Stroop Performance in Normal Control Subjects: An fMRI Study

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In an attempt to clarify regional signal intensity changes, which may accompany the performance of the Stroop Color–Word task, healthy subjects were imaged using the fMRI BOLD technique while performing a modified version of the task. Both the AAA and VOA subdivisions of the anterior cingulate cortex were significantly activated during the interference condition; however, only the signal intensity change within the VOA correlated with task performance. Additionally, signal intensity change was significantly increased in the VOA subdivision of the cingulate cortex when controlling for signal intensity change present during the performance of a color naming task. This study extends previous findings by demonstrating that in healthy adults, a subdivision of the cingulate cortex is specifically associated with the cognitive demands present in the interference condition.

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Key Words: stroop; fMRI; anterior cingulate subdivisions; cognitive challenge.

INTRODUCTION

The interference subtest of the Stroop Color–Word test has been shown to reliably produce interference effects on response latency; however, the identification of focal cortical brain regions associated with the network that underlies the interference process has been more difficult to characterize. Positron emission tomography (PET) investigations provided the first evidence for regional brain changes during the Stroop interference subtest, particularly within the anterior cingulate cortex. Many of these studies were limited in their spatial resolution, however, and generally considered the entire anterior cingulate as a single functional region. The application of functional magnetic resonance imaging (fMRI) methods in the current study allows for improved visualization of changes in cortical signal intensity during cognitive challenge paradigms enabling the further subdivision of the anterior cingulate into discrete subregions.

Considered one of the most reliable psychometric tests (Jensen et al., 1965; Uechi, 1972), and found to remain relatively unaffected by test–retest situations (MacLeod, 1991), the Stroop Color–Word task has been used both as a screening instrument and as part of a large battery of tests for the screening and detection of frontal/executive brain dysfunction. The original Stroop task is comprised of three subtests, designed to establish competing response tendencies within the study subject and assess the subject's ability to suppress interfering stimuli. In the Color Naming subtest, the subject is asked to report the color of randomly sequenced color rectangles, thus establishing the tendency to respond to color. In the Word Reading subtest, the subject is asked to read color words randomly printed in black ink, establishing a response set to reading color words. In the Interference condition, the subject is given color words which are printed in an incongruent ink color. The subject is asked to report the ink color, and therefore has to suppress the tendency to read the color word. All sections of the test are timed, and the time to complete each section is the dependent variable of interest. Difficulty with inhibition is reflected by an increase in time in the interference section relative to the color naming or word reading sections. Slowed performance on the interference subtest has thusfar been interpreted to be reflective of difficulty with the ability to resist interference, a cognitive function associated with frontal cortical integrity (Mesulam, 1987).

One advantage of the Stroop task is that it has been studied extensively by neuropsychologists and cognitive neuroscientists since it was introduced in 1935 (Stroop, 1935), and has gained prominence since the emergence of the automatic-controlled distinction in cognitive psychology (Posner and Snyder, 1975; Shiffrin and Schneider, 1977). This is largely due to the fact that the Stroop task appears to pit an automatic process (word reading) against a controlled, conscious process (color naming). It is widely used as an index of attention and executive control, as the task requires the ability to actively inhibit an overlearned response in favor of a more voluntary response. The interference effect or Stroop effect has been shown to be a robust phenomenon (Cohen et al., 1990; MacLeod, 1991), and
speed of information processing, automaticity of word reading, and parallel processing models have all been proposed to explain the slowed processing time associated with completing the incongruent condition.

Pardo and colleagues applied PET techniques to healthy adult subjects, and reported increased regional cerebral blood flow (rCBF) of the cingulate cortex during the Stroop interference subtest as compared to the color naming subtest (Pardo et al., 1990). Although a number of cortical regions were activated during this complex task, the authors concluded that the anterior cingulate cortex displayed the greatest activation associated with the executive-attentional demands of this task. In another study, which also utilized PET methodology and a different adaption of the Stroop task, Bench et al. studied healthy volunteers and varied stimulus rate, practice effects, and visual stimuli (Bench et al., 1993). Results from that study indicated that the pattern of blood flow was dependent upon the design of the paradigm. Subjects who had practiced the task exhibited changes in right orbitofrontal and midcingulate rCBF, while unpracticed subjects demonstrated increased right anterior cingulate rCBF. George et al. (1994) successfully activated the left midcingulate of nonpsychiatric control subjects during a PET study that used modifications of the Stroop task. One recent PET study investigated facilitation and interference effects related to Stroop performance, and found increased blood flow within the anterior cingulate (Carter et al., 1995).

fMRI methods provide greater spatial resolution which results in improved visualization of anatomic regions. This visualization of smaller brain regions allows for the measurement of localized changes in signal intensity in response to cognitive challenge paradigms. Initial applications of fMRI techniques using the Stroop test have produced results similar to those using PET methods. Peterson and colleagues used fMRI techniques to examine Stroop performance in healthy subjects during congruent and incongruent conditions, and concluded that given the increased signal intensity in response to the interference condition, the findings were supportive of a parallel distributed processing model for color-word interference within the anterior cingulate (Peterson et al., 1999). In a recent fMRI study by Brown which examined healthy adults during overt and covert performance of the Stroop test, investigators found increased signal intensity in the anterior cingulate during an interference condition relative to a neutral condition in both silent and out loud trials (Brown et al., 1998). Finally, in studies by Bush and Whalen, the Counting and Emotional Counting Stroop, respectively, were used and higher signal intensity was reported within the anterior cingulate regions during the incongruent or negative conditions compared to congruent or neutral conditions (Bush et al., 1998; Whalen et al., 1998). Thus, multiple imaging experiments have yielded results in support of the anterior cingulate cortex as a site involved with mediating selective attention, as measured by the Stroop task.

The cingulate cortex is described as cytoarchitecturally heterogeneous and comprised of the anterior and posterior cortices, each of which possesses different thalamic and cortical connections (Vogt et al., 1992). The anterior cingulate has been linked to a number of functions including response to painful stimulation, somatomotor function, response selection, vocalization and attention, and has been considered a key cortical area during the processing of cognitively demanding information (Corbetta et al., 1991). In fact, the anterior cingulate can be further subdivided into discrete anatomic and behavioral subdivisions, as posited by Vogt (1992); visceromotor (VMA), vocalization (VOA), nociceptive (NCA), rostral cingulate motor (CMAr), and attention to action (AAA). Recent investigations, which utilized Stroop paradigms in healthy control subjects have demonstrated changes in the AAA region of the anterior cingulate, an area hypothesized to be responsible for focused attention (Posner et al., 1988). Additional neuroimaging studies, which have examined “emotional” versions of the Stroop task or include subjects with affective disorders have reported increased activation in the VOA region (Mayberg, 1997; Whalen et al., 1998). It remains unclear, however, what role different subdivisions of the anterior cingulate might play during performance on each of the conditions of the Stroop test.

In summary, while a number of investigations have used fMRI methods to examine changes in cortical signal intensity during the Stroop test, the current study is unique in that it examines specific subdivisions of the anterior cingulate cortex during each subtest of the task, rather than considering the anterior cingulate as a single region. Additionally, unlike other neuroimaging studies which have used the Stroop task, the current investigation does not involve warping images to a preexisting template or Talairach space; each subject is examined individually using an ROI localization technique, a method which has been used to quantify localized brain activity (Constable et al., 1998). This method allows for the consideration of individual structural variability. Finally, the current study enables subjects to vocalize their responses while performing each subtest of the Stroop test, as opposed to the more common method of using a button box or other device to record subjects’ performance. Versions of the Stroop task which include vocalized responses have previously been shown to produce a more robust interference effect (MacLeod, 1991), and are theoretically closer to the original Stroop task than those that use nonvocalized response methods.

In an attempt to better characterize changes in cortical signal intensity that may accompany the performance of the Stroop task, healthy adult subjects were
imaged using the fMRI BOLD technique while performing a modified version of the Stroop task. It was hypothesized that these subjects would demonstrate significant changes in signal intensity from baseline states during the interference subtest. Further, given the multiple demands of the interference subtest, we hypothesized that increased signal intensity in the AAA and VOA subdivisions of the anterior cingulate cortex would be correlated with task performance.

Methods

Twelve right-handed, native English speaking, non-psychiatric comparison subjects were recruited for this study. All subjects received the Structured Clinical Interview for DSM-IV, Patient edition (Spitzer et al., 1996) to ensure that no history of psychiatric disorder was present. Subjects with a history of head injury, past psychotropic medication use, seizure disorder, substance abuse or dependence, or neurological disorder were excluded. Each subject was screened for color blindness, as the ability to accurately discriminate color is imperative to the current study. Additionally, subjects who expressed reticence about entering the magnet environment or who could not complete the scanning protocol due to claustrophobia were removed from the study. All subjects signed an informed consent, which described in detail the scanning procedures and which had been approved by the McLean Hospital Institutional Review Board. Finally, subjects received a small monetary compensation for their completed participation. A combined protocol, applying both conventional MR and functional MR imaging, was used to assess changes in cortical signal intensity and to generate clinical assessments of brain morphology. Additionally, a brief neuropsychological battery was administered to study subjects to control for possible verbal and performance IQ effects.

Conventional Image Acquisition

Conventional imaging data was acquired to assist with precise localization of the regions of interest. The protocol included two different MR protocols: a double-echo spin echo (SE) protocol and a 3-dimensional Fourier-transform (3-DFT) spoiled-gradient recalled acquisition. The MR images were acquired on a 1.5T Signa whole body imager (GE Medical Systems, Milwaukee). Sagittal localizer images were obtained first, followed by double echo spin-echo 3-mm coronal slices of whole brain. The imaging parameters consisted of a 256 × 256 matrix, TR = 3000 ms, TE = 30 and 8 ms, and a 24-cm field of view with two interleaved acquisitions. This resulted in 108 contiguous double echo slices. The voxel dimensions were 0.975 by 0.975 by 3mm. We used a 3-D Fourier transform spoiled gradient-recalled acquisition (3-DFT SPGR), as this sequence generates images with good contrast between gray matter and white matter (Shenton et al., 1992). The data consisted of 124 1.5-mm-thick coronal slices, collected with the following imaging parameters: TR = 35 ms, TE = 5 ms with one repetition, and flip angle = 45° with a 256 × 256 matrix. Voxel dimensions were 0.975 by 0.975 by 1.5 mm.

Regions of Interest

Boundaries for cortical regions of interest (ROI) were defined on the basis of recognizable landmarks. Additionally, the atlases of Schnizlein and Murtagh and Talairach were used as anatomical guides (Schnizlein and Murtagh, 1990; Talairach and Tournoux, 1993). The neuroanatomical regions examined included the left and right anterior cingulate cortex (Brodmann’s area 24 and 25). In order to clarify the functional specificity within discrete regions of the anterior cingulate, the anterior cingulate was further subdivided bilaterally into two areas previously defined by Vogt and colleagues (1992), which included the vocalization area (VOA), the most anterior section of the cingulate cortex, and the attention to action area (AAA), which is just posterior to the VOA (see Fig. 1A). In this study, coronal images were acquired after placement of a sagittal localizer (see Fig. 1B). The plane of section and the image thickness (6 mm) limited the number of slices that contained either AAA or VOA. In general, these regions could only be sampled from a single slice. The AAA region was identified as the area of the anterior cingulate superior to the anterior corpus callosum, while the VOA region was defined as the area immediately anterior to the genu of the corpus callosum. These areas were defined by examining individual high resolution MR coronal images for each study subject and outlining the cortical regions under study (see Fig. 2). It has recently been shown that this ROI method of localization and analyses is a sensitive method of detecting cortical activation between two regions (Constable et al., 1998). The ROIs were selected a priori, as the anterior cingulate has been shown to be associated with the anterior attentional system (Bench et al., 1993). Given the importance of reliably assessing these precise localizations, a single investigator who had completed formal reliability training and had an established intra-rater reliability of k > 0.93 and an interrater reliability of k = 0.87-0.91 for the two regions completed all of the ROI measurements.

Echoplanar Image Acquisition

Subjects completed dynamic NMR imaging with a high-speed imaging device (1.5 Tesla; General Electric Signa, modified by Advanced NMR Systems) and a quadrature head coil receiver system using a modified echoplanar imaging technique. We used a noncontrast technique, based on a T2*-weighted gradient echo
pulse sequence, which has demonstrated sensitivity to the local concentration of paramagnetic deoxyhemoglobin (Ogawa et al., 1992). For each subject, 16 coronal slices were collected with a thickness of 6 mm, and a 1-mm skip between slices. Images were acquired in continuous succession for each brain activation phase using the identical imaging pulse sequence and were localized in a plane perpendicular to the anterior/posterior comissure (AC–PC) line. The most posterior of the slices was placed just behind the central sulcus, which resulted in adequate coverage of the cingulate cortex. We collected images every 3 s using a gradient echo pulse sequence (TE = 40 ms, flip angle = 75°). An image matrix of 64 × 128 was used with a 3 × 3-mm in-plane resolution. Each challenge task included baseline and recovery conditions. A gradient echo pulse sequence was used in order to maximize the amplitude of the task induced signal intensity changes.

FIG. 1. Subdivisions of the Anterior Cingulate according to Vogt (A) and a T1 Sagittal-weighted localizing image (B). Taken from Images of Mind, Posner and Raichle, 1997.

FIG. 2. Coronal images illustrating voxels of interest in the anterior cingulate cortex.
FIGURES 3 AND 4

Baseline vs. Task Performance - AAA

Signal change (%)

Time (s)

INTF:
\[ t = 2.63 \]
\[ p = 0.027 \]

CN:
\[ t = 1.26 \]
\[ p = 0.238 \]

WR:
\[ t = -0.83 \]
\[ p = 0.426 \]

Paradigm
- INTF
- CN
- WR

Baseline vs. Task Performance - AAA

Signal change (%)

Time (s)

INTF:
\[ t = 3.02 \]
\[ p = 0.014 \]

CN:
\[ t = 1.644 \]
\[ p = 0.135 \]

WR:
\[ t = 0.992 \]
\[ p = 0.347 \]

Paradigm
- INTF
- CN
- WR

FIGURES 3 AND 4
Challenge Paradigms

Study subjects completed three imaging epochs consisting of the three Stroop subtests. To visualize signal changes, a task activation paradigm, which alternated between resting and stimulated states was utilized. This protocol allowed us to compare signal intensity changes of the images collected during activation for any paradigm against signal changes produced in images during the rest (off) conditions. We included a rest condition, although it is acknowledged that the resting state is not a completely controlled condition (Roland, 1993). The Stroop test challenges the ability to inhibit inappropriate responses and resist interference (MacLeod, 1991). We adapted a standard version of the Stroop procedure, which includes three tasks: Color Naming, Word Reading, and the Interference subtest (Stroop, 1935). These challenge paradigms are a modification of Stroop methods used by Pardo, Bench, and Carter (Bench et al., 1993; Carter et al., 1995; Pardo et al., 1990). Stimuli were presented visually from a Macintosh controlled video display. Each scanning epoch was divided into five segments; during the odd numbered segments, subjects were asked to relax. During even numbered segments, subjects were asked to perform one of three attentional tasks. Each experimental epoch, therefore, consisted of 4 off/on cycles with a 30-s baseline rest period prior to the presentation of any stimuli. During the two 30-s “on” periods within each epoch, stimuli were presented to study subjects, who in turn completed one of the three Stroop subtests. Each subject performed the following three tasks in the order described: (1) Color naming: The subject was asked to report the color of randomly sequenced color stimuli, represented as color blocks, which were printed in red, blue and green ink; (2) Word reading: The subject was asked to read color words (“red,” “blue,” and “green”) randomly printed in black ink, which established a response set to reading color words; and (3) Interference: The subject was presented with the same color words as in the Word Reading subtest, which are printed in an incongruent ink color (i.e., “red,” “blue,” “green”). The subject was asked to report the ink color; to succeed, he has to suppress the tendency to read the color word (i.e., the word blue written in red ink). Given the importance of establishing a response set for color naming and word reading, the three conditions were always administered to study subjects in the order described. For both the Color Naming and the Word Reading conditions, each 30-s activation phase consisted of the presentation of 10 lines of stimuli, each containing six targets for 2500 ms with a 500-ms interval between lines of stimuli. For the Interference subtest, each activation phase consisted of the presentation of 6 lines of stimuli, each containing six targets for 4500 ms, with a 500-ms interstimulus interval. This timing sequence was derived by testing healthy subjects off-line in order and calculating their average reaction time for the completion of six targets in each condition. This stimulus presentation rate was then set to a rate at which subjects would make errors on approximately 10% of the interference stimuli (Brown et al., 1999). The total number of lines of stimuli was therefore different for the interference subtest relative to the color naming and word reading subtests due to the increased reaction time exhibited by healthy subjects during the off-line testing.

To ensure visualization of signal changes, we collected multiple cycles of rest/activation. Each scanning epoch consisted of two trials of only one of the subtests; no mixed presentation of stimulus type was administered. Performance on the task was measured by the total percent of targets correctly identified within a scanning epoch. In order to minimize motion associated with vocalization of responses, we inserted foam cushions between the subjects’ head and the quadrate head coil for a snug fit. We also taped subjects’ across the forehead and chin during landmarking, and checked each subject’s position upon exiting the bore. While these methods do not reduce fine motion associated with vocalization a response, the amount of gross movement is minimized. Motion correction software which corrects for both in-plane rotational and translational motion was applied prior to data analyses (DART, Maas, 1997). Data which exceeded 1 degree or 1 millimeter in either the rotational or translational plane was excluded from the analyses.

RESULTS

Demographic characteristics for the study subjects are listed in Table 1. fMRI data was collected on all 12 subjects; however, two of the subjects were excluded from analyses due to excessive motion. fMRI data was successfully analyzed for the remaining subjects for each of the ROIs. The mean percent change in signal intensity from baseline was calculated separately for the AAA and VOA regions on the right and left during the color naming, word reading and interference conditions. Initially, the mean change in signal intensity

<table>
<thead>
<tr>
<th>Age</th>
<th>23.5 (± 3.93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
</tr>
<tr>
<td>Education Level</td>
<td>15.9 (± 1.22)</td>
</tr>
<tr>
<td>Handedness</td>
<td></td>
</tr>
<tr>
<td>Right (9R)</td>
<td>114.25 (± 9.56)</td>
</tr>
<tr>
<td>Left (1L)</td>
<td>125.25 (± 14.59)</td>
</tr>
</tbody>
</table>

TABLE 1

Demographic Characteristics

Study Subjects (N = 10)
for each region and all three Stroop tasks were compared to the null hypothesis of no change from baseline using one-sample t tests in SPSS 10.0.

As illustrated in Figs. 3–6, performance of the color naming and word reading subtests did not yield significant changes in signal intensity within either region of interest on the right or left side (all $P > 0.05$). In contrast, during the performance of the interference subtest, significant change from baseline occurred within the AAA region on both the left ($t(9) = 2.63; P = 0.027$) and right ($t(9) = 3.02; P = 0.014$) sides. Within the VOA region, there was also evidence of increased signal intensity and a statistically significant increase on the right ($t(9) = 2.39; P = 0.04$), and a trend towards greater change on the left ($t(9) = 1.97; P = 0.08$) side.

Given that our primary interest was to evaluate signal intensity change within these regions during the performance of the interference subtest relative to the change associated with color naming, we conducted paired t-tests between each subject’s laterized regional signal intensity change during the interference and color naming subtests. As hypothesized and illustrated in Table 2, signal intensity change within the right VOA was significantly greater during the interference subtest relative to the color naming subtest ($t(9) = 2.54; P = 0.032$), whereas activation within the left VOA did not differ significantly between the two conditions ($t(9) = 0.18$, ns). Similarly, there was no difference between the interference and color naming subtests in the AAA region for the right ($t(9) = 0.80$, ns) or left ($t(9) = 0.24$, ns) side. We also conducted paired t-tests between each subject’s laterized regional signal intensity change during the interference and word reading subtests. We found that signal change within the right VOA was significantly greater during the interference subtest relative to the word reading subtest ($t(9) = 2.23; P = 0.048$), whereas activation within the left VOA did not differ significantly between the two conditions ($t(9) = 1.33$, ns.). In addition, a significant difference between interference and word reading was detected in the AAA region on the right ($t(9) = −3.169; P = 0.011$) but not the left ($t(9) = −2.064$, ns) side.

### Correlations between Signal Intensity Changes and Task Performance

In order to more fully characterize the relationship between the signal intensity changes and the cognitive demands of each Stroop condition, Pearson correlation coefficients were performed between cortical activation and performance variables. Correlations for signal intensity change and performance are listed in Table 3. No strong correlations ($r > 0.50$) were detected for either region during any condition on the left side. However, in the right VOA region, a statistically significant correlation was demonstrated for signal change and performance on the interference subtest ($r = −0.690; P = 0.027$), although the correlations between performance and signal intensity change were weak for both the color naming ($r = 0.027$) and word reading conditions ($r = −0.348$). Within the right AAA subdivision, signal intensity change was strongly correlated with performance on the color naming subtest ($r = −0.559; P = 0.093$) but not the word reading ($r = −0.198$) or interference subtest ($r = −0.418$).

### DISCUSSION

Results from the present study indicate that significant changes in signal intensity are present in the anterior cingulate of healthy subjects when the interference subtest of the Stroop Color Word Test is compared to baseline states. Furthermore, the patterns of signal intensity change demonstrated during each condition appear to be specific to individual cortical regions. Performance of the color naming subtest, which involves discrimination and naming of colors, attention, and verbal response resulted in a nonsignificant increase in signal intensity from baseline states in both regions of interest. The interference subtest, which requires active inhibition of an overlearned response in favor of a less automatic one, resulted in a significant increase in average signal intensity in both the AAA and the VOA regions of the cingulate cortex. Moreover, only the right VOA demonstrated a significant difference in signal intensity change between the color naming and interference conditions. Given that one key processing component during the interference condition is the production of color names, the relative increase in signal intensity during the naming of incongruent colored words compared with the signal intensity change during color naming suggests the inhibition of responses is associated with the VOA region. Although both subdivisions showed increased signal intensity during the interference subtest, performance of the task was significantly inversely correlated only with the signal intensity change within the right VOA region ($P = 0.027$), further indicating that the changes measured in the VOA may be reflective of the behavioral demands of the interference subtest. Taken together, these findings underscore the specificity of the cortical response between the two subdivisions.

As the Stroop test requires the inhibition of an overlearned response (word reading) in favor of a less automatic behavior (color naming), it has been considered an appropriate model for the examination of conflict resolution and the regulation of impulse control (Matochik et al., 1996). It is of note that our findings demonstrate differential signal intensity between the word reading and interference subtests, which was significant on the right side for both regions of interest. This is not surprising, given the relative automaticity
Baseline vs. Task Performance - VOA

![Graph showing signal change versus time for different conditions.]

**FIGURES 5 AND 6**
of word reading as compared to the complex processing required for the interference subtest. The response of the anterior cingulate subregions in the present study also lend support to the hypothesis by Peterson et al. (1999), which states that the anterior cingulate coordinates multiple attentional subsystems and plays a crucial role in other cognitively demanding tasks. Moreover, Cohen et al. (1994) reported increased rCBF of the anterior cingulate in non-psychiatric subjects in response to a verbal working memory paradigm. fMRI studies by Jonides et al. (1993) and Rao et al. (1995) reported increased signal intensity within the cingulate as well as prefrontal regions in response to spatial working memory challenge paradigms. Results from these investigations suggest the role of the anterior cingulate as an area which is responsible for the organization and integration of a wide range of cognitive functions which have attentional components.

Overall, findings from the current study are in agreement with recent fMRI studies of Stroop performance. In a study by Brown (1998) which examined normal control subjects during overt and covert performance of the Stroop test, investigators found increased signal intensity changes in the anterior cingulate during an interference condition relative to a neutral condition in both silent and out loud trials. It should be noted that in this study, “covert” conditions were not silent; subjects were instructed to speak loudly enough that they could hear their own responses, yet no tongue, jaw, or lip movement was allowed, thus allowing greater comparison to the current study. Additionally, the authors reported that the interference condition evoked significantly greater pupillary dilation than the color naming condition, indicative of a greater cognitive load during interference. Peterson (1999) used fMRI to examine Stroop performance in healthy subjects during congruent and incongruent conditions with methods nearly identical to Pardo (1990). In a novel analytic approach to functional imaging data, Peterson identified regions for analytic procedures in order to model functional connectivity during Stroop performance. The authors concluded that based on the increased cortical signal change in response to the interference condition, their findings were consistent with a parallel distributed processing model for color-word interference within the anterior cingulate. Two fMRI studies, which used variants of the Stroop test, also lend support to the current study findings. In a study by Bush, the Counting Stroop was utilized, which included congruent, neutral, and incongruent conditions. The authors reported significantly higher signal intensities in posterior anterior cingulate regions during the incongruent condition than neutral or congruent conditions (Bush et al., 1998). In a study by Whalen et al. (1998), which utilized the Emotional Counting Stroop, study subjects demonstrated the greatest change in signal intensity levels in a more anterior section of the anterior cingulate as compared to the current investigation as different methodologies were used. In particular, both the Bush et al. and Whalen et al. investigations use a button press response mechanism as compared to overt vocalization and both apply Talairach warping techniques in their data analyses, which may affect localization of signal change. Further, in these two studies, visual stimuli appear on the screen in a vertical as opposed to horizontal fashion. Subjects are instructed to report the number of words which appear in each trial. The in-

### TABLE 2
Comparison of Average Signal Intensities by Task

<table>
<thead>
<tr>
<th>Task Comparison</th>
<th>AAA Right</th>
<th>AAA Left</th>
<th>VOA Right</th>
<th>VOA Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color naming vs word reading</td>
<td>t = 1.200</td>
<td>t = 1.650</td>
<td>t = 1.080</td>
<td>t = 1.120</td>
</tr>
<tr>
<td></td>
<td>P = 0.260</td>
<td>P = 0.133</td>
<td>P = 0.310</td>
<td>P = 0.290</td>
</tr>
<tr>
<td>Word reading vs interference</td>
<td>t = -3.169</td>
<td>t = -2.064</td>
<td>t = 2.284</td>
<td>t = -1.330</td>
</tr>
<tr>
<td></td>
<td>P = 0.0110</td>
<td>P = 0.069</td>
<td>P = 0.048</td>
<td>P = 0.226</td>
</tr>
<tr>
<td>Color naming vs interference</td>
<td>t = -0.800</td>
<td>t = -0.243</td>
<td>t = -2.543</td>
<td>t = -0.184</td>
</tr>
<tr>
<td></td>
<td>P = 0.444</td>
<td>P = 0.814</td>
<td>P = 0.032</td>
<td>P = 0.858</td>
</tr>
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### TABLE 3
Correlations of Signal Intensity Change and Task Performance

<table>
<thead>
<tr>
<th>Task Condition</th>
<th>AAA Right</th>
<th>AAA Left</th>
<th>VOA Right</th>
<th>VOA Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color naming</td>
<td>r = -0.559; P = 0.093</td>
<td>r = -0.198</td>
<td>r = -0.418</td>
<td></td>
</tr>
<tr>
<td>Word reading</td>
<td>r = 0.081</td>
<td>r = 0.067</td>
<td>r = -0.248</td>
<td></td>
</tr>
<tr>
<td>Interference</td>
<td>r = -0.369</td>
<td>r = -0.296</td>
<td>r = -0.690; P = 0.027</td>
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<tr>
<td></td>
<td>r = 0.035</td>
<td>r = -0.113</td>
<td>r = -0.308</td>
<td></td>
</tr>
</tbody>
</table>

* P values calculated for all “strong” correlations (r > 0.50).
interference effect which results from the conflict between reading and counting may not be equivalent to the interference effect which occurs between the competing demands of color naming and word reading of stimuli which appear horizontally. In spite of these differences, both sets of investigations report the highest levels of signal intensity within the anterior cingulate during the completion of an interference condition.

Despite the increased temporal and spatial sensitivity offered by fMRI techniques, motion artifact is often associated with changes in cortical signal intensity (Hajnal et al., 1994). A major concern of fMRI studies, which require the vocalization of responses is the possible confound produced by head motion artifact. Thus, it is not surprising that a number of previous studies which have utilized Stroop or Stroop-like tasks utilized a button box within the neuroimaging environment for subjects to indicate their response to stimuli (Bush et al., 1998; Whalen et al., 1998). Using this technique, each button is assigned a color, and each time the subject is required to respond, they simply press the button which corresponds to the color of the stimuli presented. This method attempts to solve the problem of artifact produced by subjects vocalization of responses, but introduces a number of potential confounds. In requiring subjects to press one of several buttons in response to stimuli, each of which are coded to a specific color, an additional cognitive load is added to the task. Subjects must be trained in order to learn which button corresponds to each color, which adds an undesired layer of cognitive complexity in order to recall which button to press when presented with each stimuli (Bush et al., 1998). Additionally, as several theories characterizing the cognitive processes which underlie the Stroop phenomena include two distinct pathways, one for color naming and one for word reading, each of which is competing for the response channel (vocalization), the elimination of a verbalized response would appear to alter the processes at work. Further, vocalization has been shown to produce larger interference effects in behavioral studies (MacLeod, 1991), and we have therefore included vocalized responses in our study design. To address the issue of potential motion artifact, we have implemented the DART registration algorithm which corrects for rotational and translational motion (Maas et al., 1997).

Although several investigators have utilized fMRI techniques to examine cortical signal intensity during the completion of the interference subtest of the Stroop test, the current study differs from past investigations in a number of ways. In previous studies, the anterior cingulate has frequently been treated as a single functional unit, without regard to discrete subdivisions. However, a number of cytoarchitectural studies have suggested specific roles for subdivisions of the anterior cingulate making the examination of these subregions crucial (Vogt, 1992; Devinsky and Luciano, 1993). While all previous neuroimaging studies that have utilized a Stroop task have utilized Talairach warping techniques, the current study examined each subject using individual ROI localization, without the use of warping images to a pre-existing template. Each ROI was selected individually for each study subject, thereby taking into account individual structural variability. Additionally, while a number of neuroimaging studies have collected performance data during the Stroop test, the current study is the first to examine changes in signal intensity within specific subregions of the anterior cingulate cortex with regard to individual subtest performance. Therefore, as stated previously, a strength of the current study is the ability to correlate individual performance with signal changes from discrete brain regions based on each subjects' own neuroanatomy. Finally, the current investigation required vocalization of subject responses for each subtest of the Stroop, a condition set forth in the original design of the task (Stroop, 1935).

Several factors should be considered in interpreting the study findings. First, the sample size consisted of only 10 subjects, which limits the generalizability of the study findings. Although neuroimaging studies are most often performed on a small number of subjects given the considerable time and expense of conducting such investigations, the results of such must be interpreted carefully. Additionally, this study utilized a block approach with regard to fMRI techniques as compared to single event fMRI approaches. While the single event technique can provide more precise temporal information regarding response to individual trials, the resultant cortical signal intensity change is small and may not yield sufficient signal to noise in all paradigms (Buckner et al., 1996). Further, interpretation of data still involves averaging responses over multiple stimulus presentations (Mckown et al., 1998). In this study, where the investigation of activation in the anterior cingulate was of primary importance, a block design was used in order to assure the detection of adequate cortical signal.

For the current study, we examined the anterior cingulate based on designations first made by Vogt et al., (1992), which originated from primate cytoarchitectural studies. Recent investigations have suggested that in humans, the anterior cingulate may be split into four subregions, which include an anterior or perigenual (pACC) and a midcingulate region (MCC), each of which has distinct functional properties and may be further subdivided (Vogt, 1993, 1997). The perigenual distinction encompasses the VOA, while the midcingulate cortex includes the AAA (Vogt, personal communication, 2001). A number of previous studies using Stroop tasks have reported MCC activation during variants of the “classic” or cognitive Stroop tasks, while others report increased cortical activation of the pACC during emotionally laden challenge tasks (Bush, 1997;
Whalen, 1998). As the current study utilized a different version of the Stroop task and different data analytic techniques, it is somewhat difficult to compare the activation patterns within the subdivisions to findings from previous fMRI studies. Nevertheless, the activation patterns detected in the current study are in agreement with previous investigations which reported increased signal intensity within the anterior cingulate cortex during the interference subset of the Stroop task. Given the current evolution regarding the cytoarchitecture and function of the anterior cingulate and the challenge of accurately identifying objective landmarks for specific subdivisions, future studies will likely need to consider these factors carefully.

In conclusion, the current study identified increased signal intensity change within both the AAA and VOA subdivisions of the anterior cingulate cortex during the interference condition of the Stroop test. Although both regions demonstrated significant increases in cortical signal intensity during the interference conditions, only the activation in the VOA remained significant after controlling for the signal change resulting from color naming. In addition, the signal intensity change within the VOA region was significantly correlated with performance during the interference task. The strong inverse correlation between cortical signal change within the VOA and performance of the interference condition suggest a specific role for the VOA during the completion of the interference subtest. These results are consistent with the hypothesis that anterior cingulate regions activate in response to the competing demands produced during the interference condition of the Stroop test. This study extends previous findings by demonstrating that in healthy adults, the VOA is specifically associated with the processing components present in the interference condition.

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REFERENCES


