Nonconscious Attention Bias to Threat is Correlated with Anterior Cingulate Cortex Gray Matter Volume: A Voxel-based Morphometry Result and Replication

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Nonconscious attention bias to threat is correlated with anterior cingulate cortex gray matter volume: A voxel-based morphometry result and replication

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A B S T R A C T
An important aspect of the fear response is the allocation of spatial attention toward threatening stimuli. This response is so powerful that modulations in spatial attention can occur automatically without conscious awareness. Functional neuroimaging research suggests that the amygdala and anterior cingulate cortex (ACC) form a network involved in the rapid orienting of attention to threat. A hyper-responsive attention bias to threat is a common component of anxiety disorders. Yet, little is known of how individual differences in underlying brain morphometry relate to variability in attention bias to threat. Here, we performed two experiments using dot-probe tasks that measured individuals’ attention bias to backward masked fearful faces. We collected whole-brain structural magnetic resonance images and used voxel-based morphometry to measure brain morphometry. We tested the hypothesis that reduced gray matter within the amygdala and ACC would be associated with reduced attention bias to threat. In Experiment 1, we found that backward masked fearful faces captured spatial attention and that elevated attention bias to masked threat was associated with greater ACC gray matter volumes. In Experiment 2, this association was replicated in a separate sample. Thus, we provide initial and replicating evidence that ACC gray matter volume is correlated with biased attention to threat. Importantly, we demonstrate that variability in affective attention bias within the healthy population is associated with ACC morphometry. This result opens the door for future research into the underlying brain morphometry associated with attention bias in clinically anxious populations.

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Introduction

Within our environment, biologically salient visual cues automatically capture spatial attention (Ohman et al., 2001). This location-specific attentional modulation of perceptual processing is thought to serve as a means to help prioritize the complex and continually changing visual scene. Threatening (fearful and angry) faces are salient signals of potential danger that are detected and processed by the amygdala (Adolphs et al., 1999). Threatening faces have been found to capture spatial attention both when unmasked (Armony and Dolan, 2002; Cooper and Langton, 2006; Mogg and Bradley, 1999; Pourtois et al., 2004) and when awareness has been restricted with backward masking 1 (Carlson and Reinke, 2008; Fox, 2002; Mogg and Bradley, 2002). In addition, other threatening stimuli such as predatory and poisonous animals and weapons capture spatial attention both when unmasked (Blanchette, 2006; Koster et al., 2004; Ohman et al., 2001) and masked (Beaver et al., 2005; Carlson et al., 2009a). Directing attention to potential threat is adaptive; however, elevated attentional bias to threat is commonly associated with high levels of anxiety (Fox, 2002; MacLeod and Mathews, 1988; Mogg and Bradley, 2002) and variation in genotypes associated with increased risk for anxiety (i.e., 5HTTLPR; Beever et al., 2007; Fox et al., 2009; Kwang et al., 2010; Osinsky et al., 2008; Perez-Edgar et al., 2010). Therefore, understanding the neural mechanisms underlying attention bias to threat is important not only for understanding

1 The brief presentation of an initial stimulus that is closely followed by a second “masking” stimulus is referred to as “backward masking.” This masking procedure is thought to interrupt and replace the processing of the initial stimulus with that of the second stimulus (Enns and Di Lollo, 2000).
human emotional responding in general, but also for understanding anxiety-related behaviors and disorders.

Over the past decade there has been accumulating evidence that restricted, nonconsciously processed visual threat signals activate the amygdala (Carlson et al., 2004; Liddell et al., 2005; Morris et al., 1998; Whalen et al., 1998, 2004; Williams et al., 2004, 2006) and the representations of these cues reach the amygdala via a subcortical route through the superior colliculus and pulvinar nucleus of the thalamus (Liddell et al., 2005; Morris et al., 1999, 2001). In addition to subcortical structures, the anterior cingulate cortex (ACC) also appears to be part of the neural system activated in response to nonconsciously processed threats (Liddell et al., 2005; Williams et al., 2006) and is generally involved in attention and emotion-related processing (Bush et al., 2000). Recent evidence suggests the amygdala mediates the automatic directing of spatial attention to backward masked fearful faces (Carlson et al., 2009b; Monk et al., 2008) through a network including the ACC and contralateral visual cortex (Carlson et al., 2009b). Indeed, backward masked threat stimuli elicit modulations in visual cortex within 170 ms after stimulus onset (Carlson and Reinke, 2010) and facilitate the processing of subsequent visual stimuli within the “spotlight of attention” (Carlson et al., 2010b). In sum, the amygdala is sensitive to non-conscious threats and an amygdala-ACC network modulates visual cortical processing at the location of masked threat. This functional network has been implicated in modulations of attention to masked threat; however, it is unclear whether volumes of the structures within this network relate to individual differences in attention to masked threatening stimuli. Given the association between anxiety and biased attention to threat, it is important to understand how variability in volumetric differences within this system relates to behavior.

The aim of the current investigation was to assess how volumetric variations of the amygdala and ACC are related to variability in affective attention bias. We performed two experiments using dot-probe tasks that measured individuals’ attention bias to backward masked fearful faces and collected whole-brain structural magnetic resonance images (sMRI) with 3-Tesla and 1.5-Tesla scanners in Experiments 1 and 2, respectively. We used voxel-based morphometry (VBM) for unbiased (i.e., user independent) computerized comparison of regional brain anatomy in healthy volunteers. Based on the functional neuroimaging data reviewed above, we hypothesized that reduced gray matter volumes in amygdala and ACC would be associated with reduced behavioral indices of attentional bias to backward masked fearful faces.

**Experiment 1**

**Method**

**Participants**

Forty-two individuals (25 male) between the ages of 19 and 25 ($M = 21.2, SD = 1.3$) participated in the study. Thirty-seven reported being right-handed. Potential participants were screened for metal in their bodies. The Institutional Review Board of Stony Brook University approved this study. Participants were compensated for their time ($20/h$).

**Dot-probe task**

E-Prime (Psychology Software Tools, Pittsburg, PA) was used to program and present the task, which was performed outside the scanner in a controlled testing room using a 60 Hz PC monitor for display. Fearful and neutral grayscale faces from four individual identities (half male) were used for the initial (i.e., masked) faces (Gur et al., 2002). The mask was a female (5th identity) open-mouthed happy facial expression from the same facial database. Each trial started with a white fixation cue (+) centered on a black background (1000 ms). Two face stimuli were then simultaneously presented (33 ms) to the left and right of fixation. Facial stimuli were separated by 14° of visual angle and subtended approximately 5 x 7° of visual angle. The initial faces were instantly masked with an open mouth happy expression (100 ms), which was offset by 1° of visual angle on the vertical axis. A target dot immediately followed in either the location of the left or the right face and remained on the screen until a response was made. Participants were instructed to identify the location of the dot as quickly as possible by using the numeric pad on a keyboard and pressing the “1” key with their right index finger for left-sided targets and the “2” key with their right middle finger right-sided targets. Participants were instructed to always fixate on the fixation cue, which remained in the center of the screen throughout the entirety of each trial.

Trials containing one fearful and one neutral face were considered directed attention trials. Of these trials, half were congruent (target dot presented on the same side of the screen as the fearful face) and other half was incongruent (target dot presented opposite the fearful face). Faster reaction times on congruent compared to incongruent trials indicate a facilitation of spatial attention by backward masked fearful faces (MacLeod and Mathews, 1988). Therefore, the relative level of attentional capture in a given individual can be indexed as the mean difference between congruent and incongruent reaction times across trials. Here we refer to this difference in reaction times as the **Attention Index** and individuals’ Attention Index scores were used as a regressor to assess correlations with gray matter. The task contained 40 congruent and 40 incongruent trials counterbalanced for visual field in addition to 40 neutral–neutral trials.

Participants completed an additional task to assess awareness of the backward masked faces. This task mimicked the dot-probe task in all aspects except that instead of a target dot occurring after the masked faces participants were asked to indicate with a keyboard whether they saw 1) a fearful face on the left, 2) a fearful face on the right, or 3) two neutral faces. Prior to beginning the task, participants were told that each trial would contain two sets of faces presented in rapid succession and that they were to identify the facial expressions of the first set of faces. The task included 60 trials: 20 of each type.

**MRI image acquisition and analysis**

A 3 Tesla Siemens Trio whole body scanner was used to acquire T1 images with the following parameters: **TR = 1900 ms, TE = 2.53, flip angle = 9°, FOV = 176 x 250 x 250 mm, matrix = 176 x 256 x 256, and voxel size = 1 x 0.98 x 0.98 mm**.

Voxel based morphology (VBM) permits the automated user-independent voxel-wise measurement of associations between regional brain volumes and individual differences. VBM has been extensively cross-validated with manual volumetric analysis (Woermann et al., 1999). The technique derives volume measurements from transformation of individual structural MR volume images into a common stereotactic space permitting testing of differences in sub-volumes of distinct brain regions using general linear model statistics.

Our VBM methodology was similar to those described previously (Ashburner and Friston, 2000). Volume brain images were first manually adjusted to a common orientation with the origin at the anterior commissure before all images were pre-processed using established VBM methods in SPMB (http://www.fil.ion.ucl.ac.uk/spm). Three-dimensional T1-weighted MPRAGE images were visually examined for artifacts and structural abnormalities. Images were then segmented into gray matter, white matter and cerebrospinal fluid, and again visually inspected. Gray and white matter images were normalized to standard gray matter templates. A modulation step was used to permit voxel-wise information about local tissue (Ashburner and Friston, 2000). Tissue probability maps were obtained by averaging across participant data, using an 8-mm FWHM Gaussian smoothing kernel. Measures of total brain volume were obtained from summed global signal of segmented images of gray and white matter.
Within SPM8 a multiple regression analysis was run with participants’ Attention Index (i.e., congruent–incongruent reaction times) scores as a predictor variable with gray matter volume as the dependent variable. We specifically tested whether or not increases in gray matter would correlate with faster RTs on congruent compared to incongruent trials (i.e., negatively with the Attention Index). Age and whole brain gray matter volume were included as covariates to control for potential confounding effects on regional gray matter (Ge et al., 2002; Tisserand et al., 2004). An initial whole brain threshold was set to \( p_{\text{single-tailed}} < 0.001 \) and then a family-wise error small volume correction (SVC) was applied to ACC and amygdala regions of interest (ROIs) created with the Masks for Regions of Interest Analysis software (Walter et al., 2003).

**Results**

**Behavioral responses**

All participants performed at chance (i.e., chance = 33.33%; group average \( M = 33.88\% \), \( t(41) = 0.75 \)) on the forced-choice awareness check task. Thus, backward masked faces in the current study were processed below the level of conscious awareness.

Similar to earlier work using this variant of the dot-probe task (e.g., Carlson and Reinke, 2008), analyses were performed on correct responses occurring between 150 and 750 ms after target presentation. A \( 2 \times 2 \) repeated measures analysis of variance (ANOVA) was conducted to assess the effects of visual field (left vs. right) and congruency (congruent vs. incongruent) on participants’ reaction times in the dot-probe task. As can be seen in Fig. 1a, a main effect of congruency was significant where reaction times were faster on congruent \( (M = 390.16 \text{ ms}, \ SE = 5.94) \) compared to incongruent \( (M = 396.60, \ SE = 6.24) \) trials, \( F(1, 41) = 5.79, p < 0.05 \). There were no other significant main or interaction effects.

**Attention Index correlations with sMRI**

Within the ACC ROI a cluster of voxels in the right hemisphere was found to correlate with participant’s Attention Index scores, \( k = 31 \), peak voxel at 16, 40, 20; \( t(37) = 4.75, r = −0.62, p_{\text{SVC}} < 0.05 \). This cluster was not correlated with baseline reaction times \( (r = −0.14, p > 0.1) \) and a Fisher’s z-test confirmed that the rACC–Attention Index correlation was significantly stronger than the rACC–baseline correlation \( (z = −3.69, p = 0.0001) \). This differential correlation indicates that this effect is specific to a nonconscious fear-elicited attentional facilitation in reaction times rather than differences in baseline reaction time. It should be noted that at a lower threshold \( (p < 0.01) \) a somewhat symmetrically located cluster in the left ACC was also correlated with Attention Index scores, \( k = 44 \), peak voxel at \(-8, 36, 16; t(37) = 3.69, r = −0.52, p < 0.001 \). There were no significant associations within the amygdala ROI. Table 1 provides a complete list of areas associated with Attention Index scores at \( p < 0.001 \). Of particular interest, it appears that an area consistent with the cholinergic nucleus basalis was similarly negatively associated with Attention Index scores (see Fig. 1d).

**Experiment 2**

In Experiment 2 (Exp2) we attempted to replicate the correlation in Experiment 1 (Exp1) between ACC gray matter volume and Attention Index scores in a separate independent sample. To achieve this...
aim, we utilized behavioral data from a previously published fMRI dot-probe study (Carlson et al., 2009b) that also collected anatomical T1 scans that were not previously analyzed.

Method

Participants

Twelve (7 male) right-handed individuals between the ages of 18 and 35 participated in the study. Participants were monetarily compensated for their time. Informed consent was obtained and participants were treated in accordance to the guidelines of the Institutional Review Board of Southern Illinois University Carbondale.

Dot-probe task

The task was performed in a MRI scanner during functional image acquisition. E-Prime (Psychology Software Tools, Pittsburg, PA) and the IFIS system were used to display the task on an MRI-compatible screen for 750 ms, 3.) there was a jittered (500–2000 ms) intertrial interval, 4.) there were approximately 275 trials in each trial type (congruent, incongruent, and baseline), and 5.) baseline trials were half fearful–fearful and half neutral–neutral (reaction times did not differ; Carlson et al., 2010b).

MRI image acquisition and analysis

A 1.5 T Phillips whole body scanner equipped with a head coil was used to acquire anatomical T1 images with the following parameters: TR = 2500 ms, TE = 4.60, flip angle = 30°, FOV = 230.4 x 230.4 x 160 mm, matrix = 256 x 256 x 200, and voxel size = 0.90 x 0.90 x 0.80 mm. Preprocessing was performed in SPM8 using the same steps outlined in Exp1.

A second-level regression analysis was run in SPM8 to extract gray matter volumes. Given our smaller sample and focused aim to replicate the ACC–Attention Index association from Exp1, we used the results from Exp1 to pinpoint our analysis. We extracted gray matter values from a 4 mm sphere (which at 33 voxels is approximately equal to the 31 voxel cluster found in Exp1) centered around the maximally correlated ACC voxel identified in Exp1 (i.e., 16, 40, 20) to specifically test whether or not ACC gray matter was correlated with faster reaction times on congruent vs. incongruent RTs (i.e., negatively with the Attention Index) using a single-tailed $\alpha = 0.05$.

Results

Behavioral responses

As in Exp1, correct responses occurring between 150 and 750 ms after target presentation were used for RT analyses. A 2 x 2 repeated measures ANOVA was conducted to assess the effects of visual field (left vs. right) and congruency (congruent vs. incongruent) on participants’ reaction times. As previously reported (Carlson et al., 2009b), there was a 2 way interaction ($F(1, 11) = 5.68, p = 0.05$) where reaction times were faster on congruent ($M = 382.47$ ms, $SE = 16.62$) compared to incongruent trials ($M = 412.60$ ms, $SE = 17.87$) in the left visual field (LVF; $p = 0.006$), but congruent ($M = 397.39$ ms, $SE = 20.18$) and incongruent ($M = 393.19$ ms, $SE = 15.20$) reaction times did not differ in the right visual field ($p = 0.59$). No other effects were significant. Unlike Exp1, which did not have a visual field interaction, the Attention Index used in Exp2 was computed as the difference between LVF congruent and incongruent trials, given that attention was only captured on LVF trials in this sample.

Attention Index correlations with sMRI

As depicted in Fig. 2, there was a significant correlation between participants’ rACC gray matter volume and LVF Attention Index, $r = −0.68$, $p_{\text{single-tailed}} = 0.02$ (controlling for whole brain gray matter and age). This correlation was not significant for RVF Attention Index ($r = 0.24, p > 0.1$) or baseline ($r = −0.15, p > 0.1$) trials where attention was not captured by the backward masked fearful faces. Fisher’s $z$-tests again confirmed that the correlation between LVF Attention Index and rACC gray matter was significantly stronger than rACC gray matter correlations with RVF Al ($z = −3.24, p = 0.001$) and baseline ($z = −2.06, p = 0.02$) trials.

Discussion

The results from Exp1 provide the first evidence that variability in anterior cingulate cortex (ACC) gray matter volume is associated with individual differences in attention bias to backward masked fearful faces. The nature of this relationship indicates that lower levels of ACC gray matter are associated with lower levels of attention to non-consciously processed environmental threats. In Exp2, we reproduced this effect in an independent sample. Thus, we provide both initial and replicating evidence that greater ACC volume is associated with greater attention bias to masked fearful faces. Additional regions (see Table 1) such as the basal forebrain (BF) showed similar associations between gray matter volume and attention to masked threat.

Previous work has shown that a network including the amygdala, ventral ACC, and visual cortex mediates the facilitation of spatial attention to backward masked fearful faces (Carlson et al., 2009b). Additional data from patients with amygdala lesions indicates that an intact amygdala is necessary for the recognition of fearful facial expressions (Adolphs et al., 1999) in addition to fear/emotion-related modulations in visual processing (Anderson and Phelps, 2001; Vuilleumier et al., 2004). Similarly, responsivity in ventral ACC to emotional faces is also amygdala-dependent (Vuilleumier et al., 2004). Taken together, it appears that the amygdala is critical for the initial recognition of threat and relays this information to ACC and visual cortex to facilitate attentional and perceptual processing. However, amygdala gray matter volume did not correlate with attention bias to threat. Suggesting that unlike amygdala activity, amygdala volume is not associated with biased attention to nonconscious threat. This may be explained by findings in highly extroverted individuals that the amygdala activates (Canli et al., 2002) and attention is biased (Derryberry and Reed, 1994) to positively valenced stimuli. Therefore, an enlarged amygdala might be associated with biases in attention to negative and

Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Voxels</th>
<th>t value</th>
<th>r value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle frontal gyrus (BA10)</td>
<td>L</td>
<td>−26</td>
<td>58</td>
<td>8</td>
<td>78</td>
<td>−5.15</td>
<td>−0.65</td>
</tr>
<tr>
<td>Superior frontal gyrus (BA9)</td>
<td>L</td>
<td>−20</td>
<td>46</td>
<td>32</td>
<td>41</td>
<td>−4.75</td>
<td>−0.62</td>
</tr>
<tr>
<td>Middle frontal gyrus (BA11)</td>
<td>L</td>
<td>−38</td>
<td>44</td>
<td>−16</td>
<td>55</td>
<td>−4.06</td>
<td>−0.56</td>
</tr>
<tr>
<td>Anterior cingulate cortex (BA23)</td>
<td>R</td>
<td>16</td>
<td>40</td>
<td>20</td>
<td>41</td>
<td>−4.75</td>
<td>−0.62</td>
</tr>
<tr>
<td>Basal forebrain</td>
<td>R</td>
<td>14</td>
<td>4</td>
<td>−4</td>
<td>62</td>
<td>−4.53</td>
<td>−0.60</td>
</tr>
<tr>
<td>Inferior frontal gyrus (BA47)</td>
<td>R</td>
<td>24</td>
<td>10</td>
<td>−20</td>
<td>76</td>
<td>−4.08</td>
<td>−0.56</td>
</tr>
</tbody>
</table>

Reported activations were significant at $p < 0.001$ uncorrected.
positive stimuli (i.e., when compared to neutral or non-salient stimuli). Further work is needed to directly test this speculation.

Our results demonstrate for the first time that structural variability in the ACC is associated with differential attention biases to masked threat. The ACC has been divided into emotion- and cognition-related subdivisions, in the ventral and dorsal ACC, respectively (Bush et al., 2000). The region identified here (see Fig. 1) borders these subdivisions, which suggests that this ACC region is important for emotion–cognition interactions such as the modulation of spatial attention by affective stimuli. It should be noted that it is unclear if variability in ACC volumes represents a predisposition to automatically attend to threat or a consequence of this behavior. However, given that biased attention to nonconscious threat stimuli has been linked to a polymorphism in the serotonin transporter genotype (5HTTLPR) in both general (Carlson et al., in press) and psychiatric (Bevers et al., 2007) populations, we suspect that some individuals are predisposed to have larger and denser local networks of ACC neurons, which enable facilitated attention biases toward nonconscious threat stimuli. Although we provide strong evidence that variability in ACC volume is associated with orienting to nonconscious threat, further work is needed to elucidate the causal nature of this relationship.

Prior work has noted that backward masked fearful face-elicited modulations of visual cortex may be attributed to cholinergic innervation of visual cortex (Carlson et al., 2010b). That is, acetylcholine (ACh) is thought to facilitate attention by lowering the activation threshold of neurons and thus increasing their functionally specific response (e.g., orientation specific responses of cells in striate cortex) (Sarter et al., 2003). Interestingly, in the current study we found that attentional bias to threat was correlated with variability of volumes in a region encompassing the cholinergic nucleus basalis in the BF, which houses diffuse modulatory cholinergic neurons. Therefore, greater BF gray matter may be indicative of greater ACh availability, which would presumably lead to facilitated attention processing. Consistent with this possibility, work in rodents has led to a purported affective attention system, which includes the amygdala, the cholinergic modulatory system, posterior parietal, and frontal cortical areas (Holland and Gallagher, 1999). However, future work is needed to clarify the mechanisms in which variability in BF gray matter volume leads to facilitated attention to nonconscious threat.

As stated above, individual differences in anxiety are associated with an elevated attention bias to masked and unmasked threat (Fox, 2002; MacLeod and Mathews, 1988; Mogg and Bradley, 2002; Mogg et al., 1995). Furthermore, hyper-activation within the amygdala ACC attention network (particularly the amygdala), in response to nonconscious threats, is associated with individual differences in anxiety (Etkin et al., 2004; Monk et al., 2008). In addition to functional alterations, patients with generalized anxiety disorder have been shown to have enlarged amygdala volumes (Etkin et al., 2009; Schienle et al., in press) and symptom severity associated with worry is positively correlated with ACC volumes (Schienle et al., in press). Interestingly, the ACC region implicated in anxious symptoms (Schienle et al., in press) is quite similar in location to the current ACC region associated with biased attention to threat, which may suggest that elevated levels of worry in anxiety are linked to nonconscious attentional biases to threat. This notion that early processing biases in attention influence later aspects of affective behavior is consistent with recent work demonstrating that biased attention to negative stimuli predicts increased cortisol release in stressful environments (Baldwin et al., 2007; Fox et al., 2010). Thus, early preconscious attention biases to threat appear to influence states of worry and stress reactivity, which may be associated with elevated ACC volume. In addition to anxiety, other negative emotion-related individual differences and/or disorders, may have hyper-vigilance to threat, exaggerated amygdala activity, and abnormal ACC volumes, such as post-traumatic stress disorder, depression, and heightened trait anger (Bryant and Harvey, 1997; Carlson et al., 2010a; Kasai et al., 2008; Kitayama et al., 2006; Mogg and Bradley, 2005; Mogg et al., 1995; Rauch et al., 2000, 2003; Sheline et al., 2001; van Honk et al., 2001a, 2001b). Taken together, these results highlight the close relationship between the disposition for negative emotion-related traits, the amygdala–ACC network, and biased attention to threat. Further work relating attention bias to brain anatomy is needed both at the trait level and in clinical populations to better understand how our results may relate to anxiety vulnerability.

In conclusion, the results from two experiments provide initial and replicating evidence that greater ACC volume is associated with greater attention bias to masked fearful faces. This association may help explain the neural mechanisms underlying associations between attention bias and individual differences in emotional disposition such as one’s level of anxiety. Independent of the relevance to anxiety the results importantly demonstrate that within the healthy population variability in ACC volume is associated with the degree to which one allocates attention to nonconscious threats.

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