Guanfacine Potentiates the Activation of Prefrontal Cortex Evoked by Warning Signals

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Background: Warning signals evoke an alert state of readiness that prepares for a rapid response by priming a thalamo-frontal-striatal network that includes the dorsolateral prefrontal cortex (DLPFC). Animal models indicate that noradrenergic input is essential for this stimulus-driven activation of DLPFC, but the precise mechanisms involved have not been determined. We tested the role that postsynaptic α_{2A} adrenoceptors play in the activation of DLPFC evoked by warning cues using a placebo-controlled challenge with the α_{2A} agonist guanfacine.

Methods: Sixteen healthy young adults were scanned twice with event-related functional magnetic resonance imaging (fMRI), while performing a simple cued reaction time (RT) task following administration of a single dose of oral guanfacine (1 mg) and placebo in counterbalanced order. The RT task temporally segregates the neural effects of warning cues and motor responses and minimizes mnemonic demands.

Results: Warning cues produced a marked reduction in RT accompanied by significant activation in a distributed thalamo-frontal-striatal network, including bilateral DLPFC. Guanfacine selectively increased the cue-evoked activation of the left DLPFC and right anterior cerebellum, although this increase was not accompanied by further reductions in RT. The effects of guanfacine on DLPFC activation were specifically associated with the warning cue and were not seen for visual- or target-related activation.

Conclusions: Guanfacine produced marked increases in the cue-evoked activation of DLPFC that correspond to the well-described actions of postsynaptic α_2 adrenoceptor stimulation. The current procedures provide an opportunity to test postsynaptic α_{2A} adrenoceptor function in the prefrontal cortex in the pathophysiology of several psychiatric disorders.

Key Words: Adrenergic receptors, adults, fMRI, guanfacine, prefrontal cortex, warning cues

Warning signals of impending behaviorally salient stimuli evoke an alert state of readiness that suppresses ongoing activity and lowers motor thresholds to prepare for a rapid response (1). This transient state primes a distributed brain network that includes the dorsolateral prefrontal cortex (DLPFC), which initiates and adjusts stimulus-driven control over thalamic nuclei, basal ganglia, and premotor, supplementary motor, and cingulate motor areas (2–4). The neuronal architecture of the DLPFC provides the mechanism for these regulatory functions (5). Local connections between pyramidal neurons activated by similar stimulus properties create DLPFC microcircuits that engage in recurrent excitation to maintain the response set for brief periods (4–6).

The regulatory functions of the DLPFC are intricately influenced by noradrenergic fibers of the pontine nucleus locus coeruleus (7). Phasic activation of the locus coeruleus by salient stimuli releases norepinephrine through an extensive efferent system (8,9). In the DLPFC, these noradrenergic fibers synapse on pyramidal dendritic spines that also receive synaptic inputs from sensory afferents and other pyramidal neurons (10). Postsynaptic α_{2A} adrenergic receptors are richly expressed on these trisynaptic complexes (11), co-localized with hyperpolarization-activated cyclic nucleotide-modulated (HCN) cation channels that are kept open by cyclic adenosine monophosphate (cAMP) at the resting potential (12). Stimulation of postsynaptic α_{2A} adrenoceptors inhibits cAMP production (13), thereby closing nearby HCN channels (12), increasing pyramidal excitability (14) and strengthening the connectivity of DLPFC microcircuits (12). The resultant increase in delay-related firing has been shown to reduce distractibility and improve working memory in monkeys (15–17) and humans (18), as well as enhance DLPFC perfusion in monkeys during working memory (15).

The impact of postsynaptic α_{2A} adrenoceptor stimulation on the behavioral and neural effects of warning cues is less well understood. The little available research has instead highlighted the actions of presynaptic α_{2A} autoreceptors that suppress locus coeruleus firing (19) and inhibit norepinephrine release (20). Low doses of the nonselective α_2 receptor agonist clonidine, which preferentially bind to presynaptic receptors (16), have been found to reduce the response benefits conferred by warning cues in monkeys (21) and humans (22). The latter neuroimaging study also found that clonidine diminished cue-evoked activation in parietal cortex (22), presumably secondary to reduced locus coeruleus firing (19). In contrast, low doses of the specific α_{2A} agonist guanfacine that preferentially bind to postsynaptic receptors had no impact on cue usage (21,22) and no effect on neural activity evoked by warning cues in healthy adults (22). However, this neuroimaging study employed region of interest analyses that did not assess postsynaptic α_{2A} adrenoceptor actions on cue-evoked activation in DLPFC. The current study tested the impact of postsynaptic α_2 adrenoceptor stimulation on DLPFC activation evoked by warning cues in healthy adults using event-related functional magnetic resonance imaging (fMRI) together with a pharmacological challenge with the α_{2A} adrenoceptor agonist guanfacine. The adults were scanned twice while performing a cued reaction time (RT) task following

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single oral doses of guanfacine and placebo in a double-blind, counterbalanced design. It was predicted that guanfacine stimulation of postsynaptic α_{2A} adrenoceptors would selectively enhance the activation of DLPFC evoked by warning cues.

Methods and Materials

Participants

Sixteen right-handed, healthy college students (9 female students) were recruited via campus postings for the study. The sample was 50% Caucasian, 25% African American, 19% Hispanic, and 6% Asian or mixed ethnicity. The study was approved by the institutional review boards of Queens College of City University of New York and Mount Sinai School of Medicine, and informed consent was obtained from all participants. Participants were compensated for their time.

Procedures

Participants were screened for contraindications with a physical examination, including an electrocardiogram, blood pressure readings, and a full medical history. The adults also completed the Beck Anxiety Inventory (BAI) (23), Beck Depression Inventory-II (BDI-II) (24), and Conners' Adult ADHD Rating Scale–Self-Report (CAARS–S) (25) and were given a mental status examination to rule out psychiatric disorders. Full-scale IQ was estimated with the matrix reasoning and vocabulary subtests of the Wechsler Abbreviated Scale of Intelligence (WASI) (26). A total score ≥15 on the BDI-II or the BAI, a *T* score 1 SD above the mean (i.e., >60) on the CAARS Total ADHD Symptoms index, and an estimated IQ < 80 were exclusionary for the study. Psychometric characteristics for the sample are presented in Table 1.

On both scan days, blood pressure and pulse rate were measured and 1 mg oral guanfacine or placebo was administered 90 minutes before the scheduled scan in a counterbalanced, double-blind design. Participants practiced one block of the cued RT task on an office desktop. Blood pressure was measured again at the end of the 1-hour scan session. The single dose of guanfacine had a significant depressant effect on systolic blood pressure but not diastolic blood pressure or pulse rate compared with placebo (Table 1 in Supplement 1). Mean days between scans was 7.9 days ± .6 days.

Cued RT Paradigm

The cued RT task used in this study was adapted from the well-known A-X Continuous Performance Test (27,28). The task used in this study consisted of four 300-sec blocks that began and ended with a 30-sec central fixation cross. Each block contained a series of 120 letter stimuli, including 24 (20%) targets (i.e., "X"), half of which were preceded by a cue (i.e., "A") and half by a distractor (i.e., letters "B" through "H"), yielding a total of 48 cued and 48 uncued targets across the study. The cues were always followed by a target and never by a distractor. The task temporally segregated the neural effects of warning cues and targets. The stimuli were presented individually at fixation for 200 msec. The interstimulus interval was pseudo-randomized from 1550 msec to 2050 msec (mean = 1800 msec per block) to discourage anticipatory responses. Stimuli were projected via a super video graphics array (SVGA) projector system onto a rear projection screen mounted at the head of the magnet bore that was viewed through a mirror on the head coil. Participants were instructed to respond with their right index finger as rapidly as possible to every target and were told that some targets would be preceded by the cue.

Table 1. Demographic and Psychometric Characteristics of the Sample

Variable	Mean	SD	Range
Age (Years)	25.4	4.4	21–35
Estimated IQ	113.7	9.6	99–132
BDI-II Total Score	1.8	2.5	0–9
BAI Total Score	2.4	1.6	0–14
CAARS ADHD Index	39.4	8.1	31–57

ADHD, attention-deficit/hyperactivity disorder; BAI, Beck Anxiety Inventory; BDI-II, Beck Depression Inventory-II; CAARS, Conners' Adult ADHD Rating Scale; IQ, intelligence quotient.

Image Acquisition

All participants were scanned on the same 3.0 Tesla Siemens Allegra (Siemens, Erlangen, Germany) head-dedicated magnetic resonance imaging (MRI) scanner. A high-resolution T2-weighted anatomical volume of the brain was acquired in the axial plane with a turbo spin-echo (TSE) pulse sequence (repetition time [TR] = 4500 msec, echo time [TE] = 99 msec, flip angle = 170°, field of view [FOV] = 210 mm, matrix = 512 × 336, 42 slices, slice thickness = 4 mm contiguous, in-plane resolution = .41 mm^2). Functional T2*-weighted images depicting the blood oxygenation level-dependent (BOLD) signal were acquired at the same 42 slice locations using gradient-echo echo-planar images (TR = 3000 msec, TE = 27 msec, flip angle = 85°, FOV = 210 mm, matrix = 64 × 64, slice thickness = 3 mm, gap = 1 mm, in-plane resolution = 3.75 mm × 3.75 mm). All images were acquired with slices positioned parallel to the anterior commissure-posterior commissure line. The participants all completed four runs of 300 sec each in each scan session.

Statistical Analysis

Behavior. The behavioral impact of warning cues was assessed by comparing RT for cued and uncued targets. The effects of guanfacine on performance were tested with a two-way repeated measures analysis of variance (ANOVA), in which drug (guanfacine vs. placebo) and cue condition (cued vs. uncued) served as within-subjects factors. The alpha level for these analyses was set at a liberal $p < .05$ due to the small sample.

Neuroimaging. The fMRI data were preprocessed and analyzed with SPM2 (Wellcome Department of Cognitive Neurology, London, United Kingdom). The guanfacine and placebo functional time series were separately time-corrected, realigned, and co-registered to their respective T2 images and then to each other. The time series were then conjointly normalized to the Montreal Neurological Institute (MNI) template and spatially smoothed.

First-level analyses were conducted individually for each participant with a general linear model (GLM) to determine the relationship between the observed event-related BOLD signals and regressors that represented expected neural responses to trial events. Regressors were created by convolving a train of delta functions that represented the individual trial events with the default statistical parametric mapping (SPM) basis function, which consisted of a synthetic hemodynamic response function, composed of two gamma functions and their derivatives (29). There were four regressors representing: 1) visual stimulation, including all distractor, cue, target, and error events; 2) cue effects that reflect cue-related activation; 3) targets, reflecting motor responses; and 4) errors. The six parameters created during motion correction were entered as covariates of no interest in the GLM (30). Neural activity related to visual stimulation, cues, and targets was contrasted with an implicit baseline

Table 2. BOLD Signal Responses to Warning Cues Following Guanfacine and Placebo

			Placebo					Guanfacine				
			Talairach Coordinates					Talairach Coordinates				
Region	Side	BA	x	y	z	Vol (mm^3)	*t* Value	x	y	z	Vol (mm^3)	*t* Value
Dorsolateral Prefrontal Cortex	R	46	30	47	16	1,208	5.80	36	38	17	1,688	4.71
	L	9/46	−39	36	25	1,824	4.01	−38	28	24	2,008	5.45
Primary Motor Cortex	R	4	40	−2	39	1,952	4.90	59	−3	17	1,116	3.90
	L	4	−42	−5	48	1,454	5.28	−53	−6	37	2,192	5.34
Anterior Cingulate Cortex	R	24	6	4	42	24,720	5.90	2	4	46	32,112	9.41
	L	24	−12	7	33		6.07	−6	1	50		7.40
Temporoparietal Junction	R	22	46	−42	22	1,472	4.14	48	−40	19	1,584	4.83
Intraparietal Sulcus Area	L	7						−22	−62	45	800	3.49
Visual Cortex	R	18						20	−95	10	2,472	4.99
	L	18						−28	−83	8	920	3.67
Putamen/Insula	R	—	22	0	2	14,648	5.74	20	6	0	9,304	6.09
Putamen	L	—	−26	−4	2	23,240	6.25	−22	6	0	9,128	5.53
Insula	L	—	−33	6	2		6.17					
Thalamus	R	—	12	−15	4	10,936	5.04	6	−14	−1	15,304	7.53
	L	—	−12	−16	1		6.08	−10	−16	−3		5.45
Cerebellum	R	—	14	−47	−16	6,664	5.77	32	−44	−33	7,976	6.22
	L	—	−34	−54	−23	1,040	3.95	−40	−51	−18	2,680	7.99

BA, Brodmann area; BOLD, blood oxygenation level-dependent; L, left; R, right.

modeled on the 30-sec fixation periods at the beginning and end of each block. Functional images for all participants were analyzed individually by applying appropriate linear contrasts to the parameter estimates separately for the placebo and guanfacine conditions, resulting in three contrast maps each for the guanfacine and placebo conditions per subject.

The six contrast maps for all participants were entered into second-level group analyses conducted with random-effects statistical models. The neural effects of visual stimulation, cues, and targets in the placebo condition were analyzed with separate one-sample *t* tests. Paired *t* tests were used to contrast this activation in the guanfacine and placebo conditions. The resultant voxel-wise statistical maps were thresholded for significance using a cluster-size algorithm that protects against false-positive results (31). A Monte Carlo simulation established that a cluster extent of 100 contiguous resampled voxels ($2 \times 2 \times 2$ mm^3) was necessary to correct for multiple voxel comparisons at $p < .01$. Finally, MNI coordinates were converted to the Talairach and Tournoux (32) system using a nonlinear transformation (http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html).

Results

Behavior

The presentation of a warning cue significantly decreased RT to targets for both placebo and guanfacine [$F(1,15) = 262.82$, $p < .001$]. However, there was no significant main effect of drug and no drug × cue interaction. The RTs for cued and uncued targets were 312 ± 18 msec and 495 ± 20 msec for guanfacine and 309 ± 16 msec and 489 ± 21 msec for placebo. The hit rate was consistently above 95% and the false alarm rate below 1% for all conditions.

Neuroimaging

Warning cues generated significant BOLD signal increases following both placebo and guanfacine in a distributed thalamo-frontal-striatal network that is detailed in Table 2. This network included bilateral thalamus, cerebellum, putamen, and primary motor cortex; right insular cortex and temporoparietal junction (TPJ); and bilateral anterior cingulate cortex and DLPFC. In addition, cue-evoked BOLD signal increases were seen in bilateral visual cortex and left intraparietal sulcus areas in the guanfacine condition and in the left insula in the placebo condition (Figure 1). In contrast, visual stimulation and targets produced principally left-lateralized BOLD signal responses that were most prominent in the visual cortex and the motor cortex, contralateral to the button hand, respectively. The visual- and target-related activations are listed in Tables 2 and 3 in Supplement 1.

Direct comparison of the guanfacine and placebo conditions identified drug-induced increases in the BOLD signal responses to warning cues in the left DLPFC and right anterior cerebellum (Table 3). As shown in Figure 2, guanfacine produced significantly greater cue-evoked BOLD signal responses than placebo in a cluster in the left DLPFC that was activated in both conditions. In contrast, guanfacine extended the cue-evoked BOLD signal inferiorly in the right anterior cerebellum to a region that was not activated in the placebo condition. There were no clusters with greater cue-evoked BOLD signal responses for placebo than guanfacine. Additional comparisons revealed significantly greater target-related BOLD responses for guanfacine than placebo in overlapping clusters of the right cerebellum (Table 4 in Supplement 1). However, guanfacine had no impact

Table 3. Significantly Greater BOLD Signal Responses to Warning Cues Following Guanfacine Than Placebo

			Talairach Coordinates			Vol	
Region	Side	BA	x	y	z	(mm^3)	*t* Value
Dorsolateral Prefrontal Cortex	L	46	−26	36	25	1,356	4.07
Cerebellum	R	—	26	−43	−37	1,158	4.62

BA, Brodmann area; BOLD, blood oxygenation level-dependent; L, left; R, right.

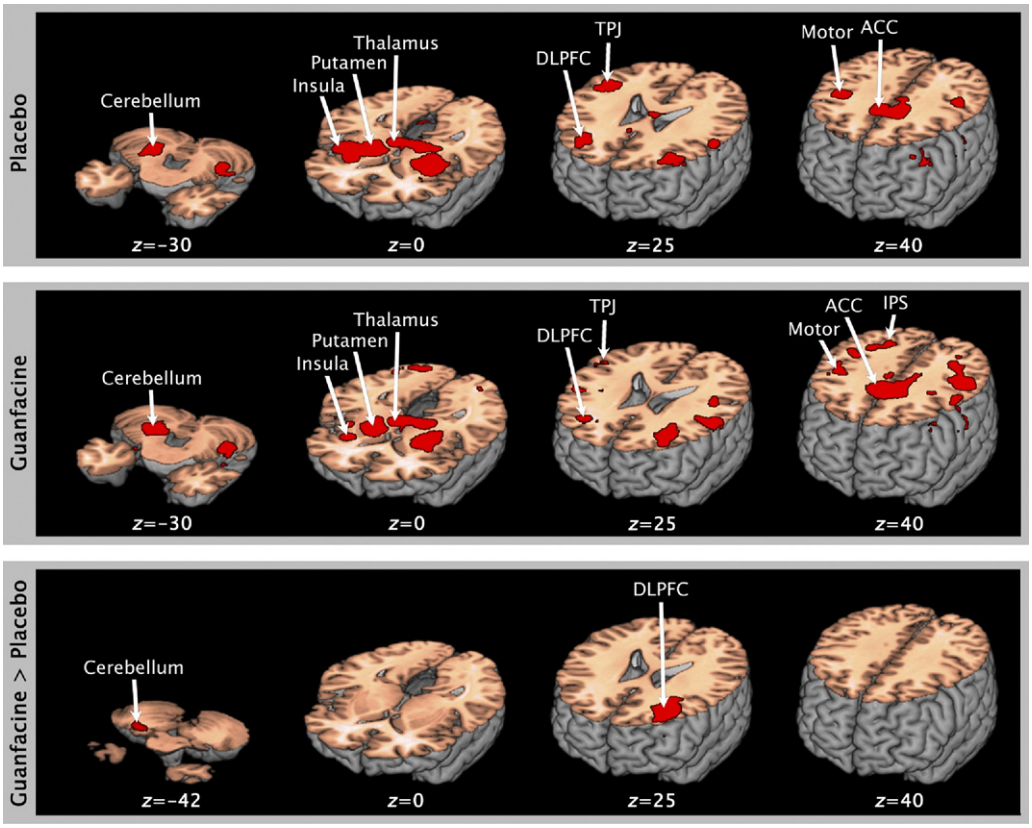

Figure 1. Blood oxygenation level-dependent (BOLD) signal increases generated by warning cues following placebo (top row) and 1 mg oral guanfacine (middle row). Cue-related BOLD signal increases were seen in bilateral thalamus, cerebellum, putamen, and primary motor cortex; right insula cortex and temporoparietal junction; and bilateral anterior cingulate cortex and dorsolateral prefrontal cortex (DLPFC). Cue-evoked BOLD signal responses were significantly greater for guanfacine than placebo in left DLPFC and right inferior cerebellum (bottom row). The figures were thresholded at $p < .01$ (one-tailed). The *z* values refer to coordinates in the Talairach and Tournoux standard anatomical space. ACC, anterior cingulate cortex; IPS, intraparietal sulcus; TPJ, temporoparietal junction.

on target-related BOLD signal responses in DLPFC. There were also no significant drug effects on visual-related BOLD signal increases.

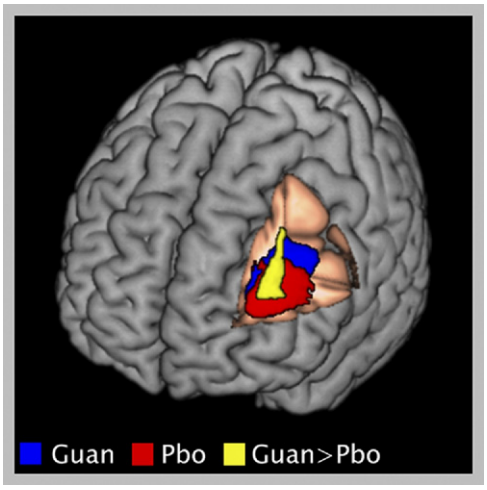

Figure 2. The anatomical overlap of the BOLD signal responses to warning in the left dorsolateral prefrontal cortex (DLPFC) following placebo and 1 mg oral guanfacine. Guanfacine produced significantly greater cue-evoked BOLD signal responses than placebo in a cluster in the left DLPFC that reflected the overlap of the two conditions. BOLD, blood oxygenation level-dependent; Guan, guanfacine; Pbo, placebo.

Discussion

The current results provide evidence that guanfacine selectively potentiates activation evoked by warning cues in DLPFC and anterior cerebellar regions that are part of a broader thalamo-frontal-striatal network specialized for response preparation. The left-lateralized impact of guanfacine on cue-evoked DLPFC activation may reflect the response demands of the task (i.e., right-handed button press) and together with the absence of similar medication actions on visual- and target-related activation, suggests that the effects of guanfacine on neural activity were task-specific rather than hemodynamic artifacts. The lack of a medication effect on the behavioral improvement conferred by warning cues is consistent with the findings of previous studies with guanfacine (22,33) and nicotine (34). Functional brain measures may be more sensitive than behavioral indexes to subtle medication effects on the detection or use of warning cues (35). Several weeks of daily treatment with guanfacine are generally needed to produce antihypertensive effects and behavioral improvements (36,37). The independence of the neurophysiological and behavioral responses to challenge doses of guanfacine makes this an ideal model to test postsynaptic α_{2A}-adrenoceptor-dependent DLPFC activation in humans without the confound introduced by performance differences (38).

The selective increase in the cue-evoked activation of the DLPFC is consistent with the neural actions of guanfacine. Guanfacine stimulation of postsynaptic α_{2A} adrenoceptors in the DLPFC suppresses an HCN inward current (12), which raises the

membrane resistance (39) and increases the excitability of target pyramidal neurons (14). These adrenoceptor actions enhance the responses of DLPFC neurons to coherent bursts of excitatory input generated by salient stimuli (39) and strengthen the connectivity of local DLPFC recurrent networks that support response set maintenance (5,12). The resultant increase in neural activity elicited by synaptic input and the processing of this input could account for the larger cue-evoked BOLD signal responses produced by guanfacine in the DLPFC in the current study (40,41).

Functionally, the strengthening of the cue-evoked activation of DLPFC by guanfacine might facilitate response anticipation in a manner similar to that reported for working memory (15,18). The increases in the responsiveness of DLPFC neurons and the activation of local recurrent networks produced by guanfacine have been shown to improve working memory in monkeys (12) and may account for the increased DPLFC perfusion in monkeys performing a working memory task (15). The finding that guanfacine enhanced cue-evoked DLPFC activation suggests that the medication may improve the detection of warning cues and the maintenance of the response sets across cue-target intervals in individuals with attention deficits and/or noradrenergic dysfunction. Suboptimal postsynaptic α_{2A} adrenoceptor regulation of DLPFC function has been implicated in the pathophysiology of attention-deficit/hyperactivity disorder (ADHD) (42) and is a promising target for pharmacological treatments for the disorder (43). The current procedures provide an opportunity to test these hypotheses.

The present results demonstrate that guanfacine produces marked increases in the cue-evoked activation of DLPFC that correspond to the well-described actions of postsynaptic α_2 adrenoceptor stimulation. The enhanced DLPFC activation may reflect an increase in the signal-to-noise ratio of pyramidal neurons and the reconnection of local recurrent networks. These findings support the use of guanfacine challenges together with fMRI as a viable measure of postsynaptic α_{2A} adrenoceptor function in DLPFC.

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Supplementary material cited in this article is available online.

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