Published in final edited form as:

Biol Psychol. 2009 March ; 80(3): 279–286. doi:10.1016/j.biopsycho.2008.10.00.

Conflict-related activity in the caudal anterior cingulate cortex in the absence of awareness

Stefan Ursu¹, Kristi A. Clark², Howard J. Aizenstein³, V. Andrew Stenger⁴, and Cameron S. Carter^{1,5}

¹ Department of Psychiatry, University of California – Davis

² Department of Neurology, University of California at Los Angeles

³ Department of Psychiatry, University of Pittsburgh

⁴ Department of Medicine, University of Hawaii at Manoa

⁵ Department of Psychology, University of California - Davis

Abstract

The caudal anterior cingulate cortex (cACC) is thought to be involved in performance monitoring, as conflict and error-related activity frequently co-localize in this area. Recent results suggest that these effects may be differentially modulated by awareness. To clarify the role of awareness in performance monitoring by the cACC, we used rapid event-related fMRI to examine the cACC activity while subjects performed a dual task: a delayed recognition task and a serial response task (SRT) with an implicit probabilistic learning rule (i.e. the stimulus location followed a probabilistic sequence of which the subjects were unaware). Task performance confirmed that the location sequence was learned implicitly. Even though we found no evidence of awareness for the presence of the sequence, imaging data revealed increased cACC activity during correct trials which violated the sequence (high conflict), relative to trials when stimuli followed the sequence (low conflict). Errors made with awareness also activated the same brain region. These results suggest that the performance monitoring function of the cACC extends beyond detection of errors made with or without awareness, and involves detection of multiple responses even when they are outside of awareness.

Previous research has shown that the caudal anterior cingulate cortex (cACC) is preferentially active during a variety of tasks that elicit cognitive interference and/or errors. The interference effects are seen during tasks that create response conflict such as: Stroop (Bush et al., 1998; Kerns et al., 2004; MacDonald, Cohen, Stenger, & Carter, 2000; Milham & Banich, 2005; Ruff, Woodward, Laurens, & Liddle, 2001; Schroeder et al., 2002), Eriksen flanker (M. Botvinick, Nystrom, Fissell, Carter, & Cohen, 1999; van Veen, Cohen, Botvinick, Stenger, & Carter, 2001), the AX version of the continuous performance task (AX-CPT, in which subjects respond to the letter X only after it follows the letter A, see Carter et al., 1998; Carter et al., 2000; Ursu, Stenger, Shear, Jones, & Carter, 2003), Go/No-go (Garavan, Ross, Kaufman, & Stein, 2003; Garavan, Ross, Murphy, Roche, & Stein, 2002; Kopp, Mattler, Goertz, & Rist,

Correspondence should be addressed to: Stefan Ursu, M.D., Ph.D., Department of Psychiatry, and Behavioral Sciences, University of California – Davis.

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1996; Menon, Adelman, White, Glover, & Reiss, 2001), oddball, and two-alternative forced choice tasks (Braver, Barch, Gray, Molfese, & Snyder, 2001). Similar effects are also observed during individual trials in which subjects commit an error (Carter et al., 1998; Dehaene, Posner, & Tucker, 1994; Falkenstein, Hoormann, Christ, & Hohnsbein, 2000; Hester, Fassbender, & Garavan, 2004; Scheffers & Coles, 2000). These studies provided converging evidence that the cACC plays a role in regulation of behavior by monitoring performance, but the precise mechanisms underlying these presumed monitoring processes remained a matter of debate.

Earlier formulations of a monitoring role for the ACC proposed an error detecting function, implemented through a comparator function in which intended response was compared to the actual response and the ACC signaled a mismatch when this occurred (Bernstein, Scheffers, & Coles, 1995; Coles, Scheffers, & Holroyd, 2001; Falkenstein et al., 2000; Gehring, Goss, Coles, Meyer, & et al., 1993). Results supporting this hypothesis are primarily based on event-related potential (ERP) studies, in which a medial negativity (error negativity, Ne or error-related negativity, ERN) is observed within 100msec of initiating an incorrect response (for a review, see Holroyd & Coles, 2002). In these ERP studies, source localization algorithms produced results consistent with a cACC generator of the Ne. While some fMRI reports have suggested that errors engage rostral areas of the ACC (Kiehl, Liddle, & Hopfinger, 2000), a number of studies have shown error-related activity in the cACC (Carter et al., 1998; Ursu et al., 2003; van Veen et al., 2001). More recently, studies combining the use of ERP and fMRI have provided more evidence that the error-related activity observed in ERP data does indeed arise from the cACC (Garavan et al., 2002; Ullsperger & von Cramon, 2001).

Further exploration of the function of the ACC led to extending its presumed monitoring function beyond error commission. For instance, detailed analyses of correct trials in ERP experiments have revealed both response-locked and stimulus-locked negative waveforms (correct-response negativity, CRN, and the N2 component, respectively, see Bartholow et al., 2005; Kopp, Rist, & Mattler, 1996; Liotti, Woldorff, Perez, & Mayberg, 2000; Van Veen & Carter, 2002). Despite some outstanding questions regarding their precise brain generators, these signals have been proposed to reflect the presence of response conflict at various stages of a correct trial (Bartholow et al., 2005; Van Veen & Carter, 2002). Similarly, studies using fMRI have shown an increase of the signal measured in the cACC in response to correct high-conflict trials (Braver et al., 2001; Carter et al., 1998; MacDonald et al., 2000; Menon et al., 2001; Milham, Banich, Claus, & Cohen, 2003; van Veen et al., 2001). Furthermore, in several studies error and conflict-related activity have co-localized in the cACC (Carter et al., 1998; Kerns et al., 2004; Ursu et al., 2003; van Veen et al., 2001; van Veen, Holroyd, Cohen, Stenger, & Carter, 2004). These results have lent support to an alternative hypothesis regarding the cACC function, which states that rather than detecting errors specifically, this region serves a more general function in performance monitoring through the detection of conflict between simultaneously active competing responses (Botvinick, Braver, Barch, Carter, & Cohen, 2001; Carter et al., 1998; Van Veen & Carter, 2002). This theory attributes the cACC activity during errors to conflict between the executed incorrect response and activation of the correct response due to ongoing stimulus evaluation. This theory is consistent with a meta-analysis by Ridderinkhof et al. (2004), who noted a high degree of overlap between conflict and error-related medial frontal activations, but with a tendency for caudal and dorsal areas (including cACC) to activate in response conflict tasks (either via suppression of prepotent tendencies or under decision uncertainty, both of which are often associated with errors), while more ventral and rostral medial frontal areas seem to be more consistently activated in response overt feedback that subjects have committed an error.

One outstanding question regarding the performance monitoring functions of the cACC is its relationship with awareness. A few studies to date have directly manipulated the subjects' awareness for their level of performance and examined the modulation of medial frontal

activity by awareness, with contradictory results. For instance, some studies reported ERP (Nieuwenhuis, Ridderinkhof, Blom, Band, & Kok, 2001) and fMRI (Hester, Foxe, Molholm, Shpaner, & Garavan, 2005) findings consistent with error-related ACC activity being independent of awareness. In contrast, one recent fMRI study suggested that cACC detects response conflict generated by supraliminal but not by subliminal stimuli (Dehaene et al., 2003).

In order to clarify these divergent results, the current study used a variation of the serial reaction time task (SRT) (Nissen & Bullemer, 1987) with a probabilistic learning rule (Cleeremans & McClelland, 1991; Jimenez & Mendez, 2001). The main goal of this task was to create an experimental condition in which response conflict is elicited outside of the subjects' awareness, by the violation of an implicitly learned rule. In this task, a visual stimulus was presented in one of four positions, and subjects responded to the position of the stimulus with a keypress. The critical manipulation in this task was that, unbeknownst to the subject, the location of the stimuli was not random but followed a probabilistic sequence. Thus, when stimuli did not follow this probabilistic sequence, subjects were not aware of the conflict between the automatic response predicted by the implicit sequence and the correct response corresponding to the actual stimulus location. A region-of-interest based analysis was conducted in order to examine the activity to implicit conflict in a specific region of the cACC, which was previously found to show conflict-related activity during a task that elicited explicit conflict (Carter et al., 2000). This study also measured cACC activity during trials in which the subjects committed errors (with awareness), in order to better address the question of whether or not these activities co-localize in the cACC and thus link the present findings to previous explorations of errors and awareness. A full-brain exploratory analysis was also conducted in order to identify other structures with conflict and error-related activity.

Methods

Subjects

Ten subjects (7 male; mean age 24.4, range 20–32 years; right-handed) participated in this study. All participants provided written consent in accordance with the Institutional Review Board and were paid for their participation. They were screened to confirm that they had no history of major Axis I psychiatric disorders (including psychotic, mood or anxiety disorders), or any medical condition that required the use of prescription medication. After pre-processing of imaging data, one subject was excluded due to head movement in excess of one voxel dimension.

Behavioral task

While in the MRI scanner, subjects performed 8 runs of a concurrent explicit/implicit learning task. The explicit component was a dual task: a forced choice delayed recognition task, and speeded responses to the location of stimuli. This dual task was relatively demanding (especially the delayed recognition component), and its role was avoid explicit detection of the the implicit component. The latter was a variation of a SRT task that used a probabilistic learning rule (detailed below).

Each run of the task consisted of three cycles of encoding/fixation phase and a recognition phase (Figure 1). During each encoding phase, 20 stimuli (words or unfamiliar faces in each half of the task, in counterbalanced order across subjects) were presented sequentially for 2 seconds each, with no inter-trial interval (ITI), in one of four possible locations (see Figure 1). Subjects received dual task instructions: 1) they were asked to respond by pressing quickly one of four keys of a response device corresponding to the location of the stimulus, using the index and middle fingers of both hands; 2) they were also instructed that, while the location of the

stimulus was random, they should try to commit each stimulus to memory, as they would be asked later to recognize them. At the end of the run, the last 20 seconds of fixation were followed immediately by the recognition phase. During recognition, all stimuli from the previous encoding phases were presented centrally, intermixed randomly with 30 novel stimuli, for 2 seconds each, with no ITI. For this phase, the instructions were to respond with a keypress “yes or no” as to whether or not they had seen the stimulus. Each stimulus (studied or novel), was presented only once during the experiment.

The implicit component of the task coincided with the encoding phase, during which stimuli were presented in one of four possible locations (see Figure 1) and subjects were required not only to encode the stimuli but also to quickly indicate their location on the screen. Unbeknownst to the subjects, the implicit learning component consisted of the location of the stimuli, which followed a probabilistic sequence pattern determined by a Markov chain rule. For example, for a 2–4–1–3 sequence, if a stimulus appeared in position 2, there was a 70% chance that the next stimulus would appear in location 4, after a stimulus in position 4, the next stimulus appeared in 70% of cases in position 1, and so on (Figure 1B). Therefore, in 30% of the trials, the stimulus violated the location sequence by appearing in a different location than that predicted by the sequence. Thus, to the extent that implicit learning of the sequence of highly probable locations took place, trials in which stimuli violated the sequence should produce response conflict between the “expected” highly probable location and the actual stimulus location, despite lack of awareness for the existence of the sequence. In this task, learning of the implicit rule was evidenced by faster reaction times and decreased error rates on trials which followed the sequence, compared to those which violated it. Errors were defined as trials in which the subject pressed the incorrect key while responding to the location of a stimulus. A location sequence was randomly assigned for each subject from the 5 possible unique 4 item sequences obtainable with 4 possible locations (the monotonic 1–2–3–4 and 4–3–2–1 sequences were not used in order to minimize the chances of explicit detection). Each subject had one location sequence throughout the entire experiment; different rules were counterbalanced across subjects. Since the current study focused on correlates of implicit conflict, trials which follow the implicit probabilistic sequence were labeled NC (non-conflict trials), while trials that deviated from the sequence were labeled C (conflict trials). The presence of the concurrent explicit encoding/recognition task and the probabilistic nature of the implicit sequence minimized the likelihood that subjects would become aware of the presence of the sequence. Subjects were also debriefed at the end of the study by asking them if they noticed any repeating pattern in the location of the stimuli. If they indicated that a pattern was noticed, they were asked to guess what the pattern was.

Two types of stimuli were included (faces and words), to collect pilot data for future analyses regarding any modulation of implicit learning processes by stimulus type, that will be explored in a different study. The 360 black-and-white faces used as stimuli were created with Faces™ 3.0 software using the random face generator and then edited to make the faces more realistic, i.e. adding shadows. Word stimuli were selected by choosing 360 words rated 90% or more as nouns in normative data (Friendly, Franklin, Hoffman, & Rubin, 1982). For each subject, each half of the experiment included only words or faces (i.e. 4 runs of each, in counterbalanced order across subjects). Stimulus presentation and response recording were done using a Power Macintosh computer, with the PsyScope software package (Cohen, MacWhinney, Flatt, & Provost, 1993).

Behavioral effects were estimated in terms of: mean reaction times and accuracy of responding to the location of the stimuli (encoding phase), and accuracy of recognition (i.e. previously seen stimulus = “yes”, novel stimulus = “no”), during the recognition phase. Effects were tested using ANOVA and planned two-tailed paired t-tests.

fMRI image acquisition and statistical analysis

MRI scanning was conducted on a 1.5 Tesla General Electric Signa Scanner. T2*-weighted functional volumes, collected using a one-shot spiral pulse sequence with TE=35msec, TR=2000msec, consisted of 26 contiguous oblique axial slices 3.8mm thick, acquired parallel to the anterior commissure – posterior commissure plane ($64 \times 64 \times 26$ voxel matrix, $3.75\text{mm} \times 3.75\text{mm} \times 3.8\text{mm}$ dimensions, FOV = 240mm). High resolution SPGR and co-planar T1-weighted images were also collected from each subject for use in spatial normalization of data.

Each subject's functional data was motion corrected with a rigid-body 6-parameter model (Woods, Grafton, Holmes, Cherry, & Mazziotta, 1998), baseline corrected (to remove trends across runs), filtered with a high-pass filter with frequency 0.025Hz, and outlier corrected. Averaging of data across subjects was done after mapping of the region of interest (ROI) onto the individual data in the case of confirmatory, ROI-based analyses, or of the individual data onto the MNI reference brain (Evans et al., 1993) in the case of exploratory, omnibus tests. These transformations used a second-order (30 parameters) nonlinear warping algorithm (Woods, Grafton, Watson, Sicotte, & Mazziotta, 1998). In the case of omnibus analyses, data were also spatially smoothed with a 3D Gaussian kernel (FWHM 8mm).

All imaging analyses were conducted using the functional volumes collected during the encoding/fixation cycles. Since behavioral effects of interest were qualitatively similar for both stimulus types (see Behavioral Results below), all imaging analyses reported here were conducted collapsed across stimulus type. For the analysis of response conflict, the effect of errors was excluded. When contrasting errors to correct trials, given the total number of trials (360) and the accuracy rates of subjects (see Results), the minimum number of observations per condition in this analysis was approximately 18 per subject. The region of interest (ROI) analysis focused on an anterior cingulate region which in a previous study displayed conflict-related activity during a Stroop task (Carter et al., 2000). The ROI, shown in Figure 2, included 17 voxels and peaked at Talairach coordinates (0, 15, 40).

Timeseries for each trial type were estimated over the 12 seconds following stimulus presentation, and were computed as a percent signal change in the 5 scans following the scan that included the stimulus. The current stimulus onset asynchrony (SOA) of 2 seconds was chosen to optimize the learning component of the task. Since this relatively rapid stimulus sequence is susceptible to non-linear summations of partly overlapping BOLD signals elicited by each stimulus, trial types were randomized and the data were analyzed using a selective averaging strategy (Burock, Buckner, Woldorff, Rosen, & Dale, 1998). Compared to deconvolution analyses using canonical hemodynamic response functions (HRF), this method makes fewer assumptions regarding the shape of partly overlapping BOLD responses to rapidly sequencing stimuli. Within each subject, for each condition (conflict, non-conflict, error, correct), the median signal change at each scan was computed, and different stimulus histories were separately averaged and weighted to account for their relative frequency. Finally, significance and directionality of condition differences were confirmed by conducting a planned paired t-test of the peak signal change between the conditions of interest (C vs. NC, error vs. correct).

An exploratory analysis was also conducted in order to explore other brain regions that showed condition differences. This analysis used a general linear model (GLM) with four covariates (errors, no responses, correct C trials and correct NC trials), and deconvolved the signal over the seven scans following each stimulus in order to generate an impulse-response function for each condition (Cox, 1996). This method was used in order to obviate the need for assumptions regarding the shape of the hemodynamic response function. In order to define an ROI, a random effects repeated measures ANOVA was conducted on the estimated coefficients for conditions of interest (e.g. C vs. NC trials, errors vs. correct trials), and the resulting condition by scan

interaction statistical map was thresholded at an alpha level of 0.05 corrected for multiple comparisons using the utility AlphaSim (Cox, 1996), after intersection with a gray matter mask (Zhang, Brady, & Smith, 2001). In each activated cluster of voxels identified by this analysis, the pattern of activity was examined by computing and testing of selectively averaged timeseries, in the same manner as for the region-based analysis.

Results

Behavioral effects

Behavioral analyses were conducted in order to explore: 1) subjects' performance on the explicit components of the task, including responding to the location of the stimuli, encoding and subsequently discriminating familiar from novel ones during the recognition test; 2) any evidence of learning the implicit probabilistic sequence, and lack of awareness of its presence; and 3) any effects of the explicit aspects of the task on implicit conflict.

Explicit components—As expected, trials in which subjects failed to respond to the location of the stimulus during encoding were extremely rare. To verify that subjects had encoded and accurately recognized the stimuli, we conducted a d' analysis of the recognition performance. This measure (also known as sensitivity index), is derived from signal detection theory and reflects the separation between the means of the signal and noise distributions, in units of the standard deviation of the noise distribution. Thus, it reflects the degree to which an observer correctly discriminates target stimuli (in our case familiar stimuli), from foils (i.e. novel stimuli). The d' is estimated using the formula: $d' = z(\text{hit rate}) - z(\text{false alarm rate})$, where function $z()$ is the cumulative distribution function rate of a gaussian probability distribution. This measure has the advantage of being independent of response biases, with nonzero positive values being indicative of efficient discrimination of signal from noise. The overall d' for the recognition performance in our experiment was 0.56, and the t-test of the subjects' d' values against 0 was significant ($p < 0.001$), indicating that subjects performed the explicit encoding/recognition task as instructed.

Implicit learning—Effects of implicit learning of the location sequence were explored by examining the subjects' performance of responding to the stimulus location, depending on whether the stimulus followed or not the location sequence. Less accurate responding was noted to sequence violations (conflict trials, C) relative to trials following the probabilistic sequence (non-conflict, NC): C vs. NC trials accuracy was 97.7% and 94.5% respectively ($t = 5.06$, $df = 8$, $p = 0.001$, paired t-test). The subjects' RTs were also significantly slower ($t = 7.53$, $df = 8$, $p < 0.001$, paired t-test) in C trials (501.9 ms) relative to NC trials (451.4 ms). When examined individually, these effects on reaction time and accuracy rates were seen in 8 of the 9 subjects. Implicit learning effects were also explored over time, by conducting a Trial type (C vs NC) $\times$ Run ANOVA of the reaction time, which showed no significant main effect of run or condition by run interaction ($p = 0.91$ and 0.22 , respectively). Therefore, in order to provide additional evidence that learning had occurred, paired t-tests were computed between C and NC trials in each run; 6 out of 8 runs showed significant learning effects (six runs $C > NC$, $0.05 < p < 0.001$; one run $p < 0.06$, one run NS).

To verify that subjects did not become explicitly aware of the sequence rule, they were debriefed at the end of the imaging experiment, by asking them if they noticed any repeating pattern in the location of the stimuli, and if yes, what the pattern was. None of them reported noticing any pattern in the location of the stimuli, and most subjects attributed this lack of knowledge to the demanding nature of the concurrent explicit task. Furthermore, in order to confirm that the implicit aspect of this task (the presence of probabilistic sequence of stimulus locations in the encoding phase) remains outside of awareness, we also conducted a behavioral

run of the experiment outside the scanner in a different sample of 19 subjects with similar demographic characteristics (mean age 25.15, range 19–30, same exclusion criteria). This experiment also included two formal tests of the subjects' conscious recognition of the location sequence, administered immediately after completion of the task, following closely the procedures used in previous studies of implicit learning in serial reaction time tasks (Curran, 1997). The first test presented subjects with five possible choices of sequences in which locations of stimuli were represented symbolically (e.g. 1–3–2–4, where 1 = leftmost location, 2 = second location from left, etc). One of these sequences was the sequence that was actually presented to the subject, and four were foil sequences. Subjects were asked to rate, on a scale of 0 to 100, their confidence that any sequence of stimuli was repeated during the experiment (0 = "sure it was not repeated", 50 = "not sure", 100 = "sure it was repeated"). A second multiple choice recognition test was then administered, this time probing the subjects' recognition of two-item fragments of the pattern. This was done by presenting subjects four 2-item fragments from the sequence together with four foil fragments, and asking them to rate them in the same way. Then, 4 recognition indices (RI) were computed, two from the whole-sequence test and 2 from the sequence fragments test, as follows: 1) RI of the target sequence (equal to the rating for the target sequence in the whole sequence test); 2) RI of the foil sequences (the average ratings for the foil sequences in the whole-sequence test); 3) RI of the sequence fragments (the average rating for the 4 sequence fragments); and 4) RI of the foil fragments (the average rating for the foil fragments).

Task performance in this experiment was similar to that in the imaging experiment: good delayed recognition (confirming that subjects were engaged in performing the explicit encoding/recognition task), and faster RT ($p = 0.028$) and higher accuracy of responding ($p = 0.02$) to stimuli which followed the probabilistic sequence compared to those which violated it (suggesting that learning of the implicit sequence of locations had occurred). However, no evidence was found that subjects had been aware of the presence of a location sequence. This was examined by conducting two paired *t* tests, one comparing the recognition indices obtained from the whole-sequence test (RI sequence vs. RI foil sequence), and the other comparing the recognition indices obtained from the sequence fragments test (RI sequence fragments vs. RI foil fragments). Neither test approached statistical significance ($p = 0.91$ and 0.33 respectively), thus increasing the confidence in the self-report data from the fMRI experiment.

Modulation of implicit conflict by other factors—Because two types of stimuli (words and faces) were used, we examined the interactions between stimulus type and implicit conflict (C or NC), and found no significant effects in either the reaction time or accuracy data ($p = 0.40$ and 0.06 , respectively). This analysis confirmed that behavioral effects of both stimulus types were qualitatively similar, and that the trend for interaction of stimulus type and implicit conflict reflected only that the accuracy effect was nominally larger for face stimuli than for words. Thus, all imaging analyses reported here were conducted collapsed across stimulus type. Furthermore, possible interactions between the implicit and explicit components were explored, by classifying the trials based on the implicit component (C vs. NC) as well as depending on performance on the subsequent recognition phase of the explicit task: correctly recalled (CR) or incorrectly recalled (IR). A two-way ANOVA showed no evidence of implicit conflict (C vs NC) by explicit recognition (CR vs IR) interactions in either the reaction time data ($p = 0.314$) or the accuracy rates ($p = 0.309$).

Imaging results

Region-Based Analysis—In the region-based analysis of correct trials, the activity of the pre-defined cACC ROI (Figure 2) showed increased activity in conflict trials (C trials, sequence violations, $p < 0.02$, paired *t*-test, Figure 2A). The same region also showed increased activity

in trials where subjects made an error vs. those trials that were performed correctly ($p < 0.004$ paired t-test, Figure 2B).

Exploratory Analysis—The multiple regression analysis (Table 1) isolated an area of increased activation to C relative to NC trials in a location (Talairach coordinates $-7, 11, 44$) similar to the one used in the region-based analysis (Talairach coordinates $0, 15, 40$). Other regions that showed increased activity to the C trials were: right precentral gyrus, another caudal anterior cingulate region, medial frontal/rostral cingulate, posterior cingulate, right inferior parietal, right dorsolateral prefrontal cortex, left insula, left and right angular gyrus/middle temporal. Regions that showed increased activity to the NC trials over the C trials were: left supplementary motor area, a more rostral anterior cingulate particle, and left postcentral gyrus (Table 1).

Similarly, the multiple regression analysis of errors vs correct responses identified significant activations in two areas of the anterior cingulate. One area, including 34 voxels with peak at Talairach coordinates $0, 1, 44$, neared both the confirmatory ROI and the cACC region isolated by the exploratory analysis of implicit conflict-related activity. In order to explore the degree of overlap between error detection and conflict detection, we examined the effects of correct responses to stimuli which violated (C trials) or followed (NC trials) the probabilistic sequence of locations. This analysis showed that, in this cluster of voxels, C trials elicited stronger activity relative to NC trials ($p = 0.054$, paired t test). A second, smaller ACC activation (9 voxels, peak at $-3, 35, 8$) was located more rostrally and extended into the medial frontal cortex (BA 10). Other brain regions with increased activity to error trials included: right angular gyrus, bilateral cuneus, bilateral visual cortex, bilateral insula, right parahippocampus, bilateral superior parietal lobe, bilateral precentral and postcentral gyri, supplementary motor cortex, medial frontal/rostral cingulate, right fusiform.

Discussion

In this study, evidence was provided that a region of the cACC shown to respond to correct high-conflict trials in a Stroop task (Carter et al., 2000) also reacts to trials in which response conflict develops in the absence of awareness, through violations of a pattern of stimulus locations implicitly learned in a serial reaction time task. The cortical activation to implicit conflict also co-localized with activity in response to errors that could be explicitly perceived, replicating findings by (Carter et al., 1998; Kerns et al., 2004; Ursu et al., 2003; van Veen et al., 2001; van Veen et al., 2004).

The current results are in agreement with those of Nieuwenhuis et al. (2001) and Hester et al. (2005), who used an antisaccade task and a response suppression task, respectively, to examine error-related neural signals in the absence of awareness for errors. The Ne (in the former study) and fMRI activation (in the latter) were observed in response to errors made with and without awareness (while another error-related ERP component, the Pe, was observed only during conscious errors). Similarly, in a PET study, Berns et al. (1997) used an implicit sequence learning task and detected increased cACC activity whenever the grammar of the learning rule changed, which can be interpreted as a conflict-generating condition somewhat analogous to the implicit sequence violations present in the current task. Our findings of co-localized activity to implicit conflict and explicit errors, help complete the link between error activity (explicit in our case, implicit in the studies of Nieuwenhuis et al. and Hester et al.), and conflict-related activity. Furthermore, our novel findings that response conflict signals elicited in the absence of awareness suggests that the Ne waveform detected in ERP studies and the cACC activity reflected in fMRI and PET signals are generated when multiple representations of incompatible responses are present, and that such signals are generated regardless of whether the subjects are aware or not of the conflict between these incompatible responses. This provides a

parsimonious mechanism for the role of the ACC in performance monitoring, that does not require information as to which of the competing responses is the correct one, as has been the case with comparator mechanisms (Coles et al., 2001; Falkenstein et al., 2000).

Our results are consistent with the model of the ACC role in performance monitoring, put forth by Ridderinkhof et al (2004). In their meta-analysis, Ridderinkhof and colleagues point out that there is considerable overlap between conflict and error-related medial frontal activations (though errors and error feedback tend to activate more dorsal and rostral areas than response conflict elicited by suppression of prepotent tendencies per se or by decision uncertainty). Indeed, in our exploratory analysis errors were associated with activations overlapping with our a-priori region of interest, as well as with more rostral and dorsal foci. Ridderinkhof et al also reviewed evidence that the performance monitoring functions implemented by the medial frontal cortex may serve as reinforcement learning signals for associative learning (Holroyd & Coles, 2002), thus extending their role to optimization of task performance triggered by monitoring signals. Indeed, the response conflict condition created by our task can be interpreted in the context of reinforcement learning theory, as a result of the presentation of a stimulus at a location which is “unexpected” relative to that that had been repeatedly reinforced by correct performance. More recently, Brown & Braver (2005) proposed a model in which the activity of the ACC should be proportional to the likelihood of performance errors. Since the latter model does not require that expectations are explicitly perceived, and the former does not distinguish between computations of error likelihood occurring with or without awareness, our findings are consistent with both models. Thus, future studies will have to directly test predictions generated by monitoring of implicit conflict versus those generated by implicit computation of error likelihood or by reinforcement learning. It should also be noted that behavioral indices on violations of the location sequence were similar to those seen, in general, in difficult trials requiring effortful performance (i.e. longer RT, decreased accuracy). Given the lack of evidence for awareness of the location sequence and its violations, it is unlikely that subjects subjectively perceived their performance on sequence violations as more effortful than on sequence trials. Thus, future experiments will have to directly examine the question whether the ACC response is dependent only on the conflict between co-activated incompatible responses, or whether it also tracks the perceived “effort” required to resolve such conflict (Critchley et al., 2003).

Interestingly, the results of this experiment are in contrast with those of Dehaene et al. (2003), in which subjects were scanned while performing a priming task in which masked and unmasked primes were either response-congruent or response-incongruent with subsequent targets. Prime congruency (i.e. the “conflict” between primes and targets) modulated the ACC activity only when the primes were unmasked, thus presumably available for conscious processing. These results were interpreted as evidence that the conflict-related ACC activity reflects conscious experience of response conflict. However, a close examination of the imaging results shows that the differential effect of conflict with masked vs unmasked primes was in fact due to robust ACC activity to *both* incongruent and congruent masked trials. This is actually not surprising, if one considers that the duration of masked primes used in that study (43ms) was significantly above the thresholds commonly used in subliminal presentations, around 20ms or less (Dehaene et al., 1998). Thus, masked primes could be perceived with some effort, as clearly indicated in a subsequent test of prime detection, when subjects detected the masked primes above chance level. Consequently, in these “subliminal” (i.e. masked) trials, the presence of partially perceived primes is likely to have introduced perceptual uncertainty and thus incorrect identification of at least some of the congruent primes, leading to co-activation activation of both possible responses, and thus increased conflict. In other words, presentation of primes that can be partially perceived consciously should lead to ambiguity as to what the correct response is, thus generating response conflict regardless whether the following target is congruent or not. Therefore, the partially perceived masked primes should

lead to some degree of response conflict even when they are congruent with the subsequent target, and consequently an artificial lack of difference in ACC activity between implicit congruent and incongruent trials. This account is consistent with previous fMRI results from tasks using degraded stimuli, which have been shown to be associated with increased activity in the ACC (Barch et al., 1997). Thus, the possibility that the ACC monitors implicit conflict had been, in fact, left open.

Another intriguing aspect of our results is the identification of activations related to implicit conflict in areas typically associated with conscious cognitive control (e.g. DLPFC) or conscious spatial attention (e.g. parietal cortex). These findings may contribute to expanding existing cognitive models which posit the dependence of active control processes on awareness. The assumption that active control is dependent on awareness has been recently questioned by a few studies reporting that fMRI activations (Lau & Passingham, 2007; Pessiglione et al., 2007) or ERP indices (van Gaal, Ridderinkhof, Fahrenfort, Scholte, & Lamme, 2008) of active cognitive control may be triggered in the absence of awareness. Therefore, further research is necessary to precisely characterize the relationship between consciousness, active cognitive control and their neural substrates.

A potential limitation of this study is its relatively small sample size. While this remains to be addressed definitively by independent replication of these results, the additional exploratory analysis provided good support for the robustness of the effects reported here. As such, the latter analysis identified a cluster of activation in the cACC that showed increased activity to conflict trials and a second area showing increased activity to error trials, both of which were in close vicinity to, and partly overlapping with the ROI used in the confirmatory analysis (see Table 1 and Figure 2). Furthermore, the exploratory analysis (Table 1) also isolated other regions more active during C trials than NC trials, such as Brodmann areas (BA) 10/46 and left superior temporal gyrus (BA 39), consistent with results obtained using a spatial conflict task by Fan, Flombaum, McCandliss, Thomas, & Posner (2003).

Interestingly, the current SRT task did not reveal a typical learning curve in the implicit reaction time effect (i.e. the learning effect was not clearly stronger in later blocks of trials, as evidenced by the interaction between trial type and run which was not significant). However, in the current task a monotonic effect of time may not necessarily be expected. Previous variants of SRT tasks used runs of “random” trials (i.e. sequence absent) interspersed with runs of “learning” trials (i.e. sequence present). In contrast, in our task the random trials were interspersed with learning trials in order to facilitate true event-related analyses of the fMRI data, and to minimize the chance that subjects would become aware of the sequence, a frequent behavioral effect noted in traditional SRT tasks. Nevertheless, despite the lack of a smooth learning curve, all other behavioral analyses suggested that significant learning effects of the implicit probabilistic sequence were present.

In conclusion, the findings of co-localized cACC activation both during violations of an implicitly learned sequence and error commission, support the hypothesis that this cortical region has a role in monitoring for the presence of response conflict, even in the absence of awareness. Future research is necessary to investigate the generality of these results, by examining the cACC’s response to implicit conflict at different levels of task representation such as stimulus conflict, and to better characterize the nature of active control processes that may be engaged in the absence of conscious awareness in response to detection of conflict.

Acknowledgements

Authors thank Shauna Gordon-McKeon and Paul Deramo for help with behavioral data collection and analysis.

References

- Barch DM, Braver TS, Nystrom LE, Forman SD, Noll DC, Cohen JD. Dissociating working memory from task difficulty in human prefrontal cortex. *Neuropsychologia* 1997;35(10):1373–1380. [PubMed: 9347483]
- Bartholow BD, Pearson MA, Dickter CL, Sher KJ, Fabiani M, Gratton G. Strategic control and medial frontal negativity: beyond errors and response conflict. *Psychophysiology* 2005;42(1):33–42. [PubMed: 15720579]
- Berns GS, Cohen JD, Mintun MA. Brain regions responsive to novelty in the absence of awareness. *Science* 1997;276(5316):1272–1275. [PubMed: 9157889]
- Bernstein PS, Scheffers MK, Coles MG. “Where did I go wrong?” A psychophysiological analysis of error detection. *J Exp Psychol Hum Percept Perform* 1995;21(6):1312–1322. [PubMed: 7490583]
- Botvinick M, Nystrom LE, Fissell K, Carter CS, Cohen JD. Conflict monitoring versus selection-for-action in anterior cingulate cortex. *Nature* 1999;402(6758):179–181. [PubMed: 10647008]
- Botvinick MM, Braver TS, Barch DM, Carter CS, Cohen JD. Conflict monitoring and cognitive control. *Psychol Rev* 2001;108(3):624–652. [PubMed: 11488380]
- Braver TS, Barch DM, Gray JR, Molfese DL, Snyder A. Anterior cingulate cortex and response conflict: effects of frequency, inhibition and errors. *Cereb Cortex* 2001;11(9):825–836. [PubMed: 11532888]
- Brown JW, Braver TS. Learned predictions of error likelihood in the anterior cingulate cortex. *Science* 2005;307(5712):1118–1121. [PubMed: 15718473]
- Burock MA, Buckner RL, Woldorff MG, Rosen BR, Dale AM. Randomized event-related experimental designs allow for extremely rapid presentation rates using functional MRI. *Neuroreport* 1998;9(16):3735–3739. [PubMed: 9858388]
- Bush G, Whalen PJ, Rosen BR, Jenike MA, McInerney SC, Rauch SL. The counting Stroop: an interference task specialized for functional neuroimaging--validation study with functional MRI. *Hum Brain Mapp* 1998;6(4):270–282. [PubMed: 9704265]
- Carter CS, Braver TS, Barch DM, Botvinick MM, Noll D, Cohen JD. Anterior cingulate cortex, error detection, and the online monitoring of performance. *Science* 1998;280(5364):747–749. [PubMed: 9563953]
- Carter CS, Macdonald AM, Botvinick M, Ross LL, Stenger VA, Noll D, et al. Parsing executive processes: strategic vs. evaluative functions of the anterior cingulate cortex. *Proceedings of the National Academy of Sciences of the United States of America* 2000;97(4):1944–1948. [PubMed: 10677559]
- Cleeremans A, McClelland JL. Learning the structure of event sequences. *J Exp Psychol Gen* 1991;120(3):235–253. [PubMed: 1836490]
- Cohen JD, MacWhinney B, Flatt M, Provost J. PsyScope: An interactive graphic system for designing and controlling experiments in the psychology laboratory using Macintosh computers. *Behavior Research Methods, Instruments & Computers* 1993;25(2):257–271.
- Coles MG, Scheffers MK, Holroyd CB. Why is there an ERN/Ne on correct trials? Response representations, stimulus-related components, and the theory of error-processing. *Biol Psychol* 2001;56(3):173–189. [PubMed: 11399349]
- Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 1996;29(3):162–173. [PubMed: 8812068]
- Critchley HD, Mathias CJ, Josephs O, O’Doherty J, Zanini S, Dewar BK, et al. Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain* 2003;126(Pt 10):2139–2152. [PubMed: 12821513]
- Curran T. Higher-order associative learning in amnesia: Evidence from the serial reaction time task. *Journal of Cognitive Neuroscience* 1997;9(4):522–533.
- Dehaene S, Artiges E, Naccache L, Martelli C, Viard A, Schurhoff F, et al. Conscious and subliminal conflicts in normal subjects and patients with schizophrenia: the role of the anterior cingulate. *Proc Natl Acad Sci U S A* 2003;100(23):13722–13727. [PubMed: 14597698]
- Dehaene S, Naccache L, Le Clec HG, Koechlin E, Mueller M, Dehaene-Lambertz G, et al. Imaging unconscious semantic priming. *Nature* 1998;395(6702):597–600. [PubMed: 9783584]

- Dehaene S, Posner MI, Tucker DM. Localization of a neural system for error detection and compensation. *Psychological Science* 1994;5(5):303–305.
- Evans, AC.; Collins, DL.; Mills, SR.; Brown, ED.; Kelly, RL.; Peters, TM. 3D statistical neuroanatomical models from 305 MRI volumes. Paper presented at the Nuclear Science Symposium and Medical Imaging Conference, 1993., 1993 IEEE Conference Record; 1993.
- Falkenstein M, Hoormann J, Christ S, Hohnsbein J. ERP components on reaction errors and their functional significance: a tutorial. *Biol Psychol* 2000;51(2–3):87–107. [PubMed: 10686361]
- Fan J, Flombaum JI, McCandliss BD, Thomas KM, Posner MI. Cognitive and brain consequences of conflict. *Neuroimage* 2003;18(1):42–57. [PubMed: 12507442]
- Friendly M, Franklin PE, Hoffman D, Rubin DC. The Toronto Word Pool: Norms for imagery, concreteness, orthographic variables, and grammatical usage for 1,080 words. *Behavior Research Methods & Instrumentation* 1982;14(4):375–399.
- Garavan H, Ross TJ, Kaufman J, Stein EA. A midline dissociation between error-processing and response-conflict monitoring. *Neuroimage* 2003;20(2):1132–1139. [PubMed: 14568482]
- Garavan H, Ross TJ, Murphy K, Roche RA, Stein EA. Dissociable executive functions in the dynamic control of behavior: inhibition, error detection, and correction. *Neuroimage* 2002;17(4):1820–1829. [PubMed: 12498755]
- Gehring WJ, Goss B, Coles MG, Meyer DE, et al. A neural system for error detection and compensation. *Psychological Science* 1993;4(6):385–390.
- Hester R, Fassbender C, Garavan H. Individual differences in error processing: a review and reanalysis of three event-related fMRI studies using the GO/NOGO task. *Cereb Cortex* 2004;14(9):986–994. [PubMed: 15115734]
- Hester R, Foxe JJ, Molholm S, Shpaner M, Garavan H. Neural mechanisms involved in error processing: a comparison of errors made with and without awareness. *Neuroimage* 2005;27(3):602–608. [PubMed: 16024258]
- Holroyd CB, Coles MG. The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. *Psychol Rev* 2002;109(4):679–709. [PubMed: 12374324]
- Jimenez L, Mendez C. Implicit sequence learning with competing explicit cues. *Q J Exp Psychol A* 2001;54(2):345–369. [PubMed: 11394051]
- Kerns JG, Cohen JD, MacDonald AW 3rd, Cho RY, Stenger VA, Carter CS. Anterior cingulate conflict monitoring and adjustments in control. *Science* 2004;303(5660):1023–1026. [PubMed: 14963333]
- Kiehl KA, Liddle PF, Hopfinger JB. Error processing and the rostral anterior cingulate: an event-related fMRI study. *Psychophysiology* 2000;37(2):216–223. [PubMed: 10731771]
- Kopp B, Mattler U, Goertz R, Rist F. N2, P3 and the lateralized readiness potential in a nogo task involving selective response priming. *Electroencephalogr Clin Neurophysiol* 1996;99(1):19–27. [PubMed: 8758967]
- Kopp B, Rist F, Mattler U. N200 in the flanker task as a neurobehavioral tool for investigating executive control. *Psychophysiology* 1996;33(3):282–294. [PubMed: 8936397]
- Lau HC, Passingham RE. Unconscious activation of the cognitive control system in the human prefrontal cortex. *J Neurosci* 2007;27(21):5805–5811. [PubMed: 17522324]
- Liotti M, Woldorff MG, Perez R, Mayberg HS. An ERP study of the temporal course of the Stroop color-word interference effect. *Neuropsychologia* 2000;38(5):701–711. [PubMed: 10689046]
- MacDonald AW 3rd, Cohen JD, Stenger VA, Carter CS. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. *Science* 2000;288(5472):1835–1838. [PubMed: 10846167]
- Menon V, Adelman NE, White CD, Glover GH, Reiss AL. Error-related brain activation during a Go/NoGo response inhibition task. *Human Brain Mapping* 2001;12(3):131–143. [PubMed: 11170305]
- Milham MP, Banich MT. Anterior cingulate cortex: an fMRI analysis of conflict specificity and functional differentiation. *Hum Brain Mapp* 2005;25(3):328–335. [PubMed: 15834861]
- Milham MP, Banich MT, Claus ED, Cohen NJ. Practice-related effects demonstrate complementary roles of anterior cingulate and prefrontal cortices in attentional control. *Neuroimage* 2003;18(2):483–493. [PubMed: 12595201]

- Nieuwenhuis S, Ridderinkhof KR, Blom J, Band GP, Kok A. Error-related brain potentials are differentially related to awareness of response errors: evidence from an antisaccade task. *Psychophysiology* 2001;38(5):752–760. [PubMed: 11577898]
- Nissen MJ, Bullemer P. Attentional requirements of learning: Evidence from performance measures. *Cognitive Psychology* 1987;19(1):1–32.
- Pessiglione M, Schmidt L, Draganski B, Kalisch R, Lau H, Dolan RJ, et al. How the brain translates money into force: a neuroimaging study of subliminal motivation. *Science* 2007;316(5826):904–906. [PubMed: 17431137]
- Ridderinkhof KR, Ullsperger M, Crone EA, Nieuwenhuis S. The role of the medial frontal cortex in cognitive control. *Science* 2004;306(5695):443–447. [PubMed: 15486290]
- Ruff CC, Woodward TS, Laurens KR, Liddle PF. The role of the anterior cingulate cortex in conflict processing: evidence from reverse stroop interference. *Neuroimage* 2001;14(5):1150–1158. [PubMed: 11697946]
- Scheffers MK, Coles MG. Performance monitoring in a confusing world: error-related brain activity, judgments of response accuracy, and types of errors. *J Exp Psychol Hum Percept Perform* 2000;26(1):141–151. [PubMed: 10696610]
- Schroeder U, Kuehler A, Haslinger B, Erhard P, Fogel W, Tronnier VM, et al. Subthalamic nucleus stimulation affects striato-anterior cingulate cortex circuit in a response conflict task: a PET study. *Brain* 2002;125(Pt 9):1995–2004. [PubMed: 12183345]
- Ullsperger M, von Cramon DY. Subprocesses of performance monitoring: a dissociation of error processing and response competition revealed by event-related fMRI and ERPs. *Neuroimage* 2001;14(6):1387–1401. [PubMed: 11707094]
- Ursu S, Stenger VA, Shear MK, Jones MR, Carter CS. Overactive action monitoring in obsessive-compulsive disorder: evidence from functional magnetic resonance imaging. *Psychol Sci* 2003;14(4):347–353. [PubMed: 12807408]
- van Gaal S, Ridderinkhof KR, Fahrenfort JJ, Scholte HS, Lamme VAF. Frontal Cortex Mediates Unconsciously Triggered Inhibitory Control. *J Neurosci* 2008;28(32):8053–8062. [PubMed: 18685030]
- Van Veen V, Carter CS. The timing of action-monitoring processes in the anterior cingulate cortex. *J Cogn Neurosci* 2002;14(4):593–602. [PubMed: 12126500]
- van Veen V, Cohen JD, Botvinick MM, Stenger VA, Carter CS. Anterior cingulate cortex, conflict monitoring, and levels of processing. *Neuroimage* 2001;14(6):1302–1308. [PubMed: 11707086]
- van Veen V, Holroyd CB, Cohen JD, Stenger VA, Carter CS. Errors without conflict: implications for performance monitoring theories of anterior cingulate cortex. *Brain Cogn* 2004;56(2):267–276. [PubMed: 15518940]
- Woods RP, Grafton ST, Holmes CJ, Cherry SR, Mazziotta JC. Automated image registration: I. General methods and intrasubject, intramodality validation. *Journal of Computer Assisted Tomography* 1998;22(1):139–152. [PubMed: 9448779]
- Woods RP, Grafton ST, Watson JD, Sicotte NL, Mazziotta JC. Automated image registration: II. Intersubject validation of linear and nonlinear models. *J Comput Assist Tomogr* 1998;22(1):153–165. [PubMed: 9448780]
- Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imaging* 2001;20(1):45–57. [PubMed: 11293691]

Figure 1.

A) General timeline of one a run, which included 3 encoding/fixation cycles followed by the recognition test. Five of the stimuli of an encoding phase and of the recognition test are also symbolically illustrated. B) Example of probabilistic sequence of stimulus locations, in this case 2–4–1–3 sequence (when a stimulus appeared in the 2nd position from the left, in 70% of trials the next stimulus appeared in the 4th location, and so on. In this example, the fourth stimulus presentation constitutes a high conflict trial, as it violates the probabilistic sequence of stimulus locations.

Figure 2.

Imaging analysis results. The area of the caudal anterior cingulate cortex (Talairach coordinates of the peak: 0, 15, 40) used in the region of interest analysis. A) This caudal anterior cingulate cortex (cACC) ROI showed increased activity when subjects responded correctly to stimuli which violated the probabilistic sequence (conflict trials, C), compared to stimuli which followed the sequence (no conflict, NC), even though subjects were unaware of the existence of the sequence. B) The same cortical ROI also showed increased activity during incorrect responses to location.

Table 1
Results of the whole-brain analysis of implicit conflict effects

Region	Brodmann's area	MNI Coord.	Talairach Coord.	Volume (nr. voxels)
Conflict > No conflict (C > NC)				
R DLPFC	BA 46	(41, 26, 30)	(40.5, 26.5, 26.5)	8
L. Inferior frontal gyrus/ insula	BA 45/47	(-43, 30, 6)	(-42.5, 29.5, 4)	19
Caudal Anterior Cingulate	BA 32	(-7, 9, 49)	(-7, 11, 44)	7
Caudal Anterior Cingulate	BA 24	(-4, 4, 34)	(-4, 5.5, 31)	7
Medial prefrontal	BA 10/32	(-7, 43, 8)	(-7, 42, 5)	25
R. Precentral Gyrus	BA 6	(32, -9, 62)	(32, -6, 57)	24
Posterior Cingulate	BA 30	(5, -55, 26)	(5, -52, 27)	18
R. Inferior Parietal	BA 40	(52, -35, 46)	(51.5, -32, 44)	16
L. Angular Gyrus/Middle Temporal Gyrus	BA 39	(-44, -65, 28)	(-43.5, -61.5, 29)	19
R. Angular Gyrus/Middle Temporal Gyrus	BA 39	(55, -65, 26)	(54.5, -62, 27)	27
No conflict > Conflict (NC > C)				
L. SMA	BA 6	(-4, -2, 68)	(-4, 1, 62.5)	8
Medial prefrontal	BA 9/32	(9, 32, 32)	(9, 35, 28)	16
L. Postcentral Gyrus	BA 43	(-46, -14, 22)	(-45.5, -12.5, 21)	7

Coordinates of the peak voxels were converted from MNI to Talairach space using the algorithm available at <http://www.mrc-cbu.cam.ac.uk/Imaging/mnispac.htm>