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N-Acetylcysteine Normalizes Glutamate Levels in Cocaine-Dependent Patients: A Randomized Crossover Magnetic Resonance Spectroscopy Study

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Treatment with *N*-acetylcysteine (NAC) normalizes glutamate (Glu) homeostasis and prevents relapse in drug-dependent animals. However, the effect of NAC on brain Glu levels in substance-dependent humans has not yet been investigated. Proton magnetic resonance spectroscopy (^{1}H MRS) was used to investigate Glu changes in the dorsal anterior cingulate cortex (dACC) after a single dose of NAC in cocaine-dependent patients and normal controls. In an open-label, randomized, crossover study, 8 cocaine-dependent patients and 14 healthy controls underwent two scan sessions: one group receiving no compound and the other following a single administration of 2400 mg NAC. The Barratt Impulsiveness Scale was administered to examine the relation between dACC Glu levels and impulsivity. In the medication-free condition, Glu levels in the dACC were significantly higher in cocaine-dependent patients compared with healthy controls. After administration of NAC, Glu levels were reduced in the cocaine-dependent group, whereas NAC had no effect in healthy controls. Higher baseline Glu levels were associated with higher impulsivity, and both were predictive of greater NAC-induced Glu reduction. The current findings indicate that NAC can normalize elevated Glu levels in cocaine-dependent patients. These findings may have important implications for treatment, because abnormal Glu levels are related to relapse, and treatment with NAC prevented relapse in animal studies. Furthermore, clinical studies have indicated beneficial effects of NAC in cocaine-dependent patients, and the current study suggests that these beneficial effects might in part be mediated by the ability of NAC to normalize glutamatergic abnormalities.

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INTRODUCTION

Traditionally, research into the neurobiological substrate of drug addiction has focused on mesolimbic dopamine reward circuitry. However, recent literature has highlighted the importance of the excitatory amino-acid glutamate (Glu) in cocaine dependence, especially its role in the continuation of and relapse into substance abuse. In rodents, protracted cocaine use induces neural changes in glutamatergic signaling, resembling neuroplasticity associated with learning and memory (Kelley, 2004). Specifically, repeated cocaine exposure has been found to result in reduced firing rates of glutamatergic projections from the medial prefrontal cortex (including the anterior cingulate cortex (ACC)) to the nucleus accumbens (Sun and Rebec, 2006) and reduced levels of extracellular Glu in the nucleus accumbens under basal conditions (Baker *et al*, 2003a). In the presence of cocaine-related cues, a large increase of synaptic Glu release derived from prefrontal afferents has been observed in the nucleus accumbens, in part resulting from reduced tone of extracellular Glu on group II metabotropic Glu (mGluR2/3) receptors that are important for regulating synaptic Glu release (Baker *et al*, 2003a; McFarland *et al*, 2003; Sun and Rebec, 2006). These neuroadaptations may be of key importance for cocaine reinstatement in self-administration animal models of relapse (Baker *et al*, 2003a; Madayag *et al*, 2007; McFarland *et al*, 2003; Sun and Rebec, 2006) and have led to the suggestion that targeting the glutamatergic system may prove effective when treating cocaine dependence (Reissner and Kalivas, 2010). A potential glutamatergic drug for

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treating substance abuse is *N*-acetylcysteine (NAC), an amino-acid cystine prodrug, which is used to treat acetaminophen overdose and sold over the counter as a mucolytic agent and nutritional supplement. Systemic administration of NAC restores extracellular Glu levels (thereby increasing tonic activation of the mGluR2/3 receptors) and prevents relapse to drug-seeking behavior in rats previously treated with cocaine (Baker *et al*, 2003b; Madayag *et al*, 2007) and heroin (Zhou and Kalivas, 2008). In humans, pilot studies have shown that NAC decreases cue-induced craving for cocaine (LaRowe *et al*, 2007), pathological gambling (Grant *et al*, 2007), number of cigarettes smoked (Knackstedt *et al*, 2009), the rewarding effect of smoking (Schmaal *et al*, 2011), and marijuana use and craving (Gray *et al*, 2010). However, whether these beneficial effects of NAC are mediated by NAC-induced Glu changes in the human brain has not yet been investigated.

A technique that allows *in vivo* assessment of Glu levels, along with other neurometabolite levels in the human brain, is proton magnetic resonance spectroscopy (^{1}H MRS). A few studies have used ^{1}H MRS to examine Glu in substance dependence. Decreased levels of Glu have been found in the rostral ACC (rACC) in cocaine-dependent patients (Yang *et al*, 2009), but both increased (Lee *et al*, 2007) and decreased (Thoma *et al*, 2011) Glu levels were found in the dorsal ACC in alcohol-dependent patients. In addition, increased Glu levels were found in the putamen in squirrel monkeys that were treated with cocaine for 9 months (Liu *et al*, 2011), although no abnormalities were found in chronic tobacco smokers (Gallinat and Schubert, 2007). With regard to the combined Glu plus glutamine signal, decreased levels have been found in the dorsal ACC in opiate addiction (Yucel *et al*, 2007). These inconsistent findings could be related to differences in patients' drug use characteristics such as time of abstinence and drug intake between the studies, as Glu abnormalities seem to be highly dependent on individual drug use characteristics (Chang *et al*, 1997; Ernst and Chang, 2008; Lee *et al*, 2007; Liu *et al*, 2011; Yang *et al*, 2009). Given these findings of Glu abnormalities in substance-dependent patients, it is important to establish whether NAC can normalize Glu alterations observed in substance-dependent individuals. Therefore, the current pilot study aimed to investigate the effect of a single dose of NAC (2400 mg) on brain Glu levels in cocaine-dependent human subjects relative to healthy controls using ^{1}H MRS. Pretreatment with a single dose of systemically administered NAC has been shown to prevent cocaine-primed reinstatement in animals (Moran *et al*, 2005). This effect was blocked by coadministration of a mGluR2/3 antagonist, indicating that this prevention of reinstatement by a single dose of NAC resulted from modulation of the Glu system (Moran *et al*, 2005). We chose the dorsal ACC (dACC) as our region of interest because most of the Glu abnormalities in previous human studies were located in the ACC, and to ensure that the ^{1}H MRS data were collected from a homogeneous tissue region that contains predominantly gray matter. Dorsal ACC dysfunction plays a key role in cocaine dependence and has been related to impaired impulse inhibition (for a review, see Garavan and Hester, 2007). For example, using functional MRI, it has been shown that decreased activation of the left dACC, as observed in cocaine-dependent patients during response inhibition tasks, is associated with increased impulsive responding (Hester and Garavan, 2004; Li *et al*, 2008). Moreover, dACC hypoactivations have been reported in response to cocaine-related cues, a finding that has been interpreted as diminished functioning of the brain's 'control network' following cue exposure (Goldstein *et al*, 2009; Volkow *et al*, 2011). Recently, a ^{1}H MRS study in subjects with borderline personality disorder demonstrated a positive association between dACC Glu levels and impulsivity (Hoerst *et al*, 2010). Therefore, we also included a self-report impulsivity questionnaire to investigate the relation between dACC Glu levels and impulsivity at baseline.

PATIENTS AND METHODS

Subjects

Ten male patients currently treated primarily for cocaine dependence (meeting DSM-IV criteria for cocaine dependence; American Psychiatry Association, 1994) were recruited from regional addiction treatment centers (CD group). Fourteen nonsmoking healthy control subjects (HC group) matched on age, sex, and education were included. Exclusion criteria were: substance use disorders (other than cocaine, alcohol, and nicotine for the CD group); current DSM-IV diagnosis (except for attention deficit hyperactivity disorder (ADHD) and antisocial personality disorder in the CD group); lifetime history of head injury with loss of consciousness for >5 min; neurological disorders; unstable medical condition; low level of education (drop-out before the age of 16 years); any use of medication affecting the central nervous system; MRI ineligibility due to nonremovable metal objects or claustrophobia. Recent drug and alcohol use was assessed with urine tests. All subjects gave written informed consent to participate in this study, which was approved by the Medical Ethical Committee of the Academic Medical Center.

Clinical Assessments

All subjects were screened for the presence of Axis I psychiatric disorders using the Mini International Neuropsychiatric Interview plus (MINI-plus; Sheehan *et al*, 1998). General intelligence (IQ) was assessed using the Dutch version of the National Adult Reading Test (NART; Schmand *et al*, 1991). Alcohol and drug consumption during the preceding 6 months was quantified using the Time Line Follow Back method (TLFB; Sobell and Sobell, 1992). The Fagerström Test for Nicotine Dependence (FTND; Heatherton *et al*, 1991) was administered to measure level of nicotine dependence. In addition, the Alcohol Use Disorder Identification Test (AUDIT), a 10-item questionnaire, was used to identify harmful patterns of alcohol consumption (Babor *et al*, 1989).

The Barratt Impulsiveness Scale (BIS-11; Patton *et al*, 1995) was administered at the start of the first session (in case of the NAC condition before medication was taken) to assess self-reported impulsivity. The BIS-11 is a 30-item questionnaire designed to assess general impulsiveness. Each item is scored on a 4-point scale (rarely/never, occasionally, often, almost always/always), with higher scores indicative of greater impulsivity. Total score as well

as scores on the cognitive, motor, and nonplanning subscales were assessed for the current study.

Pharmacologic Intervention

In an open-label, randomized, crossover design, subjects participated in two test sessions separated by 1 to 2 weeks. Before the ^{1}H MRS test sessions, subjects received either a single dose of 2400 mg NAC or no compound. The selection of the 2400 mg dose was based on previous studies showing beneficial effects of 1200 and 2400 mg/day NAC on treatment retention and drug use in cocaine and nicotine dependence (Mardikian *et al*, 2007; Knackstedt *et al*, 2009). NAC was administered 1 h before the ^{1}H MRS scan because the peak plasma concentration of NAC occurs $\sim$1–2 h after ingestion (Holdiness, 1991).

Magnetic Resonance Spectroscopy Acquisition and Processing

MRI and MRS data were obtained using a 3.0T Intera MRI scanner (Philips Healthcare, Best, The Netherlands) equipped with a SENSE eight-channel receiver head coil. Three-dimensional T1-weighted images were collected in the sagittal plane using a gradient echo sequence (TR = 9 ms; TE = 3.5 ms; 170 slices; voxel size $1 \times 1 \times 1$ mm; matrix size 256×256). Using these images, a single ^{1}H MRS voxel was placed in the left supracallosal ACC (see Figure 1). Voxel placement was done unilaterally to ensure that the ^{1}H MRS data were collected from a homogeneous tissue region that contained predominantly gray matter. The left dACC was chosen on the basis of studies showing left ACC dysfunction in cocaine-dependent patients compared with healthy controls during impulsivity tasks (Hester and Garavan, 2004; Li *et al*, 2008). MRS was performed using a short-echo point resolved spectroscopy sequence (PRESS; TR = 2000 ms; voxel size $50 \times 16 \times 10$ mm; NEX = 64) with a TE of 38 ms. A TE of 38 ms was chosen because reliable estimates of the Glu signal with this echo time were obtained previously in our lab and it approximates the echo time reported in a study that found improved detection of Glu with a TE of 40 ms (Mullins *et al*, 2008). Spectra were acquired using first-order iterative shimming and water suppression was automatically performed by the scanner.

Spectra derived from ^{1}H MRS from 4.0 to 0.2 p.p.m. were analyzed using LCModel (Linear Combination of Model spectra; Provencher, 1993). LCModel is a user-independent analysis method that estimates metabolite levels by fitting the *in vivo* spectra to a set of previously acquired *in vitro* spectra (the basis set). LCModel software provides specific basis sets for different scanners, field strengths, and echo times (Provencher, 1993). For the current study, the basis set for a Philips 3T MRI scanner was used. Results are presented in institutional units approximating millimolar level. Spectra of all subjects passed the quality control. We used the Cramér–Rao lower bounds (CRLBs), a measure of the reliability of the fit, $<$20% for each individual peak as the quality criterion (Provencher, 1993). The CRLBs for Glu in all subjects were between 7 and 12%. Additional indicators for quality of the spectra were mean (SD) signal-to-noise ratio and the mean (SD) full width half maximum (FWHM). In the medication-free condition, the signal-to-noise ratio was 16.64 (2.53) and 17.10 (1.85), and had a FWHM of 0.05 (0.02) p.p.m. and 0.05 (0.01) p.p.m. for the HC and the CD group, respectively. In the NAC condition, the signal-to-noise ratio was 16.29 (2.23) in the HC group and 16.60 (1.71) in the CD group, and had a FWHM of 0.05 (0.01) p.p.m. for both groups. LCModel estimates both metabolite concentrations referenced to the unsuppressed water signal and concentration ratios (referenced to creatine). Because concentration ratios are less sensitive to relaxation and partial volumes effects than concentrations referenced to the unsuppressed water signal, the ratios of levels of Glu, glutamate + glutamine (Glx), and *N*-acetylaspartate (NAA) to creatine plus phosphocreatine (Cr) were used in statistical analyses. To ensure that NAC-induced changes observed in concentration ratios were not caused by an effect of NAC on creatine, creatine concentrations referenced to the unsuppressed water signal were used to obtain an indication of NAC effects on creatine.

Brain morphology was assessed using a Voxel-Based Morphometry toolbox (VBM8; http://dbm.neuro.uni-jena.de/vbm/) with default settings. The VBM8 toolbox is an extension of the unified segmentation model (Ashburner and Friston, 2005) in which structural images are bias corrected, segmented into gray matter, white matter, and cerebrospinal fluid, and registered combined within the same model. The proportion of gray matter, white matter, and cerebrospinal fluid within the anatomical mask of the

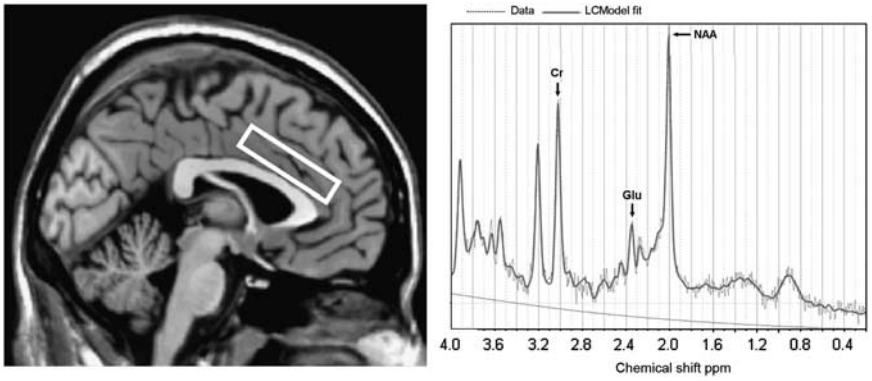

Figure 1 Voxel placement in left dorsal anterior cingulate cortex for localized single-voxel ^{1}H MRS and a representative spectrum in a healthy control subject. Chemical shift is indicated in parts per million (p.p.m.). Cr, creatine; Glu, glutamate; NAA, *N*-acetylaspartate.

Table 1 Demographic and Clinical Characteristics

Demographic variable	Cocaine-dependent group ($N=8$)		Healthy control group ($N=14$)		t(df)	*P*-value
	Mean	SE	Mean	SE		
Age	35.12	2.42	35.71	2.44	−0.16 (20)	0.88
Education (ISCED)	3.50	0.50	3.93	0.25	−0.87 (20)	0.40
IQ (NART)	93.38	2.03	97.29	3.81	−0.74 (20)	0.47
BIS-11						
Total score	72.75	1.81	57.50	2.09	4.92 (20)	<0.01
Cognitive scale	19.38	1.09	14.64	0.73	3.74 (20)	<0.01
Motor scale	26.12	0.76	21.14	0.90	3.74 (20)	<0.01
Nonplanning scale	27.25	0.84	21.71	0.89	4.14 (20)	<0.01
Cocaine use in preceding 6 months (in g)	78.69	17.27	0	NA	—	—
Alcohol in preceding 6 months (in standard units)	777.94	186.82	102.21	27.17	4.68 (7.30)	0.01
AUDIT scores	21.29	3.87	4.71	0.66	4.22 (6.35)	0.01
Number of smokers, *N* (%)	7 (87.5)	NA	0	NA	—	—
Cigarettes/day	17.25	3.49	0	NA	—	—
FTND scores	6.12	1.06	0	NA	—	—

Abbreviations: AUDIT, Alcohol Use Disorder Identification Test; BIS-11, Barratt Impulsiveness Scale version 11; FTND, Fagerström Test for Nicotine Dependence; ISCED, International Standard Classification of Education; NA, not applicable; NART, National Adult Reading Test.

ACC was calculated in order to examine group differences in tissue composition. The ACC mask was defined by merging the individually placed spectroscopy voxel position in normalized space in order to correspond to the size and placement of the MRS voxel that was used for obtaining MRS spectra in the left dACC.

Statistical Analyses

All demographic and behavioural data analyses were carried out using SPSS 16.0 (SPSS, Chicago, IL). All data were normally distributed. Differences in baseline characteristics between groups were analysed using independent *t*-tests. A repeated measures ANOVA was conducted to assess the effect of NAC treatment on metabolite levels in the dACC between the two groups. Treatment (medication-free *vs* NAC) was modeled as a within-subject factor and group (CD *vs* HC) was modeled as a between-subject factor. Administration order of NAC did not affect between-subject or within-subject differences in metabolite levels and was therefore not included as a covariate in the analyses. The *post hoc* tests were employed to examine significant differences between groups and within groups with and without NAC administration. Relationships between substance use, impulsivity measures, and Glu levels were explored using bivariate correlation and linear regression analyses. The significance criterion was set to $p<0.05$.

Table 2 NAC-Induced Changes in Glutamate (Glu), *N*-Acetylaspartate (NAA), and Glutamate+Glutamine (Glx) Referenced to Creatine and Referenced to the Unsuppressed Water Signal

Metabolite	Session	CD group	HC group	F(df)[a]	*P*-value
		Mean (SE)			
Referenced to creatine					
Glu	Medication-free	1.49 (0.07)	1.32 (0.04)	5.46 (1,20)	0.03
	NAC	1.32 (0.04)	1.35 (0.05)		
Glx	Medication-free	2.01 (0.08)	1.91 (0.06)	3.69 (1,20)	0.07
	NAC	1.94 (0.06)	2.00 (0.07)		
NAA	Medication-free	1.24 (0.04)	1.29 (0.05)	0.03 (1,20)	0.87
	NAC	1.21 (0.03)	1.25 (0.04)		
Referenced to the unsuppressed water signal					
Glu	Medication-free	8.39 (0.27)	7.36 (0.23)	4.90 (1,20)	0.04
	NAC	7.69 (0.25)	7.59 (0.21)		
Glx	Medication-free	11.33 (0.36)	10.57 (0.29)	2.51 (1,20)	0.13
	NAC	11.15 (0.37)	11.45 (0.37)		
NAA	Medication-free	6.99 (0.16)	7.12 (0.12)	0.19 (1,20)	0.66
	NAC	6.95 (0.08)	6.99 (0.18)		
Cr	Medication-free	5.66 (0.14)	5.60 (0.16)	0.01 (1,20)	0.92
	NAC	5.75 (0.11)	5.72 (0.19)		

[a]Results are presented for the treatment (medication-free *vs* NAC) × group (CD *vs* HC) interaction effect. There were no significant main effects of session or group.

RESULTS

Sample Characteristics

The demographic, clinical, and substance use characteristics are presented in Table 1. Out of 10 CD subjects, 2 tested positive for cocaine and were excluded from further analyses. The CD group did not differ from the HC group with regard to age, educational level, and IQ. The CD group had significantly higher impulsivity scores measured by the BIS-11. HC subjects did not report any cocaine use or other drug use and consumed significantly less alcohol compared with CD subjects. No adverse events were reported in the study.

Main Outcome: Effect of NAC on Glu Levels

No differences between groups were found, both in the medication-free and NAC condition, in the gray matter, white matter, and cerebrospinal fluid content of the dACC region corresponding to the ^{1}H MRS voxel (all *p*-values >0.3) and were therefore not included as covariates in subsequent analyses. A repeated measures ANOVA revealed no main effect for group ($F(1,20)=1.68$, $p=0.21$) or treatment ($F(1,20)=2.21$, $p=0.15$) on dACC Glu relative to creatine (Glu/Cr). However, a significant interaction between group and treatment was present (see Table 2). The *post hoc* tests revealed that there was a significant reduction in Glu/Cr in the NAC condition compared with the medication-free condition in the CD group ($t(7)=3.08$,

$p = 0.02$), whereas NAC had no effect on Glu/Cr in the HC group ($t(13) = -0.64$, $p = 0.53$). In the medication-free condition, significant higher Glu/Cr in the CD group compared with the HC group was found ($t(20) = 2.26$, $p = 0.04$). After administration of a single dose of NAC, differences in Glu/Cr between the two groups disappeared ($t(20) = -0.24$, $p = 0.81$). These Glu/Cr changes by NAC are graphically shown in Figure 2. Analysis with Glu concentrations referenced to the unsuppressed water signal revealed a similar group by treatment interaction (Table 2), which was driven by a significant higher Glu concentration in the medication-free condition in the CD group compared with the HC group ($t(20) = 2.80$, $p = 0.01$) and a significant reduction in Glu/Cr in the NAC condition compared with the medication-free condition only in the CD group ($t(7) = 3.48$, $p = 0.01$).

Across groups, a regression analysis revealed that medication-free Glu/Cr was predictive for the effect of NAC on Glu/Cr ($\beta = 0.70$, $t(20) = 4.43$, $p < 0.001$) and that it explained a significant and substantial proportion of variance in Glu/Cr changes by NAC ($R^2 = 0.50$, $F(1, 20) = 19.58$, $p < 0.001$; see Figure 3a).

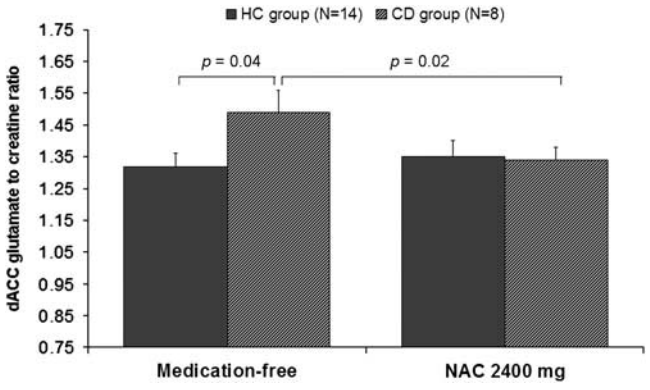

Figure 2 Effect of a single dose of NAC (2400 mg) on Glu/Cr in the left dACC in both CD and HC groups (mean ± SE). Medication-free Glu/Cr was significantly higher in the CD group when compared with the HC group. Administration of NAC reduced Glu/Cr in the CD group, whereas it had no effect on the HC group.

As NAC had an effect on Glu/Cr only in the CD group, an additional analysis was conducted within the CD group to test whether responders *vs* nonresponders to the NAC challenge in terms of Glu/Cr reduction differed in their medication-free Glu/Cr. A median split based on NAC-induced Glu/Cr changes revealed that responders had a significantly higher medication-free Glu/Cr compared with nonresponders ($t(6) = 2.90$, $p = 0.03$).

Because the measure of Glu was based on the ratio of Glu to creatine, we explored whether these results might have been caused by an effect of NAC on creatine. Creatine levels were unaffected by NAC treatment in both groups. In addition, NAC had no significant effect on other metabolite levels (Table 2).

Impulsivity and Glu Levels

Because of the involvement of the dACC in impulse control, we investigated whether Glu/Cr within the dACC was related to general impulsivity across groups. There was a significant positive correlation between medication-free Glu/Cr and impulsivity as measured by the BIS-11 (total impulsivity score, $r(22) = 0.53$, $p = 0.01$; cognitive impulsivity subscale, $r(22) = 0.65$, $p < 0.01$; nonplanning impulsivity subscale, $r(22) = 0.48$, $p = 0.02$, but not for the motor impulsivity subscale, $r(22) = 0.26$, $p = 0.25$). In addition, a regression analysis revealed that higher BIS-11 total scores were predictive of NAC-induced decreases in Glu/Cr ($\beta = 0.47$, $t(22) = 2.38$, $p = 0.03$; see Figure 3b). The total BIS-11 score explained a significant and substantial proportion of the variance in NAC-induced Glu/Cr changes ($R^2 = 0.22$, $F(1, 20) = 5.66$, $p = 0.03$).

Self-Reported Substance Use and Glu Levels

Within the CD group, there was a trend toward a negative correlation between self-reported total cocaine use during the 6 months before participation and medication-free Glu/Cr in the dACC ($r(8) = -0.67$, $p = 0.07$). No associations with other cocaine use measures such as abstinence duration or other drug use such as number of cigarettes smoked and FTND scores in the CD group were found.

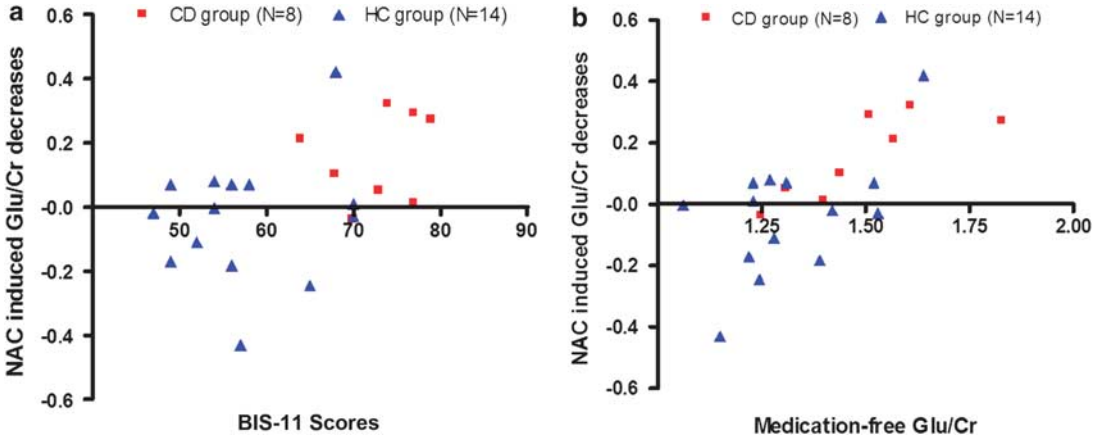

Figure 3 NAC-induced Glu/Cr decreases in the dACC were significantly predicted by (a) medication-free Glu/Cr in the dACC ($R^2 = 0.50$, $p < 0.001$) and (b) trait impulsivity measured by the BIS-11 total score ($R^2 = 0.22$, $p = 0.03$), across groups.

Moreover, alcohol use and AUDIT scores did not correlate with Glu/Cr in the CD group or in the HC group. Because both total cocaine use and BIS-11 impulsivity scores were correlated with medication-free Glu/Cr, we examined whether cocaine use and BIS-11 scores were also correlated. However, the BIS-11 total score and scores on the BIS-11 subscales were not correlated with total cocaine use.

DISCUSSION

Using ^{1}H MRS, the current study is the first to demonstrate a significant reduction in Glu/Cr in the left dACC by a single dose of NAC (2400 mg) in cocaine-dependent patients, whereas NAC had no effect on Glu/Cr in healthy controls. In the medication-free condition, significant higher Glu/Cr was found in cocaine-dependent subjects compared with healthy controls, which normalized after a single administration of NAC. The current results are in line with preclinical studies indicating that NAC restores Glu abnormalities only when Glu homeostasis is disturbed, as for example by chronic exposure to cocaine (Baker *et al*, 2003b). Furthermore, higher Glu/Cr at baseline was associated with general impulsivity ratings and both medication-free Glu/Cr and impulsivity predicted NAC-induced changes in Glu/Cr.

These findings seem to be in contrast with those of Baker *et al* (2003b) showing lower levels of extracellular Glu in the nucleus accumbens in cocaine-treated rats compared with controls. In this rodent study, administration of NAC normalized basal levels of extracellular Glu. It should be noted, however, that ^{1}H MRS is not able to distinguish extracellular from intracellular Glu and primarily reflects the more abundant intracellular Glu, which is present in neuronal and glial metabolic and neurotransmitter pools (Gruetter *et al*, 1998). In the brain, basal levels of extracellular Glu are maintained by the cystine/glutamate antiporter (system x_c^-) exchanging extracellular cystine for intracellular Glu. The NAC-induced rise in extracellular Glu in cocaine-dependent rats has been attributed to restoration of system x_c^- functioning, which is predominantly expressed on glial cells (Baker *et al*, 2003b; Moussawi *et al*, 2011). Enhancement of the exchange of intracellular Glu for extracellular cystine by NAC would be expected to reduce glial intracellular Glu and, therefore, the current findings of increased medication-free levels of Glu and the NAC-induced reduction of Glu in CD may stem from glial metabolic pools of Glu in the dACC. In addition, repeated cocaine administration has been associated with increased Glu neurotransmission in rats in medial prefrontal cortex by the reduced ability of mGluR2/3 receptors to inhibit synaptic Glu release (Xie and Steketee, 2008). Extracellular Glu stimulates presynaptic mGluR2/3 receptors and a NAC-induced increase in extracellular Glu has been found to reduce neuronal Glu transmission by increased tonic activation of the mGluR2/3 receptors (Baker *et al*, 2003b; Moussawi *et al*, 2011). Our findings of NAC-induced Glu reduction may therefore also represent neurotransmitter pools in the dACC. Studies using carbon-13 spectroscopy, a technique that can differentiate between neurotransmitter and metabolic pools of Glu, may provide more insight into the source of the currently found Glu changes by NAC (Shen *et al*, 1999). Noteworthy is that quantification of Glu is difficult partly because it largely overlaps with glutamine in its chemical shift range, which leads to increased fitting errors. Although Glu was individually quantified separately from glutamine (Gln) and Glx with reasonable fitting errors (CRLBs all below 12%) and represented ~50–80% of the total Glx signal (Pouwels and Frahm, 1998), undetected contributions of other overlapping peaks such as Gln cannot be ruled out completely, especially considering the finding that NAC had no effect on Glx that largely consists of Glu. Detection of Gln alone by means of ^{1}H MRS is even more challenging and the current study did not allow reliable evaluation of Gln changes. Quantifying Gln (separately from Glu) could be of particular interest, as synaptic Glu taken up by glial cells is converted into glutamine before returning to the presynaptic neuron for conversion back into Glu (Magistretti and Pellerin, 1999) and therefore Gln may be a more accurate index of overall glutamatergic neurotransmission than Glu (Rothman *et al*, 2003). Future studies using more advanced spectral editing techniques such as a spectrally selective refocusing method (Choi *et al*, 2006) or 2D J-resolved spectroscopy (Jensen *et al*, 2009) for improved separation of Glu from Gln are required to further characterize the effect of NAC on the Glu system in the human brain.

The current finding of an increased medication-free Glu/Cr in cocaine-dependent subjects relative to healthy controls is consistent with a recent study by Liu *et al* (2011) investigating the effects of chronic exposure to cocaine on Glu/Cr in the putamen of squirrel monkeys using ^{1}H MRS. After 9 months, Glu/Cr and Gln/Cr were significantly higher in cocaine-treated monkeys compared with baseline levels and to Glu/Cr in saline-treated monkeys. Higher ACC Glu/Cr was also found in young alcoholics (Lee *et al*, 2007). Moreover, treatment with acamprosate, a Glu-mediating compound, reduced ACC Glu/Cr whereas Glu/Cr was increased during a 4-week placebo treatment in alcohol-dependent patients (Umhau *et al*, 2010). Although some of our CD subjects met the criteria for alcohol abuse, we did not find an association between Glu/Cr and alcohol use. This could perhaps be explained by the amount of alcohol consumed in our CD group (mean of 3.5 drinks/day), which was not as high as reported in the study of Umhau *et al* (2010) (mean of ~15 drinks/day), whereas the study of Lee *et al* (2007) did not measure daily alcohol consumption. In contrast to findings of increased Glu levels in substance dependence, Yang *et al* (2009) found significantly lower levels of ACC Glu/Cr in chronic cocaine users, although they did measure Glu in a functionally different division of the ACC, namely the rACC as opposed to dACC in the current study. Whereas the dACC is more involved in cognitive processing, the rACC is mainly activated in response to emotional content (Bush *et al*, 2000). These distinct areas of the ACC have been found to be differentially affected by chronic cocaine use. Whereas hypoactivation has been observed in the dACC during response inhibition tasks and in response to drug cues (Hester and Garavan, 2004; Li *et al*, 2008; Volkow *et al*, 2011), hyperactivation (or diminished deactivation) has been detected in the rACC (Brodmann area 25) and adjacent ventromedial frontal areas especially in the presence of drug-related cues in cocaine-dependent patients (Kilts *et al*, 2001; Volkow *et al*, 2005). This interaction between a

diminished functioning of a cognitive control brain network (including the dACC) and an increased responsiveness to drug-cues in reward-processing areas (including the rACC) has been proposed to underlie compulsive drug taking (Baler and Volkow, 2006). In line with these findings of differentially affected subdivisions of the ACC, Yang *et al* (2009) found a positive correlation between years of cocaine use and Glu/Cr, that is, the longer the cocaine use, the higher the rACC Glu/Cr, whereas we found a trend towards a negative correlation between total cocaine use in the preceding 6 months before participation and dACC Glu/Cr (unfortunately, we did not have a measure of years of cocaine use in the present study). However, both correlations seem to be in the opposite direction of what would have been expected. In line with the self-medication hypothesis of cocaine use, a possible explanation might be that these Glu abnormalities are a pre-existing risk factor for the development of addiction and are actually normalized with continued cocaine use. Clearly, the relationship between brain Glu levels and cocaine dependence is not straightforward but rather complex, depending on multiple facets of individual drug use patterns, and needs further investigation.

The dACC is a key region involved in impulse inhibition (Chambers *et al*, 2009) and maladaptive high levels of impulsivity have been associated with diminished ACC functioning in substance dependence (Forman *et al*, 2004; Kaufman *et al*, 2003; Lee *et al*, 2005; Li *et al*, 2008; Meade *et al*, 2011). Preclinical literature has indicated a role for Glu in impulsivity (for a review, see Pattij and Vanderschuren, 2008). For instance, selective and nonselective NMDA receptor antagonists have been shown to increase impulsive behavior in animal models (Higgins *et al*, 2003; Mirjana *et al*, 2004). Systemic pretreatment with an mGlu2/3 receptor agonist attenuates impulsive behavior seen after serotonin receptor stimulation (Wischhof *et al*, 2011). In humans, a recent study of Hoerst *et al* (2010) examined Glu levels in the dACC in patients with borderline personality disorder and healthy controls. Irrespective of diagnosis, higher Glu/Cr was associated with higher BIS-11 total scores and cognitive impulsivity subscale scores (Hoerst *et al*, 2010). Anterior cingulate Glu/Cr was also found to be increased in untreated children with ADHD, a disorder characterised by impaired impulse control (Hammerness *et al*, 2012). In keeping with these findings, the current study revealed that medication-free Glu/Cr in the dACC was associated with (cognitive) impulsivity measured by the BIS-11. These results suggest that Glu abnormalities underlie impaired (left) dACC functioning associated with high levels of impulsivity found in substance dependence. Preclinical studies have reported that NAC treatment prevents reinstatement of drug-seeking behavior in cocaine-treated rats by restoring Glu homeostasis and thereby increasing tonic activation of the mGluR2/3 receptors (Baker *et al*, 2003b). In humans, NAC reduces the desire to use cocaine in the presence of cocaine-related cues in cocaine-dependent patients (LaRowe *et al*, 2007). Impulsivity is an important predictor of relapse into substance abuse (Bowden-Jones *et al*, 2005; Brewer *et al*, 2008; Goudriaan *et al*, 2008; Krishnan-Sarin *et al*, 2007; MacKillop and Kahler, 2009; Moeller *et al*, 2001). In the current study we found that impulsivity ratings predict NAC-induced Glu/Cr changes. Because impulsive behavior is in part regulated by mGlu2/3 receptor activation (Wischhof *et al*, 2011), and NAC increases mGluR2/3 activation resulting in prevention of cue-induced reinstatement of cocaine-seeking behavior (Baker *et al*, 2003b), one may speculate that impulsivity mediates the relation between NAC-induced Glu changes and reductions in cue-induced craving and prevention of cue-induced reinstatement by NAC treatment. However, because we did not include measures for NAC-induced changes in impulsivity and craving, future research is needed to further delineate the interrelation between Glu, impulsivity, and craving for cocaine or relapse into cocaine abuse.

As the current results were obtained in a pilot study, our results should be viewed in light of some methodological limitations. First, the current study was an open-label study, and hence we cannot rule out the possibility that subjective effects interacted with changes in Glu levels. However, we failed to observe order effects (NAC treatment *vs* no treatment) and it seems rather unlikely that expectancies have such a profound effect on Glu levels in the left dACC. Therefore, we feel that the open-label aspect of the study is not a serious threat to the validity of our findings. Second, the sample size was modest, especially with regard to the CD group. The results were similar when two more CD subjects who tested positive for cocaine were included (data not shown). However, future studies including larger sample sizes are warranted to replicate the current findings. Third, the current study was designed as a first step toward investigating the effects of NAC on Glu levels in the human brain, and for this purpose we used a single dose of NAC based on the findings of Moran *et al* (2005) showing that pretreatment with a single dose of NAC prevents relapse in cocaine-seeking behavior in an animal model of reinstatement. However, future research examining the effects of longer treatment with NAC and dose effects of NAC on Glu levels is needed to confirm the current findings. Moreover, because no clinical measures were included in the current study and the impulsivity questionnaire was only administered once, the implications of the observed NAC-induced Glu changes for clinical outcome and impulsivity remain to be elucidated. Double-blind, placebo-controlled studies implementing longer treatment durations are required to further clarify the effects of NAC on the brain Glu system and their consequences on clinical measures and state impulsivity. Another limitation is that some of our cocaine-dependent sample had secondary alcohol problems and most of them were smokers. Therefore, we cannot rule out effects of alcohol use and smoking on the current findings of the NAC-induced reduction in Glu levels. However, we did show that there was no correlation between NAC-induced Glu changes and baseline alcohol use and smoking characteristics such as alcohol consumption in the past 6 months, AUDIT scores, number of cigarettes smoked per week, and FTND scores. Finally, drawing conclusions regarding the specificity of our findings are limited by the fact that we did not acquire metabolite data from other regions than the left dACC. We chose the left dACC as our region of interest because most Glu abnormalities in previous human studies were located in the ACC. Given the findings of animal studies with NAC, it would be of particular interest to also investigate the effects of NAC on

Glu levels in the nucleus accumbens. It is, however, difficult to reliably evaluate Glu levels in this particular region because of field inhomogeneities, but future ^{1}H MRS studies performed at a higher field strength (eg, 7 Tesla) or with access to more advanced spectral editing techniques should be able to establish whether the nucleus accumbens is similarly or differently affected.

Notwithstanding these limitations, we believe that the current ^{1}H MRS study is an important step toward unraveling the mechanisms by which NAC acts on the Glu system in substance-dependent patients. NAC has been proven to successfully prevent relapse into cocaine-seeking behavior by restoring Glu homeostasis in animal models of reinstatement. Our study is the first to demonstrate a similar effect of NAC on brain Glu abnormalities in cocaine-dependent humans. Together with treatment studies indicating that NAC has a beneficial effect in patients with cocaine dependence (LaRowe *et al*, 2007; Mardikian *et al*, 2007), pathological gambling (Grant *et al*, 2007), marijuana users (Gray *et al*, 2010), and nicotine dependence (Knackstedt *et al*, 2009; Schmaal *et al*, 2011), the current study suggests that this effect might in part be mediated by the ability of NAC to normalize glutamatergic abnormalities. In addition, the current study demonstrates that baseline Glu/Cr and baseline (cognitive) impulsivity predict the ability of NAC to normalize Glu abnormalities. This is consistent with the preclinical literature and current observations of a lack of effect of NAC in controls with normal Glu levels. These findings also suggest that NAC might be especially effective in patients with high dACC Glu levels (according to a pretreatment ^{1}H MRS scan) and/or patients with high levels of self-reported (cognitive) impulsivity. However, future studies are required to determine the most predictive cutoff point of ^{1}H MRS-based Glu levels or BIS-11 impulsivity.

In conclusion, ^{1}H MRS is a valuable noninvasive tool to study Glu system functioning in cocaine dependence and to detect changes induced by Glu modulating compounds such as NAC. The current pilot study shows preliminary evidence for the ability of NAC to normalize Glu homeostasis in cocaine-dependent patients and provides a neurobiochemical rationale for future trials with NAC as a treatment for cocaine dependence. Furthermore, baseline Glu levels were predictive of NAC-induced Glu changes, suggesting that ^{1}H MRS may serve as a biological marker to predict treatment outcome.

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DISCLOSURE

The authors declare no conflict of interest.

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