RESEARCH REPORT

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