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Abnormal contingent negative variation in HIV patients receiving antiretroviral therapy

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Abstract

The contingent negative variation, an event-related potential related to neural activity in the frontal lobe and basal ganglia, neuropsychological tests and structural MRI were used to examine CNS function and structure in HIV-positive patients receiving antiretroviral therapy. Relative to controls, HIV patients had smaller thalamic volume and reduced late contingent negative variation amplitude that correlated with caudal atrophy. Behaviorally, viremic patients were more impaired than virally suppressed patients and controls on neuropsychological measures of psychomotor speed, selective attention and mental flexibility. These results suggest that antiretroviral therapy may not be effective in protecting cortical and subcortical structures against HIV-related neuropathology, regardless of immune function. However, the benefits of antiretroviral therapy on immune function appear to facilitate neurocognitive performance.

Keywords

Antiretroviral therapy; Contingent negative variation; Event-related potential; HIV; MRI

INTRODUCTION

Antiretroviral therapy has dramatically reduced the incidence and severity of opportunistic infections associated with HIV and improved neurocognitive function [1,2], but it remains unclear how effectively antiretroviral therapy protects the CNS against HIV-related disease. Many investigators have used event-related brain potentials (ERPs) to assess CNS function in HIV patients; however, most of have focused on phasic components such as the P300. Slow cortical potentials have been described and studied extensively in normals, though little attention has been paid to their use in assessing CNS function in HIV patients. In the current study, we use slow ERPs to examine the effects of HIV on CNS function in patients receiving antiretroviral therapy. In particular, we focused on the contingent negative variation (CNV) [3] because it has been linked to neural activity in the frontal lobes [4–6] and basal ganglia [7], regions of the brain most affected by HIV infection [8]. We also employed

neuropsychological tests and structural magnetic resonance imaging (MRI) to examine CNS function and structure in HIV patients receiving antiviral therapy.

The CNV is a slow cortical potential that occurs between two successive stimuli that are associated with or contingent on each other (the first stimulus serves as warning signal for the second imperative stimulus, which requires a motor response). The CNV probably reflects neuronal activity related to sensorimotor integration and the planning or execution of voluntary movements. Comprising ≥ 2 components, the early phase of the CNV is evoked by the warning stimulus and has been linked to sensory processes associated with evaluating the information contained in the warning signal [9]. The later phase of the CNV precedes the imperative stimulus and has been linked to both motor and cognitive preparation associated with the imperative stimulus [9].

The goals of the current study were to test the following hypotheses: (1) HIV-infection is associated with reduced CNV amplitude. (2) In HIV patients, CNV amplitude correlates with atrophy of frontal lobe, basal ganglia, and other subcortical structures, as measured by structural MRI. (3) Previous studies have reported associations between CNV amplitude and reaction time in normal subjects that suggest the CNV reflects some aspect of motor and cognitive preparation [9]. Thus, we also examined the hypothesis that HIV infection disrupts motor and cognitive preparation, reflected by a change in the relationship between reaction time and CNV amplitude in HIV patients compared to controls.

MATERIALS AND METHODS

Participants

Clinical and demographic characteristics of the study samples are shown in Table 1. Antiretroviral therapy was defined as either mono- or dual-drug therapy with nucleoside reverse transcriptase inhibitors (e.g. zidovudine, stavudine, or lamivudine) or combination therapy with at least one protease inhibitor (i.e. highly active antiretroviral therapy, HAART). All participants were screened to exclude those with a history of psychiatric and/or neurological disorder and substance dependence. All participants gave written informed consent approved by the Institutional Review Boards of the San Francisco Department of Veterans Affairs Medical Center and the University of California, San Francisco.

Stimuli and behavioral task

ERPs were recorded while subjects performed a reaction time task. Each trial consisted of a warning stimulus (S1, presented for 50 ms) followed 3 s later by an imperative stimulus (S2, presented for 500 ms). Subjects were instructed to press a button with the index finger of their dominant hand as quickly as possible in response to S2. The delay between S2 of one trial and S1 of the next trial varied randomly from 3 to 18 s. Failure to respond within 2 s after S2 or premature responses before onset of S2 were logged as errors and the ERPs from these trials were excluded from further analysis.

ERP recording and analysis

Electrophysiological signals, recorded with an Electro-Cap with 62 tin electrodes and four electro-oculographic electrodes, were referenced to linked ear lobes. The signal was amplified ($\times 50\,000$), filtered (0.03–40 Hz), digitized on-line with a sampling frequency of 200 Hz, and recorded continuously with the Neuroscan acquisition system. Seven midline channels (Fz, FCz, Cz, CPz, Pz, POz, Oz) were selected for further analysis. Trials containing eye movement, excessive peak-to-peak deflections, electromyographic artifact, or amplifier blocking were rejected automatically from the averaged data. Trials with correctable blinks (i.e. uncontaminated by other artifacts) were corrected by means of a regression analysis in

combination with artifact averaging method and included in the relevant average [10]. ERP averages were computed for controls, all HIV patients, and viremic HIV patients (those with viral loads >50 copies/ml; $n=23$) and virally suppressed HIV patients (those with undetectable viral loads; $n=16$). The averaging epoch started 900 ms before S1 and ended 1000 ms after S2. After ERP averages were smoothed using a low-pass filter (five points smoother), they were quantified by computing mean amplitudes in defined latency windows relative to a 900-ms prestimulus baseline (early CNV: 800–1100 ms after S1; late CNV: 300 ms prior to S2).

Separate repeated-measures ANOVAs were performed with CNV amplitude as the dependent variable, group as the between-subjects factor, and electrode as the within-subjects factor. A 2-group ANOVA was used to examine data from all HIV patients relative to controls. A 3-group ANOVA was used to examine data from viremic HIV patients, virally suppressed HIV patients, and controls. All F-ratios associated with repeated-measures factors were assessed using degrees of freedom corrected with the Greenhouse–Geisser procedure for controlling Type I error. Corrected degrees of freedom are quoted when appropriated. Tukey's honestly different significance tests were used to test for the reliability of *post-hoc* comparisons.

MRI measurements

Structural MRIs were obtained on the same day or within 1–3 days of the ERP recordings. MRIs were available for 35 control and 31 HIV patients who participated in the ERP experiment. MRI tissue segmentation was accomplished through the probabilistic segmentation of T1-weighted images similar to methods described by Van Leemput *et al.* [11], Shattuck *et al.* [12] and Ashburner and Friston [13]. To account for variations in head size, MRI measures were normalized to total intracranial volume (ICV) by summing voxels from all cortical and subcortical tissue classes (gray and white matter and cerebrospinal fluid) but excluding voxels from the cerebellum and brainstem. *t*-tests were used to compare volumetric MRI data from all HIV patients relative to controls. ANOVAs were used to examine volumetric data from viremic and virally suppressed HIV patients and controls. Tukey's honestly different significance tests were used to test for the reliability of *post hoc* comparisons. Spearman's rank order correlation was used to analyze the relationship between MRI volumetric data and ERP data.

Neuropsychological assessments

Neuropsychological tests, administered either the same day or within 1–3 days of the ERP recordings, included the Trail-Making Test A and B [14], California Verbal Learning Test [15], Grooved Pegboard Test [16], Symbol Digit Modalities Test [17], Stroop Color–Word Tests [18], and the Controlled Oral Word Association Test [19]. Group differences were assessed by a similar method as that described for MRI data.

RESULTS

CNV grand averages from the vertex are shown in Fig. 1. The amplitude of the early CNV was marginally reduced in all HIV patients relative to controls (2-group ANOVA: $F(1,76)=3.44$, $p=0.07$; Fig. 1). There was a main effect of electrode because the early CNV was more negative-going over fronto-central electrode sites (2-group ANOVA: $F(2.6,200.0)=9.64$, $p<0.001$; 3-group ANOVA: $F(2.6,196.4)=10.4$, $p<0.0001$).

The amplitude of the late CNV was significantly reduced in all HIV patients relative to controls (2-group ANOVA: $F(1,76)=17.37$, $p<0.001$; Fig. 1). The 3-group ANOVA also yielded a significant main effect of group ($F(2,75)=8.70$, $p<0.0001$). *Post-hoc* tests revealed that both viremic ($p=0.01$) and virally suppressed HIV patients ($p=0.02$) had less negative-going late CNVs than did controls; however, the amplitude of the late CNV was not significantly different

between viremic and virally suppressed HIV patients. There was a significant main effect of electrode because the late CNV was more negative-going over central electrodes (2-group ANOVA: $F(3.1,239.2) = 8.38, p < 0.001$; 3-group ANOVA ($F(3.1,236.0) = 4.21, p < 0.001$) and a significant group $\times$ electrode interaction because the amplitude difference across electrodes was greater in controls than HIV patients (2-group ANOVA: $F(3.1,229.2) = 4.88, p = 0.002$; 3-group ANOVA: $F(6.3,236.0) = 3.19, p = 0.004$).

All subjects performed the task without difficulty (accuracy in HIV patients: $97 \pm 6\%$ vs accuracy in controls: $95 \pm 9\%$, $p > 0.1$). Because previous studies reported associations between CNV amplitude and reaction time, suggesting that the CNV reflects some aspect of motor and cognitive preparation associated with S2 [10], we correlated CNV amplitude with subjects' reaction time to S2. In controls, there was a significant, positive correlation between reaction time and the amplitudes of both the early and late CNV at most central electrodes (slower reaction times were associated with less negative-going waveforms, Table 2). In contrast, there was no significant correlation between CNV amplitude and reaction time in HIV patients despite comparable reaction time with controls (HIV patients: 302 ± 76 ms; controls: 285 ± 81 ms; $p > 0.1$).

Analysis of the structural MRI data revealed significant reductions in thalamic volume (1.20 ± 0.11 vs $1.25 \pm 0.11\%$ ICV, $p = 0.04$) and trends ($p = 0.06$) of reduced white matter volume in the frontal (17.88 ± 1.81 vs $18.56 \pm 1.75\%$ ICV) and temporal (7.08 ± 0.58 vs $7.32 \pm 0.65\%$ ICV) lobes in all HIV patients relative to controls. Analysis of the two HIV subgroups and controls revealed a significant main effect of group for thalamic volume ($F(2,65) = 3.99, p = 0.02$). *Post hoc* tests revealed a significant difference in thalamic volume between virally suppressed HIV patients and controls ($p = 0.02$) but not between viremic HIV patients and controls.

Because previous findings have linked the CNV to neural activity in the frontal lobes [4–6], basal ganglia [7], and thalamus [20], we correlated CNV amplitude with frontal lobe gray and white matter volume as well as caudate, lentiform nuclei, and thalamus volumes. These analyses revealed significant, negative correlations between caudate volume and late CNV amplitude at Fz ($r = -0.48, p = 0.03$) and Cz ($r = -0.49, p = 0.03$) in all HIV patients (i.e. reduced negativity was associated with decreased volume; Fig. 2). Analysis of data from viremic and virally suppressed HIV patients revealed a similar relationship between late CNV amplitude and caudate volume; however, only the correlation coefficient between late CNV amplitude at Fz and caudate volume in virally suppressed HIV patients reached statistical significance ($r = -0.59, p < 0.02$).

Relative to controls, HIV patients were impaired on the Stroop color ($p = 0.03$), word ($p < 0.01$), and color–word ($p = 0.04$) tests (Table 3). ANOVAs examining neuropsychological data from the two HIV patient subgroups and controls revealed significant main effects of group on the oral symbol digit modalities test ($F(2,77) = 4.96, p = 0.008$), Stroop color ($F(2,77) = 3.61, p = 0.03$), word ($F(2,77) = 9.50, p < 0.001$), and color–word tests ($F(2,77) = 4.37, p = 0.02$). *Post hoc* analyses revealed that viremic patients performed worse than virally suppressed patients on the oral symbol digit modalities test ($p < 0.01$) and worse than controls on the Stroop color ($p < 0.05$), word ($p < 0.0001$), and color–word ($p < 0.05$) tests.

DISCUSSION

The first major finding of this study is that the amplitude of the late CNV was significantly less negative in HIV patients receiving antiretroviral therapy relative to seronegative controls. Negative potentials on the cortical surface have been attributed to a widespread depolarization in the dendritic trees of cortical pyramidal neurons [21]. Moreover, it has been proposed that

such potentials indicate increased cortical excitability [21]. These findings suggest that HIV reduces cortical excitability and that antiretroviral therapy does not appear to protect against this. Factors that may contribute to reduced CNV amplitudes (and decreased cortical excitability) in HIV patients include loss of neurons, dendritic arborization complexity, synaptic density, and vacuolization of dendritic spines associated with HIV infection [8]. However, it is also possible that factors in the patients' medical history preceding the onset of HIV-infection and/or the adverse effects of antiretroviral therapy may have played a role in the observed CNV differences between HIV patients and controls.

The second major finding is that there was an inverse relationship between late CNV amplitude and caudate volume in HIV patients receiving antiretroviral therapy (reduced negativity was associated with reduced volume). This is consistent with a recent intracranial study that recorded the CNV in the basal ganglia [7] and evidence that suggests the basal ganglia contain some of the brain's highest viral burden as well as HIV-infected macrophages and multinucleated giant cells during active HIV infection [22].

The third major finding is that HIV patients on anti-retroviral therapy had reduced thalamic volume relative to seronegative controls. While these results could suggest that antiretroviral therapy does not protect subcortical structures against HIV-related damage, as noted previously, factors in the patient's medical history preceding the onset of HIV-infection and/or the adverse effects of antiretroviral treatment might also explain the differences observed.

The fourth major finding is the relationship between CNV amplitude and reaction time to S2 in controls, which suggests that the CNV reflects some aspect of motor and cognitive preparation that did not exist in HIV patients. This suggests that HIV infection may disrupt the normal linkage between pre- and post-stimulus processing. This disruption may result from HIV patients adopting different task strategies from those employed by controls, as suggested by the patients' slower reaction time and slightly better performance than control subjects. Alternatively, the lack of an association between CNV amplitude and reaction time in HIV patients may reflect aspects of HIV-related CNS damage, as suggested by reductions in late CNV amplitude, inverse relationship between late CNV amplitude and caudate volume, and thalamic atrophy in HIV patients.

The fifth major finding is that viremic HIV patients were more impaired than virally suppressed HIV patients and seronegative controls on neuropsychological tests of psychomotor speed, selective attention, and mental flexibility. *Post hoc* analyses of the neuropsychological results revealed no significant differences between virally suppressed HIV patients and controls. However, *post hoc* tests showed that viremic patients were more impaired than virally suppressed patients on the symbol digit modalities test and more impaired than controls on the Stroop color, reading, and color-word tests (measures of psychomotor speed, selective attention, and mental flexibility). These results are consistent with the idea that neurocognitive improvements associated with antiretroviral therapy may be predominantly mediated by the beneficial effects of antiretroviral therapy on immune function [23].

CONCLUSIONS

Our results suggest that antiretroviral therapy benefits neurocognitive performance by improving immune function; however, it does not appear to completely protect the CNS against HIV-related damage, as evidenced by reduced CNV amplitudes.

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Fig. 1.

CNV grand averages from the vertex and schematic of the S1-S2 reaction time task. Grand averages for HIV- controls are denoted by the thick solid line, HIV + viremic patients by the dotted line, and HIV + virally suppressed patients by the thin solid line. Note the late CNV amplitude reductions in HIV + patients.

Fig. 2.

Relationship between late CNV amplitude (at Cz) and caudate volume in all HIV + patients ($r = -0.49, p < 0.03$). Similar trends were also observed in viremic HIV patients (denoted by triangles) and virally suppressed HIV patients (denoted by circles).

Table 1

Participant demographic and clinical information.

	HIV- (<i>n</i> =39)		HIV + (<i>n</i> =39)	
	Mean ± s.d.	Range	Mean ± s.d.	Range
Age (years)	43.5 ± 7.6	26–56	45.9 ± 7.3	25–57
Education (years)	16.0 ± 2.1	12–21	15.4 ± 1.9	12–20
CD4 (cells/mm ³)*	798 ± 290	276–1527	403 ± 217	25–1008
Plasma viral load (copies/ml)	—	—	18161 ± 5503	<50–32 3374

* $p < 0.0001$, HIV- vs HIV +.

Table 2
Spearman's rank correlation coefficients between reaction time and CNV amplitude at midline electrode sites.

Electrode	HIV- (n=39)		HIV + (n=39)	
	Early	Late	Early	Late
Fz	0.39*	0.38*	-0.28	0.05
FCz	0.41*	0.41*	-0.19	0.03
Cz	0.41*	0.48**	-0.19	0.08
CPz	0.39*	0.39*	-0.20	0.11
Pz	0.37*	0.34*	-0.24	0.07
POz	0.40*	0.09	-0.22	0.05
Oz	0.48**	0.15	-0.21	0.06

* $p < 0.05$;
** $p < 0.01$.

Table 3
Neuropsychological test performance (means $\pm$ s.d.).

Neuropsychological test	HIV- control (<i>n</i> =39)	All HIV + (<i>n</i> =39)	HIV +, suppressed (<i>n</i> =16)	HIV +, viremic (<i>n</i> =23)
California Verbal Learning Test				
Total	55.21 $\pm$ 9.50	55.90 $\pm$ 10.22	58.00 $\pm$ 10.80	54.43 $\pm$ 9.78
Long delay free recall	12.26 $\pm$ 2.76	11.46 $\pm$ 3.35	11.31 $\pm$ 3.63	11.57 $\pm$ 3.22
Learning slope	1.46 $\pm$ 0.50	1.29 $\pm$ 0.50	1.23 $\pm$ 0.56	1.33 $\pm$ 0.46
Digit Symbol Test				
Oral	66.36 $\pm$ 11.24	64.46 $\pm$ 14.18	71.63 $\pm$ 16.16	59.47 $\pm$ 10.30 ^{*,†}
Written	56.38 $\pm$ 10.17	55.41 $\pm$ 11.44	60.19 $\pm$ 12.85	52.09 $\pm$ 9.25 ^{*,†}
Grooved Pegboard				
Dominant	69.38 $\pm$ 14.81	70.46 $\pm$ 15.37	71.38 $\pm$ 12.44	69.83 $\pm$ 9.37
Non-dominant	74.46 $\pm$ 15.37	75.97 $\pm$ 10.52	75.69 $\pm$ 10.63	76.17 $\pm$ 10.69
Trail-making Test				
Part A	27.95 $\pm$ 11.12	24.54 $\pm$ 8.05	23.06 $\pm$ 7.78 [*]	25.57 $\pm$ 8.25
Part B	54.13 $\pm$ 20.96	56.00 $\pm$ 31.31	50.13 $\pm$ 20.66	60.09 $\pm$ 36.87
Stroop				
Color test	77.42 $\pm$ 14.00	71.89 $\pm$ 11.88 [*]	77.07 $\pm$ 13.80	68.74 $\pm$ 9.53 ^{*,†}
Word test	110.50 $\pm$ 14.92	96.43 $\pm$ 15.10 ^{**}	101.14 $\pm$ 15.57 [*]	93.57 $\pm$ 14.40 ^{**}
Color-word test	45.37 $\pm$ 11.65	40.86 $\pm$ 9.78 [*]	45.93 $\pm$ 9.88	37.78 $\pm$ 8.51 ^{*,†}
Controlled Oral Word Association Test	48.87 $\pm$ 13.27	48.05 $\pm$ 14.25	53.13 $\pm$ 18.16	44.52 $\pm$ 9.70 [†]

^{*} $p < 0.05$, HIV- vs HIV +;

^{**} $p < 0.01$, HIV- vs HIV +;

[†] $p < 0.05$, suppressed vs viremic.