Distinctive amygdala subregions involved in emotion-modulated Stroop interference

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Despite the well-known role of the amygdala in mediating emotional interference during tasks requiring cognitive resources, no definite conclusion has yet been reached regarding the differential roles of functionally and anatomically distinctive subcomponents of the amygdala in such processes. In this study, we examined female participants and attempted to separate the neural processes for the detection of emotional information from those for the regulation of cognitive interference from emotional distractors by adding a temporal gap between emotional stimuli and a subsequent cognitive Stroop task. Reaction time data showed a significantly increased Stroop interference effect following emotionally negative stimuli compared with neutral stimuli, and functional magnetic resonance imaging data revealed that the anterior ventral amygdala (avAMYG) showed greater responses to negative stimuli compared with neutral stimuli. In addition, individuals who scored high in neuroticism showed greater posterior dorsal amygdala (pdAMYG) responses to incongruent compared with congruent Stroop trials following negative stimuli, but not following neutral stimuli. Taken together, the findings of this study demonstrated functionally distinctive contributions of the avAMYG and pdAMYG to the emotion-modulated Stroop interference effect and suggested that the avAMYG encodes associative values of emotional stimuli whereas the pdAMYG resolves cognitive interference from emotional distractors.

Keywords: Stroop; cingulate cortex; emotion; neuroticism; fMRI

INTRODUCTION
Emotional stimuli often interfere with a concurrent cognitive task or immediately following a cognitive task that requires focused attention (McKenna, 1986; Vuilleumier et al., 2001; Dolcos and McCarthy, 2006; Etkin et al., 2006; Egner et al., 2008). However, the degree to which individuals are susceptible to emotional distractors is not uniform but varies substantially. Furthermore, these individual differences appear to be a key predictor of a wide range of behavioral phenotypes, including psychological vulnerability to stressful events (Winter and Kuiper, 1997; Williams et al., 2009). Individual differences in emotional susceptibility may arise from two primary causes: the detection sensitivity to emotional distractors and the ability to exert cognitive control over emotional distractors (Bishop, 2007; Arnsten, 2009). Elucidating the specific neural correlates of these two possibly independent causes of emotional susceptibility is critical because drastically different neural processes could lead to seemingly identical behavioral outcomes (Indovina et al., 2011).

The human amygdala has long been considered a key neural structure with respect to the more or less automatic detection of fear-inducing stimuli, which then trigger the interruption or interference of ongoing cognitive tasks (Vuilleumier et al., 2001; Whalen et al., 2004; Öhman, 2005; Etkin et al., 2006; Egner et al., 2008). Another line of research on the functions of the amygdala has suggested that the amygdala is responsive to and possibly takes part in the resolution of uncertainty and ambiguity regardless of valence information (Kim et al., 2003; Herry et al., 2007; Whalen, 2007). These seemingly inconsistent findings have been accounted for by the theoretical work of Whalen and his colleagues who claim that anatomically segregated distinctive subregions of the amygdala, such as its ventral and dorsal aspects, are differentially involved in processing emotional information. More specifically, the theory argues that the ventral amygdala, which primarily includes the lateral nucleus, is involved in the detection of emotional values, whereas its dorsal portion, including the central nucleus that is contiguous with the basal forebrain, contributes to encoding predictive uncertainty (Whalen et al., 2001; Kim et al., 2003). Consistent with this theory, functional segregation between distinctive subregions of the amygdala has been reported in recent human neuroimaging studies. For example, specific subregions of the amygdala have been reported to play distinctive functional roles in social conditioning (Davis et al., 2010) and in reading emotional signals from social stimuli (Etkin et al., 2004; Gamer et al., 2010; Boll et al., 2011). Furthermore, in support of the observations of previous invasive animal tracing studies (McDonald, 1998; Pitkanen et al., 2000), recent studies have successfully segregated the anterior ventral amygdala (avAMYG), which mostly includes the lateral nucleus, from the posterior dorsal amygdala (pdAMYG), which mostly contains the central nucleus of the amygdala, based on their unique anatomical connections (Bach et al., 2011), as well as on their distinctive functional connectivity patterns (Etkin et al., 2009).

In this study, we focused specifically on whether different subregions of the amygdala are involved in evaluating the associative values of emotional stimuli and resolving emotional conflict. Many previous neuroimaging studies on the emotional Stroop effect have adopted experimental paradigms that involve the simultaneous presentation of emotional distractors and a cognitive task in a single trial, such that emotional distractors are embedded in a cognitive task (Whalen et al., 1998; Etkin et al., 2006; Haas et al., 2006; Kanske and Kotz, 2011). In this type of emotional distraction Stroop task, emotional interference and its control occur simultaneously within the time frame of a single trial, which may be limited when the objective is to tease apart the separate neural processes involved in a cognitive task and in the processing of emotional distractors. More specifically, this type of design makes it difficult to conclude whether any increases in neural activation that are observed during emotional conflict are due to the process of detecting emotional distractors or to the process of the top-down control of emotional distractors. Therefore, in this study, we tried to temporally segregate emotional distractors from cognitive...
tasks within trials by presenting a negative, positive or neutral picture prior to a cognitive Stroop task. This experimental design allowed us to separate out the neural activities that were caused by the emotional distractors from those that were caused by subsequent cognitive interference, making it possible to examine the functionally distinctive systems that are uniquely involved in each process. Based on previous reports of amygdala function, we hypothesized that the avAMYG and pdAMYG would be differentially involved in the detection of the valence information of emotional stimuli and in the resolution of emotional conflict during a subsequent Stroop task, respectively.

The amygdala (particularly, its dorsal subregion) is known to have strong anatomical connections with the prefrontal cortex, or more specifically, the medial aspect of the prefrontal cortex, in non-human primates (Ghashghaei and Barbas, 2002), and a similar functional connectivity has been observed in humans (Bach et al., 2011). Emotional Stroop and emotional conflict tasks are known to induce strong activation in the medial aspect of the prefrontal cortex, that is, the cingulate cortex (Whalen et al., 1998; Bishop et al., 2004; Etkin et al., 2006; Haas et al., 2006, 2007; Mohanty et al., 2007). In particular, a subregion of the cingulate cortex, which was recently named the anterior midcingulate cortex (aMCC) (Vogt, 2005; Shackman et al., 2011), has been found to be frequently involved in encoding conflict that is caused by emotional stimuli that are mapped to specific motor responses (Etkin et al., 2006). Given that the amplitude in error-related negativity, which is a well-known neural index of the cognitive control that is presumably generated in the midcingulate cortex (MCC), has been shown to predict the degree to which participants learned from negative feedback (Frank et al., 2005; van der Helden et al., 2010), an increase in aMCC activity appears to reflect one’s effort to reduce the conflict between a cognitive goal and emotional distractors during an emotional Stroop task, particularly when the emotional distractors are closely associated to the specific motor responses (Etkin et al., 2006). A number of studies have also described an association between neuroticism and the ability to monitor and regulate cognitive conflict, in which the aMCC appears to play a key role, thus determining the individual variability of the degree to which emotional distractors interfere with cognitive processing (Bystritsky et al., 2001; Canli et al., 2001; Eisenberger et al., 2005; Haas et al., 2007; Cremers et al., 2010; Fruhholz et al., 2010). With these findings in mind, we therefore predicted that the aMCC would play a key role in mediating emotion-modulated cognitive interference and that the role of the dorsal amygdala in the resolution of emotional conflict during subsequent Stroop tasks would be mediated by top-down influences from the aMCC.

METHODS
Participants
Twenty right-handed college students were recruited as participants in this study. Six subjects were excluded from all subsequent analyses because of a failure to follow instructions (three subjects) or excessive head motions (three subjects with head motions over 3 mm). Thus, the data from 14 subjects were included in the final behavioral and neuroimaging analyses (age range, 19–24 years; mean age, 21.64; s.d., 1.645). Only female subjects participated in this experiment because of the previously reported gender differences in the processing of emotionally charged visual stimuli (Wrase et al., 2003). All participants had normal or corrected-to-normal vision. The study protocol was approved by the institutional review board of Korea University (KUCM-IRB-2006007-A-2). All participants provided written informed consents, and they were paid ~20,000 KRW (~20 US dollars) for their participation. In order to assess individual personality factor differences, we administered the translated version (Lee, 2004) of the Eysenck Personality Questionnaire-Revised (EPQ-R; Eysenck and Eysenck, 1991) prior to the functional magnetic resonance imaging (fMRI) investigations.

Materials
All visual stimuli that were used in this study were from the International Affective Picture System (Lang et al., 1997), and they consisted of three types of pictures that depicted positive, negative or neutral scenes. We tried to ensure that the pictures of each type contained an approximately equal number of humans, animals, foods or objects. All pictures were rated for arousal and valence by 15 Korean college students in order to avoid potential problems due to cross-cultural differences in the emotional responses to the pictures. Based on the collected ratings, we chose 30 pictures of each type. The mean arousal and valence ratings of the chosen pictures were 7.16 ± 0.82 and 1.91 ± 0.54, respectively, for the negative pictures; 5.07 ± 0.79 and 6.84 ± 0.58, respectively, for the positive pictures and 3.28 ± 0.62 and 5.06 ± 0.47, respectively, for the neutral pictures. The mean arousal rating of the negative pictures was significantly greater than that of the positive pictures (t(58) = 10.051, P < 0.001) or neutral pictures (t(58) = 20.663, P < 0.001). The mean valence rating of the negative pictures was also significantly greater than that of the positive pictures (t(58) = -3.4148, P < 0.001) or neutral pictures (t(58) = -24.193, P < 0.001). All visual stimuli were projected onto a screen with a LCD projector [IFIS-SA, Invivo, Gainesville, FL, USA; maximum refresh ratio, 60 Hz; display area, 640 × 480; maximum view angles, 30° field of view (FOV)] and presented to subjects through a mirror that was attached to a radio-frequency head coil.

Procedure
In the event-related fMRI experiment, participants underwent 150 trials in five successive runs. In order to avoid any problems due to potential carryover effects between different emotion trials, we decided to block the same emotion trials and alternate them in a single scan. Each run consisted of six blocks (two negative, two positive and two neutral), and each block included six trials. The block orders in the entire experiment were pseudo-randomly distributed within the run and counterbalanced across subjects. Each trial began with the display of a picture (3 s), which was followed by the display of a fixation cross (1 s). The Stroop task (1 s) was then started, and this was again followed by the display of a fixation cross (3–7 s) (Figure 1A). We considered several issues in order to determine the optimal temporal interval between the onset times of the emotional pictures and the Stroop tasks. Most importantly, we had to keep the temporal distance as short as possible in order to retain the psychological association between the emotional pictures and the subsequent Stroop task while trying to minimize the autocorrelation between the regressors of both events. In addition, we decided not to introduce jittering between the events in order to avoid the potential inclusion of unwanted expectation violation. Given all of the limitations mentioned above, we decided to use a fixed 4 s interval because it was previously reported that blood oxygen level-dependent responses to neutral events that are spaced at least 4 s apart are readily resolvable due to the low autocorrelation at that lag (Zarahn et al., 1997). In addition, a 20 s long interval was inserted between the blocks in order to allow the hemodynamic responses to return to baseline.

In order to avoid a potential motion problem when asking the participants to name or read stimuli aloud inside the scanner, we adopted a matching task (Luo, 1999), a modified version of the classic Stroop task (Stroop, 1935). In this task, subjects were required to respond by pressing a mouse button for a ‘same’ (or ‘different’) response if the meaning of a colored word (e.g. the word ‘red’ in different colors) matched (or mismatched) the color of a patch that was presented
The Stroop task consisted of congruent and incongruent trials (of a patch presented above the word in each trial. Each trial began with a picture that was presented for 3 s followed by a 1 s fixation cross, after which the Stroop task started and lasted for 1 s, which was also followed by another fixation cross (jittering ranging from 3 s to 7 s). The Stroop task consisted of four types of trials that depended on the congruency between the color and the meaning of the word and the color of the patch (Kim et al., 2005) (Figure 1B). In the match–same trial (SET 1), the color of the word and the meaning of the word matched the color of the patch, and the subjects were required to press the ‘same’ response. In the mismatch–same trial (SET 2), the color of the word did not match the color of the patch but the meaning of the word did, and the subjects were required to press the ‘same’ response. In the mismatch–different trial (SET 3), the color of the word matched the color of the patch, but the meaning of the word did not, and the subjects were required to press the ‘different’ response. Finally, in the mismatch–different trial (SET 4), the color of the word and the meaning of the word did not match the color of the patch, and the subjects were required to press the ‘different’ response. Therefore, SET 1 and SET 4 were congruent trials, whereas SET 2 and SET 3 were incongruent trials. All four types of trials were presented pseudo-randomly within each block while ensuring that no more than two trials of the same set were presented consecutively.

Prior to the fMRI experiments, all participants practiced the experimental procedure. The practice run included 18 trials (i.e. three congruent and three incongruent Stroop trials in each emotional context). The results that were obtained during the practice were not included in the main analyses. The participants were instructed to watch the presented pictures carefully and to perform the subsequent Stroop tasks. During the Stroop task, the participants were asked to focus only on the relevant information, that is, the meaning of the word and the color of the patch, and to indicate whether the color of the patch matched the meaning of the word. Subjects were required to press either a left or a right button as quickly and accurately as possible. Half of the participants used the left button for the ‘same’ responses and the right button for the ‘different’ responses, and this was reversed for the other half.

MRI data acquisition

Blood oxygenation level-dependent contrast functional images were acquired with echo-planar T2*-weighted imaging with a 3T Forte scanner (ISOL Technology Inc., Seoul, Korea) with a head coil gradient set. Each functional image volume consisted of 20 axial slices (6 mm, no gap) that were obtained with a gradient echo sequence [repetition time (TR), 2000 ms; echo time (TE), 35 ms; flip angle, 80°; field of view (FOV), 220 mm; 64 × 64 matrix acquisition]. At the end of all of the fMRI runs, T1-weighted high-resolution structural images were acquired with a Magnetization-Prepared Rapid Gradient Echo imaging sequence (TR, 3290 ms; TE, 16 ms; flip angle, 60°; FOV, 220 mm; 256 × 256 matrix acquisition; slice thickness, 1 mm).

fMRI data analyses

Preprocessing

All of the images were analyzed with SPM2 (Wellcome Department of Imaging Neuroscience, London, UK) that was implemented in Matlab 6.5 (The MathWorks, Inc., Natick, MA, USA). The first six volumes were excluded from the analysis prior to all of the preprocessing steps in order to allow for equilibration effects. All functional images were corrected for differences in slice timing and motion, normalized to a standard echo-planar imaging template that was provided by the Montreal Neurological Institute, resampled at a voxel size of 2 × 2 × 2 mm³ and smoothed with a Gaussian kernel of 8 mm full width at half maximum. A 128 s temporal high-pass filter was applied to the data in order to remove low-frequency noise.

Individual and group general linear model analyses

During the individual analyses, the onset times of nine distinctive events were identified in each run. These included the presentation of negative, positive and neutral pictures and the presentation of congruent and incongruent Stroop trials following negative, positive and neutral pictures. Each of these events was convolved with a canonical hemodynamic response function and included in the design matrix of a general linear model together with six motion regressors for each run, and contrast images were computed in order to compare the regressors of interest. Two separate one-way analysis of variance (ANOVA) tests were performed. The first was performed with the individual subjects’ beta coefficient maps of negative, neutral and positive picture presentation events, and the other was performed with the contrast maps of the incongruent vs the congruent trials for the negative, neutral and positive conditions. The former test was conducted in order to identify brain regions that were responsive to the different emotional contents of pictures, whereas the latter test focused on the investigation of neural systems that were sensitive to the cognitive conflicts that were modulated by preceding emotional context. In addition, in order to investigate the individual modulatory effects that were related to negative emotional processing, we performed simple
regression analyses, in which individual contrast maps were regressed onto participants’ neuroticism scores.

**Statistical thresholds**
For all of the fMRI findings reported in this study, we imposed a significance threshold of $P < 0.05$ that was corrected for multiple comparisons over a particular search volume size as determined by the Monte Carlo simulations that were implemented in AlphaSim with AFNI software, which generates volumetric cluster sizes that correspond to alpha levels (Cox, 1996). Given our a priori hypothesis of the role of the amygdala and the anterior cingulate cortex (ACC), we restricted the search volumes of AlphaSim for the amygdala to a sphere with a radius of 10 mm ($\sim 4200 \text{ mm}^3$) that was centered on the coordinates of the amygdala that had been acquired from a recent study that described the functionally distinctive amygdala subregions (Gamer et al., 2010). Similarly, we also restricted the search volumes of AlphaSim for the ACC to a sphere with a radius of 20 mm ($\sim 33500 \text{ mm}^3$) that was centered on the coordinates obtained from a previous study that used an emotional Stroop task (Egner et al., 2008). The coordinates that were used for the AlphaSim correction in this study were as follows: $x = -23$, $y = -3$, $z = -28$ for avAMYG; $x = 28$, $y = -9$, $z = -14$ for pdAMYG and $x = 12$, $y = 28$, $z = 24$ for aMCC. In the absence of the a priori assumption of laterality, we also mirrored the coordinates in order to examine the opposite hemisphere. In order to report all of the other areas that were found unexpectedly, we applied whole-brain corrections (i.e., 51 voxels and $P < 0.001$, uncorrected) that were based on the total gray matter volume ($\sim 673000 \text{ mm}^3$) determined by a previous volumetric MRI study (Kennedy et al., 1998), but no activation cluster survived the threshold. All active voxel locations were defined with the Montreal Neurological Institute coordinates.

**Mediation analysis**
A single-level mediation analysis was performed in order to further investigate how the interplay between the regions that were found in the main fMRI data analyses influenced the behavioral Stroop interference effect. Software that has been developed and made freely available at http://www.columbia.edu/cu/psychology/tor/ (Wager et al., 2008) was used. We tested several path models with variables that included the avAMYG ($x = -28$, $y = 2$, $z = -24$; the coefficients from the contrast of the negative vs neutral picture conditions), the aMCC ($x = 10$, $y = 18$, $z = 42$; the coefficients from the contrast of the interference effect during the negative vs neutral condition) and the pdAMYG ($x = -24$, $y = -6$, $z = -4$; the coefficients from the contrast of the interference effect during the negative vs neutral condition), and the behavioral Stroop interference effect.

**Time-course plotting**
For the time-course plots, we located three functional regions of interest (ROIs), which were the avAMYG, pdAMYG and aMCC (see above for the coordinates), in each individual participant and extracted event-related responses from the peak voxel of each ROI with a finite impulse response analysis that was implemented in SPM2. The event-related responses for all of the trials were averaged in each ROI.

## RESULTS

### Behavioral results
All participants performed the Stroop task reasonably well, with mean accuracy rate $> 95\%$ averaged across all conditions (Table 1). Differences between the correct response rates during the different conditions were tested with a 3 (emotion: negative, positive or neutral) $\times$ 2 (congruency: congruent or incongruent) repeated measures ANOVA. The analysis revealed no significant main effects for emotion ($F(2,26) = 3.062$; $P = 0.064$) or congruency ($F(1,13) = 0.570$; $P = 0.464$) or an interaction effect between emotion and congruency ($F(2,26) = 0.385$; $P = 0.684$). The same ANOVA test was performed on the reaction time (RT) data (Table 2), and this revealed a significant main effect of congruency ($F(1,13) = 25.084$; $\eta^2 = 0.26$; $P < 0.001$) and an interaction effect between emotion and congruency ($F(2,26) = 8.192$; $\eta^2 = 0.12$; $P < 0.01$) but no significant main effect of emotion ($F(2,26) = 2.090$; $\eta^2 = 0.04$; $P = 0.144$). Post hoc analyses showed that the participants were generally slower during incongruent than congruent trials following negative ($t(13) = 6.346$, $P < 0.0001$) and positive ($t(13) = 2.555$, $P = 0.024$) pictures but not following neutral ($t(13) = 0.936$, $P > 0.1$) trials (Figure 2).

Self-reports on personality that were measured with the EPQ-R questionnaire after scanning showed that the scores for neuroticism ranged from 2 to 21 (mean, 10.1; s.d., 5.0). No significant correlations were observed between the neuroticism scores and the behavioral Stroop interference effects in any of the three conditions (negative: $r = 0.361$, $P = 0.205$; positive: $r = 0.086$, $P = 0.770$ and neutral: $r = -0.074$, $P = 0.801$).

### fMRI results
An one-way ANOVA of the individual subjects’ beta coefficient maps that were made in response to the negative, neutral and positive picture presentation events revealed that the left avAMYG (Figure 3A: $x = -28$, $y = 2$, $z = -24$, $Z = 3.10$, $P < 0.05$, corrected) activated significantly more to negative than neutral pictures (Figure 3B). No such activation was observed in any other subregion within the amygdala. Similarly, the aMCC ($x = 6$, $y = 20$, $z = 40$, $Z = 3.61$, $P < 0.05$, corrected) responded significantly more to negative than neutral pictures (Figure 3C and D). The comparison of positive vs neutral pictures, as well as negative vs positive pictures, revealed no activation in the amygdala or aMCC.

Our main goal was to determine how emotional pictures modulated Stroop interference-related activity during the cognitive Stroop task immediately following picture presentation. We examined the main effects of congruency across all of the conditions, but no activation was found in the aMCC region. Assuming that this null finding may have been due to the modulatory effects of the emotional conditions and/or individual variability, we next computed statistical maps that

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<td>742 ± 75</td>
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<td>744 ± 85</td>
<td>715 ± 78</td>
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<td>768 ± 78</td>
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showed the Stroop interference-related activity (i.e. incongruent > congruent trials) for each type of picture condition and then compared the resulting contrast maps between conditions (i.e. pair-wise comparisons). This analysis revealed that the aMCC showed significantly greater interference-related activity following negative compared with neutral pictures (Figure 4: $x=10$, $y=18$, $z=42$; $Z=3.79$, $P<0.05$, corrected) or positive pictures (Supplementary Figure S1: $x=-6$, $y=16$, $z=26$; $Z=4.17$, $P<0.05$, corrected). No such activation was observed in the comparison of positive vs neutral conditions. It was noteworthy that the response pattern observed in the aMCC resembled the responses of the amygdala to the pictures and the behavioral Stroop interference effect that was modulated by emotional context.

In order to examine the neural correlates associated with the behavioral Stroop interference effects, we first calculated the behavioral Stroop interference effects (RTs) by computing the behavioral RT differences between the incongruent and congruent trials (Stroop interference effect) in all three emotional conditions. Then, we...
subtracted the scores of the Stroop interference effects in the neutral condition from those that were computed with the negative or positive emotional context, and, finally, the neural Stroop interference effect was regressed against the corresponding behavioral Stroop interference effect. This voxel-wise regression analysis revealed that left aMCC activities were positively correlated with the behavioral Stroop interference effect under the negative context (Supplementary Figure S2A and B: $x = -10$, $y = 12$, $z = 30$; $Z = 3.40; P < 0.001$, uncorrected). A similar correlation was also observed during positive context in the aMCC (Supplementary Figure S2C and D: $x = 6$, $y = 8$, $z = 28$; $Z = 3.37; P < 0.001$, uncorrected).

In addition, a significant correlation between neuroticism scores and the main effect of congruency was observed in the aMCC (Supplementary Figure S3: $x = 8$, $y = 18$, $z = 22$; $Z = 3.49; P < 0.001$, uncorrected). A number of studies have indicated that neuroticism participates in the modulation of interference-related activity in the aMCC and in the functional coupling between the amygdala and the aMCC (Cremers et al., 2010). Thus, we assessed the modulatory role of neuroticism in the emotion-modulated Stroop interference-related neural activities by running a voxel-wise regression analysis whereby the individual statistical contrast maps of the Stroop interference-related effects (i.e. incongruent – congruent trials) for the negative picture condition only. This analysis revealed that the neuroticism scores correlated positively with the Stroop interference-related activities in the amygdala on both sides (right: $x = 28$, $y = -6$, $z = -16$; $Z = 3.84; P < 0.05$, corrected; left: $x = -32$, $y = -6$, $z = -12$; $Z = 2.90; P < 0.05$, corrected). At a more lenient threshold ($P = 0.001$, uncorrected), we observed that the Stroop interference-related activities correlated with the neuroticism scores in the aMCC (Supplementary Figure S5: $x = 8$, $y = 2$, $z = 32$; $Z = 3.14; P = 0.001$, uncorrected). The avAMYG, which showed a greater response to negative vs neutral pictures, was not found in this comparison. In order to verify that these correlational findings were driven mainly by the negative condition, we ran the same voxel-wise regression analysis with the statistical contrast maps of the Stroop interference-related effects (i.e. incongruent – congruent trials) for the negative picture condition only. This analysis revealed that the neuroticism scores correlated positively with the Stroop interference-related activities in the right (Figure 5A and C: $x = 28$, $y = -2$, $z = -14$; $Z = 4.20; P < 0.05$, corrected) and left (Figure 5B and D: $x = -24$, $y = -6$, $z = -4$; $Z = 3.25; P < 0.05$, corrected) pdAMYG. However, no such correlation was observed in the amygdala or in the aMCC for the positive (vs neutral) condition, indicating that the modulatory role of neuroticism observed in these regions was mainly due to the Stroop interference effect following negative pictures.

In addition, we tested the differences between the positive and negative conditions in the correlations between the Stroop interference-related responses and the neuroticism scores. This analysis revealed significantly greater correlations for the negative vs positive conditions in both the left ($t(13) = 2.09, P = 0.018$) and right ($t(13) = 2.77, P = 0.003$) pdAMYG. In addition, a marginally significant

![Fig. 5](image.png) The individual differences in the neuroticism scores correlated positively with the Stroop interference-related activities in the right (A and C: $x = 28$, $y = -2$, $z = -14$; $Z = 4.20; P < 0.05$, corrected) and left (B and D: $x = -24$, $y = -6$, $z = -4$; $Z = 3.25; P < 0.05$, corrected) pdAMYG following negative pictures. The blue circles indicate the approximate location of the a priori search space for amygdala activation.
difference was observed ($t(13) = 1.43, P = 0.076$), even when we repeated the same test of the differences in an independently defined region of the pdAMYG ($x = 20, y = -4, z = -14; Z = 1.87; P = 0.031$, uncorrected), which showed a correlation between the contrast of positive (incongruent – congruent) – neutral (incongruent – congruent) conditions and neuroticism at a lenient threshold ($P < 0.05$, uncorrected). No such difference between the positive and negative conditions was observed in the aMCC region ($t(13) = 0.44, P = 0.33$).

It is of interest to note that the aMCC region ($x = 8, y = 12, z = 46$) that showed a positive correlation between its response to negative vs neutral pictures and neuroticism scores was located slightly anterior to the aMCC region ($x = 8, y = 2, z = 32$) that showed a positive correlation between its Stroop interference-related activity following negative vs neutral pictures and neuroticism scores, and there was a significantly positive correlation between these two subregions of the cingulate cortex ($r = 0.717, P = 0.004$).

We were specifically interested in whether the avAMYG had an influence on the behavioral Stroop interference effect and the pdAMYG and whether this effect may have been mediated by the aMCC. Therefore, we tested the following two path models with a single-level mediation analysis across participants: one with avAMYG, aMCC and the Stroop effect and the other with avAMYG, aMCC and pdAMYG as the independent variable, mediator and dependent variable, respectively (Figure 6). In both models, we found a marginally significant correlation ($P = 0.081$, two-tailed) between avAMYG and aMCC (path $a$), and both models yielded a significant mediation effect ($a*b$), indicating that the aMCC played a pivotal role in mediating the impact of the avAMYG on both the behavioral Stroop effect and the pdAMYG activity (See Supplementary Figure S6).

Additional analyses were performed in order to further check the functional dissociation between the subregions of the amygdala. First, when the two subregions were compared in terms of their correlations with neuroticism, we found a weak but significant correlation with neuroticism in the avAMYG ($r = 0.54, P = 0.046$), which was not significantly different from either the left ($z = -0.93, P = 0.35$) or the right pdAMYG ($z = -1.47, P = 0.14$). In addition, no significant differences were observed between the negative and neutral picture conditions in either the left ($t(13) = -1.598, P = 0.134$) or the right pdAMYG ($t(13) = 1.161, P = 0.266$). The tests of regional differences revealed that the avAMYG was significantly different from the left ($t(13) = 2.771, P = 0.016$) but not the right ($t(13) = 1.563, P = 0.142$) pdAMYG in terms of their responses to negative vs neutral pictures.

Finally, we performed a finite impulse response analysis in order to examine whether the pdAMYG activity simply reflected a carryover effect of its response to the emotional pictures that were presented 4s before. The event-related responses averaged across all of the trials from each ROI demonstrated that the peaks of the hemodynamic responses in the avAMYG were clearly separated from and observed ~4s after those in the pdAMYG as well as in the ACC (Figure 7).

**DISCUSSION**

Consistent with the large body of previous literature on emotion–cognition interactions, this study provided both behavioral and neural evidence that emotional information can interfere with even temporally delayed cognitive tasks. As predicted, this study revealed two distinctive subregions of the amygdala that played functionally differential roles in emotional Stroop interference. More specifically, the emotional meaning of visual stimuli appeared to be assessed by the avAMYG at an early stage of each trial, whereas the pdAMYG was responsive to cognitive conflicts, such as signaling conflicts between emotional distractors and goal-directed processes to the prefrontal cortex, and the triggering of the cognitive control necessary for the successful performance of a cognitive task (Shackman et al., 2011).

The aMCC, which receives information about the emotional content of the pictures from certain brain structures, including the avAMYG, was also responsive to incongruent vs congruent trials during the subsequent Stroop task following negative but not neutral pictures. In addition, as has already been examined in many studies of the relationships between neuroticism and the activities of the amygdala and the aMCC, highly neurotic participants showed greater activities in the pdAMYG and aMCC during incongruent vs congruent trials following negative pictures, possibly reflecting their heightened emotional interference in a negative context. Taken together, the results of this
study suggested that highly neurotic people can be characterized by a high degree of interference-related activity in both the pdAMYG and the ACC, and this could account for their increased cognitive vulnerability to emotional distractors.

In humans, the anterior ventral and posterior dorsal aspects of the amygdala correspond to the lateral nucleus and the central nucleus of the amygdala, respectively (Mai et al., 1997; Whalen et al., 2001). According to anatomical data obtained in animal studies, the former is considered the major input system of the amygdala, receiving inputs from various sensory systems (Amaral et al., 1992; LeDoux, 1996), whereas the latter is considered a major output system of the amygdala, communicating with cortical systems through its connections with various neuromodulatory systems, such as the basal forebrain (Kapp et al., 1994; Jolkkonen et al., 2002). Despite the limited spatial resolution of fMRI, a similar anatomical distinction within the amygdala has been observed in a number of recent human fMRI studies, including those with resolution levels (i.e., $3 \times 3 \times 3$ mm$^3$ voxel size) that are routinely employed by numerous neuroimaging laboratories (Morris et al., 2001; Whalen et al., 2001; Kim et al., 2003; Davis et al., 2010; Gamer et al., 2010; Bach et al., 2011). Furthermore, the regional differences within the amygdala that were observed in this study nicely corresponded to the known anatomy of the human amygdala, as well as to a recent theoretical framework about the functional dissociation between the amygdala subregions, which argues that the amygdala, particularly its dorsal subregion, is a key structure in detecting and resolving predictive uncertainty in an emotional context (Whalen et al., 2001; Kim et al., 2003).

Consistent with the theoretical framework, this study provided further evidence that supported the distinctive functional roles of each subregion of the amygdala during emotion-modulated Stroop tasks. More specifically, the avAMYG showed increased responses to negative vs neutral pictures at the onset of each trial, but the level of its activity was only weakly affected by individual variability in neuroticism. However, the pdAMYG showed greater responses to incongruent vs congruent trials during the Stroop task following negative pictures, but it remained unresponsive when emotional pictures were presented. This effect was more prominent in highly neurotic individuals. As shown in Figure 7, the time-course data from the ROIs demonstrated that the peak hemodynamic responses of the avAMYG were clearly separated from, and appeared ~4 s ahead of, those of the pdAMYG. The time-course data may indicate that the avAMYG showed relatively sustained emotional responses that were elicited by the pictures and that persisted until the onset of the Stroop cue. However, the increased activity in the pdAMYG that was elicited by the subsequent Stroop task may reflect the increased demand for cognitive control required to resolve the cognitive interference during incongruent Stroop trials, particularly following an emotional distractor.

It is of interest that the avAMYG and pdAMYG are also distinctive in terms of their anatomical connections with other neural structures, in that they have denser connections with the temporal and prefrontal cortices, respectively (Bach et al., 2011). The pdAMYG, which comprises the central nucleus of the amygdala, and the basal forebrain contain the acetylcholine-producing neurons that project to most cortical regions (Selden et al., 1998). The basal forebrain, which is located immediately above the amygdala and below the basal ganglia (Heimer, 2003), receives heavy projections from, and is indeed somewhat indistinguishable from, the central nucleus of the amygdala (Price and Amaral, 1981; Jolkkonen et al., 2002). These distinctive anatomical networks that are centered on specific amygdala subregions combined with our findings regarding their functional dissociation may suggest that the avAMYG receives sensory input signals carrying emotional information through the temporal cortex and that the pdAMYG communicates with the frontal cortex, including the aMCC, in order to detect and resolve conflicts due to emotional distractors. Furthermore, these segregated neurocircuits that are associated with different amygdala subregions may inform debates about the roles of attention and consciousness in the modulation of amygdalar responses to emotional stimuli (Vuilleumier et al., 2001; Pessoa et al., 2002; Whalen et al., 2004). More specifically, we speculate that the communication between the avAMYG and the temporal cortex may be engaged in processing emotional information, even with limited attention, whereas the communication between the pdAMYG and the prefrontal cortex may be more prone to attentional and conscious modulation.

Numerous studies have indicated that the aMCC is a key neural participant in conflict monitoring and attentional processes (van Veen et al., 2001; Botvinick et al., 2004; Egner et al., 2008), and its strong inter-relationship with the amygdala, particularly under situations with emotional conflict, has been well documented in a number of human neuroimaging studies. For example, the aMCC is known to have both anatomical (Agleton et al., 1980; Stefanacci and Amaral, 2000) and functional (Etkin et al., 2006; Egner et al., 2008) connectivity with the amygdala, and it appears to play a key role in the processing of integrating emotional signals that are conveyed through the amygdala and in the regulation of conflicts due to emotional distractors (Compton, 2003; Etkin et al., 2006; Banks et al., 2007; Egner et al., 2008). Consistent with previous studies of emotional Stroop tasks, the aMCC showed increased activity during incongruent vs congruent trials, and this differential activity was more prominent following negative vs neutral pictures. It was of particular interest to note that the emotion-modulated behavioral Stroop interference effect resembled amygdala responses to emotional pictures and subsequent aMCC activity during the Stroop task. Taken together, the increases in aMCC activity appeared to reflect increased cognitive conflicts due to signals from the amygdala that were triggered by emotional distractors, resulting in poor behavioral flexibility during the Stroop task, particularly in a negative emotional context.

This study demonstrated that increased neuroticism tended to be associated with increased activity in the cingulate cortex in response to negative vs neutral pictures and in its Stroop interference-related activity following negative vs neutral pictures. These findings appeared to be consistent with those of a large body of previous neuroimaging studies on the role of the aMCC in the regulation of cognitive interference due to emotional distractors. For example, highly neurotic individuals have shown increased activity in the aMCC and diminished control abilities over negative affect (Bystritsky et al., 2001). Recent neuroimaging studies have also provided evidence that the amygdala and aMCC are importantly associated with individual differences in neuroticism (Canli et al., 2001; Eisenberger et al., 2005; Haas et al., 2007). More specifically, the latest findings have indicated that the functional connectivity between the amygdala and the aMCC appears to be critically involved in individual differences in neuroticism (Cremers et al., 2010; Fruhholz et al., 2010). Interestingly, there was a significantly positive correlation between the two subregions of the cingulate cortex, in that one region showed a positive correlation between its response to negative vs neutral pictures and the neuroticism scores and the other showed a positive correlation between its Stroop interference-related activity following negative vs neutral pictures and the neuroticism scores. This perhaps suggested that highly neurotic participants may be characterized by an increased sensitivity of their ACC responses to negative emotional signals, which then resulted in increased interference-related activity in the aMCC and poor Stroop task performance. A similar correlation between neuroticism and Stroop interference-related activity was also observed in the pdAMYG. As can be seen with the mediation analysis in this
study, the aMCC appeared to play a pivotal role in mediating the impact of emotional distractors on the Stroop interference-related pdAMYG activity, particularly in highly neurotic individuals (Krug and Carter, 2010).

This study had a few limitations. First, the negative and positive emotional pictures were not equivalent in terms of their arousal dimensions, and this was primarily due to the difficulty of finding positive emotional pictures that were as arousing as negative emotional pictures in the International Affective Picture System database, particularly in our sample. Second, our sample size was somewhat limited as we tested only female participants. A study with additional male participants would provide a more complete picture of the neural mechanisms underlying the individual variabilities in cognitive interference due to emotional distractors. Third, a few recent studies have applied high-resolution imaging protocols with isotropic voxel sizes that range from 1.5 to 2 mm$^3$ in order to examine separate amygdala subregions (Gamer et al., 2010; Bach et al., 2011; Prévost et al., 2011, 2012). Future studies that use scan protocols with more advanced spatial resolutions are necessary to replicate and expand the present findings. Finally, although we defined SET 4 as the congruent trial in accordance with the original study (Luo, 1999), the RT data suggested that at least some trials in SET 4 may have required higher cognitive control, so that SET 4 may not be considered the same congruent trial condition as SET 1. Therefore, in order to ensure that our main findings were not driven by SET 4, we reanalyzed all of the imaging data without SET 4 and carefully examined all of the findings in the original analysis. This analysis revealed results that were highly consistent with the original results but slightly weaker, potentially because the contrasts were made between conditions with an unequal number of trials (Supplementary Figure S7).

In summary, this study demonstrated that distinctive neurocircuits may be recruited during the processes of detecting emotional information and regulating conflicts between emotional distractors and cognitive goals. In addition, this study confirmed a close functional association between the aMCC, the amygdala and individual differences in neuroticism, providing a more sophisticated neurobiological account of increased cognitive vulnerabilities to emotional distractions in highly neurotic individuals. More specifically, our findings suggested that the aAMYG assesses the emotional value of incoming stimuli at an early stage of the process, which then triggers aMCC activity. The increase in aMCC activity may result in a descending signal to the pdAMYG, which may send an ascending signal back to the aMCC in order to modulate the interference-related activity in the aMCC. This bidirectional communication between the aMCC and the pdAMYG may determine the degree to which one’s cognitive performance is influenced by emotional distractors, and this may represent one possible biological basis of neuroticism. A more sophisticated understanding of how emotion interacts with cognition in the brain and how emotional conflicts are detected and resolved would provide significant insight into the pathophysiology of various psychiatric disorders, such as depression, obsessive compulsive disorder and posttraumatic stress disorder, which are all more or less commonly characterized by emotional dysregulation (Pessoa, 2008). The present study could also be easily extended in order to include a patient population with the objective of characterizing symptoms with greater precision that are based firmly on biological constraints.

**SUPPLEMENTARY DATA**

Supplementary data are available at SCAN online.

**Conflict of Interest**

None declared.

**REFERENCES**


