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Failure of Anterior Cingulate Activation and Connectivity with the Amygdala During Implicit Regulation of Emotional Processing in Generalized Anxiety Disorder

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Abstract

Objective—Clinical data suggest that abnormalities in the regulation of emotional processing contribute to the pathophysiology of generalized anxiety disorder, yet these abnormalities remain poorly understood at the neurobiological level. We recently reported in healthy volunteers that the pregenual anterior cingulate regulates emotional conflict on a trial-by-trial basis by dampening activity in the amygdala. We also showed that this process is specific to the regulation of emotional, compared to non-emotional, conflict. Here we examined whether this form of non-instructed emotion regulation is perturbed in generalized anxiety disorder.

Methods—17 patients and 24 healthy comparison subjects, were studied using functional magnetic resonance imaging while they performed an emotional conflict task, which involved categorizing facial affect while ignoring overlaid affect label words. We compared trial-by-trial changes in conflict regulation using behavioral and neural measures.

Results—Healthy subjects effectively regulated emotional conflict from trial-to-trial, even though they were unaware of having done so. By contrast, generalized anxiety disorder patients were completely unable to regulate emotional conflict and failed to engage the pregenual anterior

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cingulate in ways that would dampen amygdalar activity. Moreover, performance and brain activation correlated with symptoms and could be used to accurately classify the two groups.

Conclusions—Our data demonstrate that patients with generalized anxiety disorder show significant deficits in the non-instructed and spontaneous regulation of emotional processing. Conceptualization of anxiety as importantly involving abnormalities in emotion regulation, particularly a type occurring outside of awareness, may open up avenues for novel treatments, such as by targeting the medial prefrontal cortex.

INTRODUCTION

Generalized anxiety disorder is characterized by frequent and difficult to control episodes of free-floating anxiety or worry (1). Cognitive models suggest that worry reflects an over-learned compensatory strategy for dulling emotional experience (2). However it is unclear why emotional experiences in these patients necessitate the use of this cognitively costly regulatory strategy. Seen from the perspective of emotion regulation, patients with generalized anxiety disorder may resort to worry because of an underlying abnormality in regulating emotional processing (3–5).

Studies in other anxiety disorders predict that the amygdalae of generalized anxiety disorder patients would be hyper-reactive to negative emotional stimuli (6). Two studies in adolescents with generalized anxiety disorder support this prediction (7, 8); two similar studies in adults fail to (9, 10). One study in adults with generalized anxiety disorder even found amygdalar hyporesponsiveness to fearful expression faces (9), though another recent study found non-specifically exaggerated amygdalar reactivity to warning cues preceding either aversive or neutral pictures (11). We have recently reported, using resting-state fMRI functional connectivity and voxel-based morphometry, on an intra-amygdalar perturbation at a subregional level in adults with generalized anxiety disorder (12).

Studies of generalized anxiety disorder also point to an important role for the prefrontal cortex. Paulesu et al. (13) found that patients were unable to normalize activation in the dorsal anterior cingulate cortex and dorsomedial prefrontal cortex after a worry induction. Pediatric studies have noted exaggerated ventrolateral prefrontal activation to emotional stimuli in patients (7, 14), with the degree of hyperactivation negatively correlated with anxiety scores (14), suggesting a compensatory role for the hyperactivation. We also found evidence for compensatory coupling of a lateral prefrontal cortical executive network with patients' amygdalae (12). Thus, while abnormalities appear to exist within both limbic and prefrontal regions in generalized anxiety disorder, the nature of these abnormalities remains poorly understood.

Use of an experimental paradigm in which emotion regulation can be tracked from trial-to-trial may therefore be a fruitful approach to understanding generalized anxiety disorder. We recently reported on a facial affect identification emotional conflict task, in which subjects were asked to identify the expression of a face (fearful or happy) while ignoring an overlying emotion word ("fear" or "happy"), which either matches (congruent) or conflicts (incongruent) with the facial expression. Reaction time interference by emotionally incongruent stimuli was seen in nearly every subject (15, 16). Interestingly, there is less

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conflict, indexed by faster reaction times, for incongruent trials if they are preceded by an incongruent trial than if they are preceded by a congruent trial (15–20), suggesting that the emotional conflict generated by incongruency on the previous trial activates a regulatory mechanism that leads to improved emotional conflict regulation on the current incongruent trial (16, 21–23) – thus optimizing task performance. We termed this across-trial effect "emotional conflict adaptation" (15, 16), in reference to the label previously applied to similar congruency sequence effects observed in non-emotional conflict tasks (21). Likewise, performance on post-congruent congruent trials is often superior to that on post-incongruent congruent trials (21).

To date, the cognitive model that best accounts for the conflict adaptation effect, after eliminating potential confounders (24), is the "conflict monitoring hypothesis" (17–23, 25, 26). According to this model, conflict is continuously evaluated, such that greater conflict regulation can be flexibly recruited as required by the amount of conflict. Thus, the conflict-monitoring hypothesis distinguishes between two important functions – conflict evaluation and conflict regulation. Many previous studies have examined the regions associated with these functions during adaptation to non-emotional conflict (e.g. color-word Stroop or flanker tasks), by comparing activity during incongruent trials that differ only with respect to whether they were preceded by a congruent or incongruent trial (17, 18, 20–23, 25, 26). Regions whose activity tracks the amount of conflict (i.e. post-congruent incongruent trials > post-incongruent incongruent trials) have been interpreted as conflict evaluation regions (17, 18, 20–23, 25, 26). Regions showing the opposite effect (post-incongruent incongruent trials > post-congruent incongruent trials), have been interpreted as conflict regulation regions (17, 18, 20–23, 25, 26), as activity in these regions is greatest when conflict is minimized through regulation. Importantly, because these contrasts compare physically identical incongruent trials, the behavioral and neural effects differ only by virtue of expectation created by conflict on the previous trial (17–23, 25, 26).

In previous studies (15, 16) we applied this logic to the analysis of adaptation in a novel emotional conflict task. Greater activity during post-incongruent incongruent trials (i.e. regulation-related) was seen in the pregenual anterior cingulate, and this was accompanied by strong negative coupling between the pregenual cingulate and the amygdala. These findings are consistent with other contexts in which emotion regulation is observed (27–29). By contrast, greater activity during post-congruent incongruent trials (i.e. evaluation-related) was seen in the amygdala and dorsal anterior cingulate/dorsomedial prefrontal cortex.

We also compared activations during emotional conflict adaptation with those during non-emotional conflict adaptation (gender identification with the same emotional faces, while ignoring gender words overlaid on the faces), to determine the specificity of activations for emotion. Pregenual cingulate activation and coupling with the amygdala was specific to emotional conflict adaptation, whereas dorsal anterior cingulate/dorsomedial prefrontal cortex activation was shared by emotional and non-emotional conflict (15, 16), consistent with the role of these latter regions in the evaluation of conflict in many other studies of non-emotional conflict adaptation (17, 20, 25, 26).

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Considering that the clinical phenomenology of generalized anxiety disorder suggests that a deficit in the regulation of emotional processing is at the core of this disorder, we hypothesized that patients would show abnormalities in adapting to emotional conflict in our task. Additionally, to better understand emotional conflict adaptation more generally, and thus enhance interpretation of abnormalities in patients, we investigated in a separate cohort of healthy subjects whether they are aware of these trial-to-trial adaptation effects, and thus whether conscious attention is required for this process. We hypothesized that subjects would not be aware of the adaptation effect, and thus that this process is carried out at an implicit level.

METHODS

Participants

A total of 41 subjects, recruited through local online advertisements, participated in the fMRI component of this study, after providing informed consent. DSM-IV-based psychiatric diagnoses (1) were determined through both an informal clinical interview with a psychiatrist, and the MINI structured diagnostic interview (30, 31). Generalized anxiety disorder was the primary diagnosis for all patients in terms of both onset and severity. Exclusion criteria were bipolar, psychotic, substance abuse or post-traumatic stress disorders. Patients with comorbid major depression were not included in this study. Other exclusion criteria included a history of a neurological disorder, head trauma or loss of consciousness, claustrophobia or regular use of benzodiazepines, opiate or thyroid medications. No patient was taking regular psychiatric medications, or used an “as needed” benzodiazepine within 48 hours of the scan. No patient had ever received an evidence-based structured psychotherapy, and only five of the patients had ever received antidepressant medication. Nine patients had no comorbidities, five had one comorbidity (dysthymia 2, social anxiety 3), three had two comorbidities (social anxiety and panic disorder 2, social anxiety and OCD 1), and none had more than two comorbidities. All controls were free of any current or past Axis I conditions or psychiatric medications. All participants completed the Spielberger State-Trait Anxiety Inventory (32), the Penn-State Worry Questionnaire (33), the Beck Anxiety Inventory (34), the Beck Depression Inventory (35), and the Mood and Anxiety Symptoms Questionnaire (36, 37), from which the anxious arousal and anhedonic depression subscales were used. Resting state data from 9 of the healthy comparison subjects and 10 of the patients were included in our recent study (12). The behavior-only study was conducted on a group of 19 healthy subjects (25.2 ± 1.0 years old, 13 females) that did not overlap with the healthy subjects involved in the fMRI study.

Experimental paradigm

The emotional conflict task was performed as previously described (15, 16). Stimuli were presented with Presentation software (Neurobehavioral Systems, http://nbs.neuro-bs.com), during fMRI scanning and displayed using a custom-built MRI-compatible projection system. The task consisted of 148 presentations of happy or fearful facial expression photographs drawn from the set of Ekman and Friesen (38), overlaid with the words “FEAR” or “HAPPY”. Stimuli were presented for 1000ms, with a varying inter-stimulus interval of 3000–5000ms (mean 4000ms), in a pseudo-random order, counterbalanced across

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trial types for expression, word, response button and gender. Subjects indicated facial affect with a button press response. Behavioral data were analyzed in SPSS (SPSS, Inc., Chicago, IL). For the behavior-only task, a questionnaire was administered after the task to assess subjects' awareness of the conflict adaptation effect.

fMRI data acquisition

Images were acquired on a 3T GE Signa scanner using a custom-built head coil. 29 axial slices (4.0 mm thickness with 0.5mm gap) were acquired across the whole brain using a T2* weighted gradient echo spiral pulse sequence (TR = 2000 msec, TE = 30 msec, flip angle = 80°, 1 interleave, FOV 22cm, 64×64 matrix) (39). To reduce blurring and signal loss arising from field inhomogeneities, an automated high-order shimming method based on spiral acquisitions was used before acquiring functional MRI scans (40). A high resolution T1 weighted spoiled grass gradient recalled (SPGR) inversion recovery 3D MRI sequence was used with the following parameters: TI = 300 msec, TR = 8 msec; TE = 3.6 msec; flip angle = 15°; 22 cm field of view; 124 slices in coronal plane; 256 × 192 matrix; 2 NEX, acquired resolution = 1.5 × 0.9 × 1.1 mm. The images were reconstructed as a 124 × 256 × 256 matrix with a 1.5 × 0.9 × 0.9 mm spatial resolution.

fMRI data analysis

The first 5 volumes were not analyzed to allow for signal equilibration effects. A linear shim correction was applied separately for each slice during reconstruction using a magnetic field map acquired automatically by the pulse sequence at the beginning of the scan (39). Functional MRI data were then preprocessed using SPM5 software (http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab (Mathworks, Inc., Natick, MA). Images were realigned to correct for motion, slice timing-corrected, spatially transformed to the Montreal Neurologic Institute coordinate system (41), resampled every 2 mm and smoothed with a 6mm full-width half-maximum (FWHM) Gaussian kernel. During preprocessing, the effects of global signal were also removed separately for each voxel (42). A 128sec temporal high-pass filter was applied to the data and temporal autocorrelation was estimated using a first-order autoregressive model. Separate regressors for the stimulus events (convolved with a canonical HRF) were created for post-congruent incongruent trials, post-incongruent incongruent trials, post-congruent congruent trials and post-incongruent congruent trials, with error and post-error trials modeled separately. Additional regressors-of-no-interest corresponding to the six motion parameters were also included. This model was applied to normalized data in the context of a generalized linear model (43), and submitted to group-level random-effects analyses using two-sample t-tests. As described previously and above (15, 16), our contrasts took advantage of the conflict adaptation effect to compare activity during incongruent (or congruent) trials for which behavior differs by virtue only of expectation created by the previous trial type (e.g. post-incongruent incongruent trials minus post-congruent incongruent trials).

For the psychophysiologic interaction analyses (44), we extracted for each subject a deconvolved time-course from the healthy subject group-level contrast of post-incongruent incongruent trials minus post-congruent incongruent trials (p=0.01). Activity within the amygdala was then regressed on a voxel-wise basis against the product of this time-course

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and the vector of the psychological variable of interest, with the physiological and the psychological variables serving as regressors of no interest, along with the six motion parameters. The results were then taken to a random effects group analysis using two-sample t-tests.

We report results within independently-defined a priori regions of interest based only on our prior data with the emotional conflict task (15, 16), using small-volume corrections (45) ($p<0.05$, family-wise error-corrected). Specifically, to determine the optimal center coordinates for spherical regions of interest, we averaged the medial prefrontal or anterior cingulate peak coordinates from our previous studies in healthy subjects scanned with the identical task and created spheres (intersected with a dilated gray matter mask) of 12mm radius around these coordinates for the dorsomedial prefrontal cortex [5 33 31] (6848 mm^3) and pregenual cingulate [−10 42 0] (5696 mm^3). In this way, our statistical inferences in the current study are directly driven by a priori hypotheses about spatial location of effects of interest from our previous studies. The amygdala region of interest corresponded to the bilateral amygdala in WFU PickAtlas (left: 12×10×18mm (1264 mm^3); right: 14×12×16mm (1288 mm^3)(46). Results are displayed within these regions of interest only.

RESULTS

Behavior

Our patient and control groups were well matched for age, gender, handedness and education (see table 1). No group difference was noted in either overall reaction times or accuracy (healthy: reaction time 793±22ms, accuracy 94.9±0.8%; patients: reaction time 872±58ms, accuracy 93.4±1.4%; $t(39)<1.4$; $p>0.15$ for accuracy and reaction time). Emotional conflict slowed reaction times similarly in both groups (incongruent minus congruent trial difference), including in every healthy comparison subject and in all but one patient (healthy: $t(23)=6.77$; $p<0.000001$; Cohen's $d=1.4$; patient: $t(16)=5.82$; $p<0.00005$; $d=1.4$); group comparison: $t(39)=0.09$; $p>0.9$; see figure 1B). There was a significant group difference in across-trial reaction time adjustment related to emotional conflict adaptation during incongruent trials ($t(39)=2.39$; $p<0.05$; $d=0.8$; see figure 1B). This effect was driven by the predicted faster performance of healthy comparison subjects on post-incongruent incongruent trials than post-congruent incongruent trials ($t(23)=2.19$; $p<0.05$; $d=0.45$, see figure 1B). Patients with generalized anxiety disorder failed to show this effect. By contrast, for congruent trials, exposure to an immediately preceding congruent trial produced similarly significant reaction time facilitation in both groups (healthy $t(23)=3.26$; $p<0.005$; $d=0.66$, patient $t(16)=2.87$; $p=0.01$; $d=0.7$; group comparison: $t(39)=0.93$; $p>0.35$; see figure 1B, and supplemental table 1).

Finally, we asked a separate group of healthy subjects whether they were aware of any pattern across trials that may help or hinder their performance. No subject mentioned previous trial conflict. In addition, discrimination in a forced-choice question of whether performance on a current incongruent trial was improved by a previous incongruent trial versus a previous congruent trial did not differ from chance ($p>0.25$), suggesting that conscious awareness of the adaptation phenomenon is not required for successful adaptation.

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Abnormal medial prefrontal responses to emotional conflict in patients

We first examined overall responses to emotional conflict (i.e. incongruent>congruent). As seen in figure 2A, healthy comparison subjects showed greater activation to emotional conflict than generalized anxiety disorder patients in the dorsomedial prefrontal cortex ([0 36 38], z=3.96, d=1.22, 2832 mm^3; [6 44 34], z=3.33, d=1.14). This difference resulted from activation by emotional conflict within this cluster in comparison subjects (t(23)=3.9; p=0.001; d=0.8) but not in patients (t(16)=1.84; p>0.05; see figure 2B). No group differences were noted in the pregenual cingulate or amygdala.

Next, we explored the neural correlates of group differences in emotional conflict adaptation, guided by our behavioral results. Based on our previous findings with the emotional conflict task in healthy subjects (15, 16), we examined the contrast of post-incongruent incongruent trials minus post-congruent incongruent trials in the pregenual cingulate in patients and healthy comparison subjects, and found a significant cluster ([−12 32 −4], z=3.49, 376 mm^3; d=1.2; see figure 3A). Average signal within this cluster was extracted for each group to further describe the effect. As predicted, in this cluster, healthy comparison subjects had greater activity during post-incongruent incongruent trials (t(23)=3.34; p<0.005; d=0.68), while in patients no difference was found (t(16)=1.6; p>0.1; see figure 3B).

Next, we examined the contrast of post-congruent incongruent trials minus post-incongruent incongruent trials in the dorsomedial prefrontal cortex and amygdalae in both groups, and found a significant cluster in the dorsomedial prefrontal cortex ([−1 36 38], z=3.26, 568 mm^3; d=1.15; see figure 3C) but not the amygdala. Extraction of average signal within this cluster revealed that the group difference was driven both by the expected greater activity in post-congruent incongruent trials in healthy subjects (t(23)=2.36; p<0.05; d=0.48), and the opposite effect in patients (t(16)=2.66; p<0.05; d=0.64; see figure 3D). Note that the inability of patients to decrease dorsomedial prefrontal activity in post-incongruent incongruent trials paralleled patients' inability to improve reaction times during these trials, when compared to healthy subjects. No group differences were noted in any of the regions of interest for the contrast of post-congruent congruent trials with post-incongruent congruent trials. Finally, comparing across all trial types, we found significantly greater activation in patients than in comparison subjects within the left amygdala ([−22 −2 −18], z=3.04, 232 mm^3; d=1.04). Using cytoarchitectonic probability maps of the basolateral, centromedial and superficial amygdalar subregions (47, 48), we found that 78% of this cluster corresponded to the superficial amygdala and 21.1% to the basolateral amygdala.

Absent pregenual cingulate-amygdalar connectivity in patients

We next examined differential functional connectivity between the pregenual cingulate and the amygdala during post-incongruent incongruent trials versus post-congruent incongruent trials using psychophysiological interaction analyses, with the pregenual cingulate as the seed and the amygdalae as the targets, while controlling for task-related activations in both regions, and task non-specific connectivity (44). As seen in figure 4A, we found a significant group difference in both the left ([−20 −4 −22], z=3.54, 536 mm^3; d=1.15) and right amygdala ([30 −4 −22], z=3.4, 168 mm^3; d=1.15). Extraction of average connectivity

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strength within these clusters revealed that the group effect resulted from the predicted significant negative pregenual cingulate-amygdalar connectivity in healthy comparison subjects during post-incongruent incongruent trials, compared with post-congruent incongruent trials (left $t(23)=4.14$; $p<0.001$; $d=0.85$; right $t(23)=3.08$; $p=0.005$; $d=0.63$), but not in patients (left $t(16)=1.23$; $p>0.2$, right $t(16)=1.44$; $p>0.1$; see figure 4B). We did not pursue further characterization of differential group cingulate-amygdala connectivity using effective connectivity methods such as dynamic causal modeling, as we had in a previous study of healthy subjects (16), since we did not feel that it would add significant new information beyond the result from the functional connectivity analysis above. Finally, we found that the majority of the left amygdala differential connectivity cluster was in the basolateral amygdala (55%), with 44.3% in the superficial amygdala, and only 0.4% in the centromedial amygdala. 100% of the right amygdalar cluster was in the basolateral amygdala.

Additional findings

We conducted several additional analyses to better understand the group differences reported above. First, for the patients, we correlated symptom scale scores with behavior and brain activity, within the group difference clusters. We found that the impairment in emotional conflict adaptation was greatest, in terms of both reaction times and dorsomedial prefrontal modulation, for the most anxious patients (see supplemental materials online). Second, we conducted multivariate pattern classification to determine if behavior and brain activation could be used to determine subjects' diagnostic group. Significant classification of patients and healthy subjects could be achieved with both behavior and brain activation data, reaching 95% when whole brain data was used (see supplemental materials online).

DISCUSSION

In this study, we investigated emotional conflict adaptation using a paradigm in which emotional processing is regulated spontaneously and in the absence of explicit instruction. We found that patients with generalized anxiety disorder were unable to adapt to emotional conflict through engagement of this regulatory process. By contrast, adaptation during congruent trials was similar in both groups, as was the overall reaction time interference due to emotional conflict, demonstrating the specificity of the deficit.

At the neural level, patients with generalized anxiety disorder failed to activate the pregenual cingulate and demonstrate negative "top-down" (16) pregenual cingulate-amygdalar connectivity during the regulation of emotional conflict. As in previous studies of emotion regulation (27, 28), regulation-related changes in activity were seen in the context of overall task-independent medial prefrontal deactivation from an implicitly modeled baseline, and this deactivation did not differ between our groups (data not shown). Moreover, since the critical contrast involves only incongruent trials, the many processes that differ between incongruent and congruent stimuli are controlled for, as are non-specific responses to task demands, leaving only the effect of previous trial conflict on processing of emotional conflict on the current trial.

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We suggest that patients' failure to show the neural effects related to previous trial conflict accounts for their behavioral regulatory deficit during emotional conflict adaptation, in accordance with predictions made by the conflict monitoring hypothesis about brain activity during conflict adaptation – a cognitive model supported by an extensive neuroimaging literature (17, 18, 20–23, 25, 26). These conclusions are also consistent with emotion regulatory roles attributed to ventromedial prefrontal regions, through connectivity with the amygdala, in other studies (27–29, 49). Moreover, we recently found functional connectivity and structural evidence for an intra-amygdalar abnormality at a subregional level in generalized anxiety disorder (12). Thus, it appears that patients have deficits in both activating relevant control regions (pregenual cingulate) and in the connectivity required for such regions to exert control over limbic structures. It is therefore interesting to speculate that the lateral prefrontal hyperactivation (7, 9, 14) or increased connectivity with the amygdala (12) previously reported in generalized anxiety disorder patients may reflect the compensatory engagement of worry, an attention-demanding cognitive process, to regulate the effects of emotional stimuli in the absence of patients being otherwise able to recruit pregenual cingulate-based regulatory mechanisms.

Finally, there is controversy regarding overall emotional responsiveness in generalized anxiety disorder, indexed largely through activation in the amygdala, with pediatric studies showing hyperactivity to negative emotional expression faces (7, 8), and adult studies showing either no difference (10) or hypoactivation (9) to similar stimuli, with another adult study showing non-specifically exaggerated amygdalar reactivity to both negative emotional and neutral cues (11). Consistent with the latter study, we found greater amygdalar activity in patients during both congruent and incongruent trials.

Our data also highlight an emerging theme in affective neuroscience – namely, that there are many ways by which emotional processing is regulated, and that deficits in these functions contribute importantly to psychopathology. To date, the neurobiology underlying the regulation of emotional processing has been primarily studied by asking subjects to deliberately alter their emotional responses to defined stimuli (i.e. "explicit" regulation)(50). Much of the normal regulation of emotional processing, however, likely occurs in the absence of explicit effort (51, 52). Far less is known about these "implicit" forms of regulation.

Based on the fact that behavioral performance in our task indicates the engagement of a mechanism for regulating emotional processing that occurs in the absence of specific regulation instructions, we and others have argued that emotional conflict adaptation is a type of implicit regulation of emotional processing (51, 52). In this study, we provide direct behavioral evidence for this in healthy subjects. The striking deficit in emotional conflict regulation in patients, in the context of otherwise intact task performance, provides the strongest evidence to date linking abnormalities in a defined form of implicit regulation and a type of psychopathology whose clinical presentation suggests emotion regulatory abnormalities.

Several limitations are important to note. First, we are unable to report on subjective ratings of emotion during emotional conflict adaptation, as asking subjects to report on subjective

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emotional states may itself lead to emotion regulation (53–57). Thus, the we infer the effects of emotion from behavioral indices, such as reaction times, and patterns of brain activation. Second, though we focused in this study primarily on the neural effects we previously found to be specific to emotional conflict (15), it would be useful in future experiments to also examine adaptation to non-emotional conflict. Finally, it is unknown whether medial prefrontal dysfunction during emotional conflict adaptation reflects a disorder-specific abnormality or a more general endophenotype of affective disorders, such as major depression. Nonetheless, the robust group differences seen at both the behavioral and neural levels suggest that the inability of patients to adapt to emotional conflict is an important aspect of the pathophysiology of generalized anxiety disorder – and potentially of other psychiatric disorders – and thus merits continued, deeper, study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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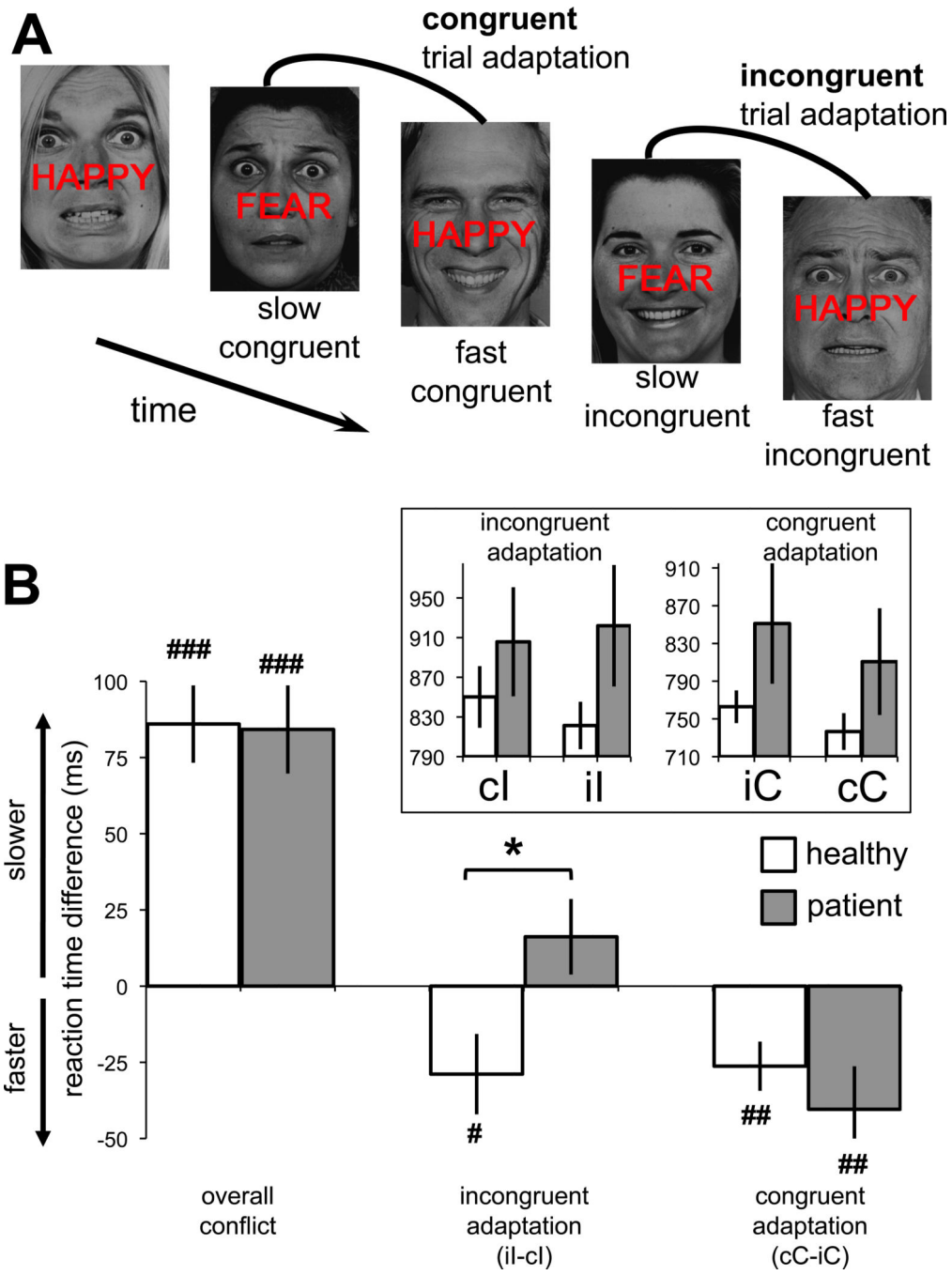

Figure 1.

Patients with generalized anxiety disorder fail to adapt to emotional conflict. (A) Example task time course, illustrating the contrasts made to examine adaptation during congruent or incongruent trials. (B) Reaction time difference scores reflecting the overall effect of emotional conflict (incongruent minus congruent trials), the facilitation in reaction times during emotional conflict adaptation (post-incongruent incongruent trials (iI) faster than post-congruent incongruent trials (cI), resulting in a negative reaction time difference score), and similar adaptation on congruent trials (post-congruent congruent trials (cC) faster than

post-incongruent congruent trials (iC)). A group difference was found only during adaptation on incongruent trials. Error bars are standard errors of the mean. Two-sample t-test: * $p<0.05$, one-sample t-test: # $p<0.05$, ## $p<0.01$, ### $p<0.001$. The inset shows reaction times for each condition (see supplemental materials online for a detailed table).

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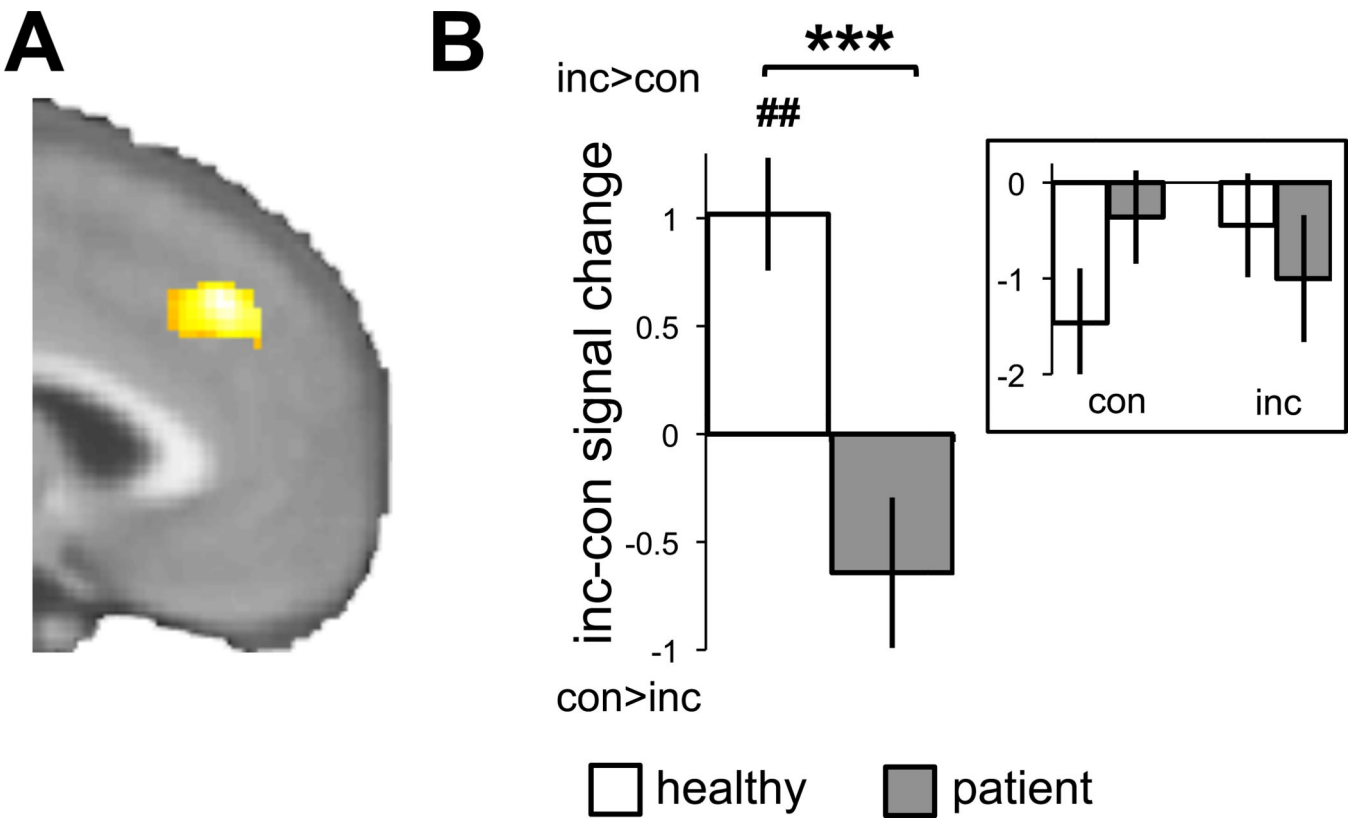

Figure 2.

Deficits in dorsomedial prefrontal activation to emotional conflict in patients. (A) Healthy comparison>patient contrast for the incongruent (inc) minus congruent (con) trial difference within the dorsomedial prefrontal region of interest, with each group's data extracted for the cluster in (B), for both difference scores and individual trial types (inset). Only healthy comparison subjects were found to activate the dorsomedial prefrontal cortex in response to emotional conflict. Error bars are standard errors of the mean. Two-sample t-test: *** $p<0.001$, one-sample t-test: ## $p<0.01$.

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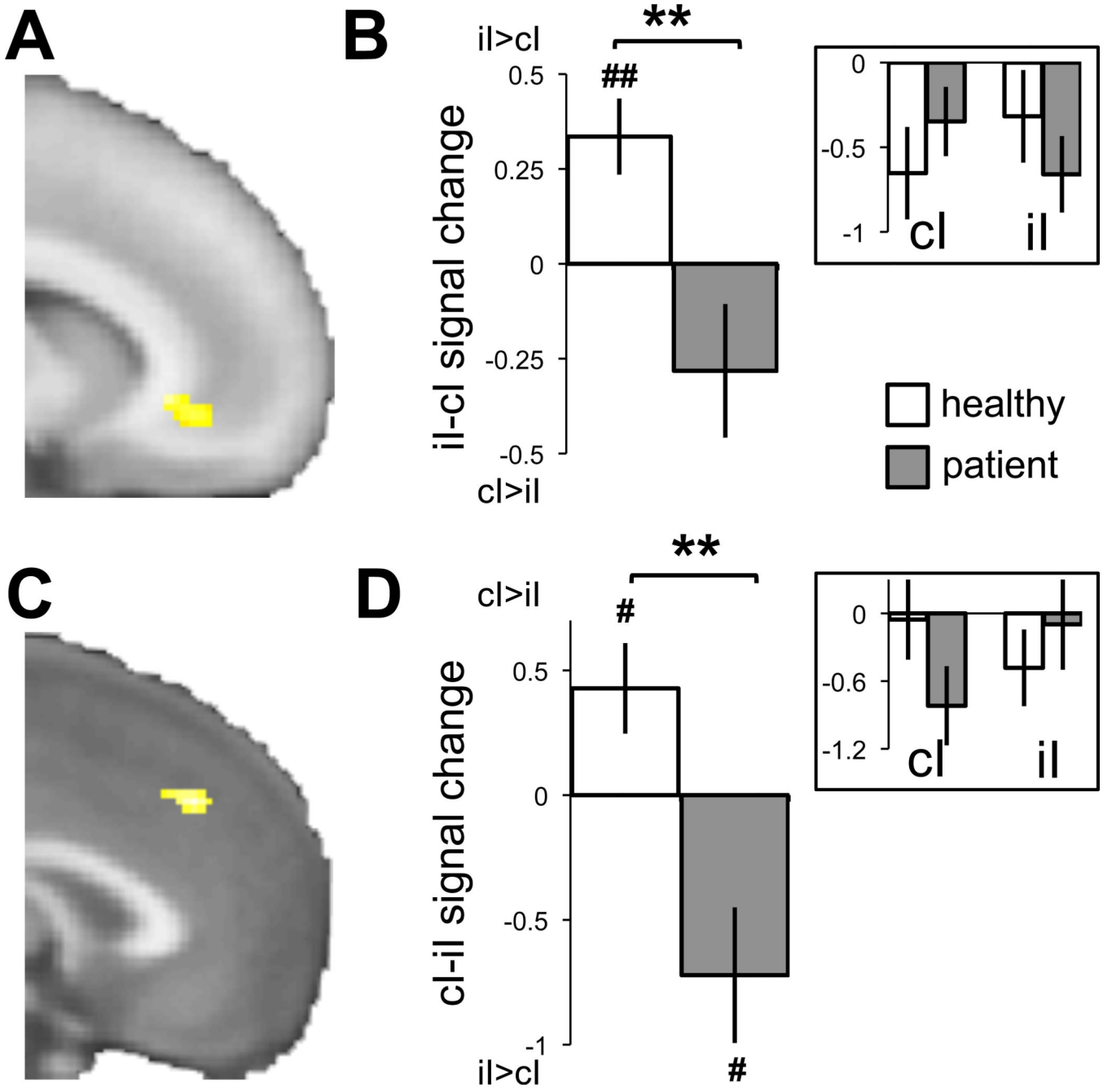

Figure 3.

Inability of patients to activate the pregenual cingulate and modulate dorsomedial prefrontal activity during emotional conflict adaptation. (A) Healthy comparison>patient contrast for the post-incongruent incongruent trial (iI) minus post-congruent incongruent trial (cI) difference within the pregenual cingulate region of interest, with each group's data extracted for the cluster in (B), for both difference scores and individual trial types (inset). Only healthy comparison subjects were found to activate the pregenual cingulate. (C) Healthy comparison>patient contrast for the post-congruent incongruent trial (cI) minus post-incongruent incongruent trial (iI) difference within the dorsomedial prefrontal region of

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interest, with each group's data extracted for the cluster in (D), for both difference scores and individual trial types (inset). Healthy comparison subjects were found to show less dorsomedial prefrontal activity in post-incongruent incongruent trials (hence a positive difference score). By contrast, in patients, there was inappropriately greater activity in the dorsomedial prefrontal cortex in response to post-incongruent incongruent trials (i.e. negative difference scores). Error bars are standard error of the mean. Two-sample t-test: ** $p<0.01$, one-sample t-test: # $p<0.05$, ## $p<0.01$.

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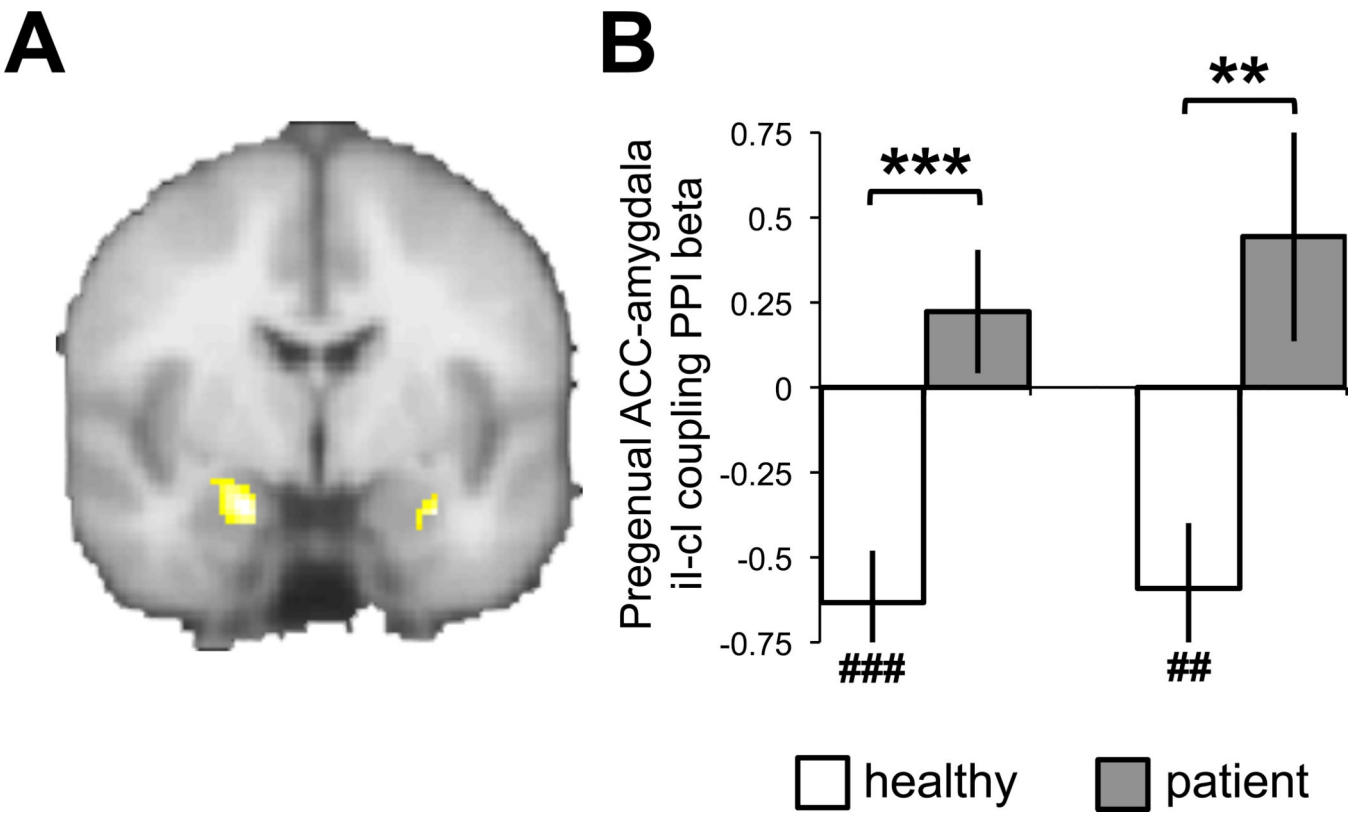

Figure 4.

Absence of negative pregenual cingulate-amygdalar connectivity during emotional conflict adaptation in patients. (A) Healthy comparison>patient contrast for the psychophysiological interaction functional connectivity analysis between the pregenual cingulate during post-incongruent incongruent trials versus post-congruent incongruent trials (iI-cI) and the amygdala, with each group's clusters in (B). Only healthy comparison subjects showed robust negative connectivity between the pregenual cingulate and the amygdalae during post-incongruent incongruent trials. Error bars are standard errors of the mean. Two-sample t-test: ** $p<0.01$, *** $p<0.001$, one-sample t-test: ## $p<0.01$, ### $p<0.001$. Coronal sections are displayed in neurological convention (i.e. left=left).

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Table 1

fMRI study subject demographics

	controls	patients	p-value
N	24	17	
age	36.5 (±11.8)	31.5 (±9.9)	n.s.
yrs educ	17.3 (±2.0)	16.6 (±2.1)	n.s.
% female	75 (N=18)	65 (N=11)	n.s.
% R handed	100 (N=24)	100 (N=17)	n.s.
STAI-T	30.6 (±5.4)	51.4 (±8.2)	1.E-09
PSWQ	32 (±8.3)	61.9 (±8.7)	<1.E-12
BAI	3.4 (±3.4)	21.6 (±11.1)	<5.E-06
BDI	3.6 (±3.4)	14.6 (±9.1)	1.E-04
MASQ-AA	18.2 (±1.5)	25.4 (±7.4)	1.E-03
MASQ-AD	48.6 (±10.3)	69.2 (±12.8)	<1.E-05

STAI-T: Spielberger trait anxiety
PSWQ: Penn-State worry questionnaire
BAI/BDI: Beck anxiety/depression inventories
MASQ: Mood and Anxiety Symptom Questionnaire anxious arousal or anhedonic depression subscales numbers in parentheses indicate standard deviations