Progress in Neuro-Psychopharmacology & Biological Psychiatry 35 (2011) 1709–1713

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

# Anterior cingulate proton spectroscopy glutamate levels differ as a function of smoking cessation outcome

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ARTICLE INFO

*Article history:*
Received 9 March 2011
Received in revised form 3 May 2011
Accepted 10 May 2011
Available online 15 May 2011

*Keywords:*
Dorsal anterior cingulate cortex
Glutamate
Nicotine
Relapse
Smoking
Spectroscopy

ABSTRACT

*Background:* Cigarette smoking is the leading preventable cause of death. Unfortunately, the majority of smokers who attempt to quit smoking relapse within weeks. Abnormal dorsal anterior cingulate cortex (dACC) function may contribute to tobacco smoking relapse vulnerability. Growing evidence suggests that glutamate neurotransmission is involved in mediating nicotine dependence. We hypothesized that prior to a cessation attempt, dACC glutamate levels would be lower in relapse vulnerable smokers.
*Methods:* Proton magnetic resonance spectra (MRS) were obtained from dACC and a control region, the parieto-occipital cortex (POC), using two-dimensional J-resolved MRS at 4 T and analyzed using LCModel. Nine nicotine-dependent women were scanned prior to making a quit attempt. Subjects then were divided into two groups; those able to maintain subsequent abstinence aided by nicotine replacement therapy (NRT) and those who slipped while on NRT (smoked any part of a cigarette after attaining at least 24 h of abstinence).
*Results:* Slip subjects exhibited significantly reduced dACC MRS glutamate (Glu/Cr) levels ($p<0.03$) compared to abstinent subjects. This effect was not observed in the POC control region.
*Conclusions:* Our preliminary findings suggest that dACC Glu levels as measured with MRS may help identify and/or be a biomarker for relapse vulnerable smokers. Future research following up on these findings may help clarify the role of dACC Glu in smoking dependence that may lead to new treatment strategies.


## 1. Introduction

Tobacco-derived nicotine dependence accounts for nearly 450,000 yearly deaths in the US (DeVita, 2005). Despite the existence of pharmacotherapies that substantially improve smoking cessation rates when combined with behavioral therapy (Gonzales et al., 2006; Jorenby et al., 2006), most smokers who quit eventually relapse (Hughes et al., 2003). Relapse vulnerability and nicotine dependence are associated with disrupted functional connectivity between the dorsal anterior cingulate cortex (dACC) and brain regions implicated in reward and smoking behavior (Hong et al., 2009; Janes et al., 2010). Additionally, smokers exhibit increased dACC activation when resisting craving to smoking cues (Brody et al., 2007), which may reflect greater effort to exert cognitive control over craving (Kerns et al., 2004). Thus, a disconnect between the dACC and reward-related brain regions could reflect a dysfunctional cognitive control network, and when present could make it more difficult for vulnerable smokers to resist craving, leading to higher relapse vulnerability. Identifying neurobiological markers of dACC dysfunction may help lead to novel smoking cessation and relapse prevention treatments.

In this preliminary study, we hypothesized that relapse vulnerable smokers would have decreased glutamate (Glu) proton metabolite levels in the dACC prior to a cessation attempt. This hypothesis is based on previous work indicating that chronic substance abusers have reduced Glu levels in the ACC. For instance, ACC Glu levels, as measured with proton magnetic resonance spectroscopy (MRS), are decreased in chronic cocaine (Yang et al., 2009) and chronic opiate users (Yücel et al., 2007). Additionally, ACC Glu levels are decreased in individuals with attention deficit hyperactivity disorder (Perlov et al., 2007), a condition associated with cognitive control dysfunction. Moreover, glutamate has been implicated as playing a role in cognitive control and in coping with cognitive challenges (Cull-Candy et al., 2001). Collectively, these findings suggest a possible link among ACC

*Abbreviations:* 2D, 2-dimensional; Cr, creatine; Cho, choline; dACC, dorsal anterior cingulate cortex; FTND, Fagerstrom Test for Nicotine Dependence; GABA, γ-Aminobutyric acid; Gln, glutamine; Glu, glutamate; JPRESS, *J*-resolved spectroscopy; MGH, Massachusetts General Hospital; MRS, magnetic resonance spectroscopy; NAA, *N*-acetylaspartate; NEX, number of excitations; NMDA, N-methyl-D-aspartate; NRT, nicotine replacement therapy; POC, parieto-occipital cortex; PRESS, point-resolved spectroscopy; TE, echo time; TR, repetition time.

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doi:10.1016/j.pnpbp.2011.05.006

Glu, cognitive control, and substance abuse. Given that our group previously reported reduced functional connectivity of the dACC in relapse-vulnerable smokers, and that the dACC mediates cognitive control (Botvinick et al., 2004; Carter et al., 1999), the present study focused on whether dACC Glu abnormalities may play a role in smoking relapse vulnerability. In our study, following pretreatment MRS measurements, all subjects attempted to quit smoking aided by nicotine replacement therapy (NRT). Glutamate levels, expressed as metabolite ratios over total creatine (Glu/Cr) then were compared between smokers who subsequently were able to maintain abstinence *vs*. those who could not. Relapse vulnerability was defined as a slip (smoking any part of a cigarette after attaining at least 24 h of abstinence), which has been shown to predict future smoking relapse (Brandon et al., 1990; Shiffman et al., 1996).

## 2. Methods and materials

### 2.1. Subjects

Smokers involved in a smoking cessation clinical trial at Massachusetts General Hospital (MGH; NCT00218465) were referred to this optional neuroimaging study at McLean Hospital. Not all neuroimaging subjects completed MRS components. Participants in the present study were those who volunteered to undergo MRS and who were able to maintain at least 24 h of abstinence after their quit date as part of the MGH trial. These subjects were a small subset of smokers who also participated in our prior fMRI study, in which we showed reduced functional connectivity between the dACC and brain regions involved in smoking behavior and smoking cue reactivity (Janes et al., 2010). Subjects enrolled in the study met DSM-IV criteria for current nicotine dependence, smoked $\geq$10 cigarettes/day in the previous six months, and had expired air carbon monoxide >10 ppmv at screening. Smokers with current unstable medical illness, pregnancy, recent drug and alcohol use (QuickTox 11 Panel Drug Test Card, Branan Medical, Irvine, California; Alco-Sensory IV, Intoximeters, St. Louis, Missouri), major depressive disorder, alcohol use disorder (prior 6 months), current psychotropic drug use, or lifetime diagnosis of organic mental or psychotic disorders were excluded. Women were exclusively enrolled because the parent clinical trial involved an investigational medication not yet FDA approved for men. The MGH and McLean Hospital Institutional Review Boards approved this study. Subjects provided written informed consent and were compensated for participation.

### 2.2. Assessment procedure

Baseline (pre-quit) smoking behavior was characterized by recording tobacco smoking pack-years, average number of cigarettes smoked per day, measuring end-expiratory CO levels (Bedfont Micro IV Smokerlyzer, Bedfont Scientific, Kent, England), and by administering the Fagerstrom Test for Nicotine Dependence (FTND; Heatherton et al., 1991; Table 1). After baseline assessments and MRS, subjects quit smoking and began the 8-week NRT smoking cessation phase of the clinical trial. Interventions involved nicotine patch (20 mg/day for 4 weeks, 14 mg/day for 2 weeks, 7 mg/day for 2 weeks), and 2 mg nicotine polacrilex gum or lozenge, up to 12 mg/day as needed, and weekly manualized individual behavioral interventions. Only subjects who quit smoking for at least 24 h were included in this analysis. Subjects who smoked any cigarettes during NRT were classified as high risk for relapse (slip group) while those who remained abstinent were classified at low relapse risk (abstinence group). This classification was based on a Society for Research on Nicotine and Tobacco's working group definition of a slip as smoking any amount following at least 24 h of abstinence (Hughes et al., 2003).

Group (slip *vs*. abstinence) differences in demographics were assessed with 2-sided two-sample Student's *t* tests while differences in baseline MRS metabolite levels were assessed with 1-sided two-sample Student's *t* tests. Data were analyzed using GraphPad Prism (Prism Version 5.0b, GraphPad Software, San Diego, California) with a statistical significance threshold of $p<0.05$.

**Table 1**
Demographic information. Age, carbon monoxide levels, and number of cigarettes smoked between awakening and shortly before the baseline spectroscopy scan. FTND, Ham-D, average number of cigarettes smoked per day, and pack-years were assessed at screening before the MRS scan day. Days on NRT are the total number of days subjects were treated with NRT during their quit attempt.

| Group | Eventual slip subjects (n = 5) | Abstinence subjects (n = 4) |
|---|---|---|
| Age (years) | 47.8 ± 11.8 | 49.3 ± 14.5 |
| Carbon monoxide (ppmv) | 16.8 ± 10.8 | 16.3 ± 9.5 |
| Cigarettes smoked prior to MRS | 4.4 ± 2.2 | 2.8 ± 2.2 |
| FTND | 6.0 ± 0.7 | 3.3 ± 1.5* |
| Ham-D | 2.8 ± 2.7 | 0.5 ± 0.6 |
| Cigarettes smoked per day | 19.9 ± 9.3 | 14.9 ± 3.7 |
| Pack-years | 31.3 ± 27.5 | 25.4 ± 18.1 |
| Days on NRT | 43.0 ± 17.6 | 41.5 ± 15.5 |

MRS, magnetic resonance spectroscopy; FTND, Fagerstrom Test for Nicotine Dependence; Ham-D, Hamilton Depression Rating Scale; NRT, nicotine replacement therapy.
Data are expressed as means ± SD.
* $p<0.01$.

### 2.3. Imaging and spectroscopy

Smoking was allowed until shortly before scanning. Subjects were scanned on a Varian Unity INOVA 4 T whole-body MR system (Varian, Palo Alto, CA) using a volumetric head coil. The unsuppressed water signal was used to manually shim the global water signal. Subsequently, $T_1$-weighted sagittal and axial structural images were obtained: echo time/repetition time (TE/TR) = 6.2 s/11.4 ms, field-of-views = 22 × 22 × 8 and 16 cm sagittal and axial, (respectively), in-plane matrix sizes = 128 × 256 × 16 (sagittal) and 256 × 256 × 64 (axial).

These images guided voxel placements in dACC (33 × 23 × 22 mm) and parieto-occipital cortex (POC) (33 × 23 × 22 mm). Manual voxel shimming resulted in global water line widths of 9–12 Hz. Proton spectroscopy utilized a 2D-JPRESS approach collecting 24 TE-stepped spectra (30–260 ms, 10-ms increments), providing 100 Hz J-resolved bandwidth, sufficient to resolve Glu from its metabolic predecessor glutamine (Gln), as well as other metabolites (Jensen et al., 2009). Additional acquisition parameters were: TR = 2 s, f1 bandwidth = 50 Hz, spectral bandwidth = 2 kHz, readout duration = 1024 ms, NEX = 16, and total scan duration = 13 min.

### 2.4. Spectral processing

MRS data analyses were undertaken using code written on-site and commercial fitting software (LCModel; Provencher, 1993). To quantify Glu, γ-Aminobutyric acid (GABA), Gln, *N*-acetylaspartate (NAA), creatine (Cr), and choline (Cho), free-induction decay series were zero-filled to 64 points, Gaussian-filtered to minimize residual ringing from NAA and Cr signals, and Fourier-transformed in the TE dimension. This produced 64 J-resolved spectra. Using GAMMA-simulated J-resolved basis sets, J-resolved spectral extractions were fit with LCModel templates (Jensen et al., 2009). Integrated areas under entire 2D surfaces for each metabolite were calculated by summing raw peak areas across all 64 J-resolved extractions, and were corrected for T2 relaxation. Metabolite ratios using total creatine (Cr, sum of creatine and phosphocreatine raw integrals) as the denominator are reported, since there were no group (slip *vs*. abstinence) Cr differences. Two POC spectra from subjects who later slipped were excluded due to low signal-to-noise and/or spectral resolution.