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ORIGINAL INVESTIGATION

The apolipoprotein E ε4 allele and memory performance in HIV-1 seropositive subjects: differences at baseline but not after acute oral lorazepam challenge

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Abstract
Rationale The APOE ε4 allele, an established genetic risk factor for late-onset Alzheimer's disease, has been linked to an increased risk for dementia especially in older individuals with HIV-1 infection. This allele has also been associated with increased memory impairment following oral lorazepam challenge in healthy elderly. Lorazepam and other benzodiazepines are widely prescribed in individuals with HIV-1 infection who are at increased risk for cognitive impairment.
Objective The aim of this study was to examine if the ε4 allele influences lorazepam-induced memory deficits in this population.
Materials and methods Forty-one non-demented, HIV-1 seropositive adults (15 ε4 carriers, mean age=43.47±8.25; 26 ε4 non-carriers, mean age=46.77±8.56) participated in a double-blind, placebo-controlled crossover design, receiving single acute oral doses of lorazepam 0.5, 1.0 mg, or placebo over three sessions, each 1 week apart. Standardized neuropsychological assessments, including measures of immediate and delayed verbal recall, were conducted at baseline and at 1, 2.5, and 5 h post-drug administration in each condition.
Results Acute lorazepam administration produced dose- and time-dependent impairments in measures of verbal recall. However, the e4 allele did not modulate these adverse effects. An APOE ε4 group by time interaction was also found such that the APOE-ε4-positive subjects had significantly better immediate and delayed verbal recall than the negative subjects at baseline assessment, but the groups did not significantly differ at any subsequent time point.
Conclusion Future studies should clarify the role of ε4 in the modulation of drug-induced cognitive toxicity and baseline performance and their relationship to progressive decline, especially in older individuals with HIV-1 infection, a group at increased risk for dementia.

Keywords HIV · AIDS · ApoE 4 · ApoE polymorphism · Lorazepam · Benzodiazepines · Memory

All analyses were repeated (not reported here, available upon request) in the subgroup of individuals currently meeting CDC surveillance case definition for AIDS (Center for Disease Control and Prevention 1992; n=30; 12 ε4+/18 ε4−). The pattern of findings was replicated.

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Introduction

Benzodiazepines (BZPs) are widely used among persons with human immunodeficiency virus (HIV). In a recent survey, they were the second most frequently prescribed class of psychotropic medications preceded only by antidepressants and used by approximately 17% of patients (Vitiello et al. 2003). The widespread use of BZPs in persons with HIV infection, who may already suffer from or be at risk for viral-induced cognitive disturbances, is problematic, since acute administration of BZPs has been shown to produce profound impairments in memory and psychomotor performance in healthy adult subjects (Block

and Berchou 1984). The high usage of BZPs in this population may also be problematic, since concomitant treatment with antiretrovirals, which are known to inhibit the activity of the hepatic P4503A (cyp3A) isoenzymes involved in the metabolism of benzodiazepines such as alprazolam, may result in higher drug levels, which in turn could contribute to even greater sedative and amnestic effects (Greenblatt et al. 2000).

Previous research has demonstrated that presence of the APOE ε4 allele (ε4), a well-established genetic risk factor for late-onset Alzheimer's disease (AD), results in increased sensitivity to the adverse cognitive effects of acute oral challenge with the commonly prescribed BZP lorazepam (Pomara et al. 2005). More specifically, healthy elderly with at least one ε4 allele were observed to experience more severe cognitive impairment and/or decreased recovery following acute oral challenge with lorazepam. The observed impairments could not be ascribed to pharmacokinetic factors, such as an increase in plasma drug levels. Thus,it is possible that pharmacodynamic factors such as previously described subtle ε4-related deleterious central nervous system (CNS) effects including reduced synaptic plasticity, increased Abeta deposition, higher oxidative stress, and other alterations may have contributed to vulnerability to drug-induced cognitive toxicity (Mahley et al. 2006).

Studies in normal or minimally impaired elderly have also indicated that this polymorphism may result in an increased risk for subjective memory complaints, subtle impairments or lower performance on delayed recall tasks, greater daytime somnolence, reductions in cerebral glucose utilization on PET, greater structural brain abnormalities including hippocampal atrophy, slower recovery, or more unfavorable outcome after various acute neurological insults, and more persistent memory deficits following cardiopulmonary bypass surgery (Small et al. 1999; O'Hara et al. 1998; Cascelli et al. 2002; Reiman et al. 2001; Cherbuin et al. 2007; Nierenberg et al. 2005; Walters and Nicoll 2005; Tardiff et al. 1997).

Importantly, HIV-1 virus is associated with a dementing illness in advanced stages of disease (Price et al. 1988), and evidence derived from preclinical and human studies has linked the ε4 polymorphism to increased neurotoxicity associated with the HIV-1 transactivator (Tat) protein, increases in peripheral neuropathy, and increased brain Aβ deposits in HIV (Turchan-Cholewo et al. 2006; Corder et al. 1998; Green et al. 2005). Some studies, but not all, have found an increased risk for HIV dementia associated with ε4, especially in individuals greater than 50 years of age (Corder et al. 1998; Dunlop et al. 1997; Valcour et al. 2004). Taken together, these findings suggest that similar to healthy or minimally impaired elderly, HIV-1-infected persons carrying an ε4 allele may be more vulnerable to drug-induced cognitive impairment.

To our knowledge, however, no studies have examined the relationship between ε4 and cognitive performance in response to acute BZP challenge in non-demented persons with HIV-1 infection. Given that the ε4 allele has a frequency of approximately 20% in the general population, its presence may be an important factor contributing to increased sensitivity to BZP-induced cognitive toxicity in HIV-1-infected individuals already at risk for compromised neurocognitive functioning. These considerations prompted us to examine the relationship between the ε4 allele and lorazepam-induced cognitive impairment in non-demented persons with HIV-1 infection using a cognitive test battery with demonstrated sensitivity in detecting these associations (Pomara et al. 2005). It was hypothesized that the presence of an ε4 allele would predispose HIV-1 seropositive individuals to greater verbal memory impairment in response to acute oral lorazepam challenge as compared to HIV-1-infected ε4 non-carriers and that these effects would be dose-dependent.

Materials and methods

Participants

HIV-1 seropositive individuals were recruited for participation in this study through the New York University (NYU) Center for AIDS Research, newspaper advertisements, other publicly posted print media, and outreach efforts to HIV support groups in the New York City Metropolitan area. The study was conducted at the NYU Bellevue General Clinical Research Center (GCRC). The Institutional Review Boards of the Nathan S. Kline Institute for Psychiatric Research in Orangeburg, NY and NYU/Bellevue Medical Center in New York, NY formally approved conduct of the study. All participants were compensated $150 for their time at study completion.

Screening evaluations were conducted 2 weeks prior to the beginning of the experiment. Exclusion criteria were: less than 18 years of age, acute medical illness as determined by results of a medical history, physical evaluation and routine laboratory tests, history of non-HIV-related neurologic disease, history of loss of consciousness (LOC) greater than 30-min duration, or LOC of any duration with consequent neurological sequelae, presence or history of psychotic illness, current alcohol or substance abuse or dementia based on DSM-IV criteria (American Psychiatric Association 1994) as determined via psychiatric interview by a board-certified psychiatrist, a positive urine drug screen test, current benzodiazepine prescription/use as confirmed by a positive urine drug screen test, or baseline plasma benzodiazepine levels, and Mini Mental State Examination (MMSE) <28 (Folstein et

al. 1975). Subjects were also free of clinically significant anxiety or depressive symptoms as determined by the Hamilton Anxiety Rating Scale and Hamilton Rating Scale for Depression (Table 1; Hamilton 1959, 1967).

In total, 120 individuals were pre-screened, either in person or via telephone. After complete description of the study to the subjects, written informed consent was obtained from 111 subjects for study participation and release of relevant medical records prior to administration of any protocol procedures. Of this group, 16 individuals were subsequently found ineligible or did not return for further participation. APOE ε4 status was determined for 95 individuals. Twenty-four subjects were lost to follow-up, 24 were unmatched, and 47 entered the acute drug challenge phase of the study. Of these 47, all were HIV-1 seropositive, medically stable, ambulatory and functionally independent, and currently attending HIV/AIDS clinics. Probable mode of HIV-1 infection was sexual transmission for 40 participants, intravenous drug use transmission for seven (one subject uncertain whether transmitted sexually or via IV drug use), and mode of transmission unknown for one participant. Thirty-five patients had a history of opportunistic infections and or CD4 T lymphocyte counts below 200 cells/μl, meeting the CDC surveillance case

Table 1 Participant demographic, biologic characteristics, and pre-study screening measures for total group and for apoE-ε4-positive and -negative subgroups

Participant characteristics	Total[a] (*N*=41)	ApoE ε4+ (*n*=15)	ApoE ε4− (*n*=26)
Gender			
Male/Female	26/15	7/8	19/7
Age (years)			
Median (range)	45 (33–67)	41 (36–62)	46.50 (33–67)
Mean±SD	44.46±8.25	43.47±7.48	46.77±8.56
Ethnic composition[b]			
African-American (*n*, %)	26 (63)	11 (73)	15 (58)
Hispanic-Latino *n* (%)	8 (20)	3 (20)	5 (19)
White/non-Hispanic (*n*, %)	6 (15)	1 (7)	5 (19)
Education (years)			
Median (range)	13 (9–18)	14 (9–18)	12.5 (9–16)
Mean±SD	13.17±2.14	13.57±2.53	12.94±1.9
Weight[c] (lbs)			
Median (range)	166 (97.1–250)	151.45 (122–250)	170 (97.1–233)
Mean±SD	166.63±38.24	161.73±31.74	169.61±31.74
Time since diagnosis			
Median (range)	283 (11–1300)	318 (11–661)	258 (20–1300)
Mean±SD	320.51±240.01	331.07±177.42	314.42±272.83
CD4+ T lymphocyte count			
Median (range)	283 (11–1300)	318 (11–661)	258 (20–1300)
Mean±SD	320.51±240.01	331.07±177.42	314.42±272.83
Mini Mental Status Exam			
Median (range)	30 (28–30)	30 (28–30)	30 (28–30)
Mean±SD	29.32±0.82	29.53±0.64	29.20±0.89
WAIS-R[d] vocabulary			
Median (range)	8 (7–16)	8 (7–14)	8 (7–16)
Mean±SD	9.05±2.18	9.40±2.38	8.84±2.08
Hamilton Depression Scale[e]			
Median (range)	1 (0–10)	0.0 (0–5)	1 (0–10)
Mean±SD	0.50±2.39	0.93±1.44	1.8±2.78
Hamilton Anxiety Scale[e]			
Median (range)	1 (0–14)	1 (0–10)	1 (0–14)
Mean±SD	2.55±3.63	2.13±3.16	2.8±3.93

WAIS-R Wechsler Adult Intelligence Scale–Revised

[a] Unless otherwise indicated, participant characteristics and pre-study screening measures are reported for the full sample (*N*=41)

[b] Ethnicity was unavailable for one ε4− participant

[c] Values for weight were missing for one ε4+ participant and three ε4− participants

[d] Value for WAIS-R vocabulary was missing for one ε4− participant

[e] Values for Hamilton Depression and Anxiety Scales were missing for one ε4− participant

definition for AIDS (Center for Disease Control and Prevention 1992). Forty-one participants were currently receiving highly active antiretroviral therapy (HAART).

Forty-seven subjects entered the acute drug challenge phase, 17 ε4 positive (ε4+; ten men and seven women) and 30 ε4 negative (ε4−; 20 men and ten women). Of this sample, six (12.77%; two ε4+ and four ε4−) have been excluded from the analyses reported here. Specifically, three participants did not complete the last of three experimental sessions, two participants had missing values on primary outcome measures due to data collection errors, and one ε4− subject was subsequently found to have a pre-screening MMSE=27. Demographic characteristics and pre-study screening measures are summarized for the total sample and by ε4 status in Table 1. Though not systematically matched on demographic characteristics, the participants did not significantly differ on any demographic or pre-study screening measures reported in Table 1 based on ε4 status as indicated by χ^2, Mann–Whitney *U*, or independent samples *t* tests (all *p* values >0.05). However, the ε4− sample, in particular, was comprised of proportionately more males (74%). Formal clinical assessment revealed that 27 of 41 subjects in the full sample (65.9%) had a history of illicit substance abuse including marijuana, cocaine, and "crack" cocaine. There were no significant differences between the ε4 groups ($p>0.05$), Fisher's exact test (two-sided). An additional 15 of 41 (36.6%) of the full sample reported a history of alcohol abuse, also without significant differences between the ε4 groups ($p>0.05$), Fisher's exact test (two-sided).

Determination of APOE status

APOE genotype/phenotype was determined at the Northwest Research Laboratories at the University of Washington using widely accepted methods with established accuracy (Hixson and Vernier 1990; Kataoka et al. 1994). Study participants and research staff were blind to participants' ε4 status throughout all experimental sessions.

Design and procedures

Following diagnostic and screening evaluation, individuals participated in three 5-h experimental sessions, each 1 week apart, during which they were administered oral doses of lorazepam 0.5, 1.0 mg, or placebo in a double-blind manner. Drug doses were counterbalanced across the experimental sessions to control for order effects, and subjects were randomly assigned to one of three treatment sequences: placebo, 0.5 mg, 1.0 mg ($n=15$); 0.5 mg, 1.0 mg, placebo ($n=12$); 1.0 mg, placebo, 0.5 mg ($n=14$). Study sessions began at approximately 9:00 A.M. under non-fasting conditions following acquisition of vital signs. Lorazepam doses and placebo were prepared by the institutional pharmacy and dispensed at the experimental sessions by research staff at the NYU GCRC. At baseline, 1, 2.5, and 5 h following oral administration of drug, a battery of neuropsychological tests was administered. The effects of single, orally administered doses of 0.5 and 1.0 mg of lorazepam on immediate and delayed verbal memory were compared to placebo across time. The presence or absence of an ε4 allele was the between-subjects factor, and dose (placebo, 0.5 and 1.0 mg lorazepam) and time (baseline, 1, 2.5, and 5 h post-dose) were repeated measures factors.

Plasma lorazepam levels

Blood for determination of plasma lorazepam levels was collected at baseline and at 1, 2.5, and 5 h post-drug administration for all study participants. Due to technical difficulties, 22% of the samples were lost at the hospital laboratory, leaving evaluable samples for 32 participants, ε4+ ($n=13$) and ε4− ($n=19$). Quantitation of plasma drug levels was determined by electron-capture gas chromatography.

Neuropsychological testing

Learning and verbal memory

The Buschke Selective Reminding Test (BSRT) is comprised of a list of 16 unrelated words read aloud by an examiner for seven consecutive trials (Buschke 1973, 1974). Participants are asked to recall as many words as possible regardless of recall order after each trial. Following the first trial, for each of six subsequent trials, the participant is *selectively* reminded of words *not* recalled from the previous presentation of the list. A total recall score is calculated by summing the correctly recalled words over the seven immediate recall trials (score range=0–112), and a delayed recall score is also obtained (score range=0–16), which is the number of words correctly recalled following a 15-min delay from the last immediate recall trial. Alternate forms of the BSRT were used at each of the four time points and within each level of the drug condition to control for practice effects. Details concerning development of the 12 alternate forms have been described elsewhere (Pomara et al. 1998).

Psychomotor functioning

The Purdue Pegboard Test was administered to measure general psychomotor functioning (Tiffin 1968). The task requires participants to place as many pegs as possible into a holed square palate for 30 s. Participants complete three pegboard trials that require (1) the sole use of a dominant

hand, (2) non-dominant hand, and (3) both hands. The number of correctly inserted pegs are counted and recorded (score range=0–25). The digit span task of the Wechsler Adult Intelligence Scale–Revised was also administered between the last BSRT immediate recall and 15-min delayed recall trials to minimize covert rehearsal of BSRT items (Wechsler 1981).

Statistical analyses

All statistical analyses were conducted using the SPSS 15.01 for Windows statistical software program (SPSS 2006). Available data on plasma lorazepam levels were examined using a three-way (ε4 status, drug dose, time) mixed model, repeated measures analyses of variance (ANOVA) to determine equivalence of drug levels between the ε4 subgroups in the 0.5- and 1.0-mg drug conditions at each assessment point. Placebo performance for total and delayed verbal recall and psychomotor functioning were examined using two-way (ε4 status, time), mixed model repeated measures ANOVAs to determine equivalence on the primary outcome measures throughout the placebo condition.

To examine the impact of ε4 status, lorazepam challenge, and time, data on verbal memory and psychomotor performance were analyzed with three-way (ε4 status, drug dose, and time), mixed model repeated measures ANOVAs. In each analysis, ε4 status served as the between-subjects factor, and the three levels of drug (placebo, 0.5 mg, and 1.0 mg lorazepam) and time since drug administration (baseline, 1, 2.5, and 5 h) served as the repeated measure factors. Follow-up tests for significant ANOVAs were conducted using pairwise comparisons and independent samples or repeated measures t tests as appropriate for significant main or interactive effects. Partial η^2 and Cohen's d are reported as effect sizes for statistically significant ANOVAs and t tests, respectively, on primary outcome measures. The criterion for statistical significance was set at $p<0.05$.

Results

Plasma lorazepam levels

Results of the three-way (ε4 status, drug dose, time), mixed model repeated measures ANOVA on available plasma lorazepam levels (ε4+ $n=13$; ε4− $n=18$) revealed no significant main or interactive effects of ε4 group status (all p values>0.05). A significant drug by time interaction [$F(3, 87)=23.30$, $p<0.001$] was found such that peak drug levels occurred at 2.5 h as shown in Fig. 1. Follow-up paired samples t tests indicated significant differences in plasma drug concentrations between the two lorazepam doses at 1, 2.5, and 5 h (all p values<0.001), but not at baseline. At each time point, plasma drug levels were

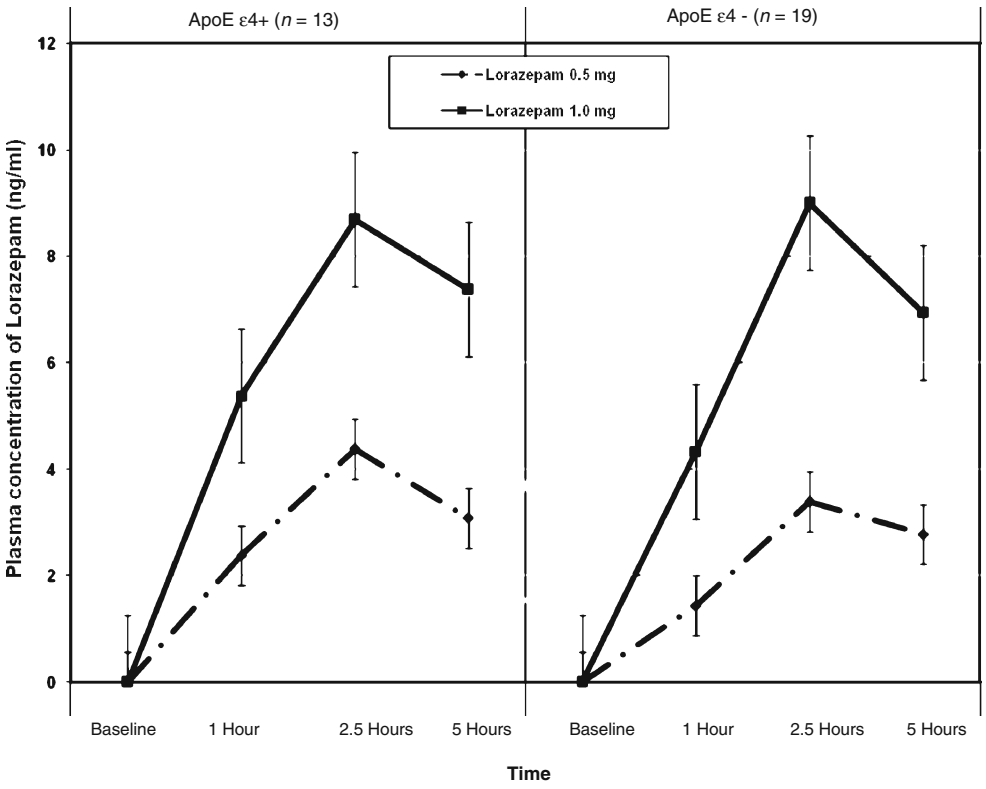

Fig. 1 Plasma lorazepam levels for ε4+ and ε4− groups in 0.5- and 1.0-mg lorazepam conditions at baseline, 1, 2.5, and 5 h (error bars = SE)

significantly higher in the 1.0-mg condition compared to the 0.5-mg condition.

Verbal memory performance during lorazepam challenge

Total immediate verbal recall

Means and standard deviations for BSRT total immediate and delayed verbal recall are presented in Table 2 as a function of ε4 group status, drug condition, and time. The three-way (ε4 status, drug dose, time), mixed model repeated measures ANOVA on total verbal recall revealed a significant main effect of drug [$F(2, 78)=12.20$, $p<0.001$, partial $\eta^2=0.24$]. Follow-up pairwise comparisons indicated that total verbal recall was significantly lower in the 0.5-mg ($p=0.013$) and 1.0-mg ($p<0.001$) lorazepam conditions as compared to placebo and that recall on 1.0 mg lorazepam was significantly lower than recall on 0.5 mg ($p=0.012$). There was also a significant main effect of time [$F(3, 117)=19.40$, $p<0.001$, partial $\eta^2=0.33$]. Follow-up pairwise comparisons indicated that recall at baseline was significantly higher than all other time points (all p values<0.001), whereas recall at 2.5 h was significantly lower than all other time points (all p values<0.001).

A significant drug by time interaction [$F(6, 234)=5.89$, $p<0.001$, partial $\eta^2=0.13$] was also observed. Performance across the three drug conditions did not significantly differ at baseline ($p>0.05$). At 1 h, however, recall was significantly lower in response to 0.5 mg ($p=0.03$) and 1.0 mg lorazepam ($p=0.004$) as compared to placebo, though the two lorazepam conditions did not significantly differ ($p=0.08$). At 2.5 h, performance was significantly worse following 0.5 mg ($p=0.02$) and 1.0 mg lorazepam ($p<0.001$) compared to placebo and significantly worse following 1.0 mg compared to 0.5 mg lorazepam ($p<0.001$). At 5 h, total recall was significantly worse on 0.5 mg ($p=0.025$) and 1.0 mg lorazepam ($p=0.003$) than on placebo; however, the two lorazepam doses did not significantly differ from one another ($p>0.05$; Table 2, Fig. 2).

A significant ε4 status by time interaction was also found [$F(3, 117)=3.03$, $p=0.032$, partial $\eta^2=0.07$] such that the ε4+ subjects had significantly higher baseline total recall ($M\pm$SD across three baseline drug conditions$=56.51\pm14.14$) than the ε4− subjects [$M\pm$SD across three baseline drug conditions$=45.77\pm12.64$, $t(39)=2.51$, $p=0.02$, $d=0.80$]. However, the ε4 groups did not differ from one another at 1, 2.5, or 5 h post-drug (all p values>0.05).

The effect of ε4 group status on baseline total recall remained statistically significant after partialing out variance accounted for by age [$F(1, 38)=4.84$, $p=0.034$, partial $\eta^2=0.11$], education, [$F(1, 38)=5.56$, $p=0.024$, partial $\eta^2=0.13$], and screening HAM-D score [$F(1, 37)=6.83$, $p=.013$, partial $\eta^2=0.16$] using analysis of covariance (ANCOVA).

Delayed verbal recall

Similar to total immediate verbal recall, the three-way (ε4 status, drug dose, and time), mixed model repeated measures ANOVA on delayed verbal recall revealed a significant main effect of drug [$F(2, 78)=7.05$, $p<0.002$, partial $\eta^2=0.15$]. Follow-up pairwise comparisons indicated that recall performance was significantly lower following 0.5 mg ($p<0.023$) and 1.0 mg of lorazepam ($p<0.002$) as compared to placebo; however, there was no difference between the 0.5- and 1.0-mg lorazepam doses ($p>0.05$) on delayed verbal recall. A significant main effect of time was also found [$F(3, 117)=30.36$, $p<0.001$, partial $\eta^2=0.44$], and follow-up pairwise comparisons indicated that delayed recall was significantly higher at baseline than all other time points (all p values<0.001) and that recall at 2.5 h was significantly lower than recall at baseline ($p<0.001$), 1 h ($p=0.001$), and 5 h ($p=0.006$).

Table 2 Means and SD of BSRT total and delayed recall by apoE ε4 allele status in placebo, 0.5-, 1.0-mg lorazepam doses at baseline, 1, 2.5, and 5 h

		Total recall			Delayed recall		
		Placebo	0.5 mg	1.0 mg	Placebo	0.5 mg	1.0 mg
ε4+	0 h	56.20±16.69	56.00±15.10	57.33±13.04	7.07±2.99	6.67±3.09	7.20±3.03
	1 h	55.60±16.88	50.20±15.06	43.40±14.51	6.27±3.47	4.73±3.39	3.33±2.23
	2.5 h	52.33±15.76	46.40±14.62	39.60±11.52	4.93±3.49	4.33±2.92	2.60±1.84
	5 h	51.60±14.13	50.07±12.38	45.93±11.94	5.00±3.00	5.00±2.88	3.87±2.80
ε4−	0 h	44.81±13.01	45.50±15.08	47.00±14.27	4.69±2.62	4.85±2.54	5.81±2.98
	1 h	46.27±1.21	43.12±13.02	42.31±14.20	4.65±2.93	4.27±3.44	4.44±2.95
	2.5 h	44.42±12.74	41.35±11.63	36.81±9.16	4.00±2.38	3.08±2.84	2.58±2.27
	5 h	46.00±11.51	41.12±11.99	41.69±11.76	4.38±2.32	3.35±2.65	3.42±2.08

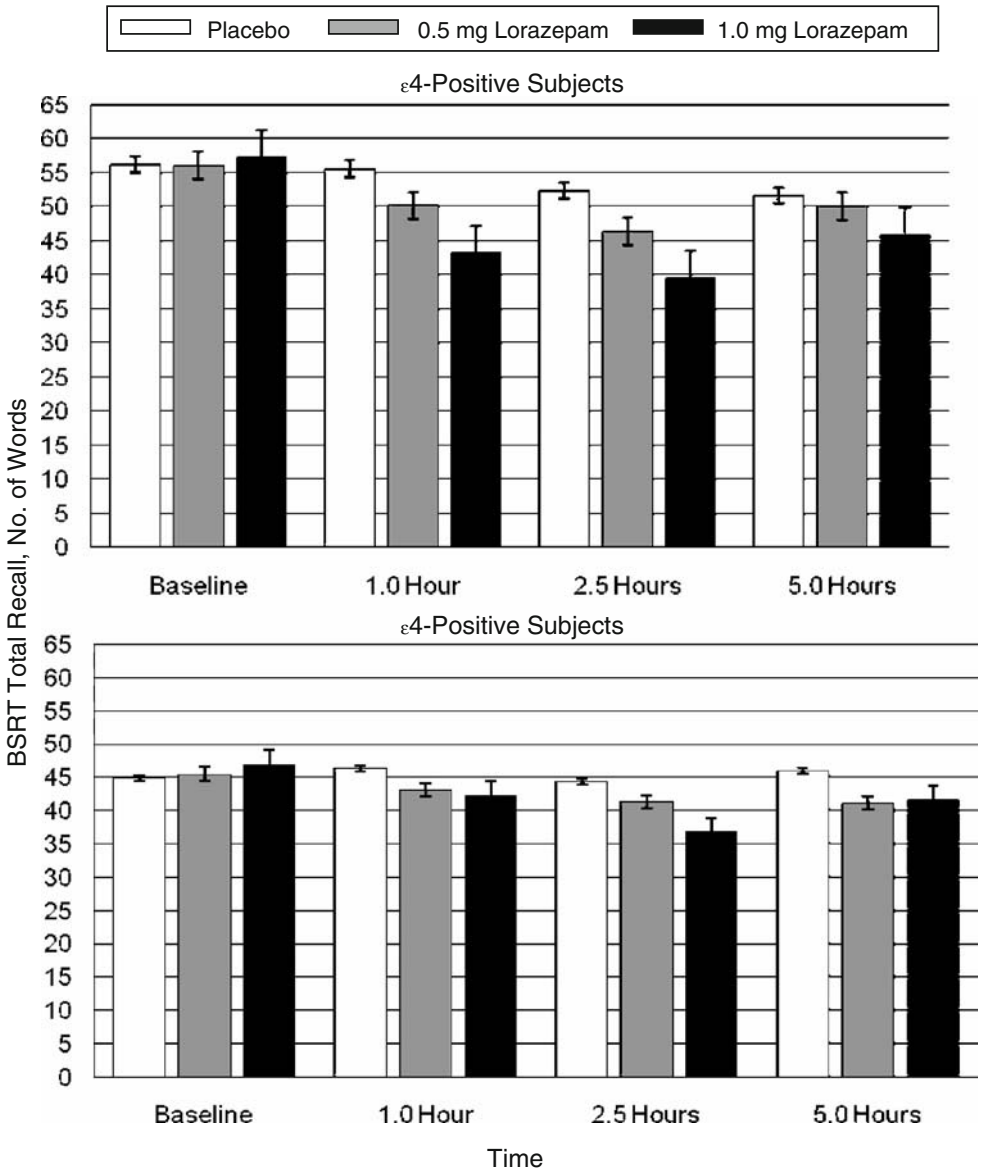

Fig. 2 Mean BSRT total immediate verbal recall scores during placebo, 0.5, and 1.0 mg lorazepam at baseline, 1.0, 2.5, and 5.0 h for ε4+ and ε4− groups (error bars = SE)

A significant drug by time interaction was also found [$F(6, 234)=4.77$, $p<0.001$, partial $\eta^2=0.11$]. Specifically, follow-up pairwise comparisons indicated that at baseline, delayed recall was significantly poorer in the placebo ($p=0.013$) and 0.5-mg ($p=0.044$) conditions as compared to the 1.0-mg lorazepam condition. However, at 1 h, delayed recall was significantly lower than placebo in the 1.0-mg lorazepam condition only ($p=0.015$), whereas the two lorazepam conditions did not significantly differ ($p>0.05$). At 2.5 h, follow-up pairwise comparisons indicated that performance was significantly worse on 0.5 mg ($p=0.036$) and 1.0 mg lorazepam ($p=0.001$) as compared to placebo, and recall was also significantly worse in the 1.0-mg as compared to 0.5-mg lorazepam condition ($p=0.047$). By 5 h, performance was significantly lower only in the 1.0-mg condition as compared to placebo ($p=0.018$), with no other differences found at this time point (Table 2, Fig. 3).

Consistent with total immediate verbal recall, results revealed a significant ε4 status by time interaction [$F(3, 117)=2.76$, $p=0.045$, partial $\eta^2=0.07$] such that the ε4+ subjects had significantly higher baseline recall ($M\pm$SD across three baseline drug conditions=6.98±2.80) than the ε4− subjects [$M\pm$SD across three baseline drug conditions=5.12±2.44), $t(39)=2.23$, $p=0.032$ ($d=0.71$)]. However, the groups did not differ at 1, 2.5, or 5 h (all p values>0.05).

The effect of ε4 group status on baseline delayed recall did not reach conventional statistical significance after partialing out variance accounted for by age [$F(1, 38)=3.69$, $p=0.062$, partial $\eta^2=0.06$], but remained statistically significant after partialing out variance accounted for by education [$F(1, 38)=4.41$, $p=0.043$] and screening HAM-D

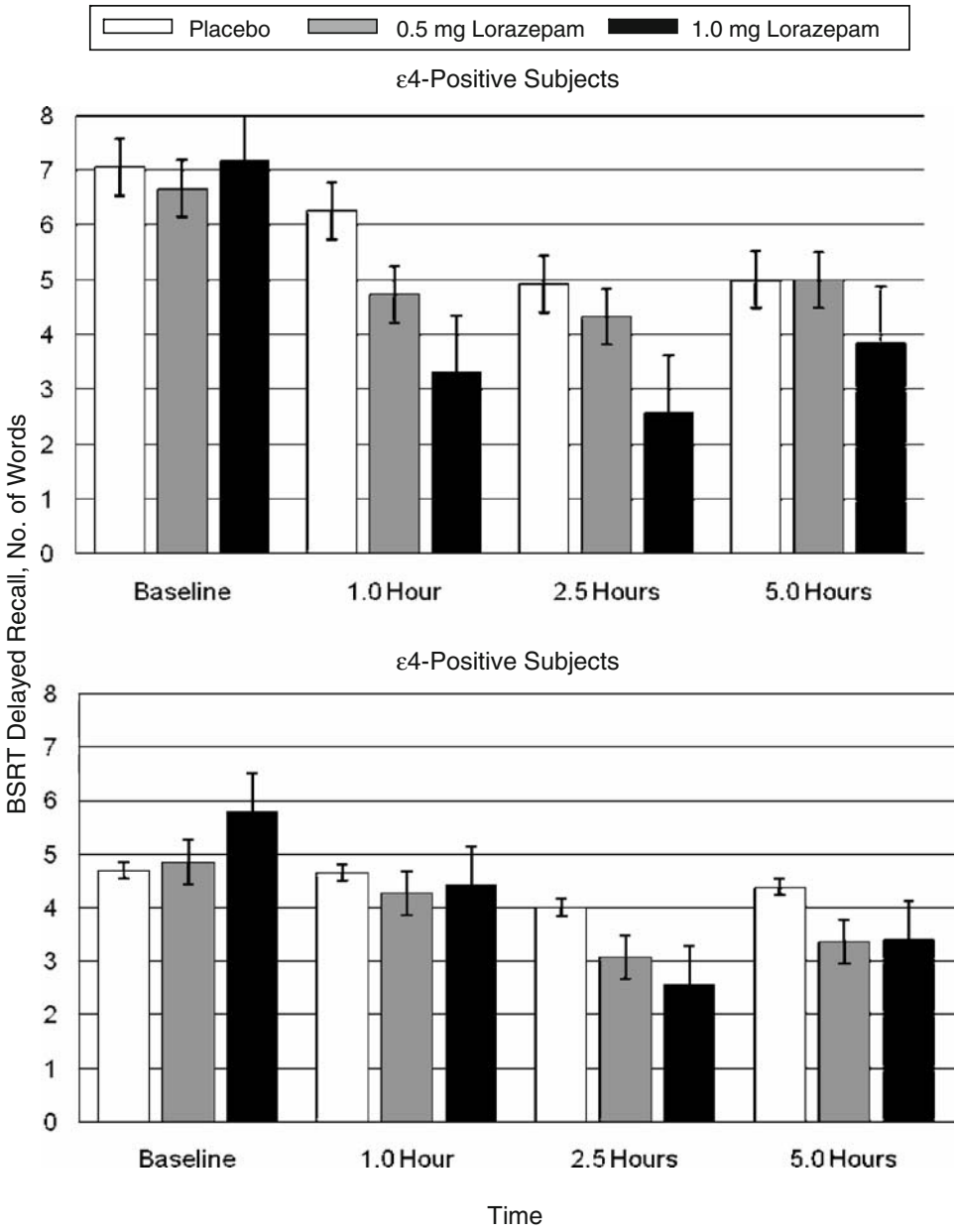

Fig. 3 Mean BSRT delayed verbal recall scores during placebo, 0.5, and 1.0 mg lorazepam at baseline, 1.0, 2.5, and 5.0 h for ε4+ and ε4− groups (error bars = SE)

score [$F(1, 37)=4.52$, $p=0.04$, partial $\eta^2=0.11$] using ANCOVA.

Psychomotor performance during lorazepam challenge

Three-way ANOVAs (ε4 status, level of drug, and time) were conducted on the Purdue Pegboard Test scores using the dominant and non-dominant hands separately and both hands together. Using the dominant hand, there were no significant main or interactive effects of ε4 status, dose, or time (all p values>0.05). With the non-dominant hand, no significant main or interactive effects of ε4 status, drug, or time emerged, but there was a significant interaction between drug and time for the full sample [$F(6, 234)=2.73$, $p=0.014$, partial $\eta^2=0.07$]. Follow-up pairwise comparisons indicated that performance across the three drug conditions was equivalent at baseline and at 1 h, but by 2.5 h, performance in the 1.0-mg lorazepam condition (M±SD=14.44±2.65) was significantly lower than performance in the placebo condition (15.27±2.32, $p<0.05$, $d=0.33$), but not significantly lower than performance in the 0.5-mg lorazepam condition. At 5 h, performance in the 1.0-mg condition (14.56±2.48) was significantly lower than performance in the 0.5-mg condition (15.24±2.36, $p=0.003$, $d=0.28$), but did not differ from placebo at this point.

Similarly, when using both hands, there were no significant main or interactive effects of ε4 status, drug, or time, but a significant drug by time interaction emerged for the full sample [$F(6, 234)=2.71$, $p=0.015$, partial $\eta^2=0.07$]. Follow-up pairwise comparisons indicated that performance within the

three levels of drug did not differ at baseline or 1 hour, but at 2.5 hours subjects performed significantly worse in the 0.5-mg (12.27±2.17, p=0.047, d=0.28) and 1.0-mg lorazepam conditions (11.83±2.50, p<0.001, d=0.39) as compared to the placebo condition (12.80±2.44). Additionally, performance at 2.5 h on 1.0 mg was significantly lower than 0.5 mg (p=0.027, d=0.19). This trend partly continued at 5 h, with subjects performing significantly worse on 1.0 mg lorazepam (12.15±2.29) than on placebo (12.71±2.54, p=0.013, d=0.23), but performance on 0.5 mg did not significantly differ from either placebo or 1.0 mg at this time point.

Secondary analyses

An examination of the placebo data suggested different levels of performance on the memory tests as a function of ε4 status. This was explored in a secondary analysis of total immediate and delayed verbal recall in the placebo condition.

Verbal memory during placebo condition

Total recall

A two-way (ε4 status, time), mixed model repeated measures ANOVA on placebo performance for total verbal recall revealed no significant main or interactive effects of time; however, a significant main effect of ε4 status was found [F(1, 39)=4.47, p<0.05, partial η^2=.10] such that those with the ε4 allele scored, on average (M±SD=53.93±14.74), significantly higher than the ε4− subjects (45.38±11.03) on total recall across the four assessments [t(39)=2.11, p=0.041, d=0.68] (Table 2, Fig. 2).

Delayed recall

The two-way (ε4 status, time), mixed model repeated measures ANOVA on placebo performance for delayed recall revealed no main or interactive effects of ε4 group status; however, a significant main effect of time was found [F(3, 117)=6.42, p<0.001, partial η^2=.14]. Follow-up pairwise comparisons indicated that delayed recall for the full group was significantly lower at 2.5 h (4.34±2.83, p=.001, d=0.42) and at 5 h, (4.61±2.57, p=0.002, d=0.34) compared with performance at baseline (5.56±2.96). Additionally, delayed recall performance for the full group was significantly lower at 2.5 h (4.34±2.83, p=.001, d=0.30) than at 1 h (5.24±3.19; Table 2, Fig. 3).

Discussion

Consistent with findings from other studies that have examined the cognitive effects of lorazepam and other BZPs in healthy populations, acute oral doses of lorazepam resulted in profound impairment in immediate and delayed recall on the BSRT (Block and Berchou 1984; Pomara et al. 1998). While the clinical significance of these findings is not known, our results call for caution in the use of this agent and perhaps other BZPs in HIV-1-infected individuals, since they already may be at risk for compromised neurocognitive functioning.

In contrast to our previous study in healthy elderly in which we found a significant interaction between ε4 and the adverse effects of acute doses of lorazepam on memory (Pomara et al. 2005), the current results in a younger population with HIV-1 infection did not reveal such an association. The basis for the discrepant findings is not entirely clear, but could be related to methodological factors including population differences, a smaller sample size, and the younger age of the HIV-1-infected sample. Several lines of evidence suggest that the deleterious effects of ε4 on the CNS, especially cognition including memory, may not emerge until later in life and proximal to onset age of AD (Jorm et al. 2007; Chen et al. 2002). This may also be true for individuals with HIV-1 infection, since a recent epidemiologic study found that the e4 allele was associated with an increased risk for dementia only in older HIV-1-infected individuals and not in younger populations (Dunlop et al. 1997). Thus, future studies examining the association between ε4 and drug-induced cognitive toxicity in subjects with HIV-1 infection should include an adequate sample of older individuals.

Interestingly, we also found the ε4 allele to be associated with enhanced baseline memory performance. While to date studies in healthy older adults have revealed no evidence of enhanced memory performance associated with this allele, our findings are consistent with data from studies in children and younger adults without HIV-1 infections in which the ε4 allele has been reported to be accompanied with a small but positive effect on cognition (Alexander et al. 2007). For example, in a study conducted by Mondadori et al. (2007), young healthy adults with the e4 allele had better delayed recall as compared to those without this allele. Similarly, the ε4 allele was associated with better cognitive performance including episodic memory in young adults with military traumatic brain injury (TBI) than a control group without this allele (Han et al. 2007).

The mechanisms by which the ε4 allele may contribute to enhanced memory performance in these younger populations are poorly understood. As suggested by Alexander et al. (2007), it is possible that these beneficial effects could be exerted by increased cholesterol which is known to play a major role in neuronal growth and synaptogenesis and/or more efficient transport within the CNS (Alexander et al. 2007). On the other hand, it is possible that these findings could reflect neuropsycholog-

ical compensatory CNS responses to subtle deleterious effects of ε4 on brain function as suggested by Han et al. for the enhanced cognitive performance, which they observed in ε4-positive individuals with TBI (Han et al. 2007).

A recent epidemiologic study in individuals with HIV-1 infection found an increased risk for dementia associated with the ε4 allele only in those 50 years and older. The mean age of the ε4-positive HIV-1-infected population in the current report was only 44.46±8.25 years, and consequently, the findings of this report may not be pertinent to older individuals with HIV-1 infection. Therefore, it would be of considerable interest to determine if baseline and drug-induced memory impairment in this population are modulated by the ε4 allele and if they are related to progressive cognitive decline or dementia.

In addition to the young age of the population, other limitations include the relatively small sample size and the absence of a group of healthy age-matched HIV-1 seronegative controls. Thus, it remains to be determined if our findings of enhanced baseline memory performance are solely related to an interaction between the ε4 allele and HIV infection. It should also be noted that the current sample comprised a group of HIV-1-infected persons without significant psychiatric symptoms. Hence, these findings may not be pertinent to anxious HIV-infected individuals who are more likely to be prescribed lorazepam or other benzodiazepines. Although HIV-infected individuals were included in this study only in the explicit absence of history of, or present psychotic illness, current alcohol or substance abuse and clinically significant symptoms of anxiety and depression, the ε4-positive and -negative groups were not matched for history of alcohol or substance abuse/dependence or prior psychiatric illness. Though there were no significant ε4 group-related differences in reported history of alcohol and substance abuse/dependence, the sample was characterized by relatively high rates of reported previous alcohol (36.6%) and illicit substance (65.9%) abuse/dependence. To the extent that history of alcohol or substance abuse and psychopathology impacts subsequent cognitive performance, the current results must be interpreted accordingly, and future studies should take these factors into account.

Finally, given difficulties encountered in recruitment of the target population, we were unable to systematically match the ε4 subgroups on subject characteristics including gender and ethnic origin, which resulted in a disproportionately larger number of ε4− males to females and an overrepresentation of African-American subjects. As such, factors associated with gender and ethnicity in HIV/AIDS including different modes of transmission, rates of engagement in various risky behaviors having differential likelihoods of an individual experiencing multiple infections with different viral strains, and different incidences of co-infections with non-HIV pathogens affecting general health and the integrity of the CNS may have impacted the observed results in ways as yet unknown to us.

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Conflict of interest None.

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