Published in final edited form as:

Alcohol Clin Exp Res. 2016 April ; 40(4): 698–705. doi:10.1111/acer.12997.

Nicotine and Nicotine Abstinence Do Not Interfere with GABA_A Receptor Neuroadaptations During Alcohol Abstinence

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Abstract

Background—Alcohol dependence and tobacco smoking are highly comorbid, and treating both conditions simultaneously is controversial. Previously, we showed that tobacco smoking interferes with GABA_A receptor neuroadaptations during alcohol withdrawal in humans, while this effect did not occur with continued nicotine use during alcohol abstinence in nonhuman primates. Here, we extend our previous work by measuring GABA_A receptor availability with positron emission tomography (PET) during drug abstinence in nonhuman primates exposed to alcohol alone, nicotine and alcohol together, and alcohol abstinence with continued nicotine exposure.

Methods—Twenty-four adolescent male rhesus macaques orally self-administered alcohol and nicotine, available separately in water and saccharin, over 20 weeks. The groups included alcohol alone ($n = 8$); nicotine and alcohol with simultaneous abstinence ($n = 8$); nicotine and alcohol with alcohol abstinence while nicotine was still available ($n = 8$); and a pilot group of animals consuming nicotine alone ($n = 6$). Animals were imaged with [¹¹C]flumazenil PET to measure binding potential (BP_{ND}), an index of GABA_A receptor availability. Imaging occurred at baseline (drug-naïve), and following alcohol and/or nicotine cessation at 1 day, 8 days, and 12 weeks of abstinence. Generalized linear mixed models were used to examine the time course of [¹¹C]flumazenil BP_{ND} during alcohol abstinence across groups.

Results—Animals consumed 3.95 ± 1.22 g/kg/d alcohol and 55.4 ± 35.1 mg/kg/d nicotine. No significant group effects were observed in [¹¹C]flumazenil BP_{ND} during alcohol abstinence; however, a main effect of time was detected. Post hoc analyses indicated that all groups abstaining from alcohol exhibited significantly increased GABA_A receptor availability at 1 day and 8 days (but not 12 weeks) of abstinence relative to baseline, while no changes in [¹¹C]flumazenil BP_{ND} during nicotine abstinence alone were observed.

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CONFLICTS OF INTEREST

All other authors have no conflicts of interest to report.

Conclusions—These data indicate that neither nicotine nor nicotine abstinence interferes with GABA_A receptor neuroadaptations during alcohol withdrawal. This conclusion is consistent with our previous study and does not contradict the use of nicotine replacement therapies or non-nicotinic-acting pharmaceuticals to quit smoking during alcohol withdrawal from a GABAergic perspective.

Keywords

Alcohol Dependence; Tobacco Smoking; GABA_A Receptors; Positron Emission Tomography Imaging

Alcohol dependence and tobacco smoking are highly comorbid conditions. Roughly half of alcohol-dependent individuals smoke (Grant et al., 2004), and these individuals have a greater severity of alcohol dependence and withdrawal symptoms (Mason and Leher, 2009). Alcohol dependence mortality is significantly increased with concurrent tobacco smoking (Hurt et al., 1996), and continued cigarette smoking during recovery from alcohol use disorders increases relapse likelihood (Weinberger et al., 2015). However, optimal treatment of comorbid alcohol dependence and tobacco smoking is not well characterized (Kodl et al., 2006). The timing of quit attempts for smoking in relation to alcohol abstinence may be critical to successful recovery (Joseph et al., 2004; Kalman et al., 2010). Additionally, a majority of smoking cessation pharmacotherapies target the nicotinic system, notably nicotine replacement therapies (Stead et al., 2012) and the medication varenicline (Chantix), which acts at nicotinic receptors (Aubin et al., 2008). In light of the prominence of these nicotinic-acting pharmacotherapies and the rapid increase of novel nicotine delivery systems such as e-cigarettes (Grana et al., 2014), the influence of both nicotine and nicotine abstinence on brain changes occurring during alcohol abstinence is a critical consideration when treating alcohol dependence.

The inhibitory γ -aminobutyric acid (GABA) system plays an important role in the neurobiology of alcohol dependence and tobacco smoking comorbidity (Cosgrove et al., 2011). Specifically, chronic alcohol reduces the functional response of GABA_A receptors (Follesa et al., 2006; Liang et al., 2007), while nicotine activates GABA neurons crucial for its rewarding properties (Tolu et al., 2013). Importantly, extrasynaptic GABA_A receptors are stimulated by alcohol and alcohol-induced GABA and neurosteroid release (Morrow et al., 1999). Neuroimaging studies of GABA_A receptor availability (chiefly influenced by receptor number, receptor occupancy, and radioligand-receptor affinity; Innis et al., 2007) during tobacco-free alcohol dependence provided evidence of short-lived increases in GABA_A receptor availability after the first week of alcohol withdrawal, which normalized to control levels after approximately a month (Cosgrove et al., 2014; Staley et al., 2005). However, GABA_A receptor availability in smokers did not normalize after extended alcohol withdrawal. Further, during the first week of withdrawal, increased GABA_A receptor availability in smokers was associated with higher craving for alcohol and cigarettes, which was not observed in nonsmokers. Thus, GABA_A receptors are likely critical to the neuroadaptations underlying the interaction of tobacco smoking and alcohol dependence. However, the role of specifically nicotine and nicotine abstinence itself interacting with alcohol withdrawal to influence the GABA system is not well characterized.

In conjunction with this previous clinical work, our group preliminarily demonstrated in nonhuman primates that nicotine alone did not block the normalization of GABA_A receptors in alcohol abstinence (Cosgrove et al., 2014), suggesting that an additive in tobacco smoke other than nicotine may block the recovery of GABA_A receptors in alcohol dependence. The goal of this work was to build upon our preliminary findings, using [¹¹C]flumazenil positron emission tomography (PET) imaging, to systematically examine the effects of both nicotine and nicotine abstinence on GABA_A receptor neuroadaptations during alcohol abstinence. Specifically, we longitudinally measured GABA_A receptor availability in 3 distinct groups of animals: alcohol abstinence alone, nicotine and alcohol with simultaneous abstinence, and nicotine and alcohol with continued nicotine during alcohol abstinence. These experiments provide important data informing smoking cessation strategies during early alcohol withdrawal.

MATERIALS AND METHODS

Animals

The animal protocol was in compliance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and was approved by the Yale University Institutional Animal Care and Use Committee. A total of 30 adolescent (3 to 4 year; 4.0 to 6.9 kg) male rhesus macaque (*Macacca mulatta*) subjects were used.

Overview

Animals first underwent 1 or 2 baseline PET scans to measure GABA_A receptor availability while alcohol and nicotine naïve. The animals then orally self-administered alcohol and/or nicotine over a 20-week period. Withdrawal-related behaviors were monitored for at least 4 weeks following substance abstinence. [¹¹C]Flumazenil PET scans were conducted for all animals at 1 day, 8 days, and 12 weeks of substance abstinence, while in a subset of 8 animals, an additional PET scan was acquired at 4 weeks of abstinence.

Drug Self-Administration

All animals had free access to water for at least 12 h/d. Drug self-administration periods lasted 20 weeks. Nicotine and alcohol, diluted in tap water and saccharin (0.03% wt/vol), were available in separate bottles attached to animals' cages for oral self-administration 23 h/d. Solutions were measured and refilled daily. The nicotine concentration gradually increased over the initial 5 weeks from 50 to 500 µg/ml. The alcohol concentration was gradually increased each week over the initial 4 weeks of drug self-administration as follows: 1, 2, 4, and 6 wt/vol.

Animals self-administered nicotine and/or alcohol in 3 groups designed to model the effects of nicotine on alcohol withdrawal in alcohol-dependent patients. Alcohol alone ("alcohol alone," $n = 8$) modeled nonsmoking drinkers. Both nicotine and alcohol with simultaneous abstinence ("nicotine and alcohol, simultaneous abstinence," $n = 8$) modeled smoking drinkers who simultaneously quit smoking and drinking. Nicotine and alcohol with continued nicotine during alcohol abstinence ("nicotine and alcohol, alcohol abstinence," $n = 8$) modeled smoking drinkers who quit drinking while maintaining nicotine intake. This

group continued to consume nicotine for a 12-week period following termination of alcohol. In addition, a pilot group of animals (“nicotine alone,” $n = 6$) consumed nicotine alone and were imaged during nicotine abstinence to mimic smokers that quit smoking.

Observing Withdrawal-Related Behavior

During the first 8 days of abstinence, food and water consumption were carefully recorded. Animal behaviors were recorded with a video camera and later assessed for withdrawal-related behaviors. A research assistant rated behaviors for fearful agitation (e.g., teeth chattering, eye blinks, yawns), general agitation (e.g., cage bangs, pacing), anxiety (e.g., scratches, foot licks, cage licks), and abdominal distress (e.g., vomiting, dry heaving). Water was available ad libitum throughout the initial 12 weeks of drug abstinence.

[¹¹C]Flumazenil PET Imaging

GABA_A receptor availability was measured with [¹¹C]flumazenil PET. [¹¹C]Flumazenil is a nonselective GABA_A receptor specific radiotracer with high affinity for α_1 , α_2 , α_3 , and α_5 subtypes of the GABA_A receptor (Sieghart, 1995). The radiotracer was produced onsite following previous methods (Sandiego et al., 2013a), yielding high specific activity [¹¹C]flumazenil (>13.5 GBq/ μ mol at time of injection). PET data were acquired with a Focus 220 small animal scanner (Siemens/CTI, Knoxville, TN), which has a field of view of 7.6 cm (axial) by 19 cm (transaxial) with a spatial resolution of 1.3 mm (Tai et al., 2005).

On the day of a PET scan, animals were initially anesthetized with ketamine hydrochloride (up to 10 mg/kg intramuscularly) at least 2 hours prior to radiotracer injection and transported to the PET suite. Animals were intubated and maintained under 1.75 to 2.25% isoflurane anesthesia with oxygen for the duration of PET scanning. A transmission scan was first acquired for attenuation correction. [¹¹C]Flumazenil (192 ± 64 MBq) was then administered as a bolus-plus-constant infusion ($K_{bol} = 90$ minutes). PET data acquisition in list mode began simultaneously with radiotracer injection and continued for 90 minutes.

Anatomical Magnetic Resonance Imaging Data Acquisition

High-resolution T₁-weighted images were acquired for image coregistration and region of interest definition. Magnetic resonance (MR) data were acquired prior to drug self-administration and within 3 months of baseline PET scans with a Siemens 3T Trio Scanner using an extremity head coil in the coronal direction and the following spin echo sequence (TE = 3.34 ms, TR = 2,530 ms, flip angle = 7°, thickness = 0.50 mm, field of view = 140 mm, image matrix = $256 \times 256 \times 176$, voxel size = $0.547 \times 0.547 \times 0.500$ mm). The brain was extracted and segmented from the MR image for each subject with Functional Magnetic Resonance Imaging of the Brain Extraction Tool (<http://www.fmrib.ox.ac.uk/fsl/bet2/index.html>).

PET Data Processing and Analysis

Dynamic PET data were histogrammed into time bins up to 5 minutes in duration and reconstructed with Fourier rebinning followed by 2-dimensional filtered back projection with a Shepp filter (cutoff at 30% Nyquist frequency). Corrections for scanner normalization, randoms, dead time, attenuation, and scatter were included. The final PET

images had a matrix size of $256 \times 256 \times 95$ with voxel dimensions of $0.95 \times 0.95 \times 0.80$ mm³. PET images were next registered to MR image space using a rigid body registration algorithm (Sandiego et al., 2013b). The MR images in native space were then normalized to an in-house rhesus monkey template using a nonlinear registration algorithm with BioImage Suite 3.01 (<http://www.bioimagesuite.org/index.html>).

Voxel-wise maps of binding potential (BP_{ND}) were calculated for each scan. We refer to [¹¹C]flumazenil BP_{ND} as GABA_A receptor availability because variations in this measure primarily reflect differences in the number of receptors available for radioligand binding, although changes in in vivo radioligand-receptor affinity are also included in BP_{ND} (Innis et al., 2007). [¹¹C]Flumazenil BP_{ND} were calculated with the simplified reference tissue model (Lammertsma and Hume, 1996) using the pons as a reference region (Klumpers et al., 2008). [¹¹C]Flumazenil BP_{ND} maps were spatially normalized to template space using the transformation matrix from the MR registration. Average BP_{ND} values were extracted from the frontal cortex, occipital cortex, temporal cortex, and cerebellum, regions of high [¹¹C]flumazenil binding.

Cytokine Blood Measurement

The GABA neurotransmitter system is closely linked with neuroinflammation (Bhat et al., 2010), which is dysregulated in chronic alcohol exposure (Mayfield et al., 2013). Therefore, as an exploratory analysis, we assayed inflammatory cytokine levels in the periphery. On the same day as PET scans, blood samples were withdrawn before administration of PET radiotracer to measure inflammatory cytokine levels. Blood samples were assayed for the inflammatory cytokines interleukin 1-beta (IL-1 β), IL-6, IL-8, IL-10, IL-12, interferon gamma, and tumor necrosis factor-alpha concentrations. Total cytokine concentrations were measured using the Meso Scale Discovery Proinflammatory Panel 1 (NHP) Kit V-Plex kit with the MESO QuickPlex SQ 120 instrument (Meso Scale Diagnostics, LLC, Rockville, MD) according to the manufacturer's suggested protocol. A 2-fold dilution was used for all samples. Calculation of cytokine concentration was performed using Discovery Workbench (v4.0) software, using the manufacturer's calculated signal intensities according to the certificate of analysis.

Statistical Analysis

GABA_A receptor availability (BP_{ND}) was assessed for differences across drug exposure groups ("alcohol alone," "nicotine and alcohol, simultaneous abstinence," "nicotine and alcohol, alcohol abstinence") and differences over time. This was carried out using a generalized linear mixed model approach. To streamline the analysis, we constructed a model focusing on drug exposure group for animals in alcohol withdrawal as a between-subjects factor and time (1 day, 8 days, 12 weeks) as a within-subjects factor. Post hoc linear and quadratic contrasts were then estimated to examine the nature of main and interaction effects. A separate analysis was also conducted to examine the time course of GABA_A receptor availability in the pilot group undergoing nicotine abstinence alone. Effect sizes were also calculated with Cohen's *d* (Cohen, 1988).

Our exploratory analysis similarly examined cytokine levels for differences in drug exposure group and differences over time. For each cytokine, we built a model focusing on drug exposure group for animals in alcohol withdrawal as a between-subjects factor and time (1 day, 8 days, 12 weeks) was a within-subjects factor.

As a secondary analysis, we investigated correlations between [^{11}C]flumazenil BP_{ND} and either the amount of drug self-administered by animals or cytokine levels. First, Pearson's correlation coefficients were calculated relating baseline [^{11}C]flumazenil BP_{ND} and % BP_{ND} for each abstinence time point with the amount of alcohol or nicotine consumed. Next, Spearman's correlation coefficients (due to skewness) were calculated relating baseline [^{11}C]flumazenil BP_{ND} and % BP_{ND} for each abstinence time point with inflammatory cytokine levels.

RESULTS

Drug Self-Administration

Animals orally self-administered clinically relevant amounts of alcohol and nicotine over 20 weeks, with average amounts consumed for each group shown in Table 1. The average daily amount of drug consumed over the final 90 days of self-administration was consistent with the 20-week average, indicating stable rates of alcohol and nicotine intake. On average, animals consumed 3.95 ± 1.22 g/kg/d of alcohol, with no group differences in the amount of alcohol consumed. Based on a standard drink containing 0.25 g/kg alcohol, these animals thus consumed on average nearly 16 drinks per day, in agreement with amounts consumed by alcohol-dependent patients. The average level of nicotine consumed during the initial 20-week drug exposure period was 55.4 ± 35.1 mg/kg/d. This is roughly equivalent to 2 packs per day of 1.25 mg/kg nicotine content cigarettes. The "nicotine and alcohol, simultaneous abstinence" group consumed significantly less nicotine than the "nicotine alone" and "nicotine and alcohol, alcohol abstinence" groups (1-way analysis of variance, $p < 0.05$). The "nicotine and alcohol, alcohol abstinence" group consumed 63.1 ± 22.6 mg/kg/d of nicotine during simultaneous drug exposure, which significantly increased to 117.8 ± 52.3 mg/kg/d during alcohol abstinence (paired t -test, $p = 0.014$).

Some animals in all treatment groups exhibited withdrawal-related behaviors following drug abstinence. No animals exhibited signs of abdominal distress or tremor during drug abstinence. Increases in pacing behavior, a proxy of general agitation, were observed in 4 subjects (2 "alcohol alone," 1 "nicotine and alcohol, simultaneous abstinence," and 1 "nicotine and alcohol, alcohol abstinence"). Similarly, increases in scratching and foot lick behavior, taken as anxiety behaviors, were observed in 5 subjects (2 "nicotine alone," 2 "nicotine and alcohol, simultaneous abstinence," 1 "nicotine and alcohol, alcohol abstinence").

Brain Imaging: GABA_A Receptor Availability During Abstinence

[^{11}C]Flumazenil BP_{ND} extracted from cortical regions were highly correlated within scans ($R^2 > 0.88$); therefore, GABA_A receptor availability was summarized by a single [^{11}C]flumazenil BP_{ND} value averaged across all cortical regions. This average cortex value

was then used for subsequent data analysis. Examination of histograms and normal probability plots revealed no skew to these data, indicating that they were sufficiently normal for parametric analysis.

There were no significant differences in [^{11}C]flumazenil BP_{ND} between alcohol abstinence groups at any time point (see Fig. 1A), consistent with our preliminary result of no significant differences in subsets of the “alcohol alone” and “nicotine and alcohol, alcohol abstinence” groups (Cosgrove et al., 2014). Thus, groups were combined for subsequent analysis. There was a main effect of time ($p = 0.01$), shown in Fig. 1B. Post hoc analysis with linear contrasts revealed significantly more GABA_A receptor availability at 1 day of abstinence ($p = 0.029$; effect size $d = 0.38$), an increase of $7.2 \pm 11.5\%$ from baseline. At 8 days of abstinence, GABA_A receptor availability further increased to $9.1 \pm 11.1\%$ of baseline values, which was highly significant ($p = 0.001$; $d = 0.59$). At the exploratory time point of 4 weeks of abstinence ($n = 8$), GABA_A receptor availability decreased by $2.5 \pm 10.2\%$ compared to baseline. At 12 weeks of abstinence, GABA_A receptor availability was $3.7 \pm 13.0\%$ higher than baseline in the whole group ($d = 0.20$). These dynamic changes in GABA_A receptor availability during alcohol abstinence are visually apparent in the average [^{11}C]flumazenil BP_{ND} maps shown in Fig. 2.

In contrast, results in a pilot cohort of 6 animals exposed to nicotine alone showed no differences between baseline and subsequent withdrawal time points, as shown in Fig. 3. The percent changes in [^{11}C]flumazenil BP_{ND} from baseline were $3.2 \pm 14.2\%$, $0.6 \pm 14.6\%$, and $0.7 \pm 4.9\%$ (corresponding to effect sizes of $d = 0.14$, 0.02 , and 0.06) at 1 day, 8 days, and 12 weeks of nicotine withdrawal, respectively.

There were no correlations between GABA_A receptor availability at baseline or change in GABA_A receptor availability with the amount of alcohol consumed ($r^2 < 0.24$ for each individual group; $r^2 = 0.14$ across all subjects) or nicotine consumed ($r^2 < 0.36$ for each individual group; $r^2 = 0.09$ across all subjects).

Inflammatory Cytokines

The cytokine data exhibited a large dynamic range with a non normal distribution; thus, nonparametric statistics were used to analyze these data. Our exploratory analysis of inflammatory cytokine levels revealed no differences by drug exposure group or by withdrawal time point. In addition, there were no correlations between cytokine levels and GABA_A receptor availability or drug amounts consumed. The small group sizes and large dynamic range of data likely precluded the observation of even trend-level findings in this exploratory analysis.

DISCUSSION

This study indicates that neither the consumption of nicotine nor the termination of nicotine alters dynamic changes in GABA_A receptor availability during early or prolonged alcohol withdrawal. Figures 1 and 2 illustrate that regardless of the presence or absence of nicotine, [^{11}C]flumazenil BP_{ND} increased from predrug levels by 7% at 1 day of alcohol abstinence, by 9% at 8 days of abstinence, and were modestly increased by 4% after 12 weeks of

abstinence. A subset of animals imaged after 4 weeks of abstinence exhibited levels that *decreased* by 3%, which when taken with the large variability in the 12-week time point preliminarily suggests a recovery of GABA_A receptors after 4 to 12 weeks of alcohol abstinence. This time course of GABA_A receptor availability during alcohol abstinence is consistent with previous clinical findings of robustly elevated GABA_A receptors in nonsmoking acute alcohol withdrawal (~10 days withdrawal) that normalize after 1 month of abstinence (Cosgrove et al., 2014; Staley et al., 2005). However, these previous studies found that smoking prevented GABA_A receptor normalization during alcohol withdrawal. Since the present data indicate that this effect of smoking is likely not due to nicotine alone, we hypothesize that an additive or combination of additives in tobacco smoke modifying GABA_A receptor function, such as the harmala alkaloids harmaline and norharmaline (Rommelspacher et al., 1981) or carbon monoxide (Benagiano et al., 2005), may prevent GABA_A receptor normalization during alcohol withdrawal in smokers.

Our finding that nicotine and nicotine abstinence do not modulate GABA_A receptor availability during alcohol abstinence has important clinical implications. The high comorbidity of alcohol dependence and tobacco smoking and worsened health and treatment outlook for concurrent tobacco smoking in alcohol use disorders demands consideration of therapies for both substances once patients are admitted to a treatment program. Tobacco smoking intervention during concurrent alcohol treatment is often low-intensity and poorly integrated (Kalman et al., 2010), but shows promise for improving both smoking cessation and alcohol relapse outlook (Burling et al., 2001). Further, heavy-drinking tobacco smokers express overall enthusiasm for combined smoking and alcohol interventions (Fucito and Hanrahan, 2015). From the perspective of the GABA_A receptor system, our findings do not contradict the use of drugs acting on the nicotinic system, such as nicotine replacement therapies and varenicline (Chantix), for tobacco smokers undergoing alcohol withdrawal. Moreover, as nicotine abstinence during alcohol withdrawal does not alter fluctuations in GABA_A receptor availability during alcohol withdrawal, our preclinical findings suggest that nicotine abstinence will not adversely affect the GABA system in patients who quit smoking and drinking simultaneously. These findings should be confirmed in a clinical population.

The hypothesis that nicotine consumption or abstinence has no effect on GABA_A receptors during alcohol abstinence is further bolstered by the preliminary finding that the consumption of nicotine alone followed by nicotine abstinence does not appear to change GABA_A receptor availability. Although our small sample size ($n = 6$) requires caution in extrapolating these data, they suggest that nicotine abstinence itself may not alter GABA_A availability. Previous studies have found that GABA_A receptor levels in limbic regions, including the hippocampus and cingulate cortex, were elevated in ex-smokers compared to never-smokers (Stokes et al., 2013) and that smoking disrupts a link between anxiety symptoms and GABA_A receptor availability (Esterlis et al., 2009). Thus, we cautiously hypothesize that these effects may also stem from tobacco smoke constituents other than nicotine, either directly or by interacting with nicotine. A direct implication of this hypothesis is that use of a nicotine patch may not provide a complete approximation of smoking in human laboratory experiments.

While a constellation of neurochemical adaptations occurs in the highly complex process of acute and prolonged alcohol withdrawal, our use of preclinical animals allowed the isolation of a single pharmacological agent, nicotine, in its ability to alter a single neuropsychopharmacological target, GABA_A receptors, during alcohol abstinence. This detailed and systematic examination represents a strength of the present work. Moreover, the use of nonhuman primate subjects, which allow for comparisons to higher primate species but provide pharmacological control over nicotine and alcohol exposure, presents a unique longitudinal setting which is not feasible with human subjects. The ability to image animals prior to any drug use is a major strength of this study. However, the present findings should be extended to human subjects to confirm the translation of our results to higher species.

The PET radioligand used for this work, [¹¹C]flumazenil, is a weak inverse agonist at GABA_A receptors (Mohler et al., 1981) that binds to receptor subtypes α_1 – α_6 , but with higher affinity for α_1 , α_2 , α_3 , and α_5 subtype-containing receptors (Sieghart, 1995). We therefore interpret [¹¹C]flumazenil specific binding (BP_{ND}) to be representative of all GABA_A receptors, although the α_1 subunit composes ~50% of GABA_A receptors (Kumar et al., 2009); thus, [¹¹C]flumazenil PET signal is likely preferential to this subtype. Quantification of [¹¹C]flumazenil was conducted using a reference region approach (Lammertsma and Hume, 1996), with the pons as a reference region (Klumbers et al., 2008). Some studies have suggested the presence of specific [¹¹C]flumazenil binding in the pons (Litton et al., 1994); thus, we carefully compared [¹¹C]flumazenil time courses in the pons (normalized to subject weight and injected radioactivity dose) within subjects. We found no significant differences in time-activity curves of [¹¹C]flumazenil in the pons nor any consistent time effect, suggesting the absence of systematic bias in the reference region. Moreover, the within-subject design minimized the possible influence of individual subject bias on the results.

In conclusion, neuroadaptations in GABA_A receptor availability during alcohol withdrawal in nonhuman primates are consistent with the changes observed in human alcohol-dependent patients (Cosgrove et al., 2014; Staley et al., 2005). Importantly, either consumption of nicotine or termination of nicotine during alcohol abstinence did not modify these GABA_A receptor neuroadaptations. We therefore hypothesize that a non nicotinic additive in tobacco smoke may block GABA_A receptor normalization in alcohol withdrawal, as previously observed in alcohol-dependent smokers. These results do not contradict the use of nicotine-based therapeutics, such as nicotine replacement therapies, to treat smoking and drinking simultaneously in patients with alcohol use disorders.

Acknowledgments

The authors wish to thank the staff at the Yale PET Center for their expert technical assistance in support of this work. We are grateful to Drs. Philip Smith and Robert Pietrzak for insightful technical discussions and Dr. Peter Jatlow for assistance with cytokine analysis. This research was supported in part by National Institutes of Health grants T32 DA022975 (ATH), K02 DA031750, R01 AA017464 (KPC), and K05 AA014715 (SSO). This publication was made possible by CTSA grant number UL1 TR000142 from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Dr. Stephanie O'Malley reports the following: AstraZeneca, Pfizer – donated study medications; Hazelden Betty Ford Foundation – Scientific Panel Member; Eli Lilly – Clinical Trial Contract; Consultant/Advisory Board Member – Alkermes, Arkeo Pharmaceuticals, Amydala, Cerecor, Inc.; American Society of Clinical Psychopharmacology Alcohol Clinical Trials Initiative Member – supported by AbbVie, Alkermes, Ethypharm, Eli Lilly, Lundbeck, and Pfizer, Inc.

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GABA_A Receptor Availability During Alcohol Abstinence

Fig. 1.

Changes in GABA_A receptor availability during alcohol abstinence. Percentage changes in [¹¹C]flumazenil BP_{ND} , an index of GABA_A receptor availability, at baseline and 1 day, 8 days, and 12 weeks of alcohol withdrawal. Panel (A) shows the individual alcohol abstinence groups as follows: “alcohol alone” ($n = 8$)—red bars; “nicotine and alcohol, simultaneous abstinence” ($n = 8$)—blue bars; “nicotine and alcohol, alcohol abstinence” ($n = 8$)—green bars. Panel (B) shows all alcohol abstinence groups averaged together ($n = 24$). * indicates a statistically significant increase in [¹¹C]flumazenil BP_{ND} observed at 1 day of abstinence relative to baseline ($p = 0.029$). ** indicates a statistically significant increase in [¹¹C]flumazenil BP_{ND} observed at 8 days of abstinence relative to baseline ($p = 0.001$). Error bars are standard error of the mean.

GABA_A Receptor Availability During Nicotine Abstinence

Fig. 2. $[^{11}\text{C}]$ Flumazenil BP_{ND} during alcohol abstinence. Dynamic changes to GABA_A receptor availability during alcohol abstinence. a magnetic resonance image is shown on the far left, followed by parametric images of $[^{11}\text{C}]$ flumazenil BP_{ND} averaged across all animals during alcohol withdrawal ($n = 24$) at baseline, 1 day, 8 days, and 12 weeks of alcohol abstinence. The color bar illustrates $[^{11}\text{C}]$ flumazenil BP_{ND} .

Fig. 3.

GABA_A receptor availability preliminarily does not change during nicotine abstinence. [¹¹C]flumazenil BP_{ND} , an index of GABA_A receptor availability, at baseline and 1 day, 8 days, and 12 weeks of alcohol withdrawal. GABA_A receptor availability appears not to change from baseline levels in this preliminary cohort ($n = 6$). Error bars are standard error of the mean.

Table 1

Drug Amounts Orally Self-Administered by Different Groups

Group	Average alcohol consumed (weeks 1 to 20) (g/kg/d)	Average nicotine consumed prewithdrawal (weeks 1 to 20) (mg/kg/d)*	Average nicotine consumed during alcohol withdrawal (weeks 21 to 32) (mg/kg/d)
Alcohol alone	4.33 ± 0.98	–	–
Alcohol and nicotine; simultaneous abstinence	3.31 ± 1.44	32.0 ± 19.4	–
Alcohol and nicotine alcohol abstinence	4.19 ± 1.09	63.1 ± 22.6	117.8 ± 52.3**
Nicotine alone	–	85.6 ± 39.0	–

* Significant differences in amount of nicotine consumed (1-way analysis of variance, $p < 0.05$).

** Significant increase in amount of nicotine consumed during alcohol withdrawal (paired t -test, $p = 0.014$).