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Guanfacine Modulates the Emotional Biasing of Amygdala-Prefrontal Connectivity for Cognitive Control

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Abstract

Functional interactions between amygdala and prefrontal cortex provide a cortical entry point for emotional cues to bias cognitive control. Stimulation of α_2 adrenoceptors enhances the prefrontal control functions and blocks the amygdala-dependent encoding of emotional cues. However, the impact of this stimulation on amygdala-prefrontal interactions and the emotional biasing of cognitive control have not been established. We tested the effect of the α_2 adrenoceptor agonist guanfacine on psychophysiological interactions of amygdala with prefrontal cortex for the emotional biasing of response execution and inhibition. Fifteen healthy adults were scanned twice with event-related functional magnetic resonance imaging while performing an emotional go/no-go task following administration of oral guanfacine (1 mg) and placebo in a double-blind, counterbalanced design. Happy, sad, and neutral faces served as trial cues. Guanfacine moderated the effect of face emotion on the task-related functional connectivity of left and right amygdala with left inferior frontal gyrus compared to placebo, by selectively reversing the functional co-activation of the two regions for response execution cued by sad faces. This shift from positively to negatively correlated activation for guanfacine was associated with selective improvements in the relatively low accuracy of responses to sad faces seen for placebo. These results demonstrate the importance of functional interactions between amygdala and inferior frontal gyrus to both

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Author K.P.S. conceived and designed the study, supervised data collection, performed the statistical analyses, and prepared the manuscript. Author S.M.C. contributed to data collection and analysis. Author J.H.N. clinically evaluated participants, supervised drug administration, and read and revised the manuscript. Author J.M.H. read and revised the manuscript. Author J.F. supervised the statistical analyses, contributed to manuscript preparation, and read and revised the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

Dr. Newcorn is a recipient of grants for research support from Eli Lilly & Co., Ortho-McNeil-Janssen, and Shire and is or has been an advisor/consultant for Alcobra, Eli Lilly & Co., NEOS, Ortho-McNeil-Janssen, Shire, and BioBehavioral Diagnostics Company. All other authors report no biomedical financial interests or potential conflicts of interest.

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bottom-up biasing of cognitive control and top-down control of emotional processing, as well as for the α_2 adrenoceptor-mediated modulation of these processes. These mechanisms offer a possible method to address the emotional reactivity that is common to several psychiatric disorders.

Keywords

prefrontal cortex; amygdala; guanfacine; emotion; go/no-go; fMRI

1. Introduction

Facial expressions and body gestures convey context-dependent emotional cues that shape social behavior (Otta et al., 1994). These emotional cues have also been found to influence cognitive function in multiple domains, including response execution and inhibition (Hare et al., 2005; Schulz et al., 2007). Facial expressions of happiness engender positive affect and approach behavior (Otta et al., 1994), which result in faster responses that are more difficult to inhibit (Hare et al., 2005; Schulz et al., 2007), while the inclusion of expressionless (neutral) faces, which are often mistakenly evaluated as negative (Lee et al., 2008), may lower the accuracy of responses to sad faces (Schulz et al., 2009). Developing interventions to modulate the neural circuits through which emotional cues bias these basic cognitive control processes has implications for addressing the impulsivity and affective instability that characterize mood (Murphy et al., 1999), personality (Silbersweig et al., 2007), and behavior disorders (Walcott and Landau, 2004).

Functional interactions between the amygdala nucleus that processes affective cues and prefrontal cortex regions that guide behavior provide a putative cortical entry point for emotion to bias cognitive control processes (Dolan, 2007). The amygdala is located deep in the medial temporal lobe, receives rich sensory input from all modalities (Stefanacci and Amaral, 2000), and has dense intrinsic connections that form the neuronal architecture to assign affective values to cues (Pitkanen et al., 1997). In turn, the amygdala may enhance the processing of affective cues via widespread projections to sensory cortices (Amaral et al., 2003) and/or influence goal-directed behavior through bidirectional connections with orbital aspects of inferior frontal gyrus (Sagvasspe et al., 2011). The inferior frontal gyrus integrates amygdala input with contextual information to compute the behavioral significance of cues (Sakagami and Pan, 2007) and engage the appropriate neural effectors for response execution and inhibition (e.g., frontal operculum). The amygdala is thus ideally positioned to bias the cognitive control functions of prefrontal cortex.

Ascending noradrenergic projections of the pontine nucleus locus coeruleus that innervate both prefrontal cortex and amygdala offer possible substrates to modulate the affective biasing of cognitive control processes (Asan, 1998; Wang et al., 2007). In prefrontal cortex, noradrenaline acts via α_2 adrenergic receptors to increase the excitability of pyramidal neurons (Carr et al., 2007) and strengthen local pyramidal recurrent networks (Wang et al., 2007), and may contribute to the gains in inferior frontal activation for response inhibition produced by the noradrenaline reuptake-inhibitor atomoxetine (Chamberlain et al., 2009). These α_2 adrenoceptor actions may enhance the inferior frontal inhibition of amygdala

activity associated with affective appraisal (Wager et al., 2008), and thereby possibly diminish the reported influence of affective cues on response execution and inhibition (Hare et al., 2005; Schulz et al., 2007; 2009). Conversely, stimulation of α_2 adrenoceptors in amygdala inhibits spontaneous and evoked firing (Buffalari and Grace, 2007), suppresses the long-term potentiation and long-term depression that support synaptic plasticity (DeBock et al., 2003), and blocks the amygdala-dependent acquisition of affective cues (Davies et al., 2004), all of which may functionally uncouple the amygdala-inferior frontal circuits that underlie the emotional biasing of response execution and inhibition (Sagaspe et al., 2011). Yet, direct evidence for the α_2 adrenoceptor modulation of the affective biasing of cognitive control is limited in humans.

This study tested the impact of α_2 adrenoceptor stimulation on the functional connectivity of amygdala and prefrontal cortex for the emotional biasing of cognitive control using event-related functional magnetic resonance imaging (fMRI) together with a pharmacological challenge with the α_{2A} adrenoceptor agonist guanfacine. Healthy adults were scanned while performing a face emotion go/no-go task following single oral doses of guanfacine and placebo in a double-blind, counterbalanced design. Functional connectivity of the amygdala for response execution and inhibition cued by happy, sad, and neutral faces was tested with psychophysiological interaction (PPI) analyses. The literature suggests that responses to happy faces will be faster and more difficult to inhibit (Schulz et al., 2007), and that the inclusion of neutral faces will result in fewer correct responses to happy and sad faces (Schulz et al., 2009). It was predicted that guanfacine stimulation of α_2 adrenoceptors would be associated with reduced functional connectivity of amygdala and inferior frontal gyrus compared to placebo, and would offset the biasing of response execution for happy and sad faces and response inhibition for happy faces expected for placebo.

2. Experimental procedures

2.1. Participants

Fifteen right-handed adults (8 females) aged 25.7 ± 1.2 years (mean $\pm$ SD) were recruited via postings for the study, and provided written informed consent after a complete description of the study was provided. Participants were compensated for their time. The study was approved by the institutional review boards of Queens College and the Icahn School of Medicine at Mount Sinai.

2.2. Procedures

Subjects were screened for contraindications to study participation with physical and mental status exams, and ratings on the Beck Anxiety Inventory (BAI) (Beck et al., 1988), Beck Depression Inventory – II (BDI-II) (Steer et al., 1999), and Conners Adult ADHD Rating Scale (CAARS) (Conners et al., 1999). Full Scale IQ was estimated with the Matrix Reasoning and Vocabulary subtests of the Wechsler Abbreviated Scale of Intelligence. 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. Mean $\pm$ SD BDI-II and BAI total scores were both 1.4 ± 0.5 , mean $\pm$ SD CAARS Total ADHD Symptoms T-score was 42.9 ± 2.5 , and mean $\pm$ SD estimated IQ was 113.7 ± 9.6 .

Blood pressure was measured and 1 mg oral guanfacine or placebo was administered 90 minutes prior to both scans in a counterbalanced, double-blind design. The 1 mg dose of guanfacine was chosen to minimize sedation and for its primarily postsynaptic binding profile (Arnsten et al., 1988). The administration of the medication was timed to capture the peak pharmacodynamic activity of guanfacine during the fMRI scan (Coull et al., 2001). Participants completed a training session that tested simple face perception and presented the face emotion go/no-go task (see below). Blood pressure was measured again at the end of each scan session. The effect of guanfacine on blood pressure was tested with repeated measures analyses of variance (ANOVA) with drug (guanfacine vs. placebo) and time (pre-scan vs. post-scan) as within-subject factors. Mean $\pm$ SD days between scans was 7.0 ± 2.5 .

2.3. Face emotion go/no-go task

The face emotion go/no-go task has previously been described (Schulz et al., 2009; Schulz et al., 2013). The task consisted of six 252-s blocks that each began and ended with a 30-sec central fixation-cross. Each block contained 72 (75%) go cues and 24 (25%) no-go cues, yielding a total of 432 go cues and 144 no-go cues. Participants had to respond rapidly with the right index finger to “go” cues and withhold responses to “no-go” cues. Stimuli were presented in the center of the screen for 500 ms with an interstimulus interval that was pseudorandomized from 1250 to 1750 ms (mean per block = 1500 ms). Face stimuli consisted of gray-scaled happy, sad, and neutral facial expressions from 18 individuals (9 female, 9 male) from the MacBrain Face Stimulus Set (Tottenham et al., 2009; available at www.macbrain.org). Alternating the valence of the face stimuli used as trial cues resulted in six blocks, as follows: 1) happy go/sad no-go; 2) sad go/neutral no-go; 3) neutral go/happy no-go; 4) happy go/neutral no-go; 5) sad go/happy no-go; and 6) neutral go/sad no-go. Trial order was counterbalanced across all conditions (e.g., trial type, facial expression, face ethnicity, face gender, face) to ensure that each trial type followed every other trial type equally often. Parallel versions of the task that differed in trial and block order were administered in counterbalanced order during the two scan sessions.

The percentage of correct inhibitions on no-go trials served as the measure of response inhibition, while the percentage of correct responses on go trials was the measure of response execution. Measures of task performance were tested with a repeated measures ANOVA, in which trial type (go vs. no-go), face emotion (happy vs. sad vs. neutral) and drug (guanfacine vs. placebo) served as within-subject factors. Reaction times (RT) for correct responses on go trials were tested with a repeated measures ANOVA with face emotion (happy vs. sad vs. neutral) and drug (guanfacine vs. placebo) as within-subject factors.

2.4. Image acquisition and preprocessing

All participants were scanned twice on the same 3.0 Tesla Siemens Allegra (Siemens Medical Systems) head-dedicated MRI scanner. Six series of 84 functional T2*-weighted images were acquired with echo-planar imaging (EPI) sensitive to the blood oxygenation level-dependent (BOLD) signal (repetition time [TR], 3000 msec; echo time [TE], 27 msec; flip angle = 85°; field of view [FOV], 210 mm; slice thickness, 2.5 mm; gap, 0.825 mm; resolution, 3.75 mm²; 42 axial slices). The TR represented a trade-off for whole-brain

coverage with thinner slices that minimized distortions and increased sensitivity in amygdala. A high-resolution T2-weighted anatomical volume of the brain was acquired at the same 42 slice locations with a turbo spin-echo (TSE) pulse (slice thickness, 3.325 mm with no gap; in-plane resolution, 0.41 mm²). All images were acquired with slices positioned parallel to the intercommisural line.

Data were preprocessed and analyzed with SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>). EPIs from the two scan sessions were separately sinc-interpolated in time to correct for the staggered acquisition of slices, realigned to the first EPI in each session to correct for head movements, and then co-registered to their respective TSE anatomical images and to each other. Next, EPIs from the two sessions were conjointly normalized to the Montreal Neurological Institute (MNI) template using parameters estimated from the first TSE image and then resampled with a 2-mm³ voxel size. Finally, EPIs were spatially smoothed with an 8-mm full width at half-maximum Gaussian kernel.

2.5. Standard convolution model for fMRI analysis

First-level analyses used a within-subjects design to identify regionally specific effects of task parameters on BOLD signal indices of activation, as previously described (Schulz et al., 2013). A general linear model was conducted to determine the relationship between event-related BOLD signals and 12 regressors that represented the 2 trial types of interest (correct no-go event vs. correct go event) $\times$ 3 face emotions (happy vs. sad vs. neutral) $\times$ 2 drugs (placebo vs. guanfacine). Contrasting the parameter estimates for each regressor versus baseline resulted in 12 contrast maps that each represented the BOLD response to a single interaction effect (e.g., no-go trials cued by happy faces in the guanfacine condition).

Preliminary analysis of the effect of trial type, face emotion, and drug on BOLD signal indices of activation in amygdala was conducted with a factorial ANOVA model (described below) that used a liberal cluster extent (50 voxels) to account for the small size of amygdala. This analysis was restricted to amygdala using a mask derived from the “aal.002” atlas for automatic anatomic labeling and generated with the PickAtlas toolbox (Maldjian et al., 2003). There was a significant main effect of trial type on the BOLD signal in left (MNI coordinates: $x = -30$, $y = -2$, $z = -20$; cluster extent = 62 voxels; $F = 14.80$; $p < 0.001$) and right amygdala (MNI coordinates: $x = 26$, $y = -4$, $z = -18$; cluster extent = 91 voxels; $F = 8.76$; $p = 0.004$), which reflected significantly greater BOLD signals for correct no-go events compared to correct go events (Figure S1). There were no other significant main or interaction effects on amygdala activation, although this is not prerequisite for testing the effect of guanfacine on PPI. These results corroborate a previous finding of amygdala activation for the correct no-go minus correct go events contrast on the same task (Schulz et al., 2009).

The seed regions of interest (ROI) in left and right amygdala were defined as the aal.002 atlas mask and data were extracted from subject-specific maxima that were within 2 mm of the group maxima for the main effect of trial type (i.e., correct no-go events vs. correct go events). The time series of the first eigenvariate of the BOLD signal in the left and right amygdala ROI, adjusted for effects of interest, were calculated from the time series of voxels passing significance for the main effect of trial type in the aal.002 atlas mask. The

mean volumes of the left and right amygdala ROI were 578 mm³ and 633 mm³, respectively. Regression coefficients (β values) for the BOLD signal indices of activation in left amygdala and inferior frontal gyrus were extracted using the aal.002 atlas mask and masks of inferior frontal gyrus created from the PPI results to illustrate significant findings of drug effects on connectivity (i.e., co-activation).

2.6. Psychophysiological interaction (PPI)

PPI is a regression-based method that tests for variations in physiological connectivity between brain regions as a function of changes in the psychological context (Gitelman et al., 2003). The method computes whole-brain connectivity between the time series of the seed ROI and the time series of all other voxels. Four PPI analyses were conducted in SPM8 for each participant to determine the functional interactions of the left and right amygdala ROI separately for correct go events and correct no-go events.

The time-series data of the first eigenvariate of the seed ROI were temporally filtered and mean corrected as in conventional SPM analysis. Bayesian estimation was used to deconvolve the time series of the BOLD signals to generate separate time series of the neuronal signal for the left and right amygdala ROI. Separate time series of the neuronal signals for correct go events and correct no-go events were then created, generating PPI regressors that represented interactions between the psychological and physiological factors, as well as separate regressors representing the main effects of correct go and no-go events (P regressors) and the baseline time courses for left and right amygdala (Y regressors). These regressors were forward-convolved with the hemodynamic response function, and then entered into four separate regression models for correct go events and correct no-go events for left and right amygdala, along with effects of no interest, including the six motion correction parameters and sessions. The specific effects of face emotion and drug on functional connectivity for correct go and no-go events were tested by applying linear contrasts to the parameter estimates for the PPI regressors versus baseline in the appropriate block, resulting in 12 contrast maps each for left and right amygdala for all participants. Each contrast map represented the change in connectivity for a single interaction effect (e.g., go trials cued by sad faces in the guanfacine condition).

The 12 contrast maps each for left and right amygdala for all participants were entered into separate second-level group analyses that used factorial ANOVA models with trial type (go vs. no-go), face emotion (happy vs. sad vs. neutral), and drug (guanfacine vs. placebo) as within-subjects factors. Treatment order was included as a covariate. This model enabled us to test all possible two-way and three-way interactions. The resultant voxel-wise statistical maps were thresholded for significance using a cluster-size algorithm that protects against false-positive results (Hayasaka et al., 2004). The height (intensity) threshold of each activated voxel was set at $p < 0.005$. A Monte Carlo simulation (procedure described in Slotnick and Schacter (2004)) that accounted for image resolution and smoothing parameters established that a cluster extent of 100 contiguous voxels corrected for multiple voxel comparisons at $p < 0.01$.

To explore the relationship of changes in behavior and connectivity between the placebo and guanfacine conditions for any significant three-way interactions, regression coefficients (β

values) for the PPI of left and right amygdala were extracted from inferior frontal gyrus using the mask created from the results of the PPI analysis (ROI peaks: $x = -44$, $y = 34$, $z = -12$). Differences in the β values for the placebo minus guanfacine conditions were calculated separately for correct go and no-go events cued by the face emotion of interest; similar difference scores were calculated for the percentage of correct responses on go trials and/or correct inhibitions on no-go trials cued the same face emotion of interest. The relationship of the β value difference scores and behavioral difference scores were tested with Pearson product-moment correlations.

3. Results

3.1. Blood pressure

Guanfacine reduced blood pressure during the scan compared to placebo (see Table S1). Repeated measures ANOVA revealed that pulse rate decreased significantly over the scan session ($F(1,15) = 8.43$, $p = 0.01$), but the reductions were greater for guanfacine than placebo ($F(1,15) = 5.67$, $p = 0.03$). Guanfacine also significantly reduced systolic blood pressure over the scan session compared to placebo ($F(1,15) = 7.17$, $p = 0.02$). There were no significant effects of time or drug on diastolic blood pressure (all $p > 0.05$).

3.2. Task performance

Guanfacine moderated the influence of face emotion on the accuracy of response execution on the go/no-go task, but had no effect on response inhibition (Figure 1). The three-way repeated measures ANOVA revealed a significant main effect of trial type ($F(1, 13) = 8.83$, $p = 0.01$) and significant face emotion $\times$ drug ($F(2, 26) = 5.26$, $p = 0.01$) and trial type $\times$ face emotion $\times$ drug ($F(2, 26) = 3.79$, $p = 0.04$) interactions on the behavioral measures of accuracy. There were no other significant main effects or interactions (all $p > 0.10$).

Follow-up testing with two-way ANOVAs yielded a significant main effect of face emotion ($F(2, 28) = 9.95$, $p = 0.001$) and a significant face emotion $\times$ drug interaction ($F(2, 28) = 14.53$, $p < 0.001$) for percent correct responses on go trials, but not for percent correct inhibitions on no-go trials (both $p > 0.05$). Pair-wise testing revealed that the percent correct responses was lower for sad faces in the placebo condition compared to both: i) neutral and happy faces in the placebo condition, both $p = 0.001$; and ii) sad faces in the guanfacine condition, $p = 0.004$, which in turn, did not differ from neutral and happy faces in either condition, both $p > 0.10$ (Figure 1).

An additional two-way ANOVA found a significant main effect of face emotion on RT ($F(2, 28) = 9.02$, $p = 0.001$), but no significant main effect of drug or face emotion $\times$ drug interaction (both $p > 0.10$). Responses were faster for happy faces (mean $\pm$ SD = 481 ± 86 ms) than neutral faces (516 ± 100 ms), $p = 0.008$, but not compared to sad faces (500 ± 86 ms), $p > 0.05$.

3.3. PPI analyses

Three-way ANOVAs revealed significant main and interaction effects of trial type, face emotion, and drug on PPI between the left and right amygdala ROI and several

frontoparietal regions that are listed in Table 1. Specifically, there was a significant main effect of trial type on the PPI of right amygdala with right dorsal anterior cingulate cortex, right primary motor cortex, and bilateral temporoparietal junction, which reflected greater connectivity for correct go events than correct no-go events. The PPI of left amygdala with bilateral posterior cingulate cortex instead showed a significant effect of face emotion that reflected increased connectivity for neutral relative to happy faces, which in turn evoked greater connectivity than sad faces. Finally, there was a main effect of drug on the PPI of left and right amygdala and overlapping regions of left posterior cingulate cortex and cuneus, with greater connectivity for placebo than guanfacine.

The ANOVAs also revealed significant trial type $\times$ face emotion interaction effects on the PPI of left and right amygdala with overlapping regions of right frontal operculum (Table 1). Post-hoc paired *t*-tests demonstrated that connectivity was greater for correct go than no-go events cued by sad faces, but was greater for no-go than go events cued by happy and neutral faces. The PPI between right amygdala and left inferior parietal lobule showed a similar trial type $\times$ face emotion interaction.

There were also significant trial type $\times$ face emotion $\times$ drug interaction effects on the PPI of left and right amygdala with overlapping clusters in left inferior frontal gyrus and several foci in middle temporal gyrus (Figure 2). Plotting the regression coefficients (β values) for the BOLD signal indices of activation in left amygdala versus left inferior frontal gyrus indicated that the three-way interactions reflected a shift from positively correlated activation for placebo to negatively correlated activation for guanfacine selectively for correct go versus no-go events cued by sad faces compared to happy and neutral faces. This shift in the functional co-activation of left amygdala and inferior frontal gyrus is exemplified by the change from a positively to a negatively sloped regression line (middle panel of Figure 2B). Further, the difference in the PPI (β values) of left and right amygdala with inferior frontal gyrus for placebo and guanfacine was positively correlated with the difference in percent correct responses on go trials cued by sad faces (Figure 3). Finally, co-varying for the change in systolic blood pressure during the scans did not significantly change the results; no amygdala connectivity was associated with the covariate.

4. Discussion

These findings provide evidence that the α_2 adrenoceptor agonist guanfacine has specific effects on the functional connectivity of amygdala for cognitive control in healthy adults, selectively modulating functional coupling with inferior frontal gyrus pars orbitalis associated with the emotional biasing of cognitive control processes. Guanfacine moderated the effect of face emotion on the task-related interaction of amygdala and inferior frontal gyrus, by selectively reversing the functional co-activation of the two regions for response execution cued by sad faces. This shift from positively to negatively correlated activation for guanfacine was associated with selective improvements in the relatively low accuracy of responses to sad faces seen for placebo. In contrast, guanfacine had no effect on valence-dependent variations in the interactions of amygdala with frontal operculum. The valence- and trial-specific differences in connectivity between discrete brain regions suggest that the effects of guanfacine were task-specific rather than hemodynamic artifacts. These results

provide clues about the role of amygdala-inferior frontal connectivity in the emotional biasing of cognitive control and the manipulation of this bias by α_2 adrenoceptor stimulation.

The behavioral results provide clear evidence that guanfacine moderated the influence of affective cues on response execution on the go/no-go task. The finding of fewer correct responses on go trials cued by sad faces in the placebo condition is partially consistent with our previous report of poorer accuracy for both sad and happy faces (Schulz et al., 2009). However, unlike previous studies, our finding of faster responses to happy faces did not reflect a broader emotional bias that interfered with the inhibition of responses to happy faces on no-go trials (Hare et al., 2005; Schulz et al., 2007). The inclusion of neutral faces as trial cues could account for the poorer accuracy of responses to sad faces in this study. Neutral expressions tend to be mistaken for sad faces (Lee et al., 2008), which was further compounded by the use of faces with closed mouths (Calvo and Nummenmaa, 2008). The selective effect of guanfacine on the emotional biasing of accuracy for sad faces confirms that these effects were task-specific rather than a general consequence of medication.

The effect of guanfacine on the functional interactions of amygdala and inferior frontal gyrus pars orbitalis supports the importance of this circuit to both the bottom-up biasing of cognitive control (Dolan, 2007) and the top-down modulation of emotional processing (Ray and Zald, 2012). Neural representations of face stimuli are processed via intrinsic amygdaloid circuits that converge in select output nuclei, including the basolateral nucleus (Pitkanen et al., 1997). The basolateral nucleus provides the bulk of the amygdala output to inferior frontal gyrus pars orbitalis (Amaral and Price, 1984), and has been shown to bias responses to affective cues (Sagase et al., 2011). In turn, the orbital aspect of inferior frontal gyrus is the only lateral prefrontal area to provide significant direct input to the amygdala (Carmichael and Price, 1995), primarily to the basolateral nucleus (Stefanacci and Amaral, 2002), and has been implicated in the regulation of emotional processing (Payer et al., 2012). The current finding of negatively correlated activation in amygdala and inferior frontal gyrus for response execution cued by sad faces for guanfacine may thus represent either a top-down suppression of emotional processing in amygdala or a bottom-up reduction in the affective bias of cognitive control, regardless of whether the positively correlated amygdala-inferior frontal connectivity in the placebo condition reflects a bottom-up bias of response execution cued by sad faces.

The depressant effect of guanfacine on blood pressure in this study confirms that the medication, which was originally developed as an antihypertensive, had the desired biochemical effect on α_2 adrenoceptors, at least peripherally. However, attempts to more precisely localize the effects of guanfacine in the brain have been frustrated by the difficulties with measuring local drug actions in humans. The effects of guanfacine on the emotional biasing of cognitive control in this study may reflect actions at α_2 adrenoceptors localized in basolateral amygdala nucleus and/or inferior frontal gyrus (Piletz et al., 2003), both of which are primarily expressed on non-noradrenergic neurons (Talley et al., 1996). Stimulation of α_2 adrenoceptors on inhibitory interneurons in basolateral amygdala results in widespread suppression of spontaneous activity, and thereby raises the signal-to-noise ratio of output-related pyramidal neurons (Buffalari and Grace, 2007), while activation of α_2

adrenoceptors in inferior frontal gyrus has been shown to enhance associative visuomotor learning (Wang et al., 2004), possibly through molecular mechanisms similar to those described for dorsolateral prefrontal cortex (Wang et al., 2007). Guanfacine may also indirectly influence amygdala and/or inferior frontal activation via α_2 adrenoceptors in thalamus (Coull et al., 2001) and dorsolateral prefrontal cortex (Schulz et al., 2013). Any of these α_2 adrenoceptor mechanisms could potentially explain the changes in functional connectivity and improvements in response execution found for guanfacine in this study.

Guanfacine had unanticipated effects on the functional interactions of amygdala with regions beyond prefrontal cortex. The adrenoceptor agonist moderated amygdala interactions with left anterior middle temporal regions that have bidirectional connections with amygdala (Iwai et al., 1987) and inferior frontal gyrus pars orbitalis (Webster et al., 1994), and have been implicated in visual object feature discrimination (Tanaka, 2003). Guanfacine also had a non-specific inhibitory effect on amygdala connectivity with posterior cingulate regions that support internally focused mentation (Andrews-Hanna et al., 2010) and function in opposition to goal-directed cognition (Mason et al., 2007). These changes in amygdala connectivity may have indirectly contributed to the effects of guanfacine on the emotional biasing of cognitive control.

4. 1. Clinical Implications

The current findings have potential implications for the α_2 adrenoceptor agonist treatment of psychiatric disorders characterized by problems with cognitive control and emotion regulation. The specific effect of guanfacine on the response bias for sad faces suggests an application for the treatment of mood-congruent biases in major depression (Blaney, 1986). However, α_2 adrenoceptor agonists have no antidepressant properties and successful antidepressant treatment does not impact adrenoceptor function (Charney et al., 1984). The lack of antidepressant effects may in part reflect the selective action of guanfacine on the connections of amygdala with cognitive control mechanisms in inferior prefrontal cortex rather than with affective mechanisms that have been implicated in both mood-congruent biases and the pathophysiology of major depression (Elliott et al., 2011). The selective effect of guanfacine on the amygdala-prefrontal connections that support the emotional biasing of cognitive control, however, offers a potential mechanism to address the emotional reactivity that is common to attention-deficit hyperactivity disorder (ADHD) (Walcott and Landau, 2004). Our results support further investigation of the use of guanfacine, already FDA-approved to treat ADHD symptoms, to target affect-related regulatory problems frequently seen in individuals with ADHD (Walcott and Landau, 2004).

4.2. Conclusions

The present results demonstrate that the α_2 adrenoceptor agonist guanfacine moderates the functional interaction of left and right amygdala with left inferior frontal gyrus pars orbitalis for the emotional biasing of cognitive control in healthy adults. Guanfacine reversed the functional co-activation of amygdala and inferior frontal gyrus selectively for response execution cued by sad faces, with the shift from positively correlated activation for placebo to negatively correlated activation for guanfacine correlated with improvements in the poor accuracy of responses to sad faces in the placebo condition. The selective action of

guanfacine on cognitive control networks centered in DLPFC may offer a possible mechanism to address the emotional reactivity and regulation deficits commonly seen in several psychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effect of face emotion and drug on go/no-go task performance in 15 healthy adults. (a) Mean percentage of correct inhibitions on no-go trials cued by happy, sad, and neutral faces following placebo and 1 mg guanfacine. (b) Mean percentage of correct response on go trials cued by happy, sad, and neutral faces following placebo and 1 mg guanfacine. Error bars indicate 1 SD. * $p = 0.004$; ** $p = 0.001$.

Figure 2.

Guanfacine modulates the influence of face emotion on the psychophysiological interaction (PPI) of amygdala and inferior frontal gyrus in 15 healthy adults. (a) Trial type $\times$ face emotion $\times$ drug interactions on the PPI of left and right amygdala with overlapping regions in left inferior frontal gyrus pars orbitalis and anterior middle temporal gyrus. Values indicate x , y , and z coordinates in Montreal Neurological Institute space. The figures are cluster corrected at $p < 0.01$. (b) Regression coefficients (β values) for the BOLD signal in left amygdala are plotted against β values for the BOLD signal in left inferior frontal gyrus for correct go and no-go trials cued by happy, sad, and neutral faces following placebo and 1 mg guanfacine.

Figure 3.

Correlation of drug effects on psychophysiological interaction (PPI) and go/no-go task performance in 15 healthy adults. The difference in regression coefficients (β values) for the PPI of left and right amygdala with inferior frontal gyrus between the placebo and guanfacine conditions are plotted against the difference in the percentage of correct responses on go trials between the placebo and guanfacine conditions for the sad face emotion condition.

Table 1
Psychophysiological Interactions with Left and Right Amygdala that Varied as a Function of Trial Type, Face Emotion, and/or Drug Condition in 15 Healthy Adults

Effects and Brain Regions	BA	Left Amygdala				Right Amygdala				F Value	F Value
		x	y	z	Vol (mm ³)	x	y	z	Vol (mm ³)		
Trial Type ^a											
R dorsal anterior cingulate cortex	24					16	12	28		3,664	17.02
R primary motor cortex	4					32	-4	24		1,240	14.03
R temporoparietal junction	40					38	-38	26		2,016	11.41
L temporoparietal junction	40					-36	-42	30		5,008	11.26
Face Emotion ^b											
R posterior cingulate cortex	23	12	-46	2	1,232						8.95
L posterior cingulate cortex	31	-4	-48	38	1,032						7.72
Drug ^c											
L posterior cingulate cortex	23	-12	-56	16	1,952	-16	-58	18		1,552	17.78
L cuneus	18	-6	-90	10	2,760	-2	-90	8		2,168	23.79
Trial Type × Face Emotion ^d											
R frontal operculum	44	44	12	28	1,584						9.42
L inferior parietal lobule	40					38	12	32		1,360	9.55
						-30	-38	46		912	10.95
Trial Type × Face Emotion × Drug ^e											
L inferior frontal gyrus	47	-44	34	-12	1,184						8.55
L middle temporal gyrus	21	-40	10	-30	1,048	-44	32	-10		1,784	13.76
						-36	2	-32		1,128	12.35
L middle temporal gyrus	21	-56	-2	-10	3,000	-36	-16	-16		2,768	11.02
L temporoparietal junction	22					-44	-50	20		936	8.40

Note: The results were generated using voxel-wise 2 (Trial: go, no-go) × 3 (Emotion: happy, sad, neutral) × 2 (Drug: guanfacine, placebo) analyses of covariance (ANCOVA). Statistical significance was set at a height threshold of $p < .005$ and a cluster volume threshold of 800 mm³ (100 voxels × 2 mm³), which corrected for multiple voxel comparisons at $p < .01$. Montreal Neurological Institute coordinates and F statistic refer to the peak voxel of activation within each cluster. There were no significant Emotion × Drug and Trial × Drug interaction effects.

BA, Brodmann area; L, left; R, right.

^a go > no-go

^b neutral > happy > sad

placebo > guanfacine
 d go > no-go for sad no-ou *my* pas go for happy and neutral
 e placebo > guanfacine (go for sad and no-go for happy)