# Lower Glutamate Levels in the Anterior Cingulate Cortex of Chronic Cocaine Users: A Proton MRS Study Using TE-Averaged PRESS at 3T with a Modified Glutamate Quantification Strategy

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## Introduction

Neuroimaging and MR spectroscopy studies have shown significant alterations in glucose metabolism [1], gray matter density [2], cognitive activities [3], and metabolite/neurotransmitter concentrations [4] in the anterior cingulate cortex (ACC) of cocaine addicts, suggesting chronic cocaine use may lead to neuroadaptations in brain structure and/or function in, among other brain regions, the ACC. Recent studies using animal models have demonstrated that chronic cocaine use produces a significant reduction in the basal levels of glutamate (Glu) transmission from the prefrontal cortex (PFC), including the ACC, to the core of the nucleus accumbens (NAc) [5], which has been thought to play a critical role in cocaine addiction and subsequent relapse to drug-seeking behavior. Therefore, measurement of Glu in the ACC may provide important information for understanding the frontal lobe alterations and the functional significance of Glu in cocaine addiction. In conventional PRESS or STEAM spectra at 1.5-3T, however, spectral overlap between Glu and glutamine (Gln), *N*-acetyl aspartate (NAA), and glutathione (GSH) can impair accurate Glu quantification. Recently developed TE-averaged PRESS, in contrast, can produce well-resolved Glu signal at 3T [6]. In this study, we used this new MRS technique with necessary modifications in Glu quantification to determine the consequences of chronic cocaine use on Glu and other metabolites in the ACC.

## Methods

***Subjects.*** Fifteen healthy volunteers (7 female; 20-48 years old, mean±S.D. of 33.8±9.7) and seventeen age-matched chronic cocaine users (4 female; 24-43 years old, mean±S.D. of 37.5±4.7; $241±176/week current cocaine use, 15±6 total years of use) participated in this study. Last use of cocaine was 42.6±21.8 hours before the MRS scan session. Subjects gave informed consent for an IRB-approved protocol. Subjects were excluded if having any major illnesses, claustrophobia, history of neurological or psychiatric disorders, drug and/or alcohol dependence besides cocaine, and pregnancy.

***MRS Data Acquisition.***. MRI and $^1$H-MRS scans were performed on a Siemens Allegra 3T scanner. Single-voxel TE-averaged PRESS data were acquired from a voxel that encompassed the ACC based on the localizer images (see Fig. 1). The sequence parameters were 128 TE increments of 2.5 ms starting at 35 ms, TR = 2 s, spectral bandwidth = 2 kHz, sampling points = 2048, NEX =2 with a two-step phase cycling, voxel size = 8 cc. Unsuppressed water signal was immediately acquired using 8 TRs following the above scan.

***Spectral Quantification***. The MRS data were quantified using LCModel [7] with a basis set of NAA, creatine (Cr), choline (Cho), Glu, Gln, and *myo*-inositol (Ins). The basis set was simulated using GAMMA [8] with the reported chemical shifts and coupling constants [9]. Because the effective TEs were rather long for this TE-averaged PRESS acquisition, relaxation effects (mainly the $T_2$ effects) were considerable and could not be omitted in spectral quantification. For correcting for the relaxation effects, the $T_1$ and $T_2$ relaxation were directly incorporating into the basis set (i.e., weighting the individual spectra at different TEs with the estimated $T_1$ and $T_2$ relaxation rates), thereby disentangling the relaxation effects from J-modulation of such strongly coupled spin systems as Glu. This modification improved the Glu quantification over the commonly-used correction approach in which the $T_1$ and $T_2$ relaxation effects are typically corrected after spectral quantification. The $T_1$ and $T_2$ values of NAA, Cr, Cho, Glu, and Gln in the ACC are 1.48, 1.21, 1.44, 1.23, and 1.23 s, and 278, 179, 282, 201, and 201 ms [10, 11], respectively. Due to lack of reported T2 values of Ins in the ACC in the literature, Ins remained uncorrected for relaxation effects. Only the quantification results with a Cramér-Rao lower bound (CRLB) less than 20% were included in the statistical analysis. Statistical analyses were performed on the ratios of NAA/Cr, Cho/Cr, Glu/Cr, and Ins/Cr between the two groups. Analysis of covariance (ANCOVA) (with group as a factor and age as a covariate) and regression analysis of Glu/Cr with age and NAA/Cr, Cho/Cr, Glu/Cr, or Ins/Cr were performed.

## Results

The groups did not differ in age ($t_{30}$=1.4, n.s.) and gender ($\chi^2$=1.9, n.s.). Fig. 1 shows the TE-averaged PRESS spectrum averaged from all the cocaine users. The Glu C4 multiplet resonance at 2.35 ppm appears as a virtual singlet peak and is well-resolved. Fig. 2 shows the metabolite levels, in ratio to Cr, in the ACC of both groups. The Glu/Cr ratio was significantly lower (12%) in the cocaine-user group compared with the controls ($F_{1,29}$=4.2; $p < 0.05$). Additionally, while the NAA/Cr significantly decreased with age ($F_{1,29}$=9.1; $p < 0.005$), there was no significant difference between two groups ($F_{1,29}$=1.5, n.s.). Finally, no significant group differences were found in Cho/Cr and Ins/Cr. Regression analyses showed a significant positive correlation between Glu/Cr and NAA/Cr (partial R = 0.379; $p < 0.05$), accounting for age (see inset of Fig. 2).

## Discussions

Creatine levels in the ACC of cocaine users have been previously reported not to differ from those in control subjects [4, 12], suggesting that the observed reduction in Glu/Cr is due to a reduction in Glu level in the ACC of cocaine addicts. A decrease in Glu is consistent with evidence from animal studies demonstrating that chronic cocaine self-administration significantly decreases the turnover rate of Glu in many reward-related brain regions including the NAc and cingulate cortex [13], suggesting a possible reduction in synaptic density or neurotransmitter synthetic capacity. As NAA is thought to be a marker of neuronal integrity, the correlation between Glu/Cr and NAA/Cr may reflect decreased ability of ACC neurons to produce Glu. A decrease in the enzyme glutamate carboxypeptidase II (CGPII) or III (CGPIII), which catabolizes *N*-acetyl-aspartylglutamate (NAAG) to NAA and Glu [14], could also lead to a reduction in Glu. In either case, a loss of ACC glutamatergic function may provide a mechanistic explanation for the impaired cognitive functions seen in cocaine addicts. Our finding of a correlation between Glu/Cr and NAA/Cr in the absence of a significant difference of NAA/Cr between addicts and controls supports a hypothesis of change in function rather than number of neurons (at least in the ACC), as has been suggested by anatomical studies [2]. In conclusion, using an improved MRS technique, the present study shows for the first time significantly lower Glu levels in the ACC of human cocaine addicts, suggesting an important role of Glu neuroadaptations in mediating cocaine addiction and perhaps supporting a strategy for novel therapeutic interventions.

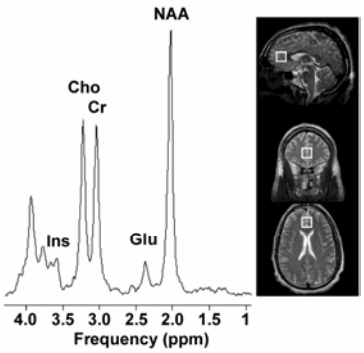


Fig. 1. TE-averaged PRESS spectrum acquired from a voxel encompassing the ACC, averaged from all cocaine users.

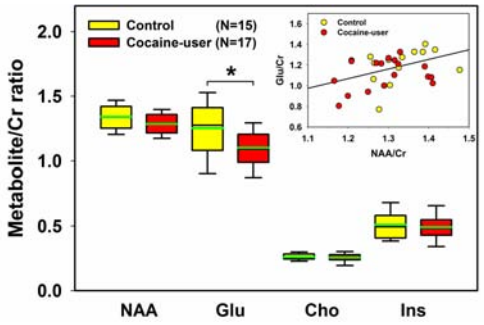


Fig. 2. Metabolite levels in the ACC of chronic cocaine users and healthy controls and positive correlation between Glu/Cr and NAA/Cr (inset). The thick light-green lines mark the mean values and the thin black lines mark the median values. * Significant ($F_{1,29}$=4.2; $p < 0.05$).

## References

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