A Role for the Human Dorsal Anterior Cingulate Cortex in Fear Expression

Mohammed R. Milad, Gregory J. Quirk, Roger K. Pitman, Scott P. Orr, Bruce Fischl, and Scott L. Rauch

Background: Rodent studies implicate the prelimbic (PL) region of the medial prefrontal cortex in the expression of conditioned fear. Human studies suggest that the dorsal anterior cingulate cortex (dACC) plays a role similar to PL in mediating or modulating fear responses. This study examined the role of dACC during fear conditioning in healthy humans with magnetic resonance imaging (MRI).

Methods: Novel analyses were conducted on data from two cohorts that had previously undergone scanning to study fear extinction. Structural and functional brain data were acquired with MRI; the functional MRI (fMRI) component employed an event-related design. Skin conductance response (SCR) was the index of conditioned responses.

Results: We found that: 1) cortical thickness within dACC is positively correlated with SCR during conditioning; 2) dACC is activated by a conditioned fear stimulus; and 3) this activation is positively correlated with differential SCR. Moreover, the dACC region implicated in this research corresponds to the target of anterior cingulotomy, an ablative surgical treatment for patients with mood and anxiety disorders.

Conclusions: Convergent structural, functional, and lesion findings from separate groups of subjects suggest that dACC mediates or modulates fear expression in humans. Collectively, these data implicate this territory as a potential target for future anti-anxiety therapies.

Key Words: Classical, conditioning, fear, galvanic skin response, gyrus cinguli, magnetic resonance imaging, memory

A plethora of data across species highlight the role of the amygdala in the acquisition and expression of learned fear (1–6). Rodent studies, however, suggest that the prelimbic (PL) division of the medial prefrontal cortex (mPFC) also plays a role in conditioned fear expression (7–10). For example, microstimulation of PL increases conditioned freezing (11), whereas inactivation of PL reduces it (12,13). The PL region in rodents seems to be homologous with the dorsal anterior cingulate cortex (dACC) in humans (14,15). The dACC in humans and non-human primates projects to the basolateral amygdala (BLA), similarly to PL in rodents. Moreover, because BLA seems to be involved in mediating conditioned fear (16) and the dACC projections to BLA are excitatory (17), it is plausible that dACC might be involved in mediating or modulating fear expression through excitation of the amygdala. Therefore, we investigated whether the human dACC is implicated in conditioned fear.

Although several human neuroimaging studies have reported incidental activation of the dACC during acquisition of fear conditioning (4,18–21), the role of the dACC in the expression of conditioned fear responses has not been well explored. To address this, we first examined whether the size of the dACC, as measured by cortical thickness, would be correlated with skin conductance response (SCR) to a conditioned stimulus signaling fear (CS+) during acquisition. Second, we examined whether dACC would be activated during fear acquisition and whether such activation would be correlated with the expression of conditioned fear responses. We conducted novel analyses on two separate cohorts of healthy humans that had been previously scanned to study the neural circuits of fear extinction (22,23). We predicted that, consistent with a role of dACC in the generation of conditioned fear, the structure and function of the dACC would be positively correlated with the SCR to conditioned stimuli during fear acquisition.

From the Department of Psychiatry (MRM, RKP, SPO, SLR); Nuclear Magnetic Resonance Center (BF), Massachusetts General Hospital and Harvard Medical School, Boston; McLean Hospital (SLR), Belmont, Massachusetts; Department of Psychiatry (GJQ), University of Puerto Rico School of Medicine, San Juan, Puerto Rico; and the Research Service (SPO), Department of Veterans Affairs Medical Center, Manchester, New Hampshire.

Address reprint requests to Mohammed R. Milad, Ph.D., Department of Psychiatry, Massachusetts General Hospital, Bldg 149, 13th Street, 2nd Floor, Charlestown, MA 02129; E-mail: milad@nmr.mgh.harvard.edu.

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Methods and Materials

Subjects were mentally healthy and ranged in age from 19 to 39. Fourteen subjects (8 men, 6 women) participated in the structural magnetic resonance imaging (MRI) component. Thirteen different subjects (7 men, 6 women) participated in the fMRI component. Written informed consent was obtained in accordance with the requirements of the Partners Healthcare System Human Research Committee.

Reported herein are novel analyses of the aforementioned two separate cohorts that underwent a fear conditioning and extinction protocol as part of previously reported studies (22–24). Here we focus on the relationship between cortical thickness and functional activation of the dACC and conditioned fear expression during the acquisition (conditioning) phase. In brief, participants underwent a differential fear conditioning paradigm in which they viewed pictures of different rooms (contexts) containing a variable colored light (cue). One color was paired with a mild electric shock (unconditioned stimulus [US]) to their fingers (CS+), whereas a different color was not (CS−). The US delivery occurred immediately at CS+ offset.

In the structural component, we used automated methods to measure thickness across the entire cerebral cortex. Thickness at each vertex (resolution unit) was correlated with SCR to the CS+ averaged across trials, and these correlations were mapped as previously described (22). This method allows for an unbiased search of correlates between SCR and cortical thickness.

The functional component used blood oxygen level-dependent (BOLD) signal to measure brain activation. The main effect of stimulus type was first assessed via a CS+ versus CS− contrast across trials. To examine the relationship between activation in the dACC and fear expression, we conducted a voxel-wise

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correlational analysis with differential SCR (CS+ − CS−). For further methodological details, see Supplement 1.

Results

All subjects showed significant increases in SCR to the CS+ relative to the CS−, indicating successful differential conditioning (23,24). We observed a significant positive correlation between thickness in the dACC region (peak vertex: $r = .70$, $p = .005$ [Talairach coordinates −3, 23, 18) and SCR to the CS+ (Figures 1A and 1B). We did not observe a significant correlation that satisfied our statistical threshold ($p = .01$, two-tailed, uncorrected) between dACC thickness and SCR to the CS− ($r = .58$, $p = .03$) or differential SCR (CS+ − CS−) ($r = .57$, $p = .03$).

In the BOLD signal analyses, we observed a significant main effect of stimulus type across conditioning trials. Specifically, dACC activation was significantly higher in response to the CS+ relative to the CS− ($p < .001$, peak at x = 1, y = 21, z = 27, Figure 1C). Voxel-wise correlational analysis revealed a significant correlation between differential SCR and dACC activation ($r = .84$, $p < .001$, x = 3, y = 33, z = 21, Figure 1D). We also examined whether dACC increased its activity to the presentation of the CS− or the US, as a control; but we did not find a significant effect for either. Nevertheless, we conducted further

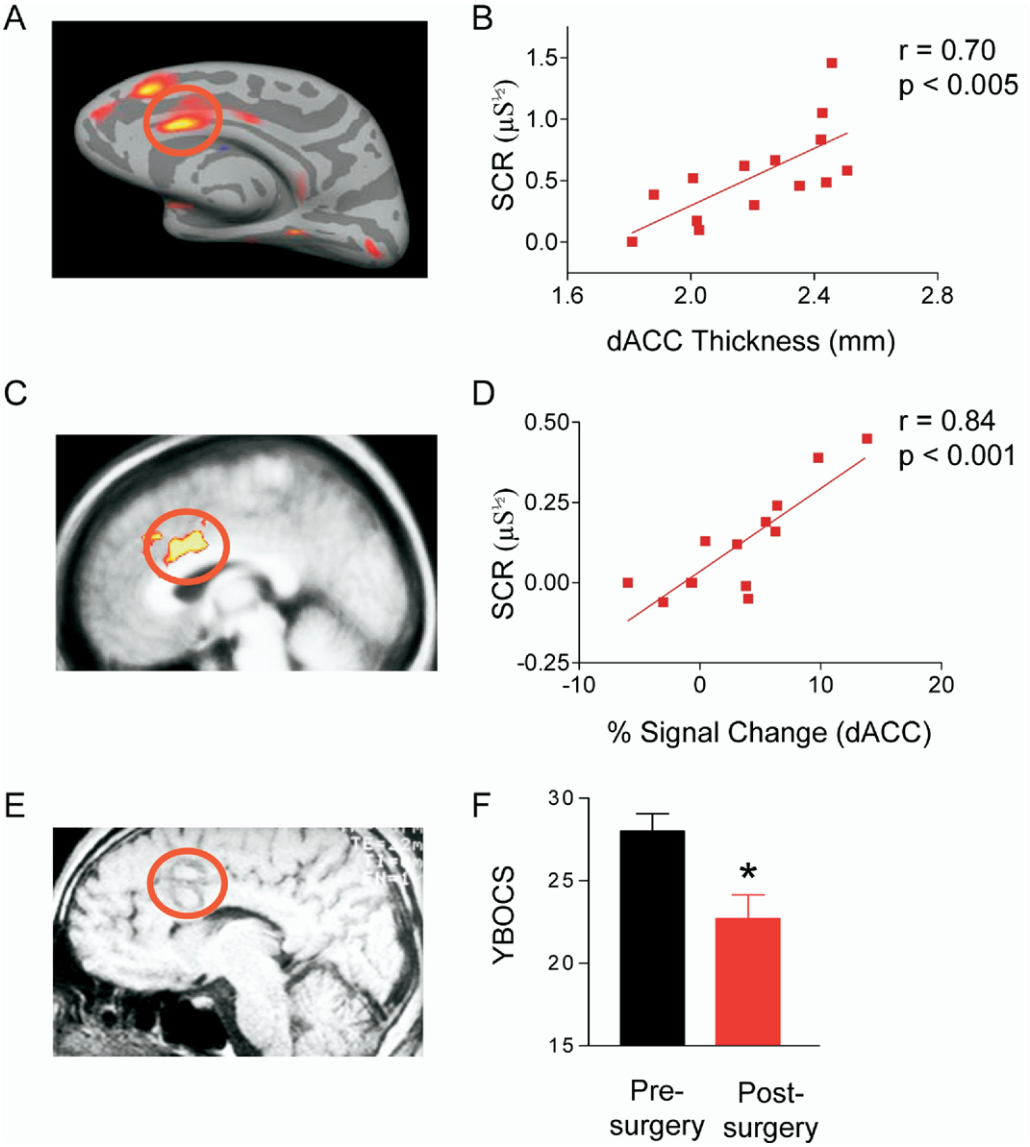

Figure 1. Correlates of dorsal anterior cingulate cortex (dACC) thickness and activation with conditioned fear responses. **(A)** Vertex-based correlational map across the medial cortical surface showing the location of the significant correlation between dACC thickness and fear conditioning (red circle). Threshold is set at $p < .01$ (dark red) to $p < .001$ (bright yellow). **(B)** Regression plot for the correlation between the dACC and skin conductance response to the conditioned stimulus signaling fear (CS+). **(C)** Activation in the dACC during fear acquisition, with the contrast of CS+ versus absence of conditioned stimulus (CS−) across all conditioning trials (red circle). Threshold is set at $p < .01$. **(D)** Regression plot for the correlation between dACC activation and differential skin conductance response (CS+ − CS−). **(E)** A typical anterior cingulotomy lesion, as visualized from parasagittal perspective via a conventional clinical T1-weighted magnetic resonance imaging (MRI) (red circle). **(F)** Reduction of symptom severity in obsessive-compulsive disorder (OCD) symptoms after cingulotomy. Post-surgery mean follow-up time was 36 months with an SD of 32 months. YBOCS, Yale-Brown Obsessive Compulsive Scale score. *$p < .05$. Error bars represent SEM. (Data from Dougherty *et al.* [36]).

analyses to examine the association between dACC response to the CS−, the US, and the corresponding SCRs. With a CS− versus pre-CS− contrast, we found a significant correlation between SCR to the CS− and dACC activation ($r = .70$, $p = .003$, x = −1, y = 30, z = 10). We also found a significant correlation between the unconditioned response (UCR) to the shock and dACC activation during its delivery ($r = .69$, $p = .004$, x = 3, y = 20, z = 24). Because the shock was delivered immediately after the offset of the CS (i.e., during fixation), here the contrast was UCR during fixation versus fixation alone.

To investigate whether dACC was activated as part of an orienting response to any stimulus, we examined whether SCR induced by presentation of the context alone (i.e., before shock) correlated with dACC activity. Here SCR was calculated as peak skin conductance level during context presentation—skin conductance level during the preceding 2 sec. The average SCR to the context was .14 ± .09 SD (square root μS, ranging from −.03 to .26). Novel contrast images for context versus fixation were created for each subject and correlated with SCR to the context on a voxel-wise basis. No significant correlations were observed.

Discussion

We found that dACC thickness was positively correlated with conditioned fear responses to the CS+, as indexed by SCR. In a separate cohort of subjects, dACC functional activation increased to the CS+ relative to the CS− during fear conditioning, and dACC activity was positively correlated with differential SCR. The convergence of structural and functional correlates of conditioned fear responding in the dACC reported herein is remarkable, considering that these two data sets were obtained from separate cohorts. Although the presentation of the CS− and the US did not induce significant activation in the dACC, the SCRs induced by these two stimuli were positively correlated with the variance in dACC activation during these two conditions. This suggests that the dACC might be involved in the expression of fear responses in general. In a finding consistent with this view, Vogt *et al.* (25) suggested that fear is associated with activation specifically in this region of the ACC. Indeed, rodent studies support the involvement of a homologous region, the prelimbic cortex, in the expression of conditioned fear (7,8,11,13).

An alternate interpretation of the present data is that the correlations observed between dACC and SCR might reflect a role of the dACC in detecting salience (i.e., the importance of the stimulus being presented). The data presented herein cannot rule out this possibility. However, presentation of the CS− and the US were also arousing, especially during the early conditioning trials. Yet, we did not observe a significant dACC response to either the CS− or US, which argues against this possibility. Also, SCR induced after the presentation of the context was not correlated with dACC activity. Nonetheless, further studies are needed to more fully delineate the involvement of the dACC in fear expression.

A role for the dACC in the generation of autonomic responses has been previously reported (26). Electrical stimulation of the dACC induces SCRs (27), whereas lesions of this brain region attenuate SCRs (28). Previous neuroimaging studies have reported positive correlations between activation in sub-regions of the ACC (including dACC) and changes in autonomic responses including SCR during high arousal states (reviewed in 29). Non-specific SCRs have been found to be positively correlated with dACC activation during aversive fear conditioning (21). These studies suggest a link between dACC and SCR in general. Other neuroimaging studies, however, have failed to find correlations between resting state or spontaneous fluctuations of SCR and dACC activation (30–32). Furthermore, frontal lesions in humans, including dACC lesions, do not affect resting or orienting SCRs (33). In the present study, the absence of a significant correlation between dACC activation and SCR to the context alone indicates that dACC activation is not associated with changes in SCR under all circumstances.

The location of the dACC region that we found to be correlated with fear expression approximates the target of anterior cingulotomy (34,35), an ablative neurosurgical treatment for patients with treatment-refractory mood and anxiety disorders (see Figure 1E). Up to 40% of patients with severe obsessive compulsive disorder (OCD) who had previously failed to respond to medications and behavioral therapy show marked improvement in OCD symptoms after cingulotomy (Figure 1F) (36). Patients receiving brief intra-operative electrical stimulation of the dACC report feelings of intense fear, whereas ablation of this brain region significantly reduces anxiety symptoms (37). The data obtained herein further suggest that the dACC could be a potential target for future anti-anxiety therapies. Whereas enhancement of function within ventromedial PFC has been proposed as a means for suppressing amygdala responses and facilitating extinction recall (3,23,38–41), neutralizing dACC function might be an effective complementary strategy for ameliorating anxiety.

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Supplementary material cited in this article is available online.

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