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The Effect of Treatment with Guanfacine, an Alpha2 Adrenergic Agonist, on Dopaminergic Tone in Tobacco Smokers: An [^{11}C]FLB457 PET Study

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Guanfacine, a noradrenergic alpha2a agonist, reduced tobacco smoking in a 4-week trial and in animal models has been shown to reduce cortical dopamine release, which is critically involved in the reinforcing effect of tobacco smoking. We measured amphetamine-induced extrastriatal dopamine release before and after treatment with guanfacine with [^{11}C]FLB457, a dopamine D_2/D_3 receptor radiotracer, and positron emission tomography (PET). Sixteen tobacco smokers had one set of [^{11}C]FLB457 PET scans on the same day, one before and one at 2.5–3 h after amphetamine (0.4–0.5 mg/kg, PO). A subset ($n = 12$) then underwent guanfacine treatment (3 mg/day for 3 weeks) and the set of scans were repeated. [^{11}C]FLB457-binding potential (BP_{ND}) was measured pre- and post amphetamine in extrastriatal brain regions. The fractional change in BP_{ND} after *vs* before amphetamine (Δ BP_{ND}) is an indirect measure of DA release and was compared between the untreated and guanfacine-treated conditions. Guanfacine treatment attenuated amphetamine-induced DA release; however, the change was due to a global 8% decrease in baseline BP_{ND} from the untreated to the guanfacine-treated condition. Chronic guanfacine treatment reduced [^{11}C]FLB457 BP_{ND} in tobacco smokers, suggesting an increase in dopaminergic tone. Guanfacine-induced normalization of dopamine signaling may be an important mesocortical mechanism contributing to its ability to aid in tobacco smoking cessation.

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INTRODUCTION

Tobacco smoking continues to be the leading cause of preventable premature disease and death in the United States, with smoking-related illnesses claiming the lives of more than 556 000 people annually and contributing to 20 million deaths since the first Surgeon General's report 50 years ago (US Department of Health and Human Services, 2014). Despite overwhelming evidence of the adverse health consequences associated with tobacco smoking, ~18% of adults in the United States continue to smoke, and the vast majority of those who attempt to quit relapse within the first month (Benowitz, 2009). The main addictive chemical in tobacco smoke is nicotine, which acts at β_2-nicotinic acetylcholine receptors (β_2-nAChRs) and facilitates the release of dopamine (Cosgrove *et al*, 2015) in limbic and cortical brain regions. Nicotine replacement therapies (NRTs), eg, the nicotine patch, which mimic the effects of nicotine, have been only moderately effective in helping smokers quit. This suggests that additional neuronal mechanisms that underlie aspects of relapse (eg, stress or cognitive dysfunction) should be targeted for more effective smoking cessation treatments.

Alpha2 adrenergic agonists, including guanfacine, have the potential to treat tobacco dependence, as indicated by their ability to attenuate nicotine-related reinforcement, cue-induced craving, and stress-related effects, as well as to improve cognitive function (Bruijnzeel *et al*, 2010; Fox *et al*, 2012; Hains *et al*, 2015; McKee *et al*, 2015; Yamada and Bruijnzeel, 2011). Currently, guanfacine is used to treat cognitive disorders with a prefrontal cortical (PFC) dysfunction, and it is approved for the treatment of attention-deficit/hyperactivity disorder (Arnsten and Jin, 2012) and hypertension. Guanfacine improves cognition (ie, working memory) and reduces stress reactivity through the inhibition of norepinephrine and the modulation of dopamine neurotransmission (Arnsten and Pliszka, 2011) particularly along the PFC–amygdala axis. Preclinical studies have demonstrated that guanfacine and clonidine, both alpha2 adrenergic agonists, reduced drug-induced dopamine release in the PFC

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(Jentsch *et al*, 2008; Jentsch *et al*, 1998). A recent study in tobacco smokers demonstrated that treatment with guanfacine *vs* placebo decreased stress-induced smoking in the laboratory, altered PFC neural activation in areas associated with attention and inhibitory control, and reduced cigarette use (McKee *et al*, 2015). Thus, guanfacine may be a potential treatment to prevent relapse during smoking cessation, however, the effect of guanfacine on dopamine neurotransmission in humans has not yet been studied.

Positron emission tomography (PET) brain imaging with the radiotracer [^{11}C]FLB457, a high-affinity dopamine D_2/D_3 receptor antagonist, can be used to quantify availability of dopamine D_2/D_3 receptors and changes in synaptic dopamine levels in extra-striatal regions of the brain (Sandiego *et al*, 2015). The objective of this study was to examine the effects of guanfacine treatment on dopamine neurotransmission in healthy tobacco smokers with [^{11}C]FLB457 and PET. We used amphetamine as a pharmacological probe to elicit dopamine release in the brain and we measured amphetamine-induced changes in [^{11}C]FLB457 binding before and at the end of 3 weeks of daily guanfacine administration. We hypothesized that guanfacine treatment would reduce amphetamine-induced dopamine release in the dorsolateral prefrontal cortex (dlPFC) and the amygdala. We chose dlPFC and amygdala as primary regions of interest based on studies indicating a role for guanfacine in modulating dopamine neurotransmission in the PFC and the PFC–amygdala axis (Arnsten and Pliszka, 2011; Jentsch *et al*, 2008). We tailored our choice of PET radiotracer accordingly, as [^{11}C]FLB457 is used to measure extra-striatal dopamine D_2/D_3 receptor availability.

MATERIALS AND METHODS

Participants

Sixteen tobacco smokers (6 female and 10 male, 37 ± 9 years old) participated in a set of [^{11}C]FLB457 PET scans, one scan before, and a second scan 3 h after, amphetamine administration. Fifteen of these subjects had their baseline and post-amphetamine scans on the same day. Due to radiochemistry issues, one subject had the amphetamine administration and post-amphetamine scan 5 days after the baseline scan. Twelve (4 female, 8 male) of the sixteen subjects came back for a second set of pre- and post-amphetamine scans, performed on the same day, at the end of 3 weeks of guanfacine treatment. On both study days all subjects were required to abstain from smoking overnight, which was verified by carbon monoxide levels <11 ppm or a carbon monoxide level that was $\leqslant 50\%$ of their intake level. All subjects had one magnetic resonance scan as previously described (Sandiego *et al*, 2015), required to delineate anatomical information from the PET data for brain region-of-interest analysis. Subjects for this imaging study were recruited from an ongoing human laboratory study in tobacco smokers investigating the effects of treatment with guanfacine on stress-precipitated smoking behavior. All subjects provided written informed consent for participation in the study. The study adhered to the Protection of Human Subjects of Research and Ethical Principles and Guidelines. The Human Investigation Committee, Yale University School of Medicine, and Yale-New Haven Hospital Radiation Safety granted approval for the study protocol.

Clinical Assessment

Subjects were medically and psychiatrically healthy. Eligibility was determined by the study PI and the study physician and included the following: a medical examination including a physical examination, electrocardiogram, serum chemistries, thyroid function studies, complete blood count, urinalysis, and urine toxicology screening. Participants had no history of significant medical illness or major head trauma. The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (SCID-IV) was administered to rule out Axis I Disorders including Substance and Alcohol Dependence but not Nicotine Dependence. Tobacco smokers were required to have been smoking at least 10 cigarettes daily for at least 1 year, and during screening to have carbon monoxide levels greater than 10 ppm and urine cotinine levels >150 ng/ml. Women had negative pregnancy tests during screening and prior to each radiotracer injection. Menstrual cycle phase was not controlled and hormonal contraception was not exclusionary.

[^{11}C]FLB457 PET Imaging

[^{11}C]FLB457 was synthesized as previously described (Sandiego *et al*, 2015). The specific activity (mean $\pm$ SD) was 32.4 ± 27.2 mCi/nmol at end of synthesis and 17.6 ± 15.4 mCi/nmol at time of injection ($n = 56$). Injected dose of [^{11}C]FLB457 across scans was 9.1 ± 1.8 mCi ($n = 56$). PET scans were performed on the ECAT EXACT HR+ (Siemens/CTI, Knoxville, TN, USA). A 6-min transmission scan was acquired prior to the start of each PET acquisition for attenuation correction. [^{11}C]FLB457 was injected intravenously as a bolus over 1-min by a computer-controlled pump (Harvard Apparatus, Holliston, MA, USA), and emission data were collected for 90 min.

Sinograms were reconstructed with all corrections (attenuation, normalization, scatter, randoms, and deadtime) into a sequence of 27 frames: 6×30 s; 3×1 min; 2×2 min; 16×5 min. Final image dimension and voxel size were $128 \times 128 \times 63$ and $2.06 \times 2.06 \times 2.43$ mm^3, respectively. Motion-correction on the dynamic data was performed by registering each frame to an early frame (ie, the first 10 min of data post injection) using a six-parameter mutual information algorithm (Viola and Wells, 1997) (FMRIB's Linear Image Registration Tool, FMRIB Software library, version 3.2). The final reconstructed image resolution was ~6 mm full-width at half maximum.

Amphetamine Administration and Plasma Levels

Amphetamine (0.4–0.5 mg/kg, PO) was administered 150–180 min prior to the second [^{11}C]FLB457 injection, in order that the peak of amphetamine levels in the plasma lined up in time with the second PET scan, based on the previous work (Narendran *et al*, 2013). Relative to amphetamine administration ($t = 0$ min), blood samples were collected at $t = 60$, 120, 180, 240, and 270 min. Of the 16 total subjects, 2 received 0.5 mg/kg and participated in both scanning

sessions, ie, before and after guanfacine treatment. Because there was evidence for an interaction between chronic guanfacine treatment and amphetamine, the dose of amphetamine was lowered to 0.4 mg/kg after the first two subjects. The details of the interaction have been previously published (Gaiser *et al*, 2015). The study was stopped after 12 subjects completed the post-guanfacine scan set because there was evidence for an interaction between guanfacine and amphetamine, even at the reduced amphetamine dose.

Guanfacine Treatment

After the first set of PET scans (untreated condition), subjects underwent treatment with guanfacine as previously described (McKee *et al*, 2015). Guanfacine was administered PO twice daily for 21 days and was titrated to steady-state levels of 3 mg/day. Subsequently, the second set of PET scans (guanfacine-treated condition) was performed to assess the effect of chronic guanfacine on amphetamine-induced dopamine release. Subjects were not asked to modify their cigarette intake during the 3-week trial. Medication compliance was assessed with a riboflavin marker, and urine florescence was assessed every 2–3 days during the 3-week period.

PET Image Processing and Analysis

Regions of interest (ROIs) were mapped from Montreal Neurological Institute (MNI) template space to PET space to compute tissue time-activity curves, as described previously (Sandiego *et al*, 2015). Our primary ROIs of dlPFC and amygdala were chosen *a priori*. We included as secondary analysis the following extrastriatal ROIs, which have measureable specific signal (ie, $BP_{ND} > 0.5$) (Narendran *et al*, 2009), for comparison to other current (Narendran *et al*, 2009) and future studies in the literature: cingulum, hippocampus, occipital cortex, parietal cortex, temporal cortex, and thalamus.

The simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996) was used for the kinetic analysis of non-displaceable binding potential (BP_{ND}) as previously described (Sandiego *et al*, 2015), using the cerebellum as the reference region. Percent change in BP_{ND} ($\%\Delta BP_{ND}$) from baseline to amphetamine challenge, an indirect measure of dopamine release, was computed as: $\%\Delta BP_{ND} = [1 - BP_{ND}(\text{challenge})/BP_{ND}(\text{baseline})] \times 100$ for both untreated and guanfacine-treated conditions in the ROIs examined.

Clinical Laboratory Measurement

There were 11 subjects who completed both imaging and laboratory sessions. Subjects completed a laboratory session examining withdrawal-precipitated smoking behavior on ability to resist smoking and ad-lib self-administration, as previously described (neutral session (McKee *et al*, 2015)). See Supplementary Figure 1 for a timeline of procedures. Subjects abstained from smoking overnight before the laboratory session, 15 h from their last cigarette. To assess smoking lapse, subjects were presented with eight cigarettes of their preferred brand and could choose to smoke at any point within 50 min (delay period). Subjects were rewarded $1 for every 5 min they resisted smoking during the 50 min delay period. Once subjects decided to start smoking, or resisted smoking for the full 50 min, a 60 min ad-libitum session commenced. Primary outcomes examined included latency to start smoking (ie, ability to resist smoking) and number of cigarettes smoked during the 60-min self-administration session.

Statistical Analysis

T-tests were used to evaluate potential differences in injected dose and injected mass between scans. Using a multiple dependent general linear model across all brain regions, we examined whether there was a significant difference between the change in BP_{ND} from the untreated to the guanfacine-treated condition. This omnibus test capped the type 1 error rate at alpha < 0.05 (one-tailed). Based on our *a priori* hypothesis that guanfacine improves cognition and reduces stress reactivity via the PFC–amygdala axis, we examined differences in the amygdala and dlPFC with *a priori* contrasts. All other brain regions were examined with *post hoc* testing correcting for multiple comparisons. Basic demographic (sex, age) and smoking variables (cigarettes per day, Fagerström Test for Nicotine Dependence (FTND) scores) were evaluated as potential covariates, and were only retained if they reduced residual variance. Sex was the only variable retained as a covariate. Using a general linear model across all brain regions, we also evaluated whether there was a significant baseline (pre-amphetamine) difference between the untreated to the guanfacine-treated conditions. Given our *a priori* hypothesis concerning the amygdala and dlPFC, Pearsons correlation coefficients were used to examine relationships between BP_{ND} values and clinical correlates of tobacco smoking and treatment outcomes.

RESULTS

Across all subjects ($n = 16$), cigarettes smoked per day and number of years smoked were 13.0 ± 5.4 no./day and 17.0 ± 6.6 years, respectively, with a mean FTND score of 5.4 ± 3.0. On the day of the scan, carbon monoxide and urine cotinine levels were 8.4 ± 3.9 ppm and 967 ± 129 ng/ml ($n = 16$) in the untreated condition and 9.1 ± 6.0 ppm and 812 ± 252 ng/ml ($n = 12$) in the guanfacine-treated condition. There were no significant carbon monoxide or cotinine level differences between pre- and post-guanfacine conditions.

PET Scan Parameters

There were no significant differences in injected dose between pre- and post-amphetamine [^{11}C]FLB457 scans, respectively, under untreated (9.2 ± 1.4 and 9.0 ± 2.0 mCi) and guanfacine-treated (9.9 ± 0.4 and 9.1 ± 1.8 mCi) conditions. No significant differences were found in injected mass between pre- and post-amphetamine [^{11}C]FLB457 scans, respectively, in the untreated (0.39 ± 0.16 and 0.44 ± 0.14 μg) and guanfacine-treated (0.31 ± 0.13 and 0.34 ± 0.20 μg) groups.

Plasma Amphetamine Levels

Amphetamine levels were measured in the plasma after amphetamine administration on both scan days, and there were no differences between the group of subjects scanned in the untreated ($n=16$) and guanfacine-treated ($n=12$) conditions (ie, 52.3 ± 2.3 and 51.3 ± 4.3 ng/ml at 180 min). The post-amphetamine [^{11}C]FLB457 PET scans started at 150–180 min after amphetamine administration, which coincided with the peak amphetamine levels which then remained elevated through the duration of the scan at 240–270 min (Figure 1).

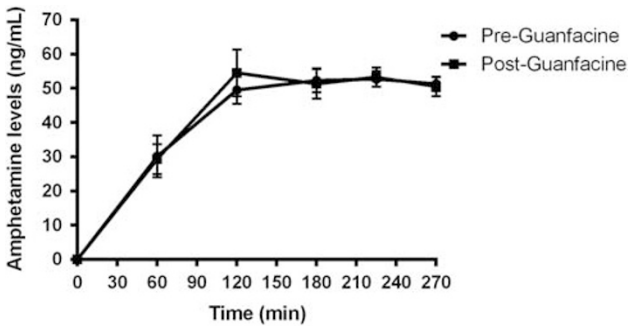

Figure 1 Time course of plasma amphetamine levels for scans before and after treatment with guanfacine. The amphetamine challenge [^{11}C]FLB457 scan started 150–180 min after amphetamine administration. Data points are the subject mean pre-guanfacine ($n=16$) and post-guanfacine ($n=12$) treatment conditions, and bars represent SEM.

Effect of Guanfacine on Extrastriatal Amphetamine-Induced Dopamine Release

For both sets of scans, amphetamine reduced [^{11}C]FLB457 BP_{ND} relative to baseline in all ROIs examined, indicative of an increase in dopamine levels (Table 1). We examined whether the ΔBP_{ND} was lower in the guanfacine-treated condition *vs* the untreated condition, ie did guanfacine treatment attenuate the amphetamine-induced dopamine release? We found that the ΔBP_{ND} was significantly reduced in the amygdala after guanfacine treatment (from 8 to 2%) [$F(1,10)=3.92$, $p=0.038$; see Table 1, Figure 2], but not in the dlPFC or other brain regions.

Effect of Guanfacine on Baseline Dopamine D_2/D_3 Receptor Availability

We examined whether the reduction in amphetamine-induced dopamine release from the untreated to the guanfacine-treated condition was due to a change in baseline BP_{ND}. We found that guanfacine treatment significantly decreased baseline BP_{ND} across the ROIs examined [$F(3,8)=6.58$, $p=0.038$], however, the BP_{ND} differences within specific regions did not reach significance (Figure 2). After treatment with guanfacine, there was a 9% reduction in baseline BP_{ND} (eg, 4% in thalamus, 8% in the amygdala, and 12% in dlPFC), averaged across all ROIs and all subjects ($n=16$ pre-treatment and $n=12$ post treatment). We had the same finding when comparing the baseline BP_{ND} in the same 12 subjects before and after treatment with guanfacine (Supplementary Figure 2).

Clinical Measures

Difference scores in baseline BP_{ND} during the untreated to the guanfacine-treated condition were calculated for all regions to explore relationships between the change in BP_{ND} with treatment outcomes. There was a significant correlation between the difference in baseline amygdala values from the untreated to treated condition with the latency to start smoking ($r=0.73$, $p=0.01$) and with number of cigarettes smoked in the self-administration phase ($r=-0.62$, $p=0.04$). A bigger change in BP_{ND} was associated with a longer latency to smoke and fewer cigarettes smoked during self-administration. There were no other relationships between BP_{ND} or ΔBP_{ND} in the regions of interest with clinical characteristics of tobacco smoking.

DISCUSSION

In the current study we found that, in tobacco smokers, treatment with guanfacine significantly reduced amphetamine-induced dopamine release in the amygdala, as measured with PET brain imaging and the radiotracer [^{11}C]FLB457. However, this reduction in the amygdala, dlPFC, and other regions examined was primarily due to a reduction in baseline BP_{ND} after chronic guanfacine treatment, which is indicative of an increase in dopaminergic tone as a result of treatment. Specifically, the reduction in BP_{ND} from pre-to post-guanfacine treatment suggests that dopamine levels have increased (Morris *et al*, 2014; Volkow *et al*, 2009). Furthermore, the increase in dopaminergic tone in the amygdala following treatment correlated with clinical outcomes, specifically longer latency to smoke and fewer cigarettes smoked during a self-administration period. This suggests that one mechanism by which guanfacine may help smokers quit smoking is by modulating dopaminergic neurotransmission.

Guanfacine is an approved medication for ADHD and has been investigated as a means to treat other cognitive and stress-related disorders such as PTSD and substance abuse (Arnsten and Pliszka, 2011; Arnsten *et al*, 2015; Fox *et al*, 2014). The dlPFC is particularly sensitive to catecholamine levels (Arnsten, 2011), and aberrant norepinephrine and dopamine levels, such as are found in cognitive and stress-related disorders, can compromise the ability to manage arousal and executive function. Arnsten *et al* (2015) have studied the neurobiology of stress extensively as it relates to cognition. In general, acute stress increases norepinephrine and dopamine release, which weakens performance of the dlPFC and impairs executive function and at the same time strengthens the amygdala so that more primitive emotional and habitual responding can take over. Guanfacine, an alpha2a adrenergic agonist, inhibits norepinephrine release, cAMP-signaling and HCN potassium channel function, which in turn is thought to normalize the balance of norepinephrine and dopamine between the amygdala and PFC (ie, the PFC–amygdala axis), thus reducing stress reactivity and improving cognitive function. Indeed, a previous study in smokers found that guanfacine treatment altered PFC activity, measured with fMRI, during a cognitive control task and reduced cigarette consumption and craving

Table 1 [^{11}C]FLB457 BP_{ND} at Baseline and After Amphetamine Challenge in the Untreated ($n=16$) and Guanfacine-Treated ($n=12$) Conditions (Mean ± SEM)

Brain Region	Untreated			Guanfacine-treated		
	Baseline	Challenge	ΔBP_{ND}(%)	Baseline	Challenge	ΔBP_{ND} (%)
dlPFC	0.73 ± 0.05	0.61 ± 0.05	15.8 ± 4.5	0.64 ± 0.05	0.58 ± 0.05	11.4 ± 3.3
Ant Cing	1.05 ± 0.06	0.95 ± 0.06	10.9 ± 3.1	0.96 ± 0.05	0.87 ± 0.07	10.0 ± 3.2
OCC	0.62 ± 0.05	0.64 ± 0.05	10.0 ± 4.6	0.55 ± 0.05	0.52 ± 0.06	9.1 ± 4.2
PAR	0.65 ± 0.05	0.50 ± 0.20	12.3 ± 4.4	0.60 ± 0.05	0.55 ± 0.07	10.9 ± 5.2
TEMP	1.23 ± 0.07	1.11 ± 0.05	9.2 ± 3.0	1.17 ± 0.06	1.10 ± 0.08	6.6 ± 2.9
Amygdala	2.59 ± 0.15	2.35 ± 0.08	8.6 ± 2.8	2.39 ± 0.09	2.34 ± 0.12	1.9 ± 3.4
Hippocampus	1.46 ± 0.11	1.22 ± 0.05	14.4 ± 4.0	1.27 ± 0.06	1.18 ± 0.07	6.4 ± 4.3
Thalamus	3.06 ± 0.12	2.80 ± 0.05	8.4 ± 3.2	2.93 ± 0.12	2.83 ± 0.14	3.5 ± 2.0

Abbreviations: Ant Cing, anterior cingulate; ΔBP_{ND}, percent change in binding potential; dlPFC, dorsolateral prefrontal cortex; OCC, occipital cortex; PAR, parietal cortex; TEMP, temporal cortex.

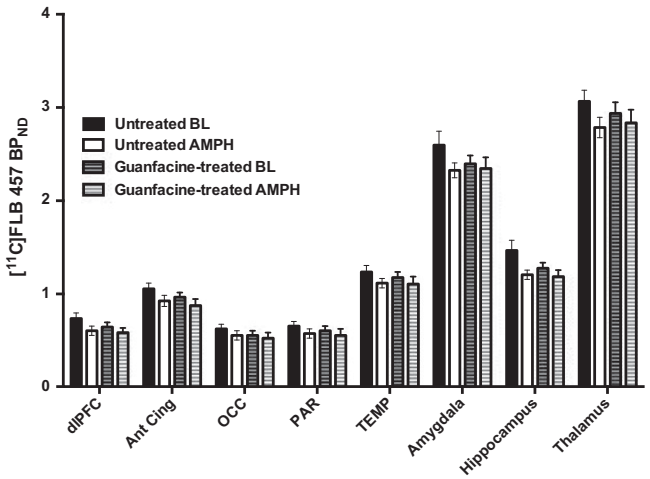

Figure 2 [^{11}C]FLB457 binding potential (BP_{ND}) is shown as the subject average during the (a) untreated baseline (black bars, $n=16$) and amphetamine (white bars, $n=16$) conditions and the guanfacine-treated baseline (black hatched bars, $n=12$) and amphetamine (white hatched bars, $n=12$) conditions across regions of interest. Baseline BP_{ND} is shown in (b) in the all 16 untreated subjects (black bars), and the same 12 subjects before (gray bars) and after (blacked hatched bars) guanfacine treatment. Error bars are SEM.

in the laboratory, compared with placebo (McKee *et al*, 2015). In line with this finding, in the current study, guanfacine treatment appears to have increased, and possibly normalized, baseline dopaminergic tone. Therefore, modulating neurochemistry in the PFC–amygdala axis, with guanfacine or a similar medication, may be an important mechanism to manage some of the adverse consequences of tobacco smoking cessation, such as the inability to manage stress and behavioral disinhibition.

In the context of tobacco smoking, nicotine is the primary addictive component and exerts its primary reinforcing effects by activating β_2-nAChRs in the mesolimbic dopamine pathway (Picciotto *et al*, 1998), but nicotine also modifies cognitive function and stress responses, through activation of nAChRs in the cortico-limbic pathway (Mansvelder *et al*, 2009). Tobacco smokers (Fehr *et al*, 2008) along with individuals dependent on other drugs of abuse (Martinez *et al*, 2004, 2005; Zijlstra *et al*, 2008), tend to have lower striatal dopamine D_2/D_3 receptor availability and, in some cases a 'blunted' striatal dopamine response (Martinez *et al*, 2005). This is thought to indicate a general reward circuit deficit, which may make it difficult for recovering substance abusers to effectively transition from drug rewards to natural reward. Dopaminergic function in extrastriatal brain regions of tobacco smokers and drug abusers has not been well characterized. This study is limited by the lack of a nonsmoker control comparison group. While we cannot determine whether tobacco smokers have a 'blunted' dopamine response compared to nonsmokers, our study does indicate that guanfacine treatment appears to increase extrastriatal dopamine tone.

While we interpret our finding of a decrease in BP_{ND} as an increase or 'normalization' of dopaminergic tone by chronic guanfacine treatment, there is a caveat. Specifically, a decrease in BP_{ND} could also be interpreted as a down-regulation of dopamine D_2/D_3 receptors. Interestingly, in nonhuman primates, there was a positive correlation between [^{11}C]raclopride binding (a radioligand to measure dopamine D_2/D_3 receptors in the striatum) and severity of self-injurious behavior (Freeman *et al*, 2015). Guanfacine treatment reduced self-injurious behaviors and improved cognition suggesting a restoration of dopamine levels. In addition, data exist to indicate that guanfacine treatment increases dopamine levels in the PFC (Arnsten, 2011). Thus, we presume that activation of noradrenergic alpha-2a receptors with guanfacine reduces dopamine D_2/D_3 receptor availability by increasing basal dopamine levels.

Additionally, with this imaging paradigm, we initially hypothesized that treatment with guanfacine would blunt amphetamine-induced dopamine release in the amygdala and dlPFC. Indeed, the amphetamine-induced reduction in [^{11}C]FLB457 BP_{ND} was less after treatment with guanfacine across all regions and the difference reached significance in the amygdala, but overall this appears to be due to a significant reduction in post-treatment baseline levels of [^{11}C]FLB457 BP_{ND}. The reduction in amphetamine-induced dopamine release in the amygdala is important because this is a region known to play a key role in regulating stress, thus chosen for investigation *a priori*. However, all other regions

examined followed the same trend, but did not reach significance after correcting for multiple comparisons. Thus, it is not completely clear whether the reduction in amphetamine-induced dopamine release by guanfacine is restricted to the amygdala.

This study was discontinued due to the interaction between amphetamine and guanfacine that increased sympathetic tone in some subjects resulting in hypertension, even when the amphetamine dose was 0.4 mg/kg (Gaiser *et al*, 2015). Thus, due to the limited number of subjects in this study, we were not able to examine sex differences. Another limitation is that the relatively high affinity of [^{11}C]FLB457 for dopamine D_2/D_3 receptors does not permit reliable measurements of binding (ie, does not achieve equilibrium during the scan duration) in the striatum, a region that is commonly studied in addiction research as part of the mesolimbic dopamine system. A recently published study found that healthy nonsmokers who underwent treatment with prazosin, an alpha 1 adrenergic antagonist, had increased [^{11}C]PHNO (D_3 receptor agonist) binding in the dorsal caudate from baseline, indicative of a decrease in dopamine levels (Le Foll *et al*, 2017). Guanfacine and prazosin act through different mechanisms (ie, alpha 2a agonist and alpha 1 antagonist, respectively) to restore the balance of norepinephrine and dopamine levels, and both have been studied for the treatment of stress related disorders (Arnsten *et al*, 2015). The effect of guanfacine treatment on striatal binding and dopamine could be examined in a future study with a radiotracer that allows stable BP_{ND} measurements in striatum, eg, [^{11}C]raclopide, [^{11}C]PHNO, or [^{18}F]fallypride.

In conclusion, guanfacine-induced normalization of dopaminergic tone may be an important treatment mechanism for smoking cessation. We have demonstrated in tobacco smokers that chronic guanfacine treatment reduces dopamine D_2/D_3 receptor availability in extrastriatal brain regions, measured *in vivo* with [^{11}C]FLB457 PET imaging. This finding is indicative of an overall increase in dopamine levels after 3 weeks of guanfacine treatment. Our study further supports targeting the noradrenergic system with guanfacine as a mechanism to regulate mesocortical activity for the treatment of tobacco smoking.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)