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Lower Glutamate Levels in Rostral Anterior Cingulate of Chronic Cocaine Users – A ¹H-MRS Study Using TE-Averaged PRESS at 3T with an Optimized Quantification Strategy

Shaolin Yang^a, Betty Jo Salmeron^a, Thomas J. Ross^a, Zheng-Xiong Xi^b, Elliot A. Stein^a, and Yihong Yang^{a,*}

^a Neuroimaging Research Branch, National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH), Baltimore, MD 21224, USA

^b Chemical Biology Research Branch, National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH), Baltimore, MD 21224, USA

Abstract

Previous studies have shown significantly lower metabolism and functional activity in the anterior cingulate cortex (ACC) of human cocaine addicts. The present study examined whether this ACC hypoactivity is associated with altered glutamate (Glu), the primary excitatory neurotransmitter in the central nervous system (CNS), which has been recently implicated in drug addiction. Fourteen chronic cocaine addicts and 14 matched healthy volunteers were examined using ¹H magnetic resonance spectroscopy at 3T. A new quantification strategy for TE-averaged point-resolved spectroscopy (PRESS) was applied to disentangle relaxation effects from *J*-evolution of coupled spin systems such as Glu. The concentrations of Glu as well as *N*-acetyl aspartate (NAA), total creatine (tCr), choline-containing compounds (tCho), and *myo*-inositol (Ins) were estimated from both groups. Glu/tCr was significantly lower in chronic cocaine users compared to control subjects and was significantly correlated with years of cocaine use. Glu/tCr was also positively correlated with NAA/tCr. NAA/tCr significantly decreased with age but was not significantly different between the two groups. These findings suggest a metabolic/neurotransmitter dysregulation associated with cocaine addiction and support a possible therapeutic intervention strategy aimed at normalizing the Glu transmission and function in the treatment of cocaine addiction.

Keywords

Drug addiction; Neurotransmitter; Relaxation; *J*-coupling

1. Introduction

A growing neuroimaging literature has shown significant alterations in brain structure (Franklin et al., 2002), function (Goldstein and Volkow, 2002; Volkow et al., 1993), and neurochemistry (Chang et al., 1999; Li et al., 1999; Meyerhoff et al., 1999; O'Neill et al.,

*Correspondence to: Yihong Yang, Ph.D., Neuroimaging Research Branch, National Institute on Drug Abuse, NIH, 251 Bayview Blvd, Suite 200, Room 07A709, Baltimore, MD 21224, Tel: 443-740-2648, Fax: 443-740-2816, yihongyang@intra.nida.nih.gov.

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2001) in active or abstinent cocaine users. Among these alterations is lower glucose metabolism in the anterior cingulate cortex (ACC) (Volkow et al., 1993). Chronic cocaine users also show impairments in attention and inhibitory control, associated with attenuated activity in the ACC (Kaufman et al., 2003). Furthermore, cocaine addicts show hypoperfusion in the rostral area of ACC (rACC) (Childress et al., 1999; Peoples, 2002). A recent functional MRI study revealed diminished functional connectivity between the rACC and amygdala in cocaine addicts compared to healthy controls (Stein et al., 2006). Together, these findings suggest that chronic use of cocaine may lead to neuroadaptations in brain structure and function in the ACC, particularly the rACC.

Glutamate (Glu) is the primary excitatory neurotransmitter in the brain. Glutamatergic neurons provide the major communication between many frontal cortical regions and, among others, the mesencephalic dopamine projection neurons in the ventral tegmental area (VTA) and some of their targets, e.g. in the nucleus accumbens (NAcc) (Kalivas and Volkow, 2005). Recent preclinical studies have demonstrated that chronic cocaine use produces a significant reduction in the basal glutamatergic transmission from the prefrontal cortex (PFC), including the ACC, to the core of the NAcc (Baker et al., 2003; Kalivas and Volkow, 2005; Thomas et al., 2001). Such changes have been thought to play a critical role in cocaine addiction and subsequent relapse to drug-seeking behavior (Kalivas and Volkow, 2005). Therefore, measurement of Glu within the ACC in humans may provide important information to better understand frontal lobe alterations and the functional significance of Glu in cocaine addiction.

¹H magnetic resonance spectroscopy (¹H-MRS) has been employed to measure metabolite concentrations, with most success on those metabolites yielding prominent, well-resolved spectral peaks, such as *N*-acetyl aspartate (NAA), total creatine (tCr), and choline-containing compounds (tCho) (Li et al., 1999; O'Neill et al., 2001). Due to *J*-modulation of its coupled spins, the proton resonances of Glu vary with echo time (TE) in a complex pattern (shown in Fig. 1) in addition to the inherent signal decay from relaxation. Compared to those in short-echo spectra (TE < 50 ms), *J*-modulation also brings the Glu proton resonances to lower intensities (Yang et al., 2008). Therefore, short-echo ¹H-MRS sequences are commonly used in Glu measurement to minimize signal loss. However, in short-echo spectra Glu signal strongly overlaps with the proton resonance signals of other metabolites such as glutamine (Gln), NAA, and glutathione at field strengths below 4T (Yang et al., 2008). The recently developed TE-averaged PRESS, essentially a two-dimensional (2D) *J*-resolved spectroscopy technique, makes it possible to measure a well-resolved Glu signal in the human brain at 3 Tesla (Hurd et al., 2004). This technique has been applied to studies of multiple sclerosis (Srinivasan et al., 2005), Alzheimer's disease (Hancu et al., 2005), Parkinson's disease (Kickler et al., 2007), normal human subjects (Sailasuta et al., 2008), and animal models (Adalsteinsson et al., 2004; Adalsteinsson et al., 2006). In those studies, the quantification of metabolite concentrations was fulfilled by fitting TE-averaged model spectra to the *in vivo* spectral data using LCModel (Provencher, 1993) or other similar analysis programs. However, when correcting for relaxation effects in quantification of Glu, a strongly coupled spin system, disentangling relaxation effects from *J*-evolution of coupled spins is important but has been obviously overlooked so far. In this study, we developed an improved strategy to quantify Glu from *in vivo* TE-averaged PRESS data by effectively disentangling relaxation from *J*-evolution of coupled spins. Using this method, we tested the hypothesis that the rACC would show an altered Glu concentration in chronic cocaine addicts compared to healthy controls.

2. Methods

2.1. Subjects

Fourteen chronic crack cocaine users (4 female; 24-43 years old, mean±S.D. of 37±4.9; \$213 ±135/wk current cocaine use, 13±5.4 total years of use; 10 cigarette smokers) and fourteen

age-matched healthy volunteers (7 female; 20-47 years old, mean±S.D. of 34±9.0; 7 cigarette smokers) participated in this study. Subjects were recruited from the general population through newspapers, flyers, and referrals, and gave written informed consent to a protocol approved by the Institutional Review Board of the National Institute on Drug Abuse. Subjects were excluded if they presented with: any major illnesses, HIV positive status, liver function tests greater than three times upper limit of normal range, claustrophobia, history of neurological disorders, current major psychiatric disorders besides cocaine and nicotine dependence, or pregnancy. All participants completed a computerized version of the SCID with follow up interview by an MS or MD level mental health clinician. Last use of cocaine was 44±21 hours before the MRS scan session. Subjects' Beck Depression Inventory-II (BDI-II) scores (Beck et al., 1996) and cigarette smoking history were also obtained. BDI-II scores consistent with mild to severe depression (16-30) were found in 9 cocaine users and were evaluated by a mental health clinician. In all cases, elevations were found to be directly related to immediate consequences of cocaine use and not consistent with major depressive disorder.

2.2. ^1H magnetic resonance spectroscopy (^1H -MRS)

Localized ^1H -MRS was performed on a Siemens Allegra 3-T MR scanner (Erlangen, Germany). Single-voxel TE-averaged PRESS data, in 128 increments of 2.5 ms starting at TE = 35 ms, were acquired from a voxel of $2\times 2\times 2\text{ cm}^3$ that encompassed the rACC (shown in Fig. 2) using a standard quadrature head coil. The other sequence parameters were TR = 2000 ms, spectral bandwidth = 2000 Hz, sampling points = 2048, and NEX = 2.

2.3. Improved spectral quantification strategy for Glu

The MRS data were quantified using the LCModel analysis program (Provencher, 1993) with a basis set consisting of the model spectra of NAA, tCr, tCho, Glu, Gln, *myo*-inositol (Ins) and a separate set of common macromolecules (MM) and lipid. The model spectra were simulated using the GAMMA library (Smith et al., 1994) and the chemical shifts and coupling constants were taken from the literature (Govindaraju et al., 2000). The spectral analysis window was set to 4.0 – 0.2 ppm. Because the effective TE (echo time at mean signal intensity during T_2 decay (Hurd et al., 2004)) is pretty long in the applied TE-averaged PRESS acquisition (For example, 178 ms when $T_2 = 278$ ms or 265 ms when $T_2 = 179$ ms), it is necessary to correct for relaxation effects for each individual metabolite. The commonly used strategy is to first fit the basis set to *in vivo* data to obtain uncorrected concentration estimates, and then correct for the relaxation effects in the post-processing. It is appropriate to use this commonly used process to quantify the data from single-TE experiments, where the difference between the model spectra in the basis set and the *in vivo* data arises from relaxation effects and the concentrations to be estimated. The process is also appropriate for singlet signals from metabolites such as tCr or the acetyl moiety of NAA in the TE-averaged PRESS data. This is illustrated by the simulated PRESS spectra of tCr at multiple TEs (TE = 35 ms, 85 ms, 135 ms, 185 ms, and 235 ms, respectively) in Fig. 1, where relaxation was ignored in the spectral simulation. The singlet resonances of tCr at 3.02 ppm and 3.92 ppm remain unchanged through different TEs when free of relaxation. Therefore, relaxation is the only TE-dependent factor for a singlet resonance in a multiple-TE acquisition. In contrast, due to the *J*-evolution of coupled spins, the C4 proton resonance of Glu at 2.35 ppm (Glu4), the C3 proton resonance of Glu around 2.08 ppm (Glu3), and the C2 proton resonance of Glu at 3.75 ppm (Glu2) demonstrate complex variation with TE. Because relaxation is inherently present, the TE dependence of resonance signal of coupled spins is governed by nonlinearly entangled *J*-evolution and relaxation effects. As such, it is not appropriate to use the same correction strategy as used for singlet resonances to quantify metabolites with coupled spins, such as Glu, because the current correction process is unable to disentangle relaxation effects from *J*-evolution of coupled spins in the TE-averaged spectroscopy. Unfortunately, this issue has been largely ignored so far in the literature.

Here we propose a new correction strategy, in which the relaxation effects are directly incorporated into the TE-averaged PRESS basis set by weighted averaging the metabolite spectra at individual TEs with their corresponding longitudinal (T_1) and transverse (T_2) relaxation effects. Specifically, in the basis set the modal spectrum of a metabolite represents the average of N spectroscopic signals observed at different TEs weighted by the respective relaxation factors, where N is the number of TE steps of the TE-averaged PRESS sequence (=128 in this study).

This new correction strategy disentangles the relaxation from the J -evolution of the strongly coupled spins, therefore improving the spectral quantification of Glu. In contrast, in the conventional quantification process, the relaxation factor is simply set to 1 when generating the modal spectra for the basis set. As such, the relaxation effects and J -evolution are nonlinearly mixed after the TE-averaging, and cannot be effectively disentangled by a composite correction factor using an “effective TE” of the TE-averaged PRESS acquisition.

To implement this correction strategy, one needs to know the T_1 and T_2 values of the metabolites of interest in each subject. However, measurement of relaxation times of the metabolites with coupled spins is not a trivial task and typically is not reliable in practice. Thus, for simplicity, we used the reported values from the literature. The T_1 values of NAA, tCr, tCho, Glu, and Gln in the rACC were 1.48¹, 1.21, 1.44 (Schubert et al., 2004), 1.23, and 1.23 s (Choi et al., 2006), and the T_2 values were 278, 179, 282 (Schubert et al., 2004), 201, and 201 ms (Choi et al., 2006), respectively. Because the T_2 value of Ins in most brain regions, including the rACC, has not yet been reported, the estimated Ins concentration remained uncorrected for relaxation. Only the quantification results with a Cramér-Rao lower bound (CRLB) less than 20% in the LCModel analysis were included in the statistical analysis.

2.4. Statistical analysis

As the rACC tCr levels have been reported not to differ between cocaine users and controls (Li et al., 1999; O'Neill et al., 2001), statistical analyses were conducted on the ratios of NAA/tCr, tCho/tCr, Glu/tCr, and Ins/tCr between the two population groups. Analysis of covariance (ANCOVA) (with group as a factor and age as a covariate) and regression analysis of Glu/tCr with age and NAA/tCr, tCho/tCr, or Ins/tCr were performed, as were regressions of the metabolite ratios with years of cocaine use and current use (with age as a covariate). All analysis used SPSS v15 (SPSS Inc., Chicago, IL, USA). An $\alpha=0.05$ was chosen as significant throughout.

3. Results

The groups did not differ in age ($t_{26}=1.37$, n.s.), gender ($\chi^2=1.35$, n.s.), or smoking status ($\chi^2=1.35$, n.s.). Figure 2 shows a TE-averaged PRESS spectrum from a single voxel that encompassed the rACC of a healthy volunteer. The Glu signal at 2.35 ppm appears as a virtual singlet and is well resolved from other metabolites. The CRLBs of NAA, tCr, tCho, Glu on all subjects were less than 20% and the data were all included in the statistical analysis, whereas the Ins data from 4 control subjects and 5 cocaine users were excluded due to excessive CRLBs. Figure 3 shows the metabolite levels, relative to tCr, in the rACC of both groups. The mean metabolite ratios in the control group are 1.27 ± 0.16 (Glu/tCr), 1.35 ± 0.10 (NAA/tCr), and 0.26 ± 0.02 (tCho/tCr). These values are consistent with the reported ratios of 1.21 ± 0.12 , 1.42 ± 0.10 , and 0.23 ± 0.02 in the ACC in healthy people (Schubert et al., 2004), in which a PRESS sequence with optimal TE = 80 ms was used for enhanced resolution of Glu. In contrast, these ratios in the cocaine-user group are 1.07 ± 0.13 , 1.27 ± 0.09 , and 0.24 ± 0.03 , respectively. The Glu/tCr

¹Personal communication with Dr. Florian Schubert

ratio was significantly lower (15.88%) in the cocaine-user group compared to the controls ($F_{1,25}=10.2$; $p < 0.005$).

To examine whether other independent or comorbid factors other than cocaine abuse contributed to the changes in Glu/tCr, we performed several secondary analyses. Although there was no significant difference in gender between the groups, a higher percentage of males were in the cocaine-user group. However, when gender was included as a second factor in the Glu/tCr analysis, there was no significant gender effect ($F_{1,23}=2.6$, n.s.) while the significant group effect remained ($F_{1,23}=5.5$, $p < 0.03$). Since the mean time between last use of cocaine and the MRS scan was 44 hours, some subjects may have been in a state of withdrawal. We investigated possible withdrawal effects by regressing Glu/tCr against the time since last cocaine use; the regression was not significant ($F_{1,12}=0$, n.s.). As both groups included cigarette smokers, we adjusted for smoking (using covariate “pack-years”, i.e., packs/day times years of smoking) in the analyses. The pack-years value was not significantly different between the two groups ($t_{26}=1.53$, n.s.). After including both age and pack-years as covariates in the group analysis, there was still no significant effect of pack-years ($F_{1,24}=0$, n.s.), however Glu/tCr remained significantly different between groups ($F_{1,24}=8.4$, $p < 0.008$). This result is consistent with a recent finding that cigarette smoking does not induce alterations in the ACC Glu levels (Gallinat and Schubert, 2007), in which a PRESS sequence with optimal TE = 80 ms was used for enhanced resolution of Glu.

Although no participants in either group met criteria for depression, chronic cocaine addicts are prone to elevated depressive measures. We included BDI-II score as an additional covariate to the ANCOVA analysis of Glu/tCr. The analysis showed that there was no significant relationship between Glu/tCr and BDI-II score ($F_{1,23}=0.56$, n.s.); however, the group effect remained significant ($F_{1,23}=7.8$, $p < 0.01$). As depression has been associated with Glx decreases in the ACC (Sanacora et al., 2003), the lack of an association in our sample is consistent with our clinical assessment that those with elevated BDI-II scores were not clinically depressed.

A recent report revealed that the quality of TE-averaged PRESS spectra may be affected by frequency drifting and phase variations resulting from eddy currents (Zhang et al., 2007). These adverse effects could be generally reflected by the spectral line width and SNR of the spectra, which are among the output parameters of the LCModel analysis. To investigate whether the lower Glu/tCr could be driven by reduced SNR or increased line width in the cocaine-user group, we did a between-group comparison of spectral line width and SNR. The spectral line width was 0.05 ± 0.01 ppm (around 6Hz at 3T) in the control group and 0.05 ± 0.01 ppm in the cocaine-user group, and SNR was 13 ± 2 in the control group and 12 ± 2 in the cocaine-user group, respectively. The spectra of the two groups did not differ in line width ($t_{26}=0.02$, n.s.) or SNR ($t_{26}=1.4$, n.s.).

Additionally, although NAA/tCr significantly decreased with age ($F_{1,25}=7.5$; $p < 0.02$), there was no significant difference as a function of group ($F_{1,25}=3.2$, n.s.). No significant group differences were found in tCho/tCr ($F_{1,25}=2.0$, n.s.) or Ins/tCr ($F_{1,25}=1.7$, n.s.). In addition, regression analyses showed a significant positive correlation between Glu/tCr and NAA/tCr ($t_{25}=2.9$; $p < 0.01$), accounting for age (see Fig. 4).

Finally, regression of the metabolite ratios with years of cocaine use and current cocaine use (with age as a covariate) showed a significant positive relationship between Glu/tCr and years of cocaine use ($t_{10}=2.25$, $p < 0.05$) (see Fig. 5) but not between Glu/tCr and current cocaine use ($t_{10}=1.42$, n.s.). The regressions of the other metabolite ratios with years of cocaine use and current cocaine use were not significant.

4. Discussion

Using a recently developed MRS technique that produced a well-resolved Glu signal and an improved quantification strategy, the present study demonstrated significantly lower Glu/tCr in the rACC of cocaine addicts compared to control subjects. This result is robust as it remained significant after accounting for other independent or comorbid factors in the data analyses, including age, gender, withdrawal effects, cigarette smoking, depressive symptoms, and spectrum quality. A significant positive correlation between Glu/tCr and NAA/tCr was also seen. Since the rACC tCr level has been previously reported to not differ between cocaine users and controls (Li et al., 1999; O'Neill et al., 2001), the lower Glu/tCr is suggested to be due to lower Glu levels in the rACC of cocaine addicts. We also found that NAA/tCr in the rACC is significantly correlated with Glu/tCr and with age, but is not significantly different between cocaine users and healthy controls.

¹H-MRS measurement of Glu as applied in this study does not distinguish between metabolic and neurotransmitter pools. Therefore, it is not possible to determine the source of the reduction of Glu in the cocaine-user group. However, the magnitude of the reduction seen in the cocaine-user group suggests involvement of the metabolic pool since this pool accounts for the major part of all CNS Glu. A decrease in the metabolic pool of Glu would be consistent with previous data indicating decreased metabolic rate of glucose in the ACC of cocaine users (Volkow et al., 1993). As the relationship between neuronal glucose oxidation and Glu/Gln cycling is thought to be linear (de Graaf et al., 2004), it is likely that some of the reduction is also due to a reduction in Glu neurotransmission in the ACC, consistent with reduced activation seen in cocaine users performing a task that normally requires ACC activation (Kaufman et al., 2003).

Future studies using other techniques may be able to determine whether altered Glu levels reflect metabolism or neurotransmission change (or both). For example, ¹³C MRS with infusion of ¹³C-labeled metabolites may be able to differentiate the neurotransmitter component from the metabolic Glu pool (Shen et al., 1999). ¹H-MRS measurement of Gln may also provide useful information on the neurotransmission status due to its important role in Glu/Gln cycling. However, an alternative technique other than TE-averaged PRESS should be resorted to for reliable measurement of Gln *in vivo*.

Given the preponderance of data from animal studies generally showing decreases in a number of measures of glutamatergic activity after cocaine use (Kalivas and Volkow, 2005; Smith et al., 2003), the positive correlation of Glu/tCr with years of cocaine use, accounting for age and current cocaine use, is intriguing. As animal models do not generally reflect the extensive cocaine-use history as seen in our cocaine-user group, one possible explanation might be the development of compensatory neurobiological mechanisms over many years of cocaine use that in some way counteracts the decrease in glutamatergic activity caused during initial cocaine use, which may be more reflected by current rodent self-administration studies. Alternatively, since the animal studies do not address vulnerability to developing cocaine addiction, their results may not be directly applicable to human addicts; only about 1 out of 18 who try the substance rapidly develop addiction to cocaine (Wagner and Anthony, 2002). It is possible that our result reflect an initial difference between control subjects and those destined to develop cocaine addiction that lessens with continued use. If true, this result might have implications for the self-medication hypothesis of drug use. This possibility, however, will require extensive further testing to fully understand its significance.

The current study has several limitations. First, in the correction for relaxation effects, we used the T₁ and T₂ values reported for normal subjects for both groups in our study. This was due to technical challenges in the measurement of T₁ and especially T₂ relaxation times of strongly

coupled spin systems such as Glu (Choi et al., 2006), and a lack of T_1 and T_2 data for cocaine users in the literature. This simplified processing might have some influence on the accuracy of the estimation of the metabolite concentrations. Second, since we used ratios, instead of absolute concentrations in the statistical analysis, we cannot completely exclude the potential confounds of an altered tCr level on the Glu/tCr ratio, although previous studies (Li et al., 1999; O'Neill et al., 2001) suggested no significant change in the tCr level in cocaine addicts compared to healthy controls. Third, we did not perform multiple statistical testing corrections due to the limited subject population. However, even with the stringent Bonferroni correction, the significant group effect of Glu/tCr between chronic cocaine users and healthy controls would survive.

In conclusion, using an improved MRS acquisition and analysis technique, the present study demonstrated significantly lower Glu/tCr in the rACC of human cocaine addicts, supporting preclinical observations of a role for Glu in mediating cocaine addiction and a mechanistic explanation for previous reports of hypoactive ACC in cocaine addicted individuals. Together these data support a novel therapeutic intervention strategy aimed at normalizing Glu transmission and function in the treatment of cocaine addiction (Baker et al., 2003; Kalivas, 2007; Mardikian et al., 2007).

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Fig. 1.

Simulated PRESS spectra of tCr and Glu at multiple TEs (TE = 35 ms, 85 ms, 135 ms, 185 ms, and 235 ms, respectively), in which relaxation was not considered in the simulation. The singlet resonances of tCr at 3.02 ppm and 3.92 ppm remain unchanged through different TEs. In contrast, due to *J*-evolution of coupled spins, the C4 proton resonance of Glu at 2.35 ppm (Glu4), the C3 proton resonance of Glu around 2.08 ppm (Glu3), and the C2 proton resonance of Glu at 3.75 ppm (Glu2) demonstrate complex variation with TE.

Fig. 2. TE-averaged PRESS spectrum acquired from a single voxel (white box) encompassing the rACC of a healthy volunteer (NAA = *N*-acetyl aspartate, tCr = total creatine, tCho = choline-containing compounds, Glu = glutamate, and Ins = *myo*-inositol).

Fig. 3. Metabolite levels (in ratio to tCr) in the rACC of chronic cocaine users (red) and healthy controls (yellow) (NAA = *N*-acetyl aspartate, tCr = total creatine, tCho = choline-containing compounds, Glu = glutamate, and Ins = *myo*-inositol). Glu/tCr is significantly lower (15.88%) ($F_{1,25}=10.2$; $p < 0.005$) in the cocaine-user group compared to the control group, after correcting for age. The thick light-green lines mark the mean values and the thin black lines mark the median values. Bars indicate the 5th/95th percentiles.

Fig. 4. Significant positive correlation between Glu/tCr and NAA/tCr (Glu = glutamate, NAA = *N*-acetyl aspartate, and tCr = total creatine) (partial $R = 0.503$; $p < 0.01$), accounting for age, in the rACC of chronic cocaine users (red) and healthy controls (yellow).

Fig. 5. Significant positive correlation between Glu/tCr and years of cocaine use (Glu = glutamate and tCr = total creatine) (partial $R = 0.579$; $p < 0.05$), accounting for age and current cocaine use, in the rACC of chronic cocaine users (red). The mean and standard deviation of Glu/tCr in the control group (blue) are also shown for comparison.