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A translational investigation targeting stress-reactivity and prefrontal cognitive control with guanfacine for smoking cessation

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Abstract

Stress and prefrontal cognitive dysfunction have key roles in driving smoking; however, there are no therapeutics for smoking cessation that attenuate the effects of stress on smoking and enhance cognition. Central noradrenergic pathways are involved in stress-induced reinstatement to nicotine and in the prefrontal executive control of adaptive behaviors. We used a novel translational approach employing a validated laboratory analogue of stress-precipitated smoking, functional magnetic resonance imaging (fMRI), and a proof-of-concept treatment period to evaluate whether the noradrenergic α2a agonist guanfacine (3 mg/day) versus placebo (0 mg/day) reduced stress-precipitated smoking in the laboratory, altered cortico-striatal activation during the Stroop cognitive-control task, and reduced smoking following a quit attempt. In nicotine-deprived smokers (n=33), stress versus a neutral condition significantly decreased the latency to smoke, and increased tobacco craving, ad-libitum smoking, and systolic blood pressure in placebo-treated subjects, and these effects were absent or reduced in guanfacine-treated subjects. Following stress, placebo-treated subjects demonstrated decreased cortisol levels whereas guanfacine-treated subjects demonstrated increased levels. Guanfacine, compared with placebo, altered prefrontal activity during a cognitive-control task, and reduced cigarette use but did not increase complete abstinence during treatment. These preliminary laboratory, neuroimaging, and clinical outcome data were consistent and complementary and support further development of guanfacine for smoking cessation.

Keywords

Guanfacine, smoking cessation, stress, fMRI, Stroop, lapse, ad-libitum smoking, craving

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Introduction

Tobacco use is a leading cause of preventable morbidity and mortality worldwide, contributing to nearly 6 million deaths yearly (World Health Organization, 2014). All current Food and Drug Administration (FDA)-approved smoking cessation medications target the nicotinic acetylcholine receptor system to some extent and attenuate nicotine-related reinforcement and withdrawal symptoms (De Biasi and Dani, 2011). However, most smokers using nicotinic acetylcholinergic agents fail to maintain long-term abstinence (Fiore et al., 2008), underscoring the need to identify novel compounds. Factors that maintain smoking and precipitate relapse are varied and complex, and the underlying biology has yet to be elucidated (Lester, 2011). Stress is a primary mechanism involved in the maintenance of and relapse to smoking (McKee et al., 2003), and targeting stress-related relapse for medications development is a critical, yet relatively unexplored, strategy for smoking cessation.

Nicotine potently activates cortico-striatal-limbic pathways (Stein et al., 1998) and significant neuro-adaptations in stress responses with chronic nicotine exposure and withdrawal have been documented. Smokers demonstrate blunted cortisol and adrenocorticotropic hormone (ACTH) responses to stress during acute abstinence (al'Absi et al., 2005; McKee et al., 2011) and in

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response to nicotine (Mendelson et al., 2008). Stress-related alterations during acute abstinence are accompanied by increased tobacco craving and reduced control over smoking behavior (McKee et al., 2011). As attention and self-control are critical executive functions required to regulate stress arousal and craving states (Sinha, 2008), treatments that target reducing stress-reactivity and which improve self-control may optimize smoking cessation outcomes. Early abstinent smokers show reduced responses in anterior cingulate cortex (ACC) and prefrontal cortex (PFC) with functional magnetic resonance imaging (fMRI) during executive function and self-control tasks, while nicotine and nicotine cues increase such activations (Kober et al., 2010; Stein et al., 1998). Decrements in executive function and self-control in smokers may contribute to increased stress-induced tobacco craving and poor regulation of stress in smokers.

Noradrenergic transmission is involved in both stress-reactivity and PFC control of cognitive function. In animal models, increasing central noradrenergic activity pharmacologically or via foot-shock stress enhances reinstatement to drugs following extinction (Shaham et al., 2003), whereas reducing noradrenergic activity with α2-adrenergic agonists attenuates stress-induced relapse to drugs (Lê et al., 2005), including stress-induced reinstatement of nicotine-seeking behavior (Yamada and Bruijnzeel, 2011). Stress exposure impairs PFC functions in both animals and humans (Arnsten, 2009), and reduced PFC-based self-control may be one mechanism by which stress induces relapse to drug-seeking (Sinha 2008), including nicotine. Stress induces high levels of cyclic AMP (cAMP) that open potassium channels on dendritic spines near PFC network synapses, weakening PFC connections underlying working memory and behavioral inhibition (Arnsten, 2009). Conversely, the α2A-adrenergic agonist guanfacine inhibits cAMP production, which closes potassium channels, strengthens PFC network connections, increases PFC neuronal firing, and improves PFC regulation of behavior (Arnsten, 2010). Targeting stress-related decrements in PFC function with guanfacine may improve self-control during stress and decrease stress-precipitated smoking relapse.

Guanfacine also improves executive functioning in non-stressed states. In monkeys, systemic guanfacine administration helped inhibit impulsive choices and wait for larger rewards, an important operation in achieving drug abstinence (Kim et al., 2012). Guanfacine also improved executive function deficits in adults with attention-deficit hyperactivity disorder (ADHD) (Taylor and Russo, 2001) and working memory deficits in adults and in those with mild traumatic brain injury (McAllister et al., 2011). The extended release formulation of guanfacine is FDA-approved for the treatment of ADHD. As PFC-based executive dysfunction exists in addictions (Everitt et al., 2008), strengthening PFC-based executive function more generally may also serve to improve smoking cessation outcomes.

In addition to attenuating stress-reactivity and improving cognitive function, there is extensive evidence that noradrenergic function is also involved in the rewarding effects of drugs (Weinshenker and Schroeder, 2007) and drug withdrawal (Semenova and Markou, 2010). Noradrenergic agents decrease nicotine-evoked dopamine release in the nucleus accumbens (Forget et al., 2010; Villégier et al., 2007), reduce conditioned place preference to nicotine (Forget et al., 2009), attenuate nicotine withdrawal-induced deficits in brain reward thresholds (Bruijnzeel et al., 2010), and reduce somatic signs of nicotine withdrawal in animals and humans (Bruijnzeel et al., 2010; Gourlay et al., 2004). Thus, there exist multiple possible mechanisms through which noradrenergic drugs may operate to influence human tobacco smoking.

The current study primarily tests whether guanfacine reduces stress-precipitated smoking and improves self-control in human subjects, while also investigating effects on PFC during a cognitive-control task. Guanfacine, approved in 1986 to treat hypertension, is an α2 adrenergic agonist that is known to preferentially bind to the α2A subtype of norepinephrine receptors, which are highly concentrated in the prefrontal cortical regions. Non-selective α2-adrenergic agonists such as clonidine have demonstrated efficacy for smoking cessation (Gourlay et al., 2004), but are limited by possible orthostatic adverse events and sedation. Guanfacine is more selective for the α2A-adrenoceptor subtype (Uhlen and Wikberg, 1991), is less sedating (Arnsten et al., 1988), has fewer pre-synaptic actions (Engberg and Eriksson, 1991), and has a longer half-life compared with clonidine (PDR, 1994), potentially improving its clinical utility.

This study used a well-validated model of smoking-lapse which evaluates the ability to resist smoking following stress and neutral conditions (McKee et al., 2011). This task is similar to a delay discounting task in that subjects are required to resist smoking to earn larger sums of money. We hypothesized that guanfacine would attenuate stress effects and associated decrements in self-control by increasing the ability to resist smoking, attenuating stress-related increases in tobacco craving, and decreasing ad-libitum smoking. Medication effects on neural activity were assessed using fMRI while subjects performed a cognitive-control Stroop task. We hypothesized that guanfacine treatment would increase activation of the insula and ACC and decrease activation of the dorsolateral PFC (dlPFC). We based this hypothesis on prior findings that: 1) Stroop-related activation in the insula and ACC was associated with better treatment outcome among adolescent smokers (Krishnan-Sarin et al., 2013); and 2) Stroop-related activation in the dlPFC decreased in substance-dependent individuals during treatment (DeVito et al., 2012). Finally, we evaluated the effects of guanfacine on clinical outcomes during a brief (four-week) proof-of-concept treatment phase, hypothesizing that guanfacine would be associated with better treatment outcomes.

Methods and materials

Design

A between-subject, double-blind, placebo-controlled design was used to compare guanfacine (3 mg/day) with placebo (0 mg/day). Following titration to steady-state medication levels, subjects (n=33) completed two laboratory sessions designed to model smoking-lapse (stress vs. neutral imagery, order counterbalanced), completed fMRI to assess cognitive control, and were then maintained on their randomized medication condition for an additional four-week period. The quit day was scheduled following the fMRI session, and subjects were provided with weekly brief behavioral treatment. Medication was tapered after the end of the treatment period (see Table 1).

Table 1. Single subject timeline.

Day	Procedure
1–21	Titration to steady-state medication levels
22	Human laboratory session to model smoking-lapse behavior (stress vs. neutral imagery, order counterbalanced). See Figure 1
24	Human laboratory session to model smoking-lapse behavior (stress vs. neutral imagery, order counterbalanced). See Figure 1
25 or 26	fMRI session to evaluate attention and inhibitory control using Stroop task
27	Quit day
34–53	1× weekly brief behavioral support during four-week treatment period
54–58	Medication taper

Note: Mean days between the two laboratory sessions = 2.24 days, SD=0.49; mean days from laboratory to fMRI session = 1.26 days, SD=0.47.

Participants

Eligible participants were 18–60 years of age, had smoked ≥10 cigarettes/day for the past year, had urine cotinine levels ≥150 ng/ml, and were normotensive (sitting BP >90/60 and <160/100 mmHg). Subjects were excluded if they met criteria for current (past six months) Axis-I psychiatric disorders (excluding nicotine dependence) (First et al., 1996), were using illicit drugs (assessed by urine toxicology), had engaged in smoking-cessation treatment in the past six months, had medical conditions or used concurrent medicine that would contraindicate guanfacine use or smoking behavior assessed by physical exam (including electrocardiogram and basic blood chemistries). The study was approved by the Yale Human Investigations Committee. All subjects signed informed consent. Subjects were recruited from the community for a smoking laboratory study, and during the consent process were informed that they could also participate in an imaging session and a brief treatment phase as part of the study. Following initial phone screening, 55 subjects completed eligibility screening and 50 were found eligible; a total of 33 subjects (17 guanfacine) completed the laboratory sessions. Of the 17 who did not complete, eight were non-starters (six lost interest, two had positive urine toxicology) and nine started medication but did not complete the study (four were dismissed for failing to comply with experimental procedures, four lost interest, and one had positive urine toxicology). Following consent, 26 subjects expressed interest in the imaging session and were consented for the optional fMRI component, and 21 subjects arrived for and completed the fMRI session (nine guanfacine). Twenty-one subjects expressed interest in the treatment phase and 18 subjects (nine guanfacine) engaged in the treatment phase (three lost interest). Subjects completing the laboratory sessions were primarily Caucasian, high-school-educated, and moderately nicotine-dependent (Fagerström Nicotine Dependence Test (FTND), Heatherton et al., 1991), with low levels of depressive symptomatology (Center for Epidemiological Studies-Depression Scale (CESD), Radloff, 1977). There were no significant differences in demographic or smoking behaviors across medication conditions (all p>.05; Table 2). Across the phases of the study (laboratory, imaging, treatment) the only difference in baseline characteristics was treatment motivation. Subjects consenting to treatment had significantly higher treatment motivation (i.e., contemplation scores: range 1–10, Biener and Adams, 1991) (mean=7.50, SE=0.49) than those not consenting (mean=4.3, SE=0.54), and this did not differ by medication.

Guanfacine treatment

The medication condition was double-blind and placebo-controlled, and randomization was stratified by gender. Effective doses for the treatment of hypertension range from 2 mg/day to 5 mg/day, with dose-dependent effects on blood pressure and adverse events (<http://www.drugs.com/pro/guanfacine.html>, accessed 1 August 2011). We evaluated 3 mg/day immediate-release guanfacine due to previous work showing that this dosing significantly reduces nicotine craving in cocaine-dependent smokers, with minimal adverse events (Fox et al., 2012). Guanfacine was administered twice daily and titrated to steady-state levels over 21 days (0.5 mg days 1–3, 1.5 mg days 4–7, 2 mg days 8–12, 2.5 mg days 13–15, 3 mg days 16–21). Subjects completing the treatment phase were maintained at their randomized dose for the additional four-week period. Thereafter, subjects received a five-day medication taper.

Laboratory assessment of stress-precipitated smoking-lapse

Procedures. Each subject completed two 6.5-h laboratory sessions (stress vs. neutral imagery; Figure 1).

Baseline assessment period: Laboratory sessions started at 9:00am. Participants were instructed to smoke a final cigarette at 10:00pm the night before. Abstinence was confirmed with a carbon monoxide (CO) reading. An IV cannula was inserted to obtain blood samples. Baseline assessments of breath CO, breath alcohol, urine drug screens, urine pregnancy screen, and vital signs were obtained. Additional measures of emotion, tobacco craving, and nicotine withdrawal were obtained. Medication administration (1.5 mg or placebo) occurred at 10:00am. Participants were provided with a standardized lunch at 11:15am to control for time since last food consumption. From 10:00am to 12:30pm, subjects were able to watch television or read.

Personalized imagery procedure: Exposure to stress and neutral imagery used personalized guided-imagery methods (McKee et al., 2011; Sinha, 2009). In a prior session, stress-imagery scripts were developed by having subjects identify and describe in detail highly stressful experiences occurring within the last six months. Only situations rated as 8 or greater (1="not at all stressful" and 10="the most stress they recently felt in their life") were accepted as appropriate for script development. A neutral-relaxing script was developed from subjects' descriptions of personal neutral/relaxing situations. Scripts were developed by a PhD-level clinician and audiotaped for presentation during the laboratory sessions. Each script was approximately 5 min in length. During the laboratory session at 12:55pm, subjects listened to scripts (stress or neutral) via headphones. See Supplementary Material for additional details.

Delay period: At 1:10pm, participants were presented with a tray containing eight cigarettes of their preferred brand, a lighter, and an ashtray. Participants were instructed that they could commence smoking at any point over the next 50 min. However, for each 5-min block of time they delayed or "resisted" smoking,

Table 2. Demographics, smoking behavior, and vitals by medication condition.

	Guanfacine (*n*=17)	Placebo (*n*=16)
Baseline		
Age, mean (SD)	35.65 (11.34)	36.13 (13.12)
Gender (male), *n* (%)	11 (64.7)	9 (56.3)
Race (Caucasian), *n* (%)	13 (76.5)	13 (81.3)
Education (high school), *n* (%)	11 (64.7)	8 (50)
Cigarettes per day, mean (SD)	19.84 (7.73)	16.30 (7.41)
Carbon monoxide (ppm), mean (SD)	30.65 (20.25)	26.75 (13.20)
FTND[a], mean (SD)	6.18 (2.35)	4.88 (2.13)
CESD[b], mean (SD)	7.47 (6.88)	4.56 (4.08)
Systolic BP (mmHg), mean (SD)	119.86 (3.72)	124.07 (14.39)
Diastolic BP (mmHg), mean (SD)	75.43 (10.01)	76.83 (9.38)
Heart rate (bpm), mean (SD)	81.58 (12.84)	80.13 (11.96)
Titration[c]		
Cigarettes per day, mean (SD)	18.98 (7.93)	15.63 (9.34)
Systolic BP (mmHg), mean (SD)	109.07 (13.67)	119.47 (12.81)
Diastolic BP (mmHg), mean (SD)	64.00 (8.60)	71.07 (8.06)
Heart rate (bpm), mean (SD)	61.81 (11.18)	69.97 (10.09)

All baseline comparisons across medication conditions were not significant ($p>.05$).
[a]Fagerström Test of Nicotine Dependence (FTND) (Heatherton et al., 1991), range 1–10 for measure.
[b]Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977), range 0–60.
[c]Values collected at the end of titration.

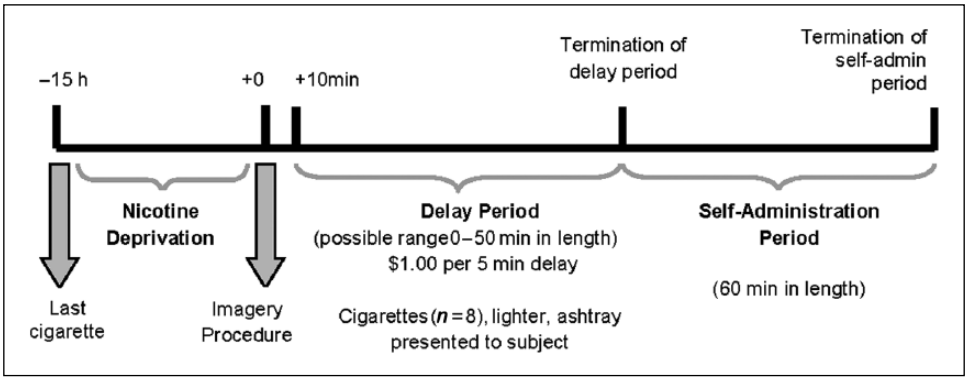

Figure 1. Timeline of laboratory procedures to evaluate smoking-lapse during stress versus neutral imagery.
Assessment of cortisol and ACTH occurred at –30 min, –15 min, +10 min, +20 min, +40 min, and +60 min from the imagery procedure and remained fixed regardless of when the termination of the delay period occurred. Assessments of craving, emotion, physiologic reactivity, and nicotine withdrawal occurred at –15 min, +5 min, and termination of the delay.

they would earn $1, up to a maximum of $10. Time when subjects announced they wanted to smoke (range 0–50 min) was recorded.

Smoking self-administration period: The ad-libitum smoking-session duration was 60 min and started once participants decided to end the delay period (or delayed for 50 min). Participants were provided with eight cigarettes of their preferred brand. Participants were instructed to "smoke as little or as much as you wish". Subjects were discharged at 3:15pm.

Assessments. Primary measures included the length of the delay period (i.e., time to initiate smoking), number of cigarettes smoked during the ad-libitum period, and tobacco craving (Questionnaire of Smoking Urges-Brief; Cox et al., 2001). Other measures were collected but not included in this report. Additional measures are described below.

Mood and nicotine withdrawal: The Differential Emotion Scale (DES), a 30-item self-report questionnaire, was used to assess current emotional state for positive (e.g., happy, joy) and negative (e.g., sadness, anger) emotion states (visual analogue scale (VAS), range 1–100; Izard, 1972). DSM-IV symptoms of nicotine withdrawal were assessed with the eight-item Minnesota Nicotine Withdrawal Scale (MNWS; Hughes and Hatsukami, 1986). Instructions were worded to assess current symptoms of withdrawal (range 0–32).

Physiologic measures: A pulse sensor was attached to the subject's forefinger to obtain a measure of pulse rate. Blood pressure was measured using a Critikon Dynamap (GE Medical Systems, Tampa, FL).

Cortisol and ACTH: Four milliliters of blood was collected at each timepoint to assess plasma ACTH and cortisol (see Supplementary Material for processing methods).

Guanfacine levels: Five milliliters of blood was collected at the start of each laboratory session to evaluate plasma-trough-guanfacine levels (see Supplementary Material for processing methods).

Timing of assessments: Tobacco craving, emotion ratings, physiologic reactivity, and nicotine withdrawal were assessed pre-imagery, post-imagery (prior to the presentation of cigarette cues), at end of delay, and at +30 min and +60 min during the ad-libitum-smoking period. ACTH and cortisol levels were assessed −30, −15, +5, +20, +40, and +60 min post-imagery.

Statistical analysis. Multivariate analyses of variance were used to examine effects of medication condition by time (week 1, week 2, week 3) on cigarettes per day, systolic BP, diastolic BP, and heart rate during the titration period.

Multivariate analyses of variance were used to examine within-subject effects of imagery condition (stress, neutral) by medication condition (guanfacine, placebo) on the primary outcomes of the length of the delay period and the number of cigarettes smoked during the ad-libitum period.

Multivariate analyses of variance were used to examine outcomes of tobacco craving, hypothalamic-pituitary-adrenal (HPA)-axis levels, emotion ratings, physiologic reactivity, and nicotine withdrawal within imagery condition (stress, neutral), within time (pre-imagery, post-imagery), and by medication condition (guanfacine, placebo). The post-imagery timepoint occurred prior to the start of the smoking-lapse task. Post-hoc analyses examined differences in stress vs. neutral imagery change scores (differences pre- to post-imagery) within each medication group. According to the predefined analytical plan, age, sex, baseline cigarettes per day, FTND scores, and CESD scores were evaluated as potential covariates (or as a between-subjects variable in the case of sex), and were retained if they reduced residual variance, or were otherwise excluded.

fMRI

Stroop. The fMRI Stroop color–word interference task has been described previously (DeVito et al., 2012; Krishnan-Sarin et al., 2013). The task involves frequent exposure of subjects to matched color–word pairs and infrequent exposure to mismatched color-word pairs (pseudo-randomly presented every 13th to 16th presentation) during fMRI. As in prior studies (Jastreboff et al., 2013), an hour prior to fMRI, subjects were given the opportunity to smoke a cigarette so that they were not in acute tobacco intoxication or withdrawal during fMRI. Subjects completed two practice runs to gain familiarity with the task and then participated in five runs, each of 168 s. Each run included seven incongruent stimuli and 105 stimuli in total. Subjects received instructions in all cases to name the color silently rather than read the word. This procedure of silent naming has been used successfully by our group to minimize subject motion during fMRI (Brewer et al., 2008; Leung et al., 2000; Potenza et al., 2003). Task performance was assessed outside the scanner immediately following fMRI. During the presentation of mismatched color–word pairs, performance of this task involves conflict monitoring and cognitive control, and specifically the inhibition of the pre-potent response to read the word (Botvinick et al., 2001; Carter et al., 2000; Kerns et al., 2004). The fMRI design is event-related, and analyses focus on activation changes related to incongruent and congruent stimuli (Brewer et al., 2008; Potenza et al., 2003).

Imaging data acquisition

Images were obtained using a 3-T Siemens Trio MRI system equipped with a standard quadrature head coil, using T2*-sensitive gradient-recalled single-shot echo planar pulse sequence. Subjects were positioned in the coil and head movements were restrained using foam pillows. Functional, blood oxygen level dependent (BOLD) images were acquired with a single-shot gradient-echo echo planar imaging (EPI) sequence. Twenty-five 4 mm axial slices parallel to the AC-PC line (1 mm skip) were acquired with TR = 1500 ms, TE = 27 ms, flip angle = 60 degrees, field of view = 220 × 220 mm, matrix = 64 × 64. In addition, a high-resolution 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) image was acquired for each subject (TR=2530 ms; TE =3.34 ms; flip angle = 7 degrees; slice thickness=1 mm; field of view=256 × 256 mm; matrix=256 × 256).

Imaging data preprocessing. Functional images underwent preprocessing using SPM5 (Wellcome Trust Center for Neuroimaging, London, UK) following published methods (Kober et al., 2010), including the following: slice scan-time correction to the middle slice of each volume; realignment of all functional images to the first image of the first functional scan; co-registration of the anatomical image and the average of these realigned functional images; co-registration of all functional images using parameters obtained from co-registration of the mean image; application of the SPM Unified Segmentation process to the anatomical scan, using prior information from the ICBM Tissue Probabilistic Atlas and estimation of non-linear warping parameters (Ashburner and Friston, 2005); and warping the functional images to the Montreal Neurological Institute (MNI) template space, followed by smoothing of functional images using a 6 mm Gaussian kernel.

Imaging data analysis. First-level robust regression was performed on each participant's preprocessed images, using the standard general linear model but with iteratively reweighted least squares using the bisquare weighting function for robustness (Kober et al., 2010), as implemented in MATLAB 7.3 (Mathworks, Natick, MA; robust.m). Motion parameters and high-pass filter parameters were added as additional regressors of no interest. Activity during congruent and incongruent trials was estimated as percent signal change from baseline. Next, a second-level, random effects analysis was performed to compare activity between condition and between groups, using NeuroElf (New York, NY, NeuroElf.net). Findings were Family-Wise-Error-corrected for multiple comparisons at $p<.05$ using Monte-Carlo simulation.

Treatment phase evaluating medication effects on clinical outcomes

Procedures. The quit day was scheduled within a week of completing the laboratory and fMRI sessions. Participants attended weekly appointments to receive brief behavioral support

Table 3. Relative frequencies of treatment emergent adverse events commonly associated with guanfacine (3 mg/day) versus placebo during the three-week titration period and the four-week treatment phase.

Adverse event	Three-week titration period		Four-week treatment phase	
	Guanfacine (%)	Placebo (%)	Guanfacine (%)	Placebo (%)
Dry mouth	88.2*	18.8	88.9*	0
Drowsiness	47.1	18.8	11.1	0
Dizziness	6.3	24.5	11.1	0
Headache	29.4	25.0	33.3	11.1
Impotence	0	6.3	0	0
Constipation	23.5	6.3	11.1	0
Fatigue	47.1*	6.3	11.1	11.1

All events were rated as minimal or mild on a five-point scale (0=absent, 1=minimal, 2=mild, 3=moderate, 4=severe). A chi-square comparison across adverse events revealed higher incidence of dry mouth during the titration and treatment phases, and higher incidence of fatigue during the titration period only (*p<.05).

(15–20 min), and to complete research assessments (adverse events, timeline followback to assess cigarette use, CO levels, mood ratings, craving, and withdrawal). Following standard clinical guidelines (Fiore et al., 2008), basic behavioral support was provided by a PhD-level clinician following the Mayo Clinic's manual *Smoke-Free and Living It* (Mayo Clinic, 2000).

Statistical analysis. The primary outcomes were cigarettes/day and retention over the four-week treatment period. Complete abstinence and percent days abstinent (arcsine transformed) were also calculated. The effect of guanfacine on cigarettes/day was evaluated using linear mixed models with a conservative approach for missing values. Missing values were left as missing rather than imputing baseline values based on the assumption that missing subjects had returned to baseline levels of smoking. Cigarettes/day represented the dependent variable, medication was included as a between-subjects explanatory variable, and time was represented a within-subjects factor. Covariates were handled similarly to the procedure described for the laboratory sessions. Treatment and time interactions were modeled and followed by appropriate post-hoc tests. Similar analyses were conducted for weekly measures of CO levels, positive mood ratings, negative mood ratings, craving, and withdrawal.

Results

Human laboratory analogue evaluating medication effects on stress-precipitated smoking-lapse

Medication compliance and adverse events: Compliance was 100% as assessed by a riboflavin marker (Del Boca et al., 1996) and pill counts. Plasma-trough-guanfacine levels collected at the start of each laboratory session did not differ (stress condition mean=4.25 ng/mL, SE=0.44; neutral condition mean=3.97 ng/mL, SE=0.34, p>.05 paired comparison). Levels were consistent with those expected following steady-state dosing (Sorkin and Heel, 1986). Adverse events were assessed twice weekly during titration and once weekly during treatment (Levine and Schooler, 1986). Common adverse events associated with guanfacine are reported (Table 3). All were rated as minimal or mild. No subject discontinued or required dosing adjustment due to adverse events.

Titration phase: Cigarettes per day did not significantly change by medication during the titration period. Systolic blood pressure significantly decreased following guanfacine administration (main effect of medication: $F_{1,27}$=4.74, p<.05), whereas diastolic blood pressure (p=.10) and heart rate (p=.11) demonstrated reductions following guanfacine administration that did not reach statistical significance (Table 2).

Latency to smoking, ad-libitum smoking, craving, and withdrawal: As expected, there was a significant medication-by-imagery-condition interaction on time to resist smoking ($F_{1,28}$=4.45, p<.05; Cohen d=0.80, large effect), indicating that stress reduced smoking resistance in the placebo group, and this effect was eliminated in the guanfacine group (p<.05; Figure 2(a)). Once subjects started smoking, there was a significant medication-by-imagery interaction on cigarettes smoked during the 60-min ad-libitum period ($F_{1,28}$=4.42, p<.05, Cohen d=0.79, large effect; Figure 2(b)). Stress increased the number of cigarettes smoked in the placebo group (p<.05), and this effect was absent in the guanfacine group. For tobacco craving, there was a significant medication-by-imagery-condition interaction on tobacco craving ($F_{1,29}$=4.54, p<.05, Cohen d=0.80, large effect; Figure 2(c)). Post-hoc analysis demonstrated that stress versus neutral imagery increased tobacco craving in the placebo group (pre to post-imagery), and this effect was reduced in the guanfacine group (p<.05). Further, stress-related increases in tobacco craving were greater in the placebo versus guanfacine-treated subjects (p<.05). Additional analyses demonstrated that baseline levels in craving did not differ across imagery or medication groups (grand mean = 41.92, SE=4.52), and did not differ by the end of the self-administration session (grand mean = 17.38, SE=2.66). There were no main effects of medication on latency, smoking, or craving. There were no effects of medication or imagery condition on nicotine withdrawal ratings. At the start of the laboratory session, subjects demonstrated withdrawal symptoms consistent with overnight deprivation (guanfacine mean=4.24, SE=1.06, placebo mean=5.75, SE=1.09).

Physiologic measures: Systolic blood pressure demonstrated a significant main effect of medication ($F_{1,31}$=18.72, p<.0005), with lower values in the guanfacine group (guanfacine mean=101.37 mmHg, SE=2.28; placebo mean=115.53 mmHg, SE=2.35). While stress increased systolic blood pressure in the placebo group (p<.05), the overall interaction across medication, imagery, and time (pre-post imagery) was not significant (p>.05).

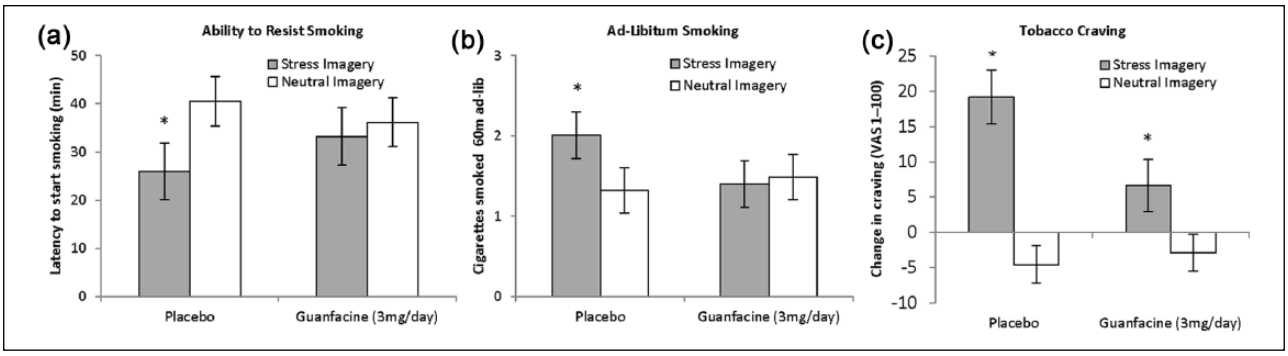

Figure 2. Stress increases the ability to resist smoking (a), ad-libitum smoking (b), and tobacco craving (c) during a human laboratory experiment, and these effects are absent or reduced in guanfacine-treated subjects (guanfacine *n*=17; placebo *n*=16).
(a) Stress (versus neutral imagery) reduced the ability to resist smoking in the placebo group, and this effect was absent in the guanfacine group (**p*<.05 stress vs. neutral imagery within medication group). (b) Stress increased the number of cigarettes smoked in the placebo group, and this effect was absent in the guanfacine group (**p*<.05 stress vs. neutral imagery within medication group). (c) Stress (versus neutral imagery) increased tobacco craving (change from pre to post-imagery) in the placebo group, and this effect was reduced in the guanfacine group. During the stress session, the increase in craving was greater in the placebo vs. guanfacine group (**p*<.05 stress vs. neutral imagery within medication group; and guanfacine vs. placebo within stress imagery).
Note: The post-imagery timepoint occurs prior to the smoking-lapse task.

Post-hoc analysis demonstrated that placebo-treated but not guanfacine-treated subjects showed an increase in systolic blood pressure during stress vs. neutral imagery sessions (Figure 3(a)). Diastolic blood pressure demonstrated a main effect of medication ($F_{1,31}$=15.04, p<.001), with lower values in the guanfacine group (guanfacine mean=57.92 mmHg, SE=1.71; placebo mean=67.44 mmHg, SE=1.76). Heart rate demonstrated a trend for a main effect of medication ($F_{1,31}$=3.20, p<.10), with lower values in the guanfacine group (guanfacine mean=59.06 bpm, SE=2.40; placebo mean=65.23 bpm, SE=2.48). There were no significant interactions across medication or imagery condition for diastolic blood pressure or heart rate.

Cortisol and ACTH: For cortisol levels, the multivariate interaction of medication by imagery (stress, neutral) by time (pre and post-imagery) was significant ($F_{1,26}$=7.19, p<.02). Post-hoc analysis examining change scores (pre- to post-imagery) of imagery condition by guanfacine or placebo found that cortisol levels were greater following stress for guanfacine-treated subjects (p<.05), but did not differ for placebo-treated subjects. Following stress, cortisol levels decreased in the placebo-treated subjects and increased in the guanfacine-treated subjects (p<.05) (Figure 3(b)). Additional analyses demonstrated that baseline levels of cortisol did not differ across imagery or medication groups (grand mean = 10.00 ng/mL, SE=0.86), but differed by imagery condition 60 min following the imagery manipulation ($F_{1,25}$=6.49, p<.02; stress imagery mean=11.45 ng/mL, SE=1.42, neutral imagery=9.46 ng/mL, SE=1.21). It should be noted that the majority of subjects had commenced smoking by the +60 cortisol timepoint, which increased cortisol levels. ACTH levels did not demonstrate significant effects of medication or imagery condition, although examination of mean values demonstrated that guanfacine tended to alter ACTH values in an opposite pattern to cortisol.

Manipulation check on stress and neutral imagery: Significant time-by-imagery-condition interactions were observed for positive ($F_{1,31}$=53.93, p<.0005) and negative ($F_{1,31}$=18.12, p<.0005) mood ratings. Following stress imagery, positive mood decreased and negative mood increased. There were no effects of medication on mood ratings.

fMRI session

Stroop performance: Overall reaction times were similar to those in our prior published work (congruent reaction time (RT) mean = 580.40 ms, SD = 62.18 ms; incongruent RT mean = 816.11 ms, SD= 112.15 ms) (DeVito et al., 2012; Krishnan-Sarin et al., 2013). A repeated measures ANOVA with condition as a within-subjects factor and group as a between-subjects factor revealed the anticipated effect of condition (incongruent vs. congruent; $F_{1,19}$=139.67, p<.001). There was no effect of guanfacine on Stroop RT ($F_{1,19}$=.01, p=.9) and no interactive effects. An independent sample t-test revealed no medication differences with respect to accuracy of responses (t_{19}=.52, p=.61).

fMRI: CO levels did not significantly differ across guanfacine (mean=27.33, SE=5.76) and placebo (mean=29.83, SE=4.99) groups at the start of the scanning session. Consistent with prior studies (Leung et al., 2000; Potenza et al., 2003), incongruent versus congruent stimuli presentation ("Stroop Effect") was associated with activation of ventrolateral and dorsolateral PFC, insula, striatum, thalamus, anterior cingulate/dorsomedial PFC, and parietal cortex (Figure S1, Supplementary Material). In the guanfacine group relative to the placebo group, increased activation was observed in the anterior cingulate, sensorimotor cortex, ventromedial PFC (vmPFC), insula, and middle and superior temporal gyri, and decreased activation was observed predominantly in the dorsolateral PFC (dlPFC) and parietal cortex (Table 4; Figure 4; Figure S2, Supplementary Material).

Treatment phase

During the four-week treatment phase, guanfacine decreased cigarette use (estimate of fixed effect for medication, t_{46}=5.15, p<.0005; Cohen d=1.52, large effect; Figure 5(a)) and improved treatment retention (z=2.65, p<.01; Figure 5(b)). No subject achieved complete abstinence for the four-week phase; however, there was a trend towards increased percent days abstinent in the guanfacine group (24%) compared with the placebo group (1%) (p=.10). Measures of CO levels, positive mood ratings, negative

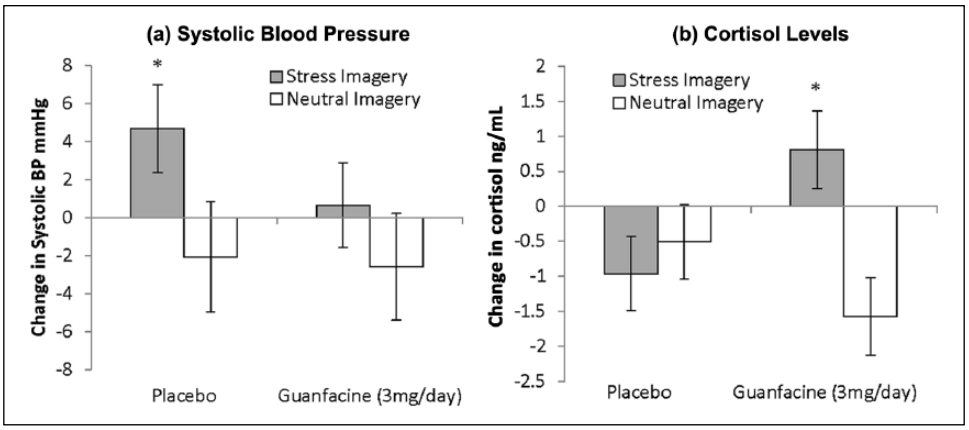

Figure 3. Stress increases systolic blood pressure in placebo but not guanfacine-treated subjects (a) and stress increases cortisol response in guanfacine but not placebo-treated subjects (b).
(a) Effect of medication (guanfacine vs placebo) and imagery (stress vs neutral) on systolic blood pressure during the smoking-lapse paradigm (guanfacine n=17; placebo n=16). Main effect of guanfacine on decreasing systolic blood pressure. Post-hoc analysis of change (pre to post-imagery) demonstrated that stress vs. neutral imagery increased systolic blood pressure in placebo but not guanfacine-treated subjects (*p<.05 stress versus neutral imagery within medication). (b) Effect of medication (guanfacine vs. placebo) and imagery (stress vs. neutral) on cortisol levels during the smoking-lapse paradigm (guanfacine n=13; placebo n=14). Post-hoc analysis of change (pre to post-imagery) demonstrated that cortisol levels differed in guanfacine across stress and neutral imagery sessions but not in placebo-treated subjects (*p<.05 stress versus neutral imagery within medication) and differed across medication groups within stress condition (*p<.05 guanfacine vs. placebo within stress). Note: The post-imagery timepoint occurs prior to the smoking-lapse task.

mood ratings, craving, and withdrawal did not demonstrate medication effects during the treatment phase.

Discussion

Laboratory, neuroimaging, and clinical outcome data were consistent and complementary. Results suggest that, in overnight nicotine-deprived daily cigarette smokers, guanfacine reduced stress-precipitated lapse by increasing the ability to resist smoking in the laboratory, increased ventromedial prefrontal brain activity during the cognitive-control task, and decreased smoking during a brief treatment phase. The α2A-agonist guanfacine may ameliorate stress responses through multiple inter-related mechanisms, including reductions in norepinephrine and dopamine release (Jentsch et al., 1998) and strengthening PFC network connections via post-synaptic α2A inhibition of cAMP-sensitive potassium channel signaling in PFC neurons (Arnsten, 2010). Guanfacine may also "replace" nicotine's enhancing effects at α7-nicotinic receptors in the PFC, which are permissive for N-methyl-D-aspartate (NMDA) receptor actions (Yang et al., 2013). Consistently with these ideas, guanfacine increased self-control in the laboratory (i.e., increased the latency to start smoking), reduced stress-related increases in tobacco craving, and decreased smoking behavior in the laboratory and during short-term treatment.

Using a validated human laboratory analogue of smoking-lapse which targets the first instance of smoking during a quit attempt (McKee et al., 2012), we demonstrated that personalized stress imagery decreased the ability to resist smoking, and increased tobacco craving and subsequent cigarette smoking in placebo-treated subjects, supporting prior findings (McKee et al., 2011). The effect of stress on these outcomes was eliminated or reduced in subjects who received guanfacine. Importantly, the magnitude of these effects was large, suggesting that guanfacine potently counteracted known stress-effects on smoking behavior.

Our human laboratory findings extend preclinical results demonstrating that α2 adrenergic agonists attenuate stress-precipitated relapse behavior (Lê et al., 2005; Yamada and Bruijnzeel, 2011). Associations between stress and tobacco relapse episodes are well documented (McKee et al., 2003; Shiffman and Waters, 2004), and we have hypothesized that stress promotes ongoing use and relapse by increasing craving and decreasing self-control for rewarding substances in addicted individuals (Sinha, 2008). The finding of relatively diminished activation of dlPFC and parietal cortices in the guanfacine versus placebo groups in the absence of between-medication-group differences in Stroop performance suggests that guanfacine may facilitate function of executive control circuitry by promoting more efficient processing in these regions. The current findings on reduced smoking and craving response also extend preclinical results documenting that noradrenergic agents attenuate both nicotine taking (Forget et al., 2010) and nicotine seeking (Forget et al., 2010; Yamada and Bruijnzeel, 2011). As documented with varenicline, pharmacological targets that effectively address both consumption and craving have the potential to be highly effective treatments (see Rollema et al., 2007).

Consistent with the laboratory findings, guanfacine increased reduction in cigarette use from baseline by 70%, and increased retention to 100% during the brief treatment period. These results, while preliminary, compare favorably with clonidine, which has been found to increase quit rates by 63% (Gourlay et al., 2004). It is possible that guanfacine's specificity for the α2A-adrenoceptor subtype (Uhlen and Wikberg, 1991) in comparison with clonidine, which is non-specific for α2 receptors, may be mediating differences in effects on smoking behavior. However, effects on complete abstinence, CO levels, mood, craving, and withdrawal were not demonstrated, which may have been due, in part, to the sample recruited for this study. While subjects agreed to engage in a treatment phase as part of the protocol and had reasonably high treatment motivation, they were initially recruited for a laboratory

Table 4. Brain regions showing significant group differences in Stroop effect.

Regions of activation	Peak coordinates					
	R/L	*x*	*y*	*z*	*k*	Max statistic
Guanfacine (Inc>Con) > Placebo (Inc>Con)						
Superior temporal gyrus/posterior insula	R	69	−15	12	113	4.64
Superior temporal gyrus/posterior insula	L	−42	−21	18	239	4.56
Superior and middle temporal gyrus/mid insula	L	−42	−6	−27	176	4.29
Mid cingulate/post central gyrus/sensorimotor	R	24	−21	45	107	3.81
Ventromedial PFC	R	12	45	−9	116	3.78
Placebo (Inc>Con) > Guanfacine (Inc>Con)						
Superior/middle frontal gyrus	L	−18	45	24	230	4.10
Superior parietal	L	−42	−54	42	231	3.87
Superior/middle frontal gyrus	R	36	33	42	214	3.81
Superior temporal/thalamus/caudate	R	30	−42	21	105	3.57
Superior parietal	R	42	−42	36	104	3.50

Results are whole-brain family-wise-error-corrected at $p<0.05$. *k*: number of 3 × 3 × 3 voxels; L: left; Max statistic: value at peak voxel; R: right. *x,y,z* coordinates are in MNI space.

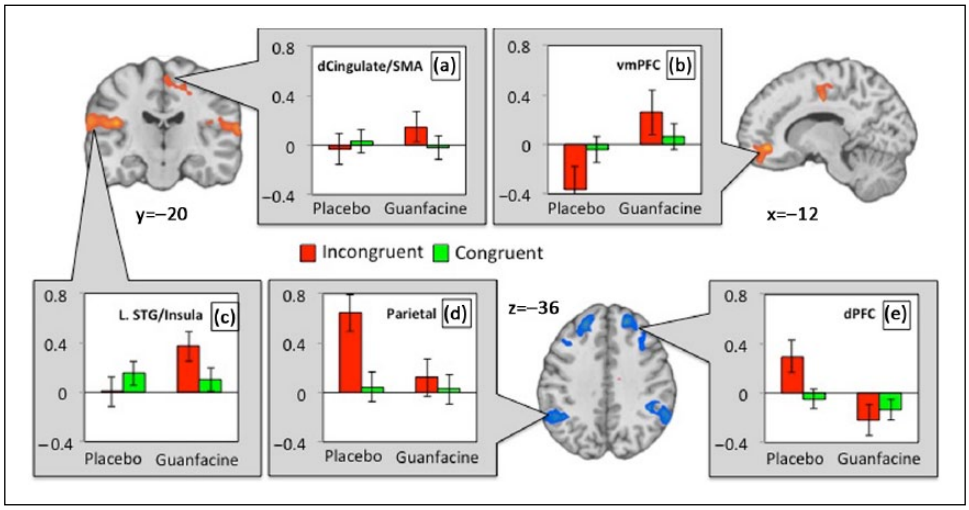

Figure 4. Guanfacine alters prefrontal activation to the incongruent stimuli in a Stroop task during the fMRI session (guanfacine *n*=9; placebo *n*=12).

Guanfacine also attenuated demand on dorsal attention networks during the task. During this Stroop task, participants see color words (e.g., BLUE) presented in either congruent (blue) or incongruent color fill (green). Participants are asked to silently name the color fill while ignoring the word itself, and their ability to do so is thought to reflect attention, conflict resolution, and inhibitory control during incongruent stimuli presentations. Percent signal-change values during incongruent (red) and congruent trials (green) are displayed in regions that showed significant between-group difference in the incongruent> congruent contrast in whole brain analysis (family-wise-error corrected $p<.05$). Figure shows increased activity in (a) dorsal cingulate/ sensorimotor area (SMA), (b) ventromedial prefrontal cortex (vmPFC), and (c) superior temporal gyrus (STG)/insula. Attenuated activity is shown in (d) superior parietal and (e) dorsal prefrontal cortex (dPFC).

study. In future work it will be important to determine specific mechanisms underlying guanfacine's efficacy in clinical trial evaluations, recruiting smokers specifically interested in smoking cessation.

During the titration period, guanfacine significantly reduced systolic blood pressure, and also tended to reduce diastolic blood pressure and heart rate. Mean values of blood pressure were approaching definitions outlined for hypotension, suggesting that it may not be appropriate to treat smokers with existing hypotension with guanfacine. Future work should investigate whether lower doses of guanfacine would be efficacious for smoking cessation while minimizing reductions in blood pressure, heart rate, and other orthostatic adverse events.

Placebo-treated subjects demonstrated reductions in stress-precipitated changes in cortisol levels, whereas guanfacine-treated subjects demonstrated increased levels. When compared with non-smokers, smokers typically demonstrate a blunted HPA-axis activation in response to stress, and this blunted response has been associated with smoking relapse (al'Absi et al., 2005). No significant effects of imagery condition or medication were observed for ACTH levels. These findings suggest that guanfacine may normalize the cortisol response to stress in overnight-deprived daily smokers. Chronic nicotine alters corticotrophin releasing factor (CRF) and noradrenergic signaling in stress-reactive extrahypothalamic circuits such as the amygdala and the bed nucleus of the stria terminalis (BNST;

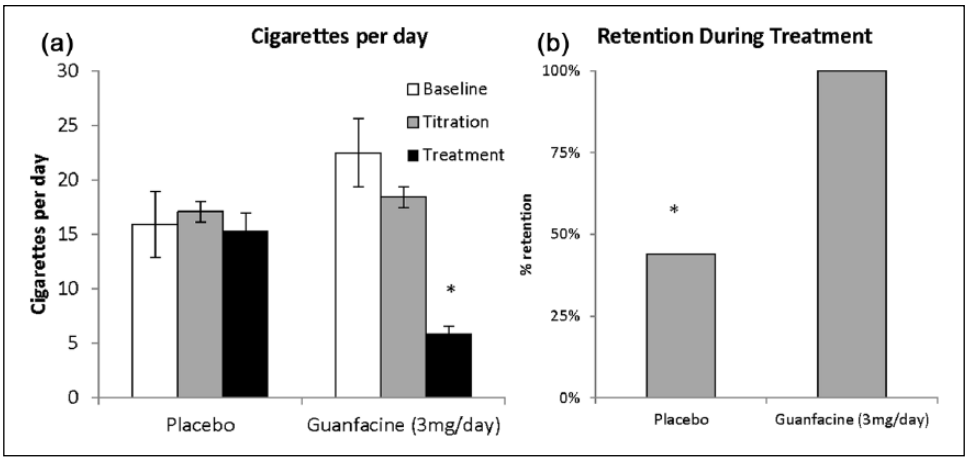

Figure 5. Guanfacine reduces cigarette use and improves retention during a brief treatment phase (guanfacine n=9; placebo n=9).
(a) Effect of medication (guanfacine vs. placebo) on cigarette use during baseline (mean 30-day average), titration (mean of final titration week), and the four-week treatment period (four-week average adjusted for baseline). Guanfacine significantly reduces cigarette use during the treatment phase (*p<.0005). (b) Effect of medication (guanfacine vs. placebo) on retention in treatment during the four-week quit attempt. Guanfacine significantly increases retention during the treatment phase (*p<.01).

Buczek et al., 1999), regions involved in regulation of the HPA axis and cortisol responses (Forray and Gysling, 2004). The chronic nicotine-related alterations in these circuits may contribute to blunted cortisol responses to stress and to nicotine in daily smokers. Guanfacine has been shown to have its effects on stress-induced nicotine reinstatement via effects on the amygdala and the BNST (Yamada and Bruijnzeel, 2011), which may in turn affect amygdala and BNST efferents to the HPA axis and to autonomic regulation. However, it should be noted that this hypothesis regarding the normalization of blunted cortisol response is speculative, as we did not include a non-smoker control group to evaluate the strength of the stress manipulation used in the current study.

Neuroimaging results demonstrated altered activation in brain areas associated with attention and inhibitory control, consistent with findings that guanfacine treatment is associated with improvement in attention and PFC-based executive functioning tasks (Kim et al., 2012). Guanfacine's enhancement of Stroop-related activation in the anterior cingulate, vmPFC, and bilateral temporal gyri/insula, while decreasing activity in dlPFC and parietal cortices, suggests improved engagement of PFC-based cognitive and impulse-control systems and decreased reliance on dorsal attentional circuits while attending to incongruent versus congruent stimuli. A parsimonious explanation for this pattern of neural activation is that guanfacine may increase efficiency within dorsal attention networks engaged during Stroop performance, and further study is needed to examine this possibility directly. Interestingly, among treatment-seeking adolescent smokers, Stroop-related activations in the insula and anterior cingulate positively correlated with treatment-related reductions in cotinine levels (Krishnan-Sarin et al., 2013); thus, these and the current findings indicate a relationship between increased Stroop-related insula and anterior-cingulate activation and better treatment outcome for smokers. However, insula lesions have been associated with smoking cessation (Naqvi et al., 2007) and insula activation to smoking cues are associated with smoking slips (Janes et al., 2010), suggesting a context-dependent mechanism for the relationship between insula activation and smoking. A pattern of Stroop-related increased vmPFC activation and diminished dlPFC activation similar to that observed in this study was previously associated with improved outcome in cocaine dependence (Brewer et al., 2008). Relatively diminished activation of dorsal PFC was also observed following behavioral treatment for substance abuse (DeVito et al., 2012).

Additional limitations to those already mentioned include a modest sample size and attrition. Although the sample size and rates of attrition were comparable to other laboratory studies of stress in smokers, and medication effects on our primary outcomes were robust, it will be important to replicate findings. We did not collect data on time of last dose of guanfacine/placebo during the fMRI session, although this concern might be mitigated by the scanning being performed at steady-state dosing levels of guanfacine. Stress was not specifically measured during the treatment phase, although measures of negative mood (including anxiety and distress) did not demonstrate significant findings.

Overall, results point to guanfacine as a potential pharmacotherapy for smoking cessation. Using a novel translational approach, we report for the first time that guanfacine significantly reduced smoking-lapse and craving using a well-validated laboratory analogue of stress-precipitated smoking, altered brain activity during a cognitive-control task, and reduced smoking and improved retention during a subsequent treatment period. Our findings are consistent with preclinical results that guanfacine attenuates stress-related relapse and rescues decrements in self-control, and support further development of guanfacine as a potential pharmacotherapy for smoking cessation.

Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Drs Arnsten and Yale receive royalties from the sales of Intuniv (extended-release guanfacine) for the treatment of pediatric ADHD. They do not receive royalties from the sales of generic guanfacine, which is used to treat adults. Generic guanfacine was used in the current study.

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Dr Potenza has received financial support or compensation for the following: Dr Potenza has consulted for and advised Ironwood, Lundbeck, Shire, and iNSYS; has received research support from the National Institutes of Health, Mohegan Sun Casino, the National Center for Responsible Gaming, and Psyadon pharmaceuticals; has participated in surveys, mailings, or telephone consultations related to drug addiction, impulse control disorders, or other health topics; has consulted for law offices and gambling entities on issues related to impulse control disorders; provides clinical care in the Connecticut Department of Mental Health and Addiction Services Problem Gambling Services Program; has performed grant reviews for the National Institutes of Health and other agencies; has guest-edited or edited journals or journal sections; has given academic lectures in grand rounds, continuing medical education (CME) events, and other clinical or scientific venues; and has generated books or book chapters for publishers of mental health texts.

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