Boosting impulse control in addiction: Pharmacological neuroimaging studies

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PART III

CHAPTER 4

N-ACETYLCYSTEINE NORMALIZES GLUTAMATE LEVELS IN COCAINE DEPENDENT PATIENTS: A RANDOMIZED CROSS-OVER MAGNETIC RESONANCE SPECTROSCOPY STUDY

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ABSTRACT

BACKGROUND Treatment with N-acetylcysteine (NAC) normalizes glutamate homeostasis and prevents relapse in drug dependent animals. However, the effect of NAC on brain glutamate levels in substance dependent humans has not yet been investigated. Proton Magnetic Resonance Spectroscopy (\(^1\)H MRS) was used to investigate glutamate changes in the dorsal anterior cingulate cortex (dACC) after a single dose of NAC in cocaine dependent patients and normal controls.

METHODS In an open-label, randomized cross-over study, 8 cocaine dependent patients and 14 healthy controls underwent two scan sessions: one receiving no compound and one following a single administration of 2400 mg NAC. The Barratt Impulsiveness Scale was administered to examine the relation between dACC glutamate levels and impulsivity.

RESULTS In the medication-free condition, glutamate levels in the dACC were significantly higher in cocaine dependent patients compared to healthy controls. After administration of NAC, glutamate levels were reduced in the cocaine dependent group, whereas NAC had no effect in healthy controls. Higher baseline glutamate levels were associated with higher impulsivity, and both were predictive of greater NAC induced glutamate reduction.

CONCLUSION The current findings indicate that NAC can normalize elevated glutamate levels in cocaine dependent patients. These findings may have important implications for treatment, because abnormal glutamate 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.
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 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) to the nucleus accumbens (Sun and Rebec 2006) and reduced levels of extracellular glutamate in the nucleus accumbens under basal conditions (Baker et al. 2003a). In the presence of cocaine-related cues, a large increase of synaptic glutamate release derived from prefrontal afferents has been observed in the nucleus accumbens, in part resulting from reduced tone of extracellular glutamate on group II metabotropic glutamate (mGluR2/3) receptors which are important for regulating synaptic glutamate 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 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 glutamate 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 glutamate changes in the human brain has not yet been investigated.

A technique that allows in vivo assessment of glutamate 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 glutamate in substance dependence. Decreased levels of glutamate have been found in the rostral anterior cingulate cortex (ACC) in cocaine dependent patients (Yang et al. 2009), but both increased (Lee et al. 2007) and decreased (Thoma et al. 2011) glutamate levels were found in the dorsal ACC in alcohol dependent patients. In addition, increased glutamate levels were found in the
putamen in squirrel monkeys that were treated with cocaine for nine months (Liu et al. 2011), although no abnormalities were found in chronic tobacco smokers (Gallinat and Schubert 2007). With regard to the combined glutamate 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 glutamate 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 glutamate abnormalities in substance dependent patients, it is important to establish whether NAC can normalize glutamate 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 glutamate levels in cocaine dependent human subjects relative to healthy controls using ¹H MRS. Pre-treatment 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 co-administration of an mGluR2/3 antagonist, indicating that this prevention of reinstatement by a single dose of NAC resulted from modulation of the glutamate system (Moran et al. 2005). We chose the dorsal ACC (dACC) as our region of interest because most of the glutamate abnormalities in previous human studies were located in the ACC, and to ensure that the ¹H 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. 2008a). 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 ¹H MRS study in subjects with borderline personality disorder demonstrated a positive association between dACC glutamate levels and impulsivity (Hoerst et al. 2010). Therefore, we also included a self-report impulsivity questionnaire to investigate the relation between dACC glutamate levels and impulsivity at baseline.

**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 centres (CD group). Fourteen non-smoking 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 ADHD and antisocial personality disorder in the CD group); lifetime history of head injury with loss of consciousness for more than 5 minutes; neurological disorders; unstable medical condition; low level of education (drop-out before the age of 16); any use of medication affecting the central nervous system; MRI ineligibility due to non-removable 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 ten-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 non-planning subscales were assessed for the current study.

Pharmacologic intervention
In an open label, randomized cross-over design, subjects participated in two test sessions separated by one to two weeks. Before the ¹H MRS test sessions, subjects received either a single dose of 2400 mg N-acetylcysteine (NAC) or no compound. The selection of the 2400 mg dose was based on previous studies showing beneficial effects of 1200 mg/day and 2400 mg/day NAC on treatment retention and drug use in cocaine and nicotine dependence (Knackstedt et al. 2009; Mardikian et al. 2007). NAC was administered one hour before the ¹H MRS scan because the peak plasma concentration of NAC occurs approximately 1–2 hours after ingestion (Holdiness 1991).
**Magnetic Resonance Spectroscopy acquisition and processing**

MRI and MRS data were obtained using a 3.0 T 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 1x1x1mm; matrix size 256 x 256). Using these images, a single ¹H MRS voxel was placed in the left supracallosal anterior cingulate cortex (see Figure 1). Voxel placement was done unilaterally to ensure that the ¹H 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 to healthy controls during impulsivity tasks (Hester and Garavan 2004; Li et al. 2008a). MRS was performed using a short-echo point resolved spectroscopy sequence (PRESS; TR=2000 ms; voxel size 50x16x10 mm; NEX=64) with a TE of 38 ms. A TE of 38 ms was chosen because reliable estimates of the glutamate 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 glutamate 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.

![Figure 1](image.png)

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

Spectra derived from ¹H MRS from 4.0 to 0.2 ppm 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 (CRLB), a measure of the reliability of the fit, less than 20% for each individual peak as the quality criterion (Provencher 1993). The CRLBs for glutamate 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) ppm and 0.05 (0.01) ppm 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) ppm 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 glutamate (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 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 Inc., Chicago, Illinois). All data was 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 modelled as a within-subject factor and group (CD vs. HC) was modelled as a between-subjects factor. Administration order of NAC did not affect between-subjects or within-subjects differences in metabolite levels and was therefore not included as a covariate in the analyses. 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 glutamate levels were explored using bivariate correlation and linear regression analyses. The significance criterion was set to $p<0.05$. 

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RESULTS

Sample characteristics

Demographic, clinical and substance use characteristics are presented in Table 1. Two out of ten CD subjects 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 to CD subjects. No adverse events were reported in the study.

Table 1: Demographics and clinical characteristics

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


Main outcome: effect of NAC on glutamate levels

No differences between groups were found, both in the medication-free and NAC condition, in gray matter, white matter and cerebrospinal fluid content of the dACC region corresponding to the ¹H MRS voxel (p values all >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 glutamate relative to creatine (Glu/Cr). However, a significant interaction between group and treatment was present, see Table 2. Post hoc tests revealed that there was a significant reduction in Glu/Cr in the NAC condition compared to 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 to 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 to the HC group ($t(20)=2.80, p=0.01$) and a significant reduction in Glu/Cr in the NAC condition compared to 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. Since 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 versus non-responders 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 to non-responders ($t(6)=2.90, p=0.03$). Because the measure of glutamate was based on the ratio of glutamate 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).
Table 2: NAC-induced changes glutamate (Glu), N-acetylaspartate (NAA) and glutamate+glutamine (Glx) referenced to creatine and referenced to the unsuppressed water signal

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Session</th>
<th>CD group Mean (SE)</th>
<th>HC group Mean (SE)</th>
<th>F (df)¹</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>NAC</strong></td>
<td><strong>NAC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Medication-free</td>
<td>1.49 (0.07)</td>
<td>1.32 (0.04)</td>
<td>5.46 (1,20)</td>
<td>0.03</td>
</tr>
<tr>
<td>Glu</td>
<td>NAC</td>
<td>1.32 (0.04)</td>
<td>1.35 (0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glx</td>
<td>Medication-free</td>
<td>2.01 (0.08)</td>
<td>1.91 (0.06)</td>
<td>3.69 (1,20)</td>
<td>0.07</td>
</tr>
<tr>
<td>Glx</td>
<td>NAC</td>
<td>1.94 (0.06)</td>
<td>2.00 (0.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA</td>
<td>Medication-free</td>
<td>1.24 (0.04)</td>
<td>1.29 (0.05)</td>
<td>0.03 (1,20)</td>
<td>0.87</td>
</tr>
<tr>
<td>NAA</td>
<td>NAC</td>
<td>1.21 (0.03)</td>
<td>1.25 (0.04)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Results are presented for the treatment (medication-free versus NAC) x group (CD versus HC) interaction effect. There were no significant main effects of session or group.

Impulsivity and glutamate 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; non-planning 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 (β=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 (R²=0.22, F(1,20)=5.66, p=0.03).

Self reported substance use and glutamate levels
Within the CD group, there was a trend towards a negative correlation between self-reported total cocaine use during the six 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. 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.

**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.

**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 to healthy controls, which normalized after a single administration of NAC. The current results are in line with preclinical studies indicating that NAC restores glutamate abnormalities only when glutamate 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 glutamate in the nucleus accumbens in cocaine treated rats compared to controls. In this rodent study, administration of NAC normalized basal levels of extracellular glutamate. It should be noted, however, that $^1$H MRS is not able to distinguish extracellular from intracellular glutamate and primarily reflects the more abundant intracellular glutamate, which is present in neuronal and glial metabolic and neurotransmitter pools (Gruetter et al. 1998). In the brain, basal levels
of extracellular glutamate are maintained by the cystine/glutamate antiporter (system x_c) exchanging extracellular cystine for intracellular glutamate. The NAC induced rise in extracellular glutamate 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 glutamate for extracellular cystine by NAC would be expected to reduce glial intracellular glutamate and, therefore, the current findings of increased medication-free levels of glutamate and the NAC induced reduction of glutamate in CD may stem from glial metabolic pools of glutamate in the dACC. In addition, repeated cocaine administration has been associated with increased glutamate neurotransmission in rats in medial prefrontal cortex by the reduced ability of group II metabotropic glutamate (mGluR2/3) receptors to inhibit synaptic glutamate release (Xie and Steketee 2008). Extracellular glutamate stimulates presynaptic group II metabotropic glutamate (mGluR2/3) receptors and a NAC induced increase in extracellular glutamate has been found to reduce neuronal glutamate transmission by increased tonic activation of the mGluR2/3 receptors (Baker et al. 2003b; Moussawi et al. 2011). Our findings of NAC induced glutamate 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 glutamate, may provide more insight into the source of the currently found glutamate changes by NAC (Shen et al. 1999).

Noteworthy is that quantification of glutamate is difficult partly because it largely overlaps with glutamine in its chemical shift range, which leads to increased fitting errors. Although glutamate was individually quantified separately from glutamine (Gln) and glutamate plus glutamine (Glx) with reasonable fitting errors (CRLB’s all below 12%) and represented approximately 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 which largely consists of Glu. Detection of Gln alone by means of ¹H 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, since synaptic glutamate 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 glutamate 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 to 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 glutamate 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 around 15 drinks/day), whereas the study of Lee et al. (2007) did not measure daily alcohol consumption.

In contrast to findings of increased glutamate levels in substance dependence, Yang et al. (2009) found significantly lower levels of ACC Glu/Cr in chronic cocaine users, although they did measure glutamate in a functionally different division of the ACC, namely the rostral ACC opposed to the dorsal ACC in the current study. Whereas the dorsal ACC is more involved in cognitive processing, the rostral ACC (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. 2008a; 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, i.e. 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 prior to 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 glutamate 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 glutamate 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. 2008a; Meade et al. 2011). Preclinical literature has indicated a role for glutamate in impulsivity (for a review see Pattij and Vanderschuren 2008). For instance, selective and non-selective NMDA receptor antagonists have been shown to increase impulsive behavior in animal models (Higgins et al. 2003; Mirjana et al. 2004). Systemic pre-treatment 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 glutamate 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 attention deficit hyperactivity disorder (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 glutamate 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 glutamate 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. 2001b). 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 glutamate 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 glutamate, impulsivity and craving for cocaine or relapse into cocaine abuse.
Since 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, so we cannot rule out the possibility that subjective effects interacted with changes in glutamate 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 glutamate 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 towards investigating the effects of NAC on glutamate 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 pre-treatment 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 glutamate 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 glutamate 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 glutamate 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 glutamate levels. However, we did show that there was no correlation between NAC induced glutamate changes and baseline alcohol use and smoking characteristics such as alcohol consumption in the last six 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 dorsal ACC (dACC) as our region of interest because most glutamate 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 glutamate levels in the nucleus accumbens. It is, however, difficult to reliably evaluate glutamate levels in this particular region because of field inhomogeneities, but future ¹H MRS studies performed at a higher field strength (for example 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 towards unravelling the mechanisms by which NAC acts on the glutamate system in substance dependent patients. NAC has been proven to successfully prevent relapse into cocaine seeking behavior by restoring glutamate homeostasis in animal models of reinstatement. Our study is the first to demonstrate a similar effect of NAC on brain glutamate 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 glutamate abnormalities. This is consistent with the preclinical literature and current observations of a lack of effect of NAC in controls with normal glutamate levels. These findings also suggest that NAC might be especially effective in patients with high dACC glutamate levels (according to a pre-treatment $^1$H MRS scan) and/or patients with high levels of self-reported (cognitive) impulsivity. However, future studies are required to determine the most predictive cut-off point of $^1$H MRS based glutamate levels or BIS-11 impulsivity.

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