Hernan M, *et al*. *Causal Inference: What If*. Boca Raton, FL: Chapman & Hall/CRC; 2020.
56. Maldarelli F, *et al*. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. *PLoS Pathog*. 2007;**3**(4):e46.

Downloaded from https://academic.oup.com/sleep/article/44/8/zsab058/6204183 by guest on 26 December 2022