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GABA_A-benzodiazepine receptor availability in smokers and nonsmokers: Relationship to subsyndromal anxiety and depression

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Abstract

Many smokers experience subsyndromal anxiety symptoms while smoking and during acute abstinence, which may contribute to relapse. We hypothesized that cortical gamma aminobutyric acid _A – benzodiazepine receptor (GABA_A-BZR) availability in smokers and nonsmokers might be related to the expression of subsyndromal anxiety, depressive, and pain symptoms. Cortical GABA_A-BZRs were imaged in 15 smokers (8 men; 7 women), and 15 healthy age and sex-matched nonsmokers, and 4 abstinent tobacco smokers (3 men; 1 woman) using [¹²³I]iomazenil and single photon emission computed tomography (SPECT). Anxiety and depressive symptoms were measured using the Spielberger's State-Trait Anxiety Index (STAI) and the Center for Epidemiology Scale for Depressive Symptoms (CES-D). The cold pressor task was administered to assess pain tolerance and sensitivity. The relationship between cortical GABA_A-BZR availability, smoking status and subsyndromal depression and anxiety symptoms, as well as pain tolerance and sensitivity, were evaluated. Surprisingly, there were no statistically significant differences in overall GABA_A-BZR availability between smokers and nonsmokers, or between active and abstinent smokers; however, cortical GABA_A-BZR availability negatively correlated with subsyndromal state anxiety symptoms in nonsmokers but not in smokers. In nonsmokers, the correlation was seen across many state anxiety brain areas [parietal ($r=-.47$, $p=.03$), frontal ($r=-.46$, $p=.03$), anterior cingulate ($r=-.47$, $p=.04$), temporal ($r=-.47$, $p=.03$), occipital ($r=-.43$, $p=.05$) cortices, and cerebellum ($r=-.46$, $p=.04$)], trait anxiety [parietal ($r=-.72$, $p=.02$), frontal ($r=-.72$, $p=.02$), and occipital ($r=-.65$, $p=.04$) cortices] and depressive symptoms [parietal ($r=-.68$; $p=.02$), frontal ($r=-.65$; $p=.03$), anterior cingulate ($r=-.61$; $p=.04$), and temporal ($r=-.66$; $p=.02$) cortices]. The finding that a similar relationship between GABA_A-BZR availability and anxiety symptoms was not observed in smokers suggests that there is a difference in GABA_A-BZR function, but not number, in smokers. Thus, while subsyndromal anxiety and depressive symptoms in nonsmokers may be determined in part by GABA_A-BZR availability, smoking

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disrupts this relationship. Aberrant regulation of GABA_A-BZR function in vulnerable smokers may explain why some smokers experience subsyndromal anxiety and depression.

Keywords

Tobacco smoke; brain; SPECT; GABA-A-benzodiazepine receptor; anxiety; depression

INTRODUCTION

Tobacco is the most widely abused substance in our society with numerous social, economic, and medical consequences. Despite the well-recognized harmful effects of tobacco, approximately 20% of the American population smokes. One reason smokers report they continue to smoke is that smoking relieves anxiety and depressive symptoms (Cooney et al., 1998; Covey et al., 1990; Glassman, 1993). Smokers who report subsyndromal anxiety or depression have a more difficult time quitting smoking than those who do not (Anda et al., 1990; Glassman, 1993). The experience of subsyndromal anxiety and depressive symptoms is not simply a continuum of major depression, thus, these symptoms warrant investigation on their own, especially in women smokers (Borrelli et al., 1999). The specific neurochemical mechanisms underlying subsyndromal anxiety and depression in tobacco smokers are not known. Understanding the neural substrates mediating these symptoms may assist in the development of more effective treatments to assist this vulnerable group of smokers in their efforts to quit smoking.

GABA is the primary inhibitory neurotransmitter in brain, and has been widely implicated in the pathophysiology of anxiety (Lydiard, 2003; Nemeroff, 2003; Vaiva et al., 2004) and depressive disorders (Kugaya et al., 2003) and may contribute to the expression of subsyndromal anxiety and depressive symptoms in tobacco smokers. Nicotine, the principal addictive constituent in tobacco smoke, stimulates GABA release through its actions on nicotinic acetylcholine receptors (nAChRs) on GABAergic neurons in the thalamus, hippocampus, and throughout the cerebral cortex (Domino et al., 2000; Erhardt et al., 2002; Fedele et al., 1998; Ghatan et al., 1998; Mansvelder et al., 2002; Meshul et al., 2002; Reid et al., 2000). In recently abstinent tobacco smokers (4–7 days), nAChR numbers are higher (Staley et al., 2006), whereas cortical GABA levels are lower in recently abstinent women smokers (2 days) compared to nonsmokers (Epperson et al., 2005). While nAChR numbers are higher, the actual number of functional nAChR are likely lower, with a majority of the higher number of nAChR including a larger number of desensitized or inactivated receptors (Picciotto et al., 2008). This appears to be due the effects of nicotine, since cortical GABA levels are lower in rodents chronically treated with nicotine (Porcu et al., 2003). However, cortical GABA-BZR numbers increase in response to chronic nicotine treatment in rodents compared to controls (Magata et al., 2000). Thus, while nicotine may lead to changes in GABA levels and GABA_A-BZR numbers, the effect of tobacco smoking on GABA-BZRs has not been studied.

Tobacco smoke contains over 4000 chemicals in addition to nicotine. Thus, GABAergic function in smokers also may be affected by other chemical constituents in tobacco smoke, specifically the β -carbolines harman and norharman. These are purported to be inverse agonists at the benzodiazepine (BZ) site on the GABA_A receptor (Rommelspacher et al., 1981) and may promote anxiety in smokers (Poindexter and Carpenter, 1962). Administration of BZ antagonists enhances anxiety symptoms in patients with panic disorder or social phobia suggesting that low GABA_A-BZR number may be associated with anxiety (Malizia et al., 1995; Nutt et al., 1990). Direct GABA receptor agonists, and indirect GABA agonists (inhibitors of GABA uptake and metabolism), have demonstrated

antinociceptive qualities in animal models of pain (Levy and Proudfit, 1977; Vaught et al., 1985; Zoen and Enna, 1985), suggesting that alterations in GABA function may contribute to altered pain sensitivity in smokers. During periods of heightened anxiety, the response to pain stimuli is greater (Uman et al., 2006). These findings suggest that in smokers who experience increased anxiety symptoms during abstinence, painful stimuli may be experienced at greater intensity and these ex-smokers may relapse in order to alleviate pain, as well as anxiety.

In the present study, we evaluated GABA_A-BZR availability in smokers and nonsmokers with no history of or current DSM-IV mental disorder using [¹²³I]iomazenil single photon emission computed tomography (SPECT). We also examined the relationship between GABA_A-BZR availability and symptoms of anxiety and depression and response to painful stimuli. We hypothesized that individuals with lower GABA_A-BZR availability would exhibit greater subsyndromal anxiety and depressive symptoms and, lower pain tolerance and greater sensitivity to pain.

METHODS AND MATERIALS

Subjects

Fifteen (8 men, 7 women) smokers and fifteen (8 men, 7 women) nonsmokers provided written informed consent to participate in this study (Table I). Four smokers (3 men, 1 woman) signed an additional consent to abstain from smoking for five weeks and participate in a second SPECT scan. This study was approved by the Human Investigation and Radiation Safety Committees at the Yale University School of Medicine (New Haven, CT), and screening appointment was approved by the Human Subjects Subcommittee at the West Haven VACHS (CT). Eligibility was evaluated via structured interview, physical examination, laboratory blood tests, urine drug screen, and an electrocardiogram. None of the subjects had a history or evidence of a serious medical or neurological illness, psychiatric disorder, or substance abuse (except for nicotine dependence in smokers). Subjects had not used psychotropic substances other than alcohol and tobacco for at least one year and reported no marijuana use for at least one month preceding the study. None of the subjects tested positive for drugs, other than nicotine, on a urine drug screen. All subjects were advised to abstain from alcohol for one week preceding the SPECT scan and smokers were asked to maintain their normal smoking routine prior to the scan day. All women had a regular menstrual cycle and did not use hormonal contraceptives. Nonsmokers were defined as individuals that smoked less than 40 cigarettes in their lifetime and none for two years before the study.

Assessments

At the baseline screening appointment (about 2 weeks prior to SPECT scan), all subjects had a physical exam, blood and urine laboratory tests, and were administered the Structural Clinical Interview (SCID-I) for the Diagnostic and Statistical Manual – TR (DSM-IV-TR), and questionnaires to assess nicotine dependence and smoking behavior Fagerstrom Test of Nicotine Dependence – FTND (Heatherton et al., 1991), Tiffany Questionnaire for Smoking Urges (Tiffany and Drobes, 1991), Minnesota Nicotine Withdrawal Scale – MNWS (Hughes, 1991), and subsyndromal depression (Center for Epidemiological Studies Depression Scale - CES-D (Radloff, 1977). These assessments were repeated on the day of the SPECT scan (about 2 hours after the last cigarette in active smokers), in addition to measurement of state and trait anxiety symptoms (Spielberger's State-Trait Anxiety Inventory – STAI) (Spielberger et al., 1983). State anxiety is defined as unpleasant emotional arousal in the face of threatening demands or danger, whereas trait anxiety reflects the existence of stable individual differences in the tendency to respond with state

anxiety in anticipation of threat (Lazarus, 1991). CES-D was repeated on the day of the 2nd SPECT scan for four abstinent smokers. STAI and CES-D were not administered to the first 5 nonsmokers participating in the study.

The cold pressor task was administered on the day of the SPECT scan to measure sensitivity to pain, tolerance of which has been related to elevated symptoms of anxiety. Subjects were required to keep their dominant hand in warm water for 60 seconds. The subjects were then instructed to put the same hand in cold water for 90 seconds, but were allowed to remove it if they started to experience intolerable pain. The subjects were instructed to rate their pain when they initially started feeling pain (rating of sensitivity) on a scale of 0 (no pain) to 100 (worst pain ever felt); the subjects also made a pain rating at the time of hand withdrawal (rating of tolerance) (Walsh et al., 1989).

Plasma and urine samples were obtained at the screening appointment and on the day of the SPECT scan to measure cotinine (the primary metabolite of nicotine) levels. Cotinine levels were measured in plasma as described previously (Staley et al., 2006). In brief, on each scan day, concentrations were measured in plasma obtained from EDTA anti-coagulated blood samples obtained in the middle of the [¹²³I]iomazenil SPECT scan. Samples were centrifuged at room temperature and promptly frozen. Cotinine and nicotine concentrations in serum were assayed using reversed-phase HPLC. Urinary cotinine levels were monitored daily during the 8 d nicotine withdrawal period using NicAlert cotinine test strips (Nymox Pharmaceutical Corporation, Hasbrouck Heights, NJ).

Contingency Management for Smoking Cessation

Contingency management techniques were used to achieve 5-weeks of abstinence in four smokers as described previously (Staley et al., 2006). In brief, carbon monoxide (CO) levels <11 ppm and urine cotinine levels < 100 ng/mL were used to define abstinence from cigarettes. Smokers who agreed to quit smoking were compensated twice daily (morning and afternoon) for the first 8 days of abstinence and less frequently (2–3 times weekly) thereafter based on a breath CO reading and urine cotinine level indicating abstinence.

[¹²³I]iomazenil SPECT Imaging and Magnetic Resonance Imaging

Fifteen smokers and fifteen nonsmokers participated in one [¹²³I]iomazenil SPECT scan. Four of the smokers abstained from smoking and nicotine replacement products for 5 weeks, and participated in an additional [¹²³I]iomazenil SPECT scan to determine if there were any changes in GABA_A-BZR with abstinence. [¹²³I]iomazenil was prepared as described previously (Zoghbi et al., 1992). The average yield was a mean $\pm$ SD of 59.6% $\pm$ 12.7% (n=34 preparations) and its radiochemical purity was a mean $\pm$ SD of 98.6% $\pm$ 1.7%. All participants were pretreated with saturated potassium iodide to reduce thyroid uptake of ¹²³I. The duration of infusion was 6.5 hours with a mean $\pm$ SD total injected dose of 220.8 $\pm$ 10.0 MBq for nonsmokers and 223.1 $\pm$ 0.9 MBq for smokers (n=15) and 225.3 for smokers (N=4) at 5 weeks abstinence, with a bolus to infusion ratio for all subjects of 3.83 $\pm$ 0.02 hours. The infusion rate was 19.1 $\pm$ 0.3 MBq/h (nonsmokers); 19.1 $\pm$ 0.4 MBq/h (smokers) and 19.1 $\pm$ 0.3 MBq/h smokers at 5 weeks abstinence. Prior to imaging, five external fiducial markers containing 0.037 MBq of ¹²³I were placed on the scalp to provide a common reference for co-registration with emission images. Smokers smoked their last cigarette before coming to SPECT imaging lab and thus were imaged about 7h after the last cigarette. Simultaneous transmission and emission scans (STEP) were acquired with a line source containing 740 MBq of cobalt 57 on a PRISM 3000XP 3-headed camera (Picker International, Cleveland, Ohio). Three consecutive 12-minute emission scans were acquired in continuous mode starting at 366.9 $\pm$ 15.2 min for nonsmokers and 365.6 $\pm$ 9.0 min for smokers of [¹²³I]-iomazenil infusion. Three venous blood samples were collected at the

midpoint of the SPECT scan (385.1 ± 11.9 min for nonsmokers and 384.3 ± 13.7 for smokers) for metabolic assessment of [^{123}I]iomazenil (Zoghbi et al., 1992). [^{123}I]iomazenil SPECT has 83% to 90% reproducibility (Abi-Dargham et al., 1995).

Magnetic resonance imaging was performed on a Sonata 1.5T Siemens (Siemens AG, Wittelsbacherplatz 2; D-80333 Munchen Germany). Axial images were acquired parallel to the anteroposterior commissural line with an echo time of 5 milliseconds; repetition time of 24 milliseconds; matrix 256×192 ; number of excitations of 1; field of view of 24 cm; and 128 contiguous slices with a thickness of 1.3 mm.

Image Analysis

SPECT images were processed and volume of interest analyses were performed as described previously (Staley et al., 2005). Emission data from SPECT scans were reconstructed from counts acquired in the ^{123}I photopeak (159 keV) with a 20% symmetric window using a Butterworth filter (power factor=10, cutoff= 0.24 cm). An attenuation map was reconstructed from the transmission and flood data and was used for non-uniform attenuation correction (Rajeevan et al., 1998). The magnetic resonance image was co-registered to the emission image and reoriented to the inter-commissural plane and standardized 2-dimensional region of interest templates were placed on the parietal, frontal, anterior cingulate, occipital, temporal cortices, thalamus, striatum and cerebellum. Three-dimensional volumes of interest were transferred to co-registered SPECT images. Regional activities from right and left hemispheres were averaged, decay corrected, and expressed as kilobecquerels per cubic centimeter using a calibration factor of $0.000912 \mu\text{Ci}/\text{cpm}$ derived from a ^{123}I distributed source phantom. Regional activities (kilobecquerels per cubic centimeter) were normalized to free [^{123}I]iomazenil (kilobecquerels per milliliter) plasma levels (Abi-Dargham et al., 1994). Equilibrium distribution volume (V_T/f_p) was the main outcome measure, which is proportional to brain region activity divided by free parent (Innis et al., 2007). Two raters analyzed the data and the mean outcome measure is used for all analyses. Interrater was <10% variability between raters across regions.

Statistical Analyses

Univariate analyses of variance (ANOVA) were conducted comparing smokers and nonsmokers. The relationship between [^{123}I]iomazenil V_T/f_p and various clinical measures of mood was examined in the total sample and in the smoker and nonsmoker groups. The relationship with various clinical facets of nicotine dependence was examined in smokers.

Region of interest data were analyzed using SPSS version 16.0 (SPSS Inc. Headquarters, Chicago, IL). Differences in V_T/f_p between smokers and nonsmokers were assessed using a fixed-effects regression model with [^{123}I]iomazenil V_T/f_p as the dependent variable and with group (smokers or nonsmokers), region of interest, and interactions as fixed factors and unstructured variance-covariance matrix across regions. Within-subject differences in V_T/f_p between smokers imaged at 7 h abstinence and again at 5 weeks abstinence were evaluated using a paired student's t-test.

Spearman correlations coefficient was employed to assess associations between clinical variables (smoking, region of interest, mood) and [^{123}I]iomazenil V_T/f_p for the total sample, as well as for smokers and nonsmokers.. Different slopes for smokers and nonsmokers were estimated to illustrate the relationship between significant clinical variables and brain receptor availability. No correlations were done for smokers at 5 weeks abstinence because of the small sample size.

RESULTS

Fifteen smokers (8 men, 7 women) and fifteen age- and sex-matched nonsmokers participated in the study. Smokers had significantly higher plasma cotinine levels (299.9 ± 98.9 ng/ml) than nonsmokers (0.0 ± 0.0 ng/ml) ($p=0.000$) on the day of the scan. Nonsmokers and smokers demonstrated similar levels of alcohol consumption (1.3 ± 2.2 and 1.2 ± 2.4 standardized drinks/week respectively) the week prior to the SPECT scan. Smoking characteristics, including the age at which subjects began smoking, the total number of years they smoked, the number of cigarettes smoked per day, and family history of smoking, are reported in Table I.

Effect of Smoking on GABA_A-BZR Availability

There were no statistically significant differences in [123 I]iomazenil uptake between smokers ($n=15$) and nonsmokers ($n=15$) (Figure 1). Furthermore, in the four smokers who abstained from smoking and nicotine replacement treatments for five weeks, there was no statistically significant differences in [123 I]iomazenil uptake between the two time-points: prior to smoking cessation and at five weeks of abstinence (for parietal, frontal, anterior cingulate, temporal and occipital cortices and the cerebellum the paired t- test resulted in $t = 0.22$ and $p = 0.84$) suggesting that GABA_A-BZR availability was not different between active smoking and 5 weeks of abstinence.

Correlational analyses between regional [123 I]iomazenil uptake and severity of nicotine dependence (FTND), nicotine craving (Tiffany Desire and Withdrawal subscales), and nicotine withdrawal (Minnesota Withdrawal Scale) demonstrated no significant associations in any of the brain regions studied (parietal, frontal, anterior cingulate, temporal, and occipital cortices, and cerebellum).

Relationship Between GABA_A-BZ Receptor Availability and Subsyndromal Anxiety and Depressive Symptoms, and Pain Tolerance

Self-reported trait (stable individual trait) and state (current unpleasant emotional arousal) anxiety symptoms were evaluated using the STAI on the day of scan. In the total sample, there was a significant negative correlation between [123 I]iomazenil uptake and state anxiety symptoms in all regions examined: parietal ($r=-.47, p=.03$), frontal ($r=-.46, p=.03$), anterior cingulate ($r=-.47, p=.04$), temporal ($r=-.47, p=.03$), occipital ($r=-.43, p=.05$) cortices, and cerebellum ($r=-.46, p=.04$). When these correlations were evaluated by smoking status, the correlations were significant only for the nonsmokers (Figure 2).

Trait anxiety correlated negatively with [123 I]iomazenil uptake in the total sample in the cerebellum ($r=-.46, p=.04$) with similar trends noted in the temporal and occipital cortices. When evaluated by smoking status, the correlation between [123 I]iomazenil uptake and symptoms of trait anxiety was significant in the parietal ($r=-.72, p=.02$), frontal ($r=-.72, p=.02$), and occipital ($r=-.65, p=.04$) cortices of nonsmokers (Figure 3), but this effect was lost in the smokers.

A measure related to state anxiety, the association between smokers' pain tolerance and GABA_A-BZR availability was evaluated using the cold pressor task. There was no significant correlation between ability to keep hand in cold water or rating of associated pain and [123 I]iomazenil uptake in any of the brain regions assessed. Additionally, at the time of these assessments, we did not detect significant associations between pain tolerance or sensitivity and anxiety ($p>.3$).

Self-reported depressive symptoms were evaluated using the CES-D on the day of scan. In the total sample there was no significant association between GABA_A-BZR availability and

subsyndromal depressive symptoms. However, in nonsmokers, brain [123 I]iomazenil uptake and CES-D scores were negatively correlated in the parietal ($r=-.68$; $p=.02$), frontal ($r=-.65$; $p=.03$), anterior cingulate ($r=-.61$; $p=.04$), and temporal cortices ($r=-.66$; $p=.02$), with lower uptake reflecting more depressive symptoms (Figure 4). There was no correlation between [123 I]iomazenil uptake and CES-D scores in smokers, suggesting that smoking modifies the relationship between GABA_A-BZR and depressive symptomatology.

DISCUSSION

The present study evaluated GABA_A-BZR availability in smokers and age and sex-matched nonsmokers using [123 I]iomazenil SPECT. Our findings demonstrated no statistically significant differences in GABA_A-BZR availability in smokers compared to nonsmokers. However, important differences were observed in the relationship between smoking status, GABA_A-BZR availability, and measures of anxiety and depression. Specifically, low GABA_A-BZR availability was related to self-reported feelings of anxiety and depression in nonsmokers, but not in smokers.

The lack of effect on receptor availability in human smokers was initially surprising given the preclinical finding that chronic nicotine treatment resulted in an upregulation in BZR numbers (Magata et al., 2000). These findings could be due to a higher amount of endogenous benzodiazepine, such as diazepam binding inhibitor produced by nicotine exposure (Katsura et al., 1994), that could block [123 I]iomazenil from binding to the receptor. Alternatively, since our scans were conducted after only 7 hours of abstinence, the presence of nicotine and other substances like harmala alkaloids in tobacco smoke, that are purported benzodiazepine inverse agonists, may have interfered with [123 I]iomazenil binding to the receptor (Rommelspacher et al., 1981). We also examined GABA_A-BZR availability in 4 smokers who remained abstinent for 5 weeks and did not observe any abstinence-induced changes. However, these results need to be replicated with larger samples of smokers during acute (<1 week) and prolonged (>1 month) abstinence before we can conclusively eliminate the possibility of change in GABA_A-BZR availability during acute withdrawal. We also observed no significant relationships between GABA_A-BZR availability and various facets of smoking behavior including nicotine dependence and self-reported symptoms of desire for a cigarette or withdrawal from smoking, suggesting no direct relationship between available GABA_A-BZ receptors and nicotine craving, withdrawal symptoms, or severity of nicotine dependence in nondeprived smokers.

Regarding measures of anxiety, an inverse correlation was noted between GABA_A-BZR availability and subsyndromal state and trait anxiety in nonsmokers. Benzodiazepines, the primary pharmacological treatment for anxiety disorders, decrease anxiety by facilitating GABAergic function through actions at GABA_A-BZR. Our findings are also consistent with the evidence from other clinical studies demonstrating GABA_A-BZR involvement in anxiety disorders: lower cortical GABA_A-BZR availability has been shown in patients with panic disorder (Kaschka et al., 1995; Kaschka et al., 1992; Schlegel et al., 1994) and in women with anxiety disorder (Tiihonen et al., 1997). All the above evidence and our results suggest that low GABA_A-BZR availability may be a neural marker for the expression of anxiety in nonsmokers. Similarly, we observed an inverse association between subsyndromal depressive symptoms and GABA_A-BZR availability in nonsmokers. The relationship between GABAergic function and depressive symptoms has been long established (Brambilla et al., 2003; Petty, 1995; Sanacora et al., 2004), but evidence of a relationship between GABA_A-BZR availability and depressive symptoms is less consistent - lower GABA_A-BZR availability appears to be a marker of comorbid depressive and anxiety disorder (Cameron et al., 2007), but is not evident in depressed patients without comorbid anxiety (Kugaya et al., 2003).

We did not find any evidence of relationships between GABA_A-BZ receptors and anxiety/depression among smokers. The literature on the relationship of smoking to anxiety and depression is rather mixed. It has been suggested that anxiety may precede smoking (Merikangas et al., 1998), and also that smoking may precede an anxiety disorder (Kessler, 2004). Past history of depression and heightened anxiety predict relapse within 7 days of smoking cessation (Brown et al., 2001). On self-report, smoking individuals state that smoking reduces anxiety symptoms (Jarvik et al., 1989; Pomerleau et al., 1984). Nicotine, the most well studied component of tobacco smoke, has been shown to have anxiolytic effects (Brioni et al., 1994; Zarrindast et al., 2008), and nicotine withdrawal is associated with increases in anxiety (Costall et al., 1989). However, nicotine use also increases most physiological measures of arousal (i.e., heart rate, blood pressure; (Perkins, 1995), and higher doses of nicotine exacerbate anxiety (Cheeta et al., 2001). Similar to effects on anxiety, nicotine has been suggested to have an antidepressant effects in nonsmokers (Gilbert, 1996), and some studies show higher levels of nicotine dependence in depressed versus non-depressed smokers (Pomerleau et al., 2004). Further, a relapse to mood disorders is observed during smoking cessation in smokers with past history of major depressive disorder (Gilbert et al., 2002; Hatsukami et al., 1988).

There are several potential explanations for the lack of an association between GABA_A-BZR availability and anxiety and depressive symptoms among smokers in the current study, one of which being potential disruptions in the relationship between GABA_A-BZ receptors and the expression of anxiety or depressive symptoms. This disruption could be a direct effect of exposure to either nicotine or to other chemicals in tobacco smoke such as the harmala alkaloids, which could have altered the functionality of the GABA_A-BZ receptors. Although the literature is limited on the relationship between GABA_A-BZ receptors availability or functionality and nicotine or tobacco smoke, a preclinical study of the effects of chronic (18 days) nicotine exposure on expression of GABA receptor subunits and transporters reported increases in the α_1 subunit (but not α_3 , α_4 , α_5) and GAT3 in the nucleus accumbens and decreases in GAT1 in the medial prefrontal cortex (Pickering et al., 2008), providing evidence for neuroadaptive region-specific changes due to nicotine exposure on expression and possibly functionality of different components of the GABA system. Alternatively, these differences could be related to complex interactions between tobacco use and GABA-BZ receptor levels and genetic predispositions to being a smoker or having anxiety or depressive symptoms. Further research is warranted to determine the reasons for lack of findings between GABA_A-BZ receptor availability and subsyndromal mood symptoms in the current smoker sample.

Implications Regarding Smoking Cessation Treatments

The current findings suggest a regulatory mechanism that mediates the relationship between GABA_A-BZR availability and subsyndromal symptoms of depression and anxiety. Upon abstaining from smoking, many smokers, especially women smokers, report feelings of depression (Kessler, 2003) or anxiety (Pomerleau et al., 2005) immediately after quitting as well as long-term (Catley et al., 2005). The findings from the present study, which clearly demonstrate a relationship between the GABA_A-BZR availability and depressive symptoms in nonsmokers, also indicate that this relationship is disrupted in smokers. However, smoking status and prolonged abstinence was not observed to produce any significant changes in GABA-BZ receptor levels. These findings suggest that the relationship between depression and anxiety smoking and GABA_A-BZ receptors is complex and may be dependent on other vulnerability factors such as sex and/or genetic predisposition or alterations in other GABA-related systems. The inverse correlation between GABA_A-BZR availability and anxiety symptoms in nonsmokers, coupled with the lack of an overall difference in GABA_A-BZR availability between smokers and nonsmokers, suggests that

aberrant GABA_A-BZR function, rather than overall number, may be responsible for the lack of correlation between receptor availability and anxiety and depression seen in smokers. Potential mechanisms underlying this dissociation could be increased internalization or desensitization of GABA_A-BZR in smokers that leads to altered function in the absence of altered overall receptor numbers. Future evaluations need to examine these complex relationships further to identify vulnerable groups of smokers who may benefit from unique treatments for symptoms of subsyndromal anxiety and depression.

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Fig. 1. Scatterplots illustrating individual [^{123}I]iomazenil regional distribution volume (V_T/f_p) values for region of interest analyses. Regional distribution volume (regional activity/free plasma parent) determined for each individual subject is illustrated for the parietal, frontal, anterior cingulate, temporal and occipital cortices, and cerebellum. All smokers smoked $\sim 7\text{h}$ prior to the scan. There were no significant differences between groups.

Fig. 2.

Smokers and nonsmokers completed the STAI State Anxiety Questionnaire on the day of the [^{123}I]iomazenil SPECT Scan. In the overall sample, there was a significant negative associations between [^{123}I]iomazenil uptake and state anxiety. However, when evaluated by smoking status, in nonsmokers there were significant negative associations between brain uptake of [^{123}I]iomazenil and self-reported symptoms of state anxiety. In the smokers, there were no significant associations.

Fig. 3.

Smokers and nonsmokers completed the STAI Trait Anxiety Questionnaire on the day of the [^{123}I]iomazenil SPECT Scan. In the overall sample, there were no significant associations between [^{123}I]iomazenil uptake and trait anxiety in any cortical regions assessed. When evaluated by smoking status, in nonsmokers there were significant negative associations between brain uptake of [^{123}I]iomazenil and self-reported symptoms of trait anxiety. In the smokers, there were no significant associations.

All subjects

Nonsmokers

Smokers

Fig. 4.

In the total sample, there was no significant correlation between CES-D response and $[^{123}\text{I}]\text{iomazenil}$ uptake. However, in nonsmokers, $[^{123}\text{I}]\text{iomazenil}$ uptake was correlated to symptoms of subsyndromal depression as assessed by CES-D on the scan day in all cortical regions assessed and cerebellum. In smokers, no associations between brain $[^{123}\text{I}]\text{iomazenil}$ uptake and depressive symptoms were observed.

Table I**Demographics of Nonsmokers and Smokers***

	Nonsmokers	Smokers	Abstinent Smokers
	N=15	N=15	N=4
<i>Age (SD)</i>	38.1 (± 12.8)	38.6 (± 9.8)	44.3 (± 8.5)
<i>Race**</i>	5AA	6AA	2AA
	1AS	1HS	2C
	9C	8C	
<i>Age first cigarette</i>	N/A	21.5 (± 7.7)	28.0 (± 6.7)
<i>Years smoked</i>	N/A	19.2 (± 9.1)	16.3 (± 3.9)
<i>Cigarettes/day</i>	N/A	16.0 (± 7.1)	0
<i>FH+</i>	5	9	2
Baseline Assessments			
<i>FTND</i>	0.0	5.7	6.0
<i>MNWS</i>	N/A	10.9 (± 11.7)	14.3 (± 8.3)
<i>CES-D</i>	4.6 (± 3.3)	5.8 (± 5.1)	7.8 (± 6.1)
SPECT Scan Day Assessments			
<i>Tiffany Desire</i>	N/A	57.6 (± 18.3)	26.5 (± 14.5)
<i>Tiffany Withdrawal</i>	N/A	24.2 (± 14.3)	11 (± 0)
<i>MNWS</i>	N/A	4.7 (± 5.8)	4.3 (± 7.8)
<i>CES-D</i>	4.1 (± 3.1)	5.0 (± 3.9)	3.5 (± 6.4)
<i>STAI State</i>	23.1 (± 4.8)	26.3 (± 7.0)	N/A
<i>STAI Trait</i>	27.6 (± 5.8)	29.5 (± 7.7)	N/A

Abbreviations: AA – African American, AS – Asian, HS – Hispanic, C – Caucasian; FH+ - family history positive for smoking; FTND – Fagerstrom Test for Nicotine Dependence; MNWS – Minnesota Withdrawal Scale; CES-D - Center for Epidemiological Studies Depression Scale; STAI – Spielberger's State/Trait Anxiety Inventory

* Data are presented as mean $\pm$ SD.

** Race was self-reported by each subject.

All of nonsmokers smoked less than 40 cigarettes in lifetime.