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GABA Concentrations in the Anterior Cingulate and Dorsolateral Prefrontal Cortices: Associations with Chronic Cigarette Smoking, Neurocognition and Decision-Making

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Abstract

Chronic cigarette smoking is associated with regional metabolite abnormalities in choline-containing compounds, creatine-containing compounds, glutamate, and *N*-acetylaspartate. The effects of cigarette smoking on anterior frontal cortical gamma-aminobutyric acid (GABA) concentration is unknown. This study compared chronic smokers (n=33) and non-smokers (n=31) on anterior cingulate cortex (ACC) and right dorsolateral prefrontal cortex (DLPFC) GABA+ (the sum of GABA and co-edited macromolecules) concentrations and associations of GABA+ levels in these regions with neurocognition in seven different domains of functioning, decision-making and impulsivity measures. Smokers had significantly lower right DLPFC GABA+ concentration than non-smokers, but groups were equivalent on ACC GABA+ level. Across groups, greater number of days since end of menstrual cycle were related to higher GABA+ level in the ACC, but not right DLPFC GABA+ concentration. In exploratory correlation analyses, higher ACC and right DLPFC GABA+ levels were associated with faster processing speed and better auditory-verbal memory, respectively in the combined group of smokers and non-smokers; in smokers only, higher ACC GABA+ was related to better decision-making and auditory-verbal learning. This study contributes additional novel data on the adverse effects of chronic cigarette smoking on the adult human brain and demonstrated ACC and DLPFC GABA+ concentrations were associated with neurocognition and decision-making/impulsivity in active cigarette smokers. Longitudinal studies on the effects of smoking cessation on regional brain GABA levels, with a greater number of female participants, are required to determine if the observed metabolite abnormalities are persistent or normalize with smoking cessation.

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AUTHOR CONTRIBUTION

Dr. Durazzo was responsible for study concept and design, participant recruitment and screening, clinical, neuropsychological and behavioral assessments, MR data acquisition, MR data processing, statistical analyses, data interpretation and writing the manuscript. Dr. Meyerhoff was involved in participant recruitment, MR data processing, data interpretation, manuscript editing, and contributed significant intellectual content to the manuscript. The authors approved the final version of the manuscript.

INTRODUCTION

Abundant evidence indicates chronic cigarette smoking in adolescents and adults is related to neurobiological and neurocognitive abnormalities, which are not specifically attributable to common medical conditions associated with smoking (Azizian et al., 2009; Durazzo et al., 2010; Elbejjani et al., 2019; Sharma and Brody, 2009). Studies of the neurobiological consequences of cigarette smoking over the past 20 years have largely focused on magnetic resonance imaging-based morphometric studies, which have consistently indicated structural abnormalities in multiple cortical and subcortical regions [see (Durazzo et al., 2018; Durazzo et al., 2017; Elbejjani et al., 2019; Vnukova et al., 2017) and references therein]. Collectively, structural aberrations in the anterior frontal cortex appear to be the most consistently reported morphometric finding.

Magnetic resonance (MR) spectroscopy enables the interrogation of brain tissue functional integrity. Single volume proton MR spectroscopy (SVS) and spectroscopic imaging (SI) methods allow the non-invasive quantitation of several brain metabolites that collectively provide information on the neurophysiological viability of tissue (Meyerhoff et al., 2011; Ross and Bluml, 2001). Abnormalities in brain metabolite concentrations (e.g., gamma-aminobutyric acid, GABA; glutamate, Glu or Glu/glutamine, Glx; *N*-acetylaspartate, NAA; choline-containing compounds, Cho; creatine-containing compounds creatine and phosphocreatine, Cr) may precede macroscopic structural and/or neurocognitive changes observed in some diseases/conditions [see (Meyerhoff et al., 2013; Öz et al., 2013) for details on functional and clinical relevance of the foregoing brain metabolites].

The few MR spectroscopy studies with adult chronic smokers primarily quantitated NAA, Cho, Cr and Glu. Gallinat and associates reported smokers showed lower NAA levels than non-smokers in the left hippocampus, and higher pack-years were associated with higher Cho levels in the anterior cingulate gyrus (Gallinat et al., 2007); however, in an ensuing study, these researchers found no differences between active-smokers, former-smokers, and non-smokers on Glu levels in the anterior cingulate cortex (ACC) and left hippocampus (Gallinat and Schubert, 2007). O'Neill and colleagues reported no differences between smokers and non-smokers in thalamic Glu concentration, but greater number of cigarettes smoked per day and higher pack-years of smoking were strongly related to lower thalamic Glu level in smokers (O'Neill et al., 2014). Mennecke and associates (Mennecke et al., 2014) reported higher left ACC Glu/glutamine (Glx) and Cho concentrations in smokers than non-smokers; following 3 days of smoking cessation, anterior cingulate Glx level was equivalent to non-smoker levels, but Cho concentration remained elevated. In a combined SVS and SI study, we (Durazzo et al., 2016) reported smokers exhibited lower dorsolateral prefrontal cortex (DLPFC) NAA, Cr, myoinositol and Glu concentrations and lower lenticular nuclei NAA level compared to non-smokers; smokers also had greater age-related decreases of DLPFC NAA and ACC and DLPFC Glu levels. In the combined sample of smokers and non-smokers, higher NAA and Glu in the DLPFC and higher NAA concentrations in multiple lobar gray matter and white matter regions and subcortical nuclei were associated with better neurocognition and lower impulsivity.

GABA is formed by the decarboxylation of glutamate and is the major inhibitory neurotransmitter in the human brain, and, depending on region, up to 25% of cortical interneurons are GABAergic (Schmidt-Wilcke et al., 2018). GABAergic neurons are fundamentally implicated in the synchronization of neuronal activity across the mammalian brain (Clancy et al., 2010; Schmidt-Wilcke et al., 2018). MR spectroscopy methods measure the overall GABA intracellular and extracellular concentration in the defined volume of interest (Meyerhoff et al., 2013). Lower DLPFC GABA level in adults was associated with poorer performance on working memory components (Yoon et al., 2016) and greater self-reported impulsivity (Boy et al., 2011). In adolescents and young adults, lower ACC GABA concentration was related to greater self-reported impulsivity and poorer response inhibition (Silveri et al., 2013). In the sole preliminary human study comparing cortical GABA in smokers and non-smokers, Epperson and colleagues (Epperson et al., 2005) found no differences between male smokers and non-smokers on occipital cortex GABA concentration. GABA levels in female smokers were lower than in male smokers, but female non-smokers had higher GABA concentration than male non-smokers. Follicular-phase GABA levels were significantly higher in female non-smokers than in female smokers. Occipital cortex GABA levels showed no changes after 48 hours of smoking cessation in both males and females. Epperson and colleagues demonstrated occipital cortex GABA concentrations were moderated by smoking status and sex, but the occipital cortex is typically not considered a critical node in brain circuits associated with the development and maintenance of substance use disorders. Neurobiological abnormalities of the ACC and DLPFC are implicated in the development and maintenance of all substance use disorders, including nicotine dependence (Volkow et al., 2012; Volkow et al., 2013). Therefore, comparison of GABA level in these regions in smokers and non-smokers may further elucidate the neurobiological consequences of chronic cigarette smoking in regions associated with the persistence of addictive disorders, and correlations with neurocognitive, decision-making and impulsivity measures will assess potential functional correlates of anterior frontal cortical GABA concentrations. Therefore, the primary goals of this study were to: 1) compare GABA levels in the ACC and DLPFC in smokers and non-smokers; 2) relate ACC and DLPFC GABA levels to multiple domains of neurocognition and measures of decision-making and self-reported impulsivity.

Based on the previous collective MRS literature, we predicted:

1. Smokers demonstrate significantly lower GABA concentration in the ACC and right DLPFC as well as greater age-related decline in GABA levels in both regions compared to non-smokers.
2. Across smokers and non-smokers, higher ACC and right DLPFC GABA levels are related to better neurocognitive functioning and decision-making as well as lower self-reported impulsivity.
3. In smokers, greater lifetime years of smoking and cigarette pack years are related to lower ACC and right DLPFC GABA concentrations.

METHODS

Participants

Healthy, community-dwelling participants were recruited via electronic billboards, and word-of-mouth. Participants were between the ages of 24 and 69 and gainfully employed at the time of study (see Table 1). All participants provided written informed consent according to the Declaration of Helsinki prior to engaging in procedures, and the consent document and procedures were approved by the University of California San Francisco and the San Francisco VA Medical Center.

Detailed inclusion/exclusion criteria are described elsewhere (Durazzo et al., 2012). In summary, non-smoking (n=32) and smoking (n=34) participants were excluded for history of neurologic (e.g., head trauma with loss of consciousness > 5 min, seizure disorders, neurodegenerative disorders), general medical (e.g., diabetes, hypertension, chronic obstructive pulmonary disease), and psychiatric (i.e., mood, schizophrenia spectrum, anxiety, substance/alcohol use disorders) conditions known or suspected to effect neurocognition or brain neurobiology. All females were pre-menopausal, and all were taking oral hormone-based contraception, by self-report. While most non-smokers never smoked, four smoked less than 40 cigarettes during their lifetime but had no cigarette/tobacco use in the 10 years prior to study. All smoking participants were actively smoking at the time of assessment, smoked 10 or more cigarettes/day for at least 5 years, with no smoking cessation greater than 1 month in the 5 years prior to study. At the time of study, no smoker was engaged in any pharmacological or behavioral smoking cessation intervention or used any other forms of tobacco or electronic cigarettes/vaping. Smokers could smoke ad libitum prior to initiation of all procedures, and take smoke breaks, if requested.

Psychiatric, medical, and substance/alcohol consumption assessment

Participants were administered the screening section of the Structured Clinical Interview for DSM-IV Axis I disorders, Patient Edition, Version 2.0, and an in-house questionnaire designed to screen for medical, neurological, psychiatric and developmental conditions known or suspected to neurocognition or brain neurobiology. Participants were also administered semi-structured interviews for lifetime alcohol consumption (Lifetime Drinking History, LDH) and substance use (in-house questionnaire assessing substance type, and quantity and frequency of use). From the LDH, we calculated average number of drinks/month (defined as containing 13.6 grams of pure ethanol) over 1 year prior to enrollment and average number of drinks/month over lifetime. Participants also completed self-report measures of anxiety (State-Trait Anxiety Inventory, form Y-2, STAI) and depressive (Beck Depression Inventory, BDI) symptomatologies, and family history of problem drinking. Smokers completed a measure of nicotine dependence level [Fagerström Test for Nicotine Dependence (FTND)], and information on the total number of cigarettes currently smoked per day, and the total number of years of smoking over lifetime. From this information, pack-years [i.e., (typical number of cigarettes per day/20) x total number of years of smoking] were calculated for smokers. All participants' urine was assessed for common illicit substances (e.g., THC, opiates, cocaine, and amphetamines) prior to assessment, and recent ethanol consumption was tested via breathalyzer. No participant was positive for the

above common illicit substances or ethanol consumption at the time of assessment. See (Durazzo et al., 2013) for corresponding references for the above measures.

Neurocognitive and behavioral assessment

Participants completed a comprehensive neurocognition battery on the same day as the magnetic resonance scan and was composed of measures commonly employed in clinical and research settings in North America (Strauss et al., 2006) and those adversely affected by chronic cigarette smoking (Durazzo et al., 2010, 2012). Smokers were allowed to smoke *ad libitum* before and during neurocognitive testing to reduce the potential confound of nicotine withdrawal symptoms on test performance [for review see (Sacco et al., 2004)]. Forty percent (40%) of smokers took one smoke break during testing and all smoked one cigarette. The neurocognitive domains assessed, and the constituent measures were as follows: *Executive functions:* Short Categories Test, Stroop Test Color-Word subtest, Trail Making Test B, Wechsler Adult Intelligence Scale 3rd Edition (WAIS-III) Similarities, Wisconsin Card Sorting Test-64: Computer Version 2-Research Edition non-perseverative errors, perseverative errors, and perseverative responses. *General intelligence:* Ward-7 Full Scale IQ, based on WAIS-III Arithmetic, Block Design, Digit Symbol, Digit Span, Information, Picture Completion, and Similarities subtests. *Processing speed:* Trail Making Test A, WAIS-III Digit Symbol and Symbol Search, and Stroop Test Color and Word subtests. *Visuospatial skills:* Luria-Nebraska Item 99 and WAIS-III Block Design. *Working memory:* WAIS-III Arithmetic and Digit Span. A domain composite z-score was calculated from the arithmetic mean of z-scores for all individual domains. See Durazzo and colleagues (Durazzo et al., 2012) for full description of the neurocognitive battery, associated references and results of comparisons of smokers and non-smokers on the above neurocognitive battery. *Decision-making*: Iowa Gambling Task (IGT) (Bechara, 2007)]. *Impulsivity:* Barrett Impulsivity Scale-11 (BIS-11) (Patton et al., 1995). Scores for all the above measures were converted to z-scores based on the performance of the non-smokers in this study. See (Durazzo et al., 2016) for comparisons between smokers on non-smokers from this cohort on the IGT and BIS-11 total score and attentional, motor and non-planning subscales.

Magnetic resonance imaging acquisition and processing

Magnetic resonance imaging (MRI) data were acquired on a 4 Tesla (4T) Bruker MedSpec system; 3D T1-weighted images were obtained with Magnetization Prepared Rapid Gradient (MPR) imaging, and 3D T2-weighted images via turbo spin-echo [see for (Durazzo et al., 2018) full MRI acquisition details]. The 4T structural images were segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) via the Expectation Maximization Segmentation (EMS) method (Van Leemput et al., 1999) and co-aligned with the SVS volumes of interest for determination of their tissue contribution (i.e., GM, WM, CSF) (Mon et al., 2012; Studholme et al., 2001).

SVS acquisition and processing

T1- and T2-weighted images were displayed and used to position each volume-of-interest (VOI) for the anterior cingulate cortex (ACC; $35 \times 25 \times 20$ mm^3) and right dorsolateral prefrontal cortex (DLPFC; $40 \times 20 \times 20$ mm^3). We had no hypotheses about hemispheric laterality, so we choose the right DLPFC at the outset of the project because high

quality GABA and other neurometabolite spectra were consistently obtained from the right hemisphere. The ACC and DLPFC VOIs were oriented parallel to an imaginary line connecting the anterior and posterior commissures. The caudal boundary of the ACC volume was positioned on a plane parallel to the inferior aspects of the genu and splenium of the corpus callosum that situated the VOI in the midsagittal perigenual region and included both the left and right ACC. The caudal boundary of the right DLPFC was positioned superior to the horizontal ramus of the Sylvian fissure, and the entire volume was placed anterior to the precentral gyrus; the DLPFC volume was then oriented along the contour of the cortex to maximize inclusion of GM (see Fig. 1 for VOI spatial locations and representative example of spectral quality).

After VOI-specific 3-D shimming and optimization of water suppression, J-edited GABA spectra were acquired from the ACC and DLPFC with a MEGA PRESS sequence [TR/TE = 2000/71, 90 degree flip angle, 2000 Hz spectral bandwidth, editing frequencies at 1.9 and 7.5 ppm, approximately 12 minutes total acquisition time (Kaiser et al., 2008)], followed by acquisition of an unsuppressed water spectrum for each VOI. All SVS data were processed using established routines from in-house Matlab program and IDL (Research Systems, Inc., Boulder, CO) with SITOOLS (Soher et al., 1996), including a Lorentz-Gaussian apodization (−3Hz, +4 Hz, respectively) yielding peak areas for GABA+ (the sum of GABA and co-edited macromolecules) signal and molar concentrations, relative to water, in institutional units (i.u.). GABA was quantitated as GABA+ because the J-editing pulse sequence co-edited for macromolecules that resonate close to the saturated GABA resonance at 1.9 ppm. See previous reports (Mon et al., 2012, Meyerhoff et al., 2014) for further details on SVS data processing, quality control and quantitation. Spectra were excluded from fitting for a low signal-to-noise ratio, broad line widths that would prohibit separate fitting of Cho and Cr resonances (i.e., metabolite line width >25 Hz), and significant residual water signal that would modulate the baseline of the GABA difference spectrum and preclude proper spectral peak fitting. After application of these quality control measures, 30 non-smokers and 29 smokers had acceptable ACC GABA+ spectra, while 31 non-smokers and 33 smokers had satisfactory right DLPFC GABA+ spectra. There was no significant difference between non-smokers and smokers on percent GM contributing to ACC (non-smokers 46%, smokers 47%) or right DLPFC (non-smokers 40%, smokers 42%) volumes. Please see Durazzo et al., 2016 for information on NAA, Cho, Cr myoinositol and Glu concentrations in this cohort of smokers and non-smokers.

Statistical analyses

Group comparisons: Generalized linear modeling compared smokers and non-smokers on ACC and right DLPFC GABA+ concentrations. Predictors in all models included smoking status (smoker and non-smoker), age, body mass index (BMI), lifetime average drinks/month and the smoking status x age interaction. BMI was as a covariate because it was related to SVS metabolite concentrations in healthy controls (Gazdzinski et al., 2008; Gazdzinski et al., 2010). Lifetime average drinks/month was used as a covariate since smokers consumed significantly more than non-smokers. All main effects and interactions were considered statistically significant at $p<.05$. Significant main effects for smoking status were followed-up with t-tests (two-tailed, comparing smokers and non-smokers on mean

ACC and right DLPFC GABA+ levels), and p<.025 was considered statistically significant after standard Bonferroni correction for multiple comparisons. Effect sizes were calculated with Cohen's *d* (Cohen, 1988). There was a strong positive linear association (r = 0.83) between the number of days since end of menstrual cycle and ACC GABA+ concentration in the combined cohort of female smokers (n=4) and non-smokers (n=4). Given the small number of females in each group, using sex as a covariate would likely result in model overfitting and spurious results. To address the strong linear relationship between ACC GABA+ and menstrual cycle phase, we employed linear regression with age and days since last menstrual cycle as predictors of GABA+ level and used the predicted values from this model as ACC GABA+ concentrations for females. There was no association between the number of days since end of menstrual cycle and right DLPFC GABA+ level.

Associations of ACC and DLPFC GABA+ levels with decision-making, impulsivity, neurocognitive and smoking severity measures:

Associations of ACC and DLPFC GABA+ concentrations with the IGT, BIS-11 measures and neurocognitive domains were examined in the total sample (i.e., smokers and non-smokers) and within each group separately with linear regression (semi-partial correlations reported) controlling for age, education, and lifetime average drinks/month. Relationships of ACC and DLPFC GABA+ concentrations with lifetime years of smoking and pack-years were examined in smokers with linear regression (semi-partial correlations reported), controlling for age and lifetime average drinks/month. These exploratory associations were considered significant at p<.05.

Statistical software:

All analyses were conducted with SPSS v24.

RESULTS

Participant characteristics:

No significant differences were observed between smokers and non-smokers on age, BDI, STAI-trait, BMI, and frequency of White race and males (all p > .10). Smokers had greater 1-year average drinks/month and fewer years of formal education than non-smokers (both p < .01; see Table 1).

Group comparisons of ACC and DLPFC GABA+ concentrations:

ACC: There was no main effect for smoking status [χ^2 (1) = .034, p = .85]. Age, BMI and lifetime average drinks per month were not significant predictors of ACC GABA+ concentration (all p > .06); there were no interactions between smoking status and the foregoing covariates (all p > .40).

Right DLPFC:

A significant main effect was observed for smoking status [χ^2 (1) = 6.55, p = .01]. Age, BMI and lifetime average drinks per month were not significant predictors of right DLPFC

GABA+ concentration (all p > .30); there were no interactions between smoking status and the aforementioned covariates (all p > .45).

Pairwise comparison indicated smokers had significantly lower GABA+ level (−18%) than non-smokers (p = .01), with a moderate effect size for this mean difference (Cohen's d = 0.66; see Fig. 2).

Associations of SVS and SI metabolite levels with neurocognitive, decision-making, risk-taking, impulsivity, and smoking severity measures

ACC: In the combined group of non-smokers and smokers, higher GABA+ level was related to better processing speed (r = .39, p = .02); the magnitudes of the associations demonstrated by non-smokers and smokers were highly congruent (data not shown), so the best fit for the combined group is presented in Fig. 3a. In smokers only, higher GABA+ concentration was associated with better auditory-verbal memory (r = .40, p = .021; see Fig. 3b) and higher IGT net total (higher score indicative of better decision-making; r = .36, p = .036; see Fig. 3c). No significant relationships were observed between ACC GABA+ concentration and BIS-11 measures in non-smokers or smokers. No significant associations between ACC GABA+ level and lifetime years of smoking, pack-years or interval of last cigarette smoked to scan initiation were observed in smokers.

Right DLPFC:

In the combined group of non-smokers and smokers, higher GABA+ level was related to better auditory-verbal memory (r = .30, p = .017); the magnitudes of the associations demonstrated by non-smokers and smokers were highly congruent (data not shown), therefore, the best fit for the combined group is presented in Fig. 3d. No significant relationships were observed between right DLPFC GABA+ concentration and BIS-11 measures in non-smokers or smokers. In smokers, no associations were observed between right DLPFC GABA+ level and lifetime years of smoking, pack-years or interval of last cigarette smoked to scan initiation.

DISCUSSION

The main findings from the current study were: 1) Smokers demonstrated significantly lower GABA+ concentration in the right DLPFC than non-smokers, but groups were equivalent on ACC GABA+ level. 2) No group x age interactions were observed for the ACC or right DLPFC, which indicated similar age-related effects for smokers and non-smokers for GABA+ levels in these volumes of interest. 3) In the combined group of smokers and non-smokers, higher ACC and right DLPFC GABA+ levels were associated with better processing speed and auditory-verbal memory, respectively; in smokers only, higher ACC GABA+ was related to better decision-making and auditory-verbal learning.

The effects of chronic smoking on right DLPFC GABA+ levels measured in this study may be related to structural and functional compromise of DLPFC tissue containing GABAergic cortical neurons (Durazzo et al., 2010; Wang et al., 2015). The GABA concentrations reported in this study reflect both the intracellular and extracellular transmitter and non-transmitter GABA levels in the volumes of interest (Meyerhoff et al., 2013). Given that

GABA transmitter levels are in the low micromolar range (0.02. – 2.50 μM) (Roth and Draguhn, 2012), MRS methods largely quantitate non-transmitter levels. On a microcellular level, chronic cigarette smoking may indirectly influence GABA concentration, as measured by MR spectroscopy, through modulation [ultimately down-regulation of nicotinergic receptor numbers (Benowitz, 2008)] of nicotinic cholinergic receptor activity of GABAergic interneurons throughout the cortex (Aracri et al., 2010); this may promote perturbations in $GABA_A$ receptor activity and/or GABA synthesis (Epperson et al., 2005). Cigarette smoking may also influence GABA concentration through compromised integrity of astrocyte structure/function and its critical role in the glutamate/GABA-glutamine cycle (Seo et al., 2017). The lower right DLPFC GABA+ concentration in smokers in the current study is consistent with our previous report of metabolite level abnormalities in this cohort (Durazzo et al., 2016), where we found smokers demonstrated lower NAA, Cr, myoinositol and Glu concentrations than non-smokers in the right DLPFC, but not in the ACC.

The lower right DLPFC GABA+ level in this study may reflect dysfunction in circuits involved in top-down regulation of goal directed behavior (Williams 2016). Combined with previous structural MR-based studies, the results suggest the DLPFC is particularly vulnerable to the adverse effects of smoking in this cohort. In smokers only, higher ACC GABA+ concentration was related to better decision-making and auditory-verbal learning; the absence of such a significant correlation in non-smokers was not related to restriction of range for ACC GABA+, IGT or auditory-verbal learning measures in non-smokers (data not shown). ACC subserves aspects of auditory-verbal learning and memory (Grasby et al., 1993) and is strongly implicated in decision-making and impulsivity (Posner et al., 2007; Rudebeck et al., 2008), which are directly interrogated by the IGT (Stocco et al., 2009; Weller et al., 2009). In adolescents and young adults, higher ACC GABA+ was related to better response inhibition and lower self-reported impulsivity (Silveri et al., 2013). In this combined sample of smokers and non-smokers, higher pregenual ACC GABA+ concentration was associated with better processing speed and higher right DLPFC GABA+ was related to better auditory-verbal memory. Multiple cortical-cortical/cortical-subcortical circuits subserve human information processing speed, and subdivisions of the ACC contribute to various processing aspects of cognitive and emotional/affective information processing (Kolb and Whishaw, 2009; Mohanty et al., 2007), while the right DLPFC subserves components of auditory-verbal memory (Grasby et al., 1993; Johnson et al., 2001). Given the role of GABA interneurons as modulators of circuit activity in all cortical regions, cortical GABA is highly involved in synchronizing network activity and establishing functional oscillations (e.g., gamma and alpha) that are associated with information processing and learning (Duncan et al., 2014; Schmidt-Wilcke et al., 2018).

Days since end of menstrual cycle were related to ACC but not DLPFC GABA+ concentration. Previous studies have indicated menstrual cycle phase in smokers and non-smokers was related to occipital cortex (Epperson et al., 2002; Epperson et al., 2005) and anterior frontal (voxel appeared to be primarily located in the anterior frontal white matter) GABA levels (De Bondt et al., 2015). De Bondt and colleagues (De Bondt et al., 2015), reported higher GABA level was observed during ovulation, but only in those not taking hormone-based contraceptives. In Epperson et al., 2002, all participants had not used hormone-based contraceptives for at least 10 months prior to study, but in Epperson and

colleagues 2005, use of hormonal or other contraceptives was not specified. In the current study, the small number of females were all taking hormone-based oral contraceptives and greater number of days since end of menstrual cycle was related to higher ACC GABA+ level. The factors contributing to the lack of an association between menstrual cycle phase and right DLPFC GABA+ concentration is unclear. Additional research with larger numbers of female participants is required to better understand the influence of menstrual cycle phase and hormone-based contraceptive use on regional cerebral GABA+ levels.

This study has limitations that may affect the generalizability of the findings. The sample size was modest and unrecorded premorbid/comorbid group differences in lifestyle or biomedical conditions (e.g., exercise, diet/nutrition, subclinical pulmonary or cardiovascular dysfunction) and genetic polymorphisms influencing MRS detectable GABA concentrations may have affected the reported findings. Measures of smoking severity were not related to ACC or right DLPFC GABA+ concentrations, which contrasts with our previous study (Durazzo et al., 2016), where we found greater years of smoking were related to lower ACC and right DLPFC Glu levels; this also may indicate the group differences in right DLPFC GABA+ concentration are, at least partially, related to factors not assessed in this study. The perigenual location of the ACC volume may have yielded different GABA+ concentrations and functional associations than studies with more rostral ACC placement; our ACC volume also combined tissue from the left and right hemisphere, which barred examination for lateralized differences between smokers and non-smokers. The small number of female participants precluded assessment for sex effects. GABA was quantitated as GABA+ given the J-editing pulse sequence co-edited for macromolecules that resonate close to the saturated GABA resonance at 1.9 ppm. Therefore, there is a remote possibility that the right DLPFC GABA+ differences observed may be influenced by group differences in macromolecular concentrations. Our spectral fitting program did not generate Cramer-Rao lower bounds used by some fitting programs to assess spectra quality; however, we believe our QC methods were conservative, based on decades of experience with in-vivo MRS data of this kind, and yielded reliable and accurate spectral fits. The significant associations between our GABA+ and neurocognitive measures support the functional relevance of the GABA+ measures obtained in this report.

This study contributes additional novel findings on the adverse effects of chronic cigarette smoking on the human adult brain. To our knowledge, this is the largest sample to date and sole study that concurrently acquired ACC and DLPFC GABA+ concentrations, as well as to demonstrate associations with neurocognition and decision-making/impulsivity in adult non-smokers and smokers. The observation that GABA+ levels in these anterior frontal cortical brain regions were functionally relevant reinforces the importance of GABA in multiple aspects of neurocognition and behavior. Longitudinal studies on the effects of smoking cessation on regional brain GABA levels, with a greater number of female participants, are required to determine if the observed metabolite abnormalities are persistent or normalize with smoking cessation.

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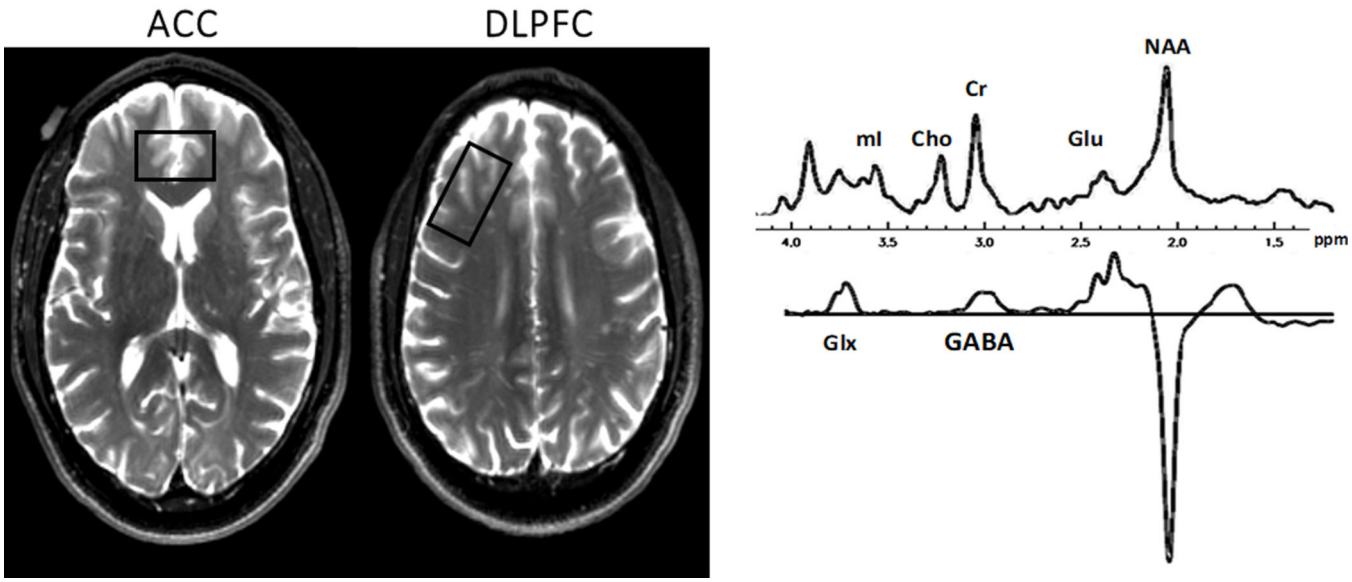

Figure 1.
ACC and DLPFC spatial placement. Spectrum is representative of quality obtained from ACC and DLPFC in non-smokers and smokers.

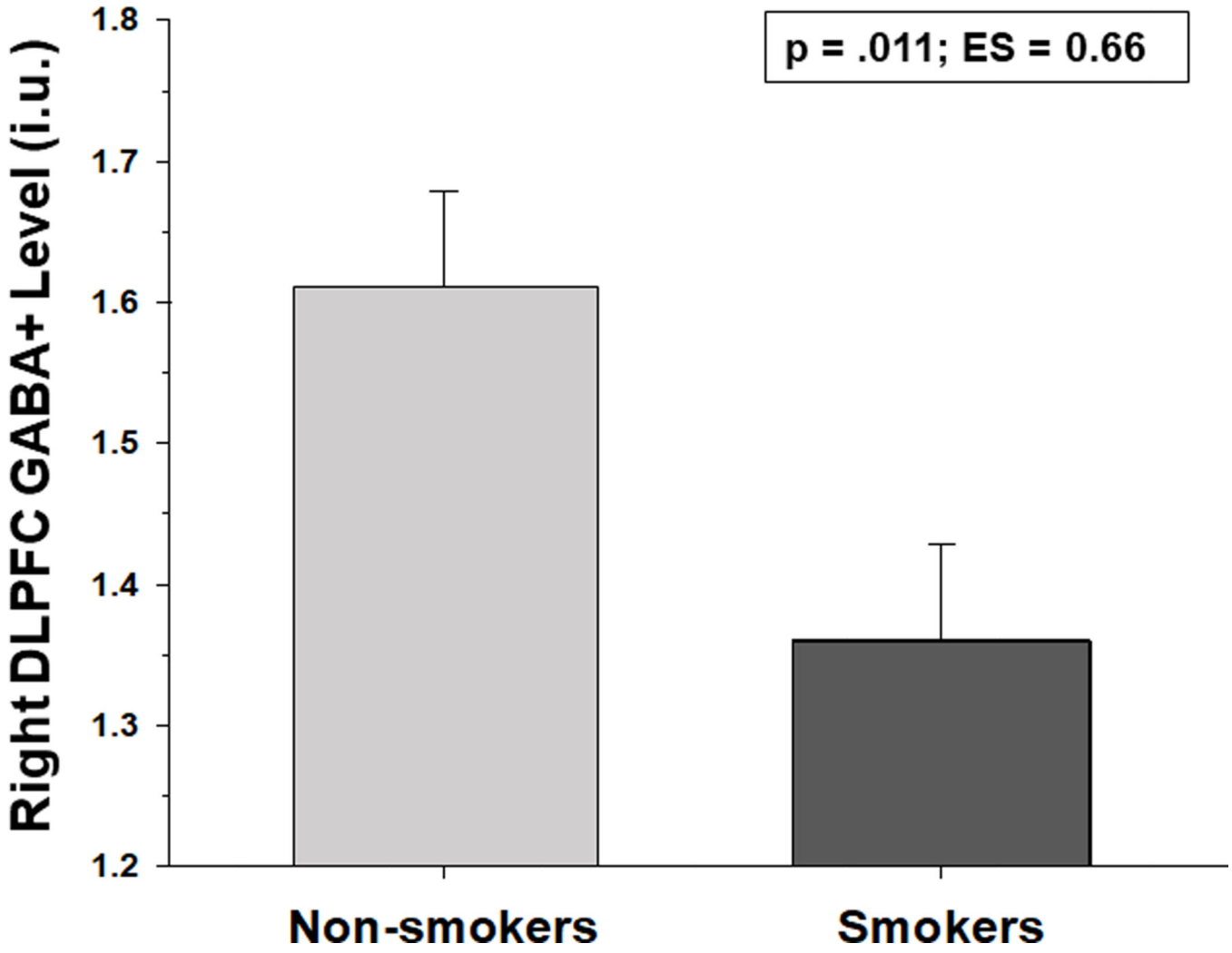

Figure 2.
Right DLPFC GABA+ level (i.u., institutional units) in non-smokers and smokers. Error bars are standard error of the mean.

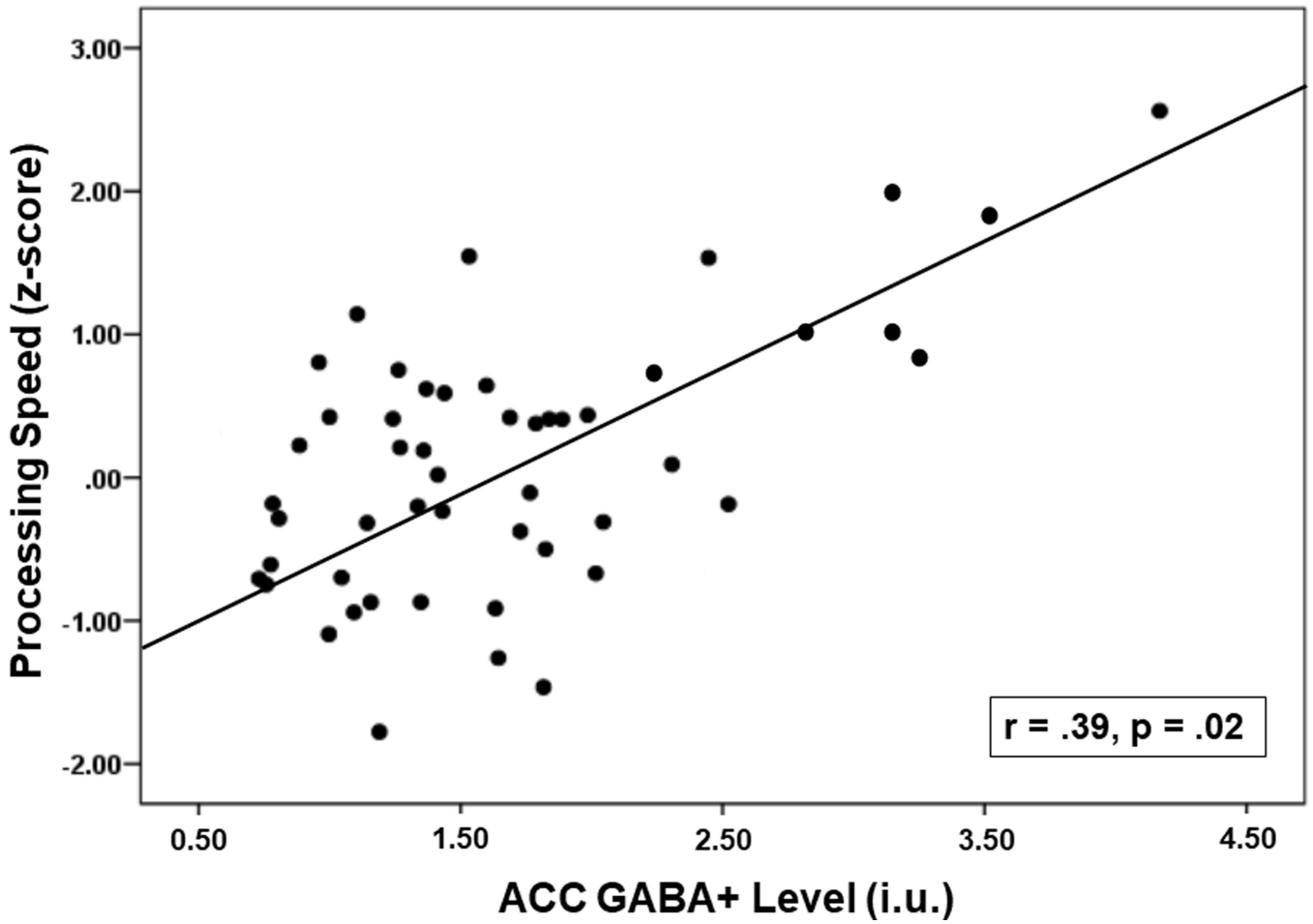

Figure 3a.

Association between processing speed and ACC GABA+ level (i.u., institutional units) in the combined sample of non-smokers and smokers.

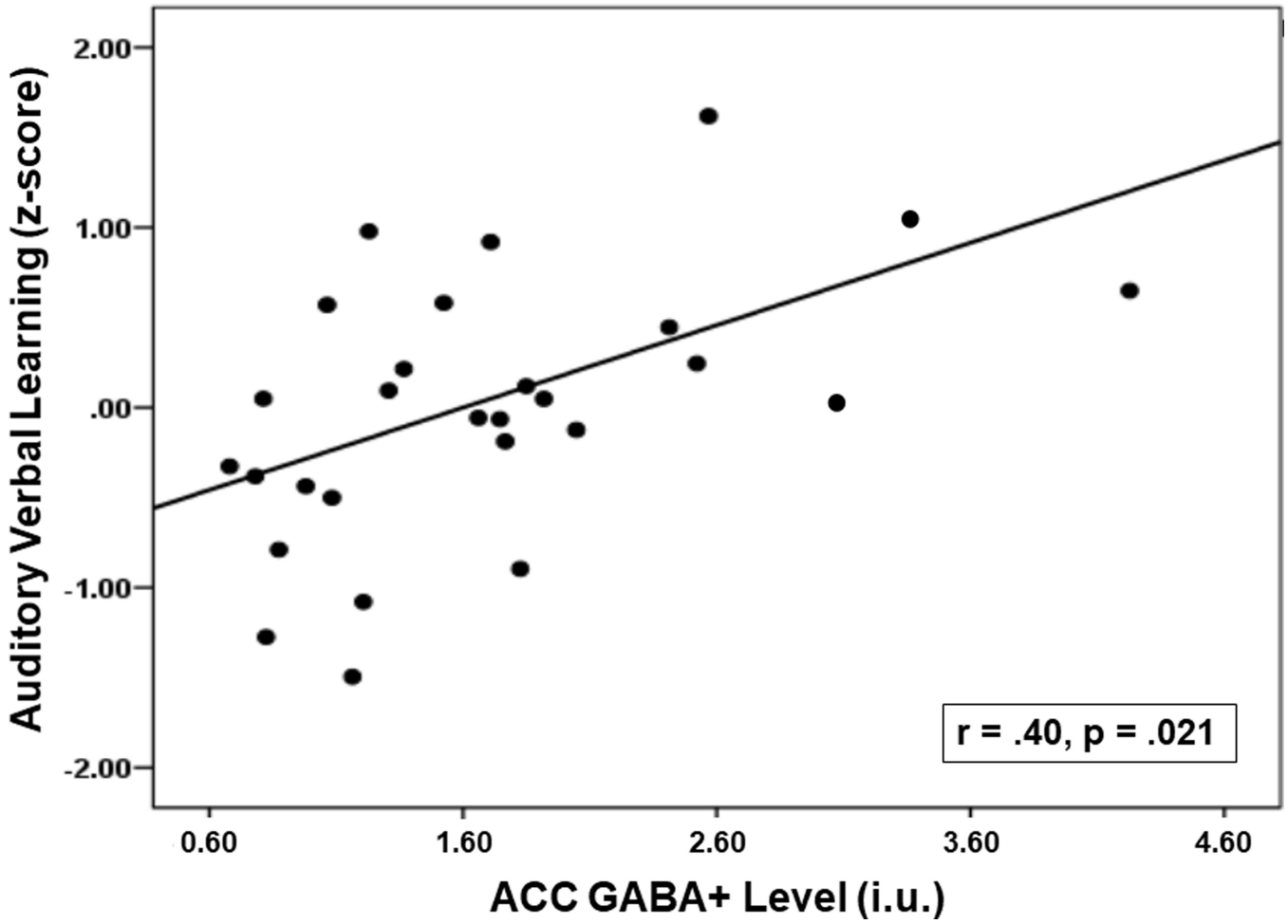

Figure 3b.
Association between auditory-verbal learning and ACC GABA+ level (i.u., institutional units) in smokers.

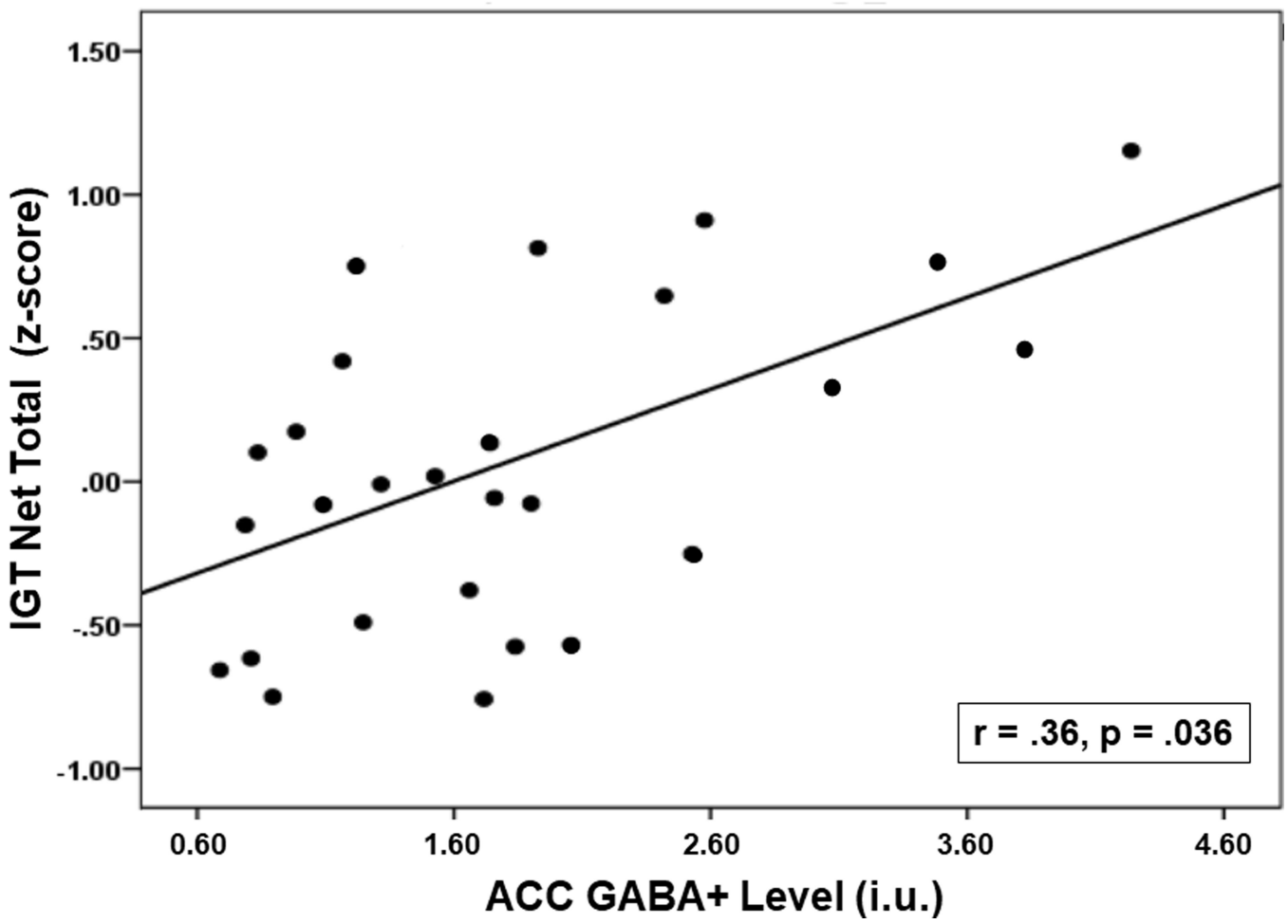

Figure 3c.
Association between IGT net total score and ACC GABA+ level (i.u., institutional units) in smokers.

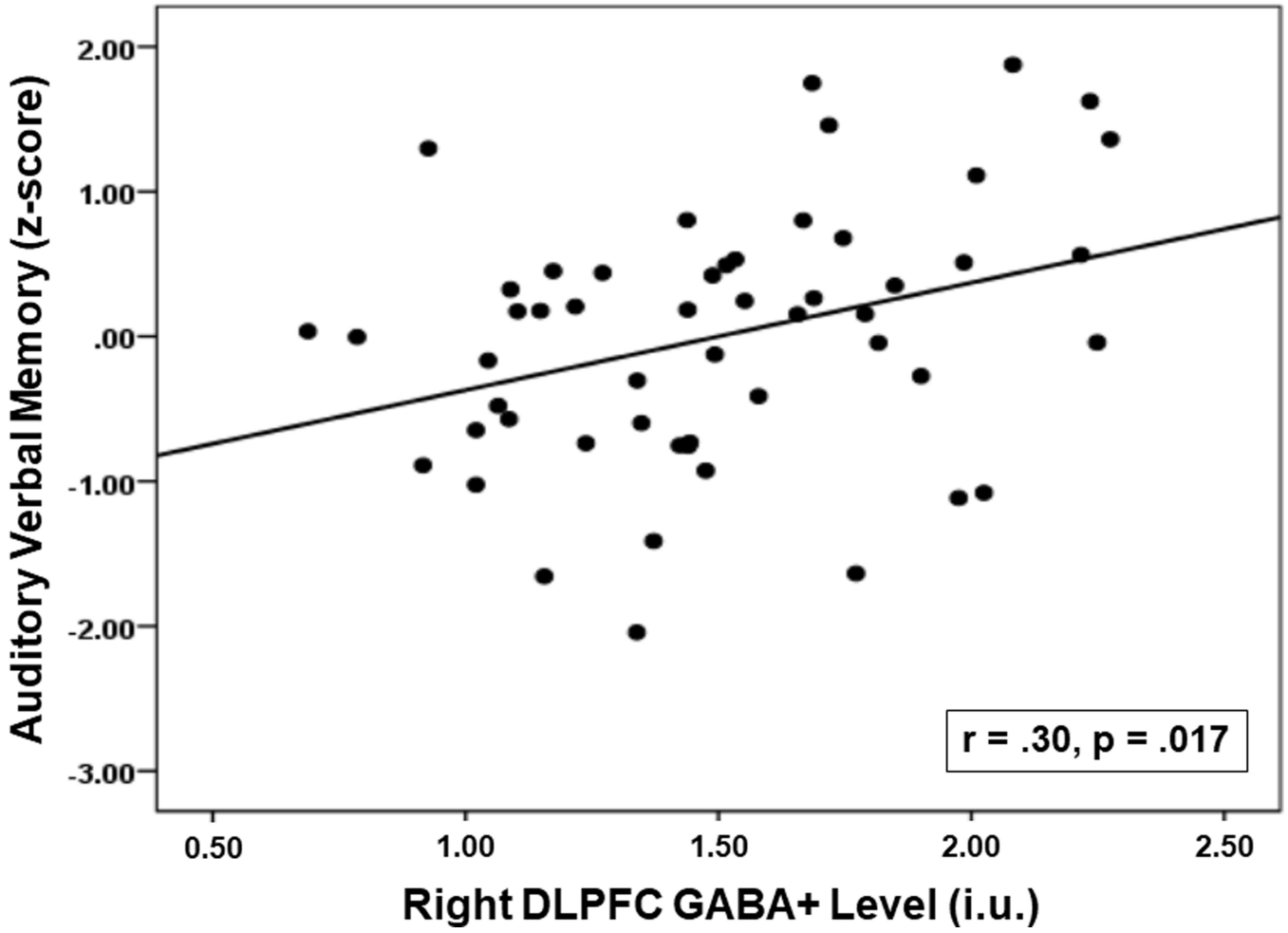

Figure 3d.

Association between auditory-verbal memory and right DLPFC GABA+ level (i.u., institutional units) in the combined sample of non-smokers and smokers.

Table 1.

Group Demographics, Alcohol and Cigarette Use Histories, and Self-Report Questionnaires

Measure	Non-smokers (n = 31)	Smokers (n=33)
Age (years)	50 (12)	49 (10)
Education (years)	16.5 (2.0)	14.8 (2.0)[&]
Male (%)	88	89
White race (%)	60	71
1-year average drinks/month	14 (14)	22 (20)
Lifetime average drinks/month	18 (13)	26 (14)[*]
FTND	NA	5 (2)
Pack-years	NA	27 (15)
BDI	3 (4)	5 (4)
STAI	33 (7)	34 (9)
Body mass index	25 (4)	26 (4)

Note. BDI: Beck Depression Inventory. FTND: Fagerstrom Tolerance Test for Nicotine Dependence. NA: not applicable. STAI: State -trait Anxiety Inventory – Trait.

[&]Non-smokers > Smokers, $p < .05$. Mean (SD).

[*]Non-smokers < Smokers, $p < .05$.